EFFECT OF REDOX CONDITIONS ON THE FATE OF EMERGING ORGANIC MICROPOLLUTANTS DURING ARTIFICIAL RECHARGE OF GROUNDWATER: BATCH EXPERIMENTS

Ph.D. Thesis

Hydrogeology Group (GHS)

Dept. Geotechnical Engineering and Geosciences, Universitat Politecnica de Catalunya, UPC-BarcelonaTech

Institute of Environmental Assessment and Water Research (IDAEA), Spanish Research Council (CSIC)

Author:

Manuela Barbieri

Supervisors:

Dr. Jesús Carrera

Dr. Xavier Sànchez-Vila

Dr. Carlos Ayora

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alla vita, shuk pacha kaypak kan

Abstract

Artificial recharge of groundwater, consisting in infiltrating water into aquifers by means of properly designed facilities, represents an important tool in water resources management. Beside its quantitative benefits (augmentation of groundwater resources, long term underground storage, etc.), a great interest for this technique is related to the natural treatment provided to water by subsurface passage. The processes occurring in the soil-aquifer system (filtration, sorption, mixing, redox reactions, biodegradation, etc.) have indeed proven to yield an overall improvement of water quality, removing effectively also a number of organic contaminants. At present time, the issue is to understand whether emerging organic micropollutants, i.e. pharmaceuticals, personal care products, hormones, illicit drugs, pesticides and surfactants, can also be removed. A number of these compounds are not completely removed by conventional water treatments, being thus introduced continuously into surface water bodies by the discharge of wastewater treatment plants effluents. In spite of their low concentration (ng/L and µg/L), their ubiquitous presence in the environment is currently a cause of concern for aquatic life and human health. In this context, artificial recharge may represent a potential alternative or complementary treatment for the removal of organic micropollutants from water. Evidences showed that, among the factors influencing the fate of conventional organic contaminants in the aquifer, the predominant redox conditions could play an important role. Yet, in the case of emerging micropollutants the knowledge on this topic is still limited.

The main objective of this thesis, motivated by artificial recharge practices using Llobregat river water (Barcelona, Spain), is to investigate on the potential effect of redox conditions on the fate of

selected organic micropollutants, most of them being emerging contaminants, during artificial recharge of groundwater. The study is based on batch experiments involving natural aquifer material, micropollutants at environmental concentrations (1 µg/L each compound), and settings feasible at artificial recharge sites. Different anaerobic redox conditions (namely, nitrate-, manganese-, iron- and sulphate-reducing conditions) were promoted and sustained in each set of microcosms by adding adequate quantities of electron donors and acceptors. The experiments included biotic and abiotic series to separate contaminant's biodegradation (i.e. biotic mineralization or transformation) from sorption and other abiotic processes. An experiment at higher pollutants concentration (1mg/L each compound) was also carried out, to check the representativeness of studies at concentrations easier to be tested and analysed. The ultimate aim of the work is to identify 1) the most favourable redox conditions for the removal of the target compounds from water, for their following stimulation in the field test site, and 2) pollutants' removal rates, to predict their behaviour in the aquifer.

In the first part of the thesis, the experimental design criteria and methodology are thoroughly described. The assessment of the prevailing redox conditions and the description of the overall chemical evolution of the experiment are also presented, jointly with results from some numerical modelling. In the remaining part of the thesis, results for the studied micropollutants are presented. The ubiquitous beta blocker atenolol exhibited indeed an increasing rate of degradation as redox state evolves from nitrate to manganese, iron and sulphate reducing conditions. A reversible unreported phenomenon could be also identified for the amine-containing compounds diclofenac and sulfamethoxazole, showing their transformation into a nitrite-bearing transient compound coinciding with the peaking of nitrite. Finally, some of the results showed no impact of redox on the fate of pollutants, e.g. for the drug carbamazepine, the pesticide chlorphenvinfos, and the estrogen estrone.

Resum

La recarrega artificial de les aigües subterrànies consisteix en infiltrar aigua als aqüífers per mitjà de les instal·lacions dissenyades per a tal fi i representa una eina important en la gestió dels recursos. A més de l'augment dels recursos d'aigües subterrànies, la recàrrega pot suposar una millora natural de la seva qualitat durant el seu pas pel subsòl. Els processos que tenen lloc en el sistema sòl-aqüífer (filtració, adsorció, reaccions de mescla , redox, biodegradació, etc) han demostrat produir una millora integral de la qualitat de l'aigua, eliminant també de manera efectiva una sèrie de contaminants orgànics. En l'actualitat, la qüestió és saber si la contaminació pels microcontaminants orgànics emergents, és a dir, productes farmacèutics, cosmètics, hormones, drogues il·lícites, pesticides i tensioactius, també es poden atenuar de forma natural. Alguns d'aquests compostos no són completament eliminats pels tractaments d'aigua residual convencionals, essent introduïts contínuament en els cursos d'aigua superficials pels efluents de les plantes de tractament. Malgrat la baixa concentració (ng/L i μg/L), la seva presència en el medi ambient és actualment un problema per a la vida aquàtica i la salut humana. En aquest context, la recàrrega artificial pot esdevenir una alternativa potencial o un tractament complementari per a l'eliminació de microcontaminants orgànics de l'aigua. Hi ha evidències que les condicions redox predominants podrien influir en el comportament d'aquests productes en l'aqüífer. No obstant, en el cas dels microcontaminants emergents, el coneixement sobre aquest tema és encara molt limitat.

L'objectiu principal d'aquesta tesi és doncs investigar el possible efecte de les condicions redox sobre el destí de microcontaminants orgànics, la majoria dels quals són contaminants emergents.

El treball està motivat pels projectes de recàrrega artificial amb aigua del riu Llobregat (Barcelona, Espanya). L'estudi es basa en experiments de laboratori (batch) utilitzant materials naturals de l'aqüífer, microcontaminants en concentracions ambientals (1 µg/L de cada compost), i unes condicions ambientals similars als llocs de recàrrega artificial. Afegint les quantitats adequades d'acceptors i donadors d'electrons s'han obtingut i mantingut les diferents condicions redox anaeròbies en cada conjunt de microcosmos. Els experiments inclouen sèries biòtiques i abiòtiques per separar la biodegradació de contaminants de l'adsorció a la superfície dels sòlids i altres processos abiòtics. També s'ha dut a terme un experiment amb una major concentració de contaminants (1 mg/L de cada compost), per comprovar la representativitat dels estudis convencionals. Els objectius finals del treball són: 1) identificar les condicions redox més favorables per eliminar de l'aigua els compostos seleccionats, a fi de ser promogudes en l'assaig de camp; i 2) mesurar les velocitats de les reaccions de degradació per tal de preveure el comportament dels micrcontaminants en el aqüífer.

En la primera part de la tesi, s'han descrit de manera detallada els criteris del disseny experimental i la metodologia. També es presenta l'avaluació de les condicions redox predominants, la descripció de l'evolució química orgànica i inorgànica de l'experiment, així com també els resultats de la seva modelització numèrica. En els restants capítols es presenten els resultats per a alguns dels microcontaminants estudiats. Les dades no utilitzades s'han inclòs en un apèndix. L'omnipresent beta blocker atenolol va incrementar la seva velocitat de degradació a mida que l'estat redox canviava des de condicions de nitrat a manganès, ferro i sulfato-reducció. També és destacable un fenomen fins ara no descrit on els compostos amb amina, com diclofenac i sulfometoxazol, mostren una transformació reversible a un metabòlit coincidint amb el pic d'aparició del nitrit a expenses de la reducció del nitrat. Finalment, alguns dels compostos no van mostrar cap influència de l'estat redox en la seva estabilitat, com la droga carbamazepina, el pesticida clorofenvinfos i l'estrogen estrona.

Resumen

La recarga artificial de acuíferos, que consiste en la infiltración de agua subterránea en instalaciones diseñadas para tal fin, constituye una importante herramienta en la gestión de recursos hídricos. Más allá de aumentar los recursos de aguas subterráneas, suscita gran interés la capacidad de tratamiento natural que confiere el tránsito sub-superficial a las aguas. Los procesos que tienen lugar en el sistema suelo-acuífero (filtración, adsorción, mezcla, reacciones redox, biodegradación, etc.) permiten una mejora general de la calidad del agua, eliminando incluso diversos contaminantes orgánicos. En la actualidad, el reto es entender si los microcontaminantes orgánicos emergentes, es decir farmacéuticos, productos de cuidado personal, hormonas, drogas ilícitas, plaguicidas y tensioactivos, puedan también ser atenuados. Muchos de ellos no son completamente eliminados en tratamientos de agua convencionales, siendo introducidos constantemente en aguas superficiales por los efluentes de las plantas de tratamiento de aguas residuales. A pesar de sus baja concentraciones (ng/L and µg/L), su omnipresencia en el medioambiente es actualmente causa de preocupación para la vida acuática y la salud humana. En este contexto, la recarga artificial puede representar un tratamiento alternativo o complementario para la eliminación de microcontaminantes orgánicos de las aguas. Hay evidencias de que las condiciones redox dominantes pueden influenciar el comportamiento de contaminantes orgánicos convencionales en los acuíferos. Sin embargo, en el caso de los microcontaminantes emergentes los conocimientos sobre este tema es todavía limitado.

El principal objetivo de esta tesis, motivada por proyectos de recarga artificial con aguas del río Llobregat (Barcelona, España), es investigar el efecto potencial de las condiciones redox sobre el

comportamiento de microcontaminantes orgánicos seleccionados (la mayoría de los cuales son contaminantes emergentes) durante la recarga artificial.

El estudio se basa en experimentos batch de laboratorio usando material natural del acuífero, microcontaminantes en concentraciones ambientales (1 μg/L cada compuesto), y condiciones verosímiles en enclaves de recarga artificial. Añadiendo cantidades adecuadas de aceptadores y donadores de electrones, fueron establecidas y se mantuvieron diferentes condiciones redox anaeróbicas (condiciones nitrato-, manganeso-, hierro- y sulfato-reductoras) en cada grupo de microcosmos. Se llevaron a cabo series bióticas y abióticas para separar la biodegradación de los contaminantes (mineralización biótica o transformación) de los procesos de adsorción y de otros procesos abióticos. También fue realizado un experimento para alta concentración de contaminantes (1 mg/L cada compuesto), para comprobar la representatividad de los estudios convencionales. El objetivo final de este trabajo es identificar 1) las condiciones redox más favorables para la eliminación del agua de los contaminantes seleccionados, para su posterior simulación en el sitio piloto, y 2) las tasas de eliminación de esos contaminantes, para predecir sus comportamientos en el acuífero.

En la primera parte de la tesis, se describen en detalle los criterios del diseño experimental y la metodología. Se presentan también la evaluación de las condiciones redox dominantes, la descripción de la evolución química general de los experimentos, y los resultados de su modelación numérica. En la parte restante de la tesis se presentan los resultados para los microcontaminantes estudiados. El ubicuo beta bloqueador atenolol manifestó un incremento de la tasa de degradación al evolucionar el estado redox desde condiciones nitrato-reductoras, hacia manganeso-, hierro- y sulfato-reductoras. Un fenómeno reversible no descrito anteriormente pudo ser identificado en los compuestos con aminas, como diclofenaco y sulfametoxozol, mostrando su transformación en un compuesto transitorio con nitrito. Finalmente, algunos compuestos mostraron no estar afectados por las condiciones redox; es el caso del fármaco carbamazepina, el plaguicida clorfenvinfos, y el estrógeno estrona.

Riassunto

La ricarica artificiale delle acque sotterranee, che consiste nell'indurre l'infiltrazione di acque di varia origine nelle formazioni acquifere mediante installazioni appositamente progettate, rappresenta un importante strumento di gestione delle risorse idriche. Accanto ai suoi benefici quantitativi (incremento delle risorse idriche sotterranee, immagazzinamento per un lungo periodo nel sottosuolo, etc.) un grande interesse per questa tecnica è connesso al trattamento naturale che l'acqua subisce per effetto del suo passaggio nel sottosuolo. E' ormai dimostrato che i processi che avvengono nel sistema suolo-acquifero (filtrazione, adsorbimento, miscelazione, reazioni di ossidoriduzione, biodegradazione, etc.) producono un generale miglioramento della qualità delle acque, rimuovendo efficacemente anche un gran numero di contaminanti organici. Allo stato attuale delle conoscenze, il problema è comprendere se i microinquinanti organici emergenti, quali i prodotti farmaceutici, i prodotti destinati alla cura personale, gli ormoni, le droghe illegali, i pesticidi e i tensioattivi possono anche essi essere rimossi. Un gran numero di questi composti non vengono completamente eliminati dai trattamenti convenzionali delle acque, venendo così continuamente introdotti nei corpi idrici superficiali dallo scarico degli effluenti degli impianti di trattamento delle acque di rifiuto. Nonostante la loro bassa concentrazione (ng/L e µg/L), la loro presenza ubiquitaria nell'ambiente è attualmente causa di preoccupazione per la vita acquatica e la salute umana. In questo contesto, la ricarica artificiale può rappresentare una potenziale alternativa o un trattamento complementare per rimuovere i microinquinanti organici dalle acque. Si é riscontrato che, tra i fattori che influenzano il destino dei contaminanti organici convenzionali negli acquiferi, lo stato redox predominante puó giocare un ruolo importante. Tuttavia, nel caso di microinquinanti emergenti le conoscenze su questa tematica sono ancora limitate.

Il principale obbiettivo di questa tesi, motivata da pratiche di ricarica artificiale che utilizzano le acque del fiume Llobregat (Barcellona, Spagna), è investigare il potenziale effetto delle condizioni di ossidoriduzione, durante la ricarica artificiale delle acque sotterranee, sul destino di microinquinanti organici selezionati, la maggior parte dei quali costituiscono contaminanti emergenti. Lo studio è basato su esperimenti batch di laboratorio, utilizzando materiali naturali degli acquiferi, microinquinanti in concentrazioni ambientali (1 µg/L per ciascun composto) e configurazioni praticamente realizzabili nei siti di ricarica artificiale. Differenti condizioni redox anaerobiche sono state create e mantenute in ciascun set di microcosmi, aggiungendo adeguate quantità di donatori e accettori elettronici. Negli esperimenti sono state incluse serie biotiche e abiotiche per separare la biodegradazione dei contaminanti (cioè la mineralizzazione o la trasformazione biotica) dall'adsorbimento e da altri processi abiotici. E' stato anche effettuato un esperimento a maggiore concentrazione di inquinanti (1 mg/L per ciascun composto), per controllare la rappresentatività degli studi a concentrazioni più facili da testare e analizzare. L'obbiettivo finale del lavoro mira a identificare le condizioni di ossidoriduzione più favorevoli alla rimozione dall'acqua dei composti selezionati, per la loro successiva implementazione nei siti di prova in campagna.

Nella prima parte della tesi vengono ampiamente descritti i criteri e la metodologia sperimentale. Vengono anche presentate la valutazione delle condizioni redox prevalenti e la descrizione dell'evoluzione chimica globale dell'esperimento, unitamente ai risultati di alcune modellazioni numeriche.

Nella parte restante della tesi, vengono presentati i risultati ottenuti per alcuni dei microinquinanti studiati, rinviando ad una appendice per la rimanente parte dei dati. Per l'ubiquitario betabloccante atenolol si è riscontrato un incremento del tasso di degradazione al passare da condizioni di riduzione del nitrato a quelle di riduzione del manganese, ferro e solfato. Si é identificato un fenomeno reversibile sinora non osservato per composti contenenti ammine, come il diclofenac ed il sulfametoxazol, per i quali è stata rilevata una trasformazione reversibile a metaboliti in corrispondenza con il picco di apparizione del nitrito. Infine, alcuni altri microinquinanti non si sono dimostrati sensibili alle condizioni di o ssidoriduzione.

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Chapter 1

Introduction

Artificial recharge of groundwater, also known as managed aquifer recharge (MAR), represents an increasingly important tool in water resources management. This technology consists in intentionally infiltrating water into aquifers by means of properly designed facilities. It is based on surface, deep and vadose zone recharge schemes (Bouwer, 2002; Custodio and Llamas, 2001. Figure 1.1). Surface systems are addressed to the recharge of unconfined aquifers, and require the presence of quite high permeable material near the surface. They include dams placed across the streambeds and T-leeves inside flat channels (in-channel systems), as well as infiltration ponds, lagoons, spreading channels, and controlled flooding of flat plains (off-channel systems). When permeable soils or sufficiently large land surfaces are not available, aquifer recharge could be achieved with trenches and dry wells in the vadose zone or, in the case of deep and/or confined aquifers, with conventional injection wells and aquifer storage and recharge (ASR) systems.

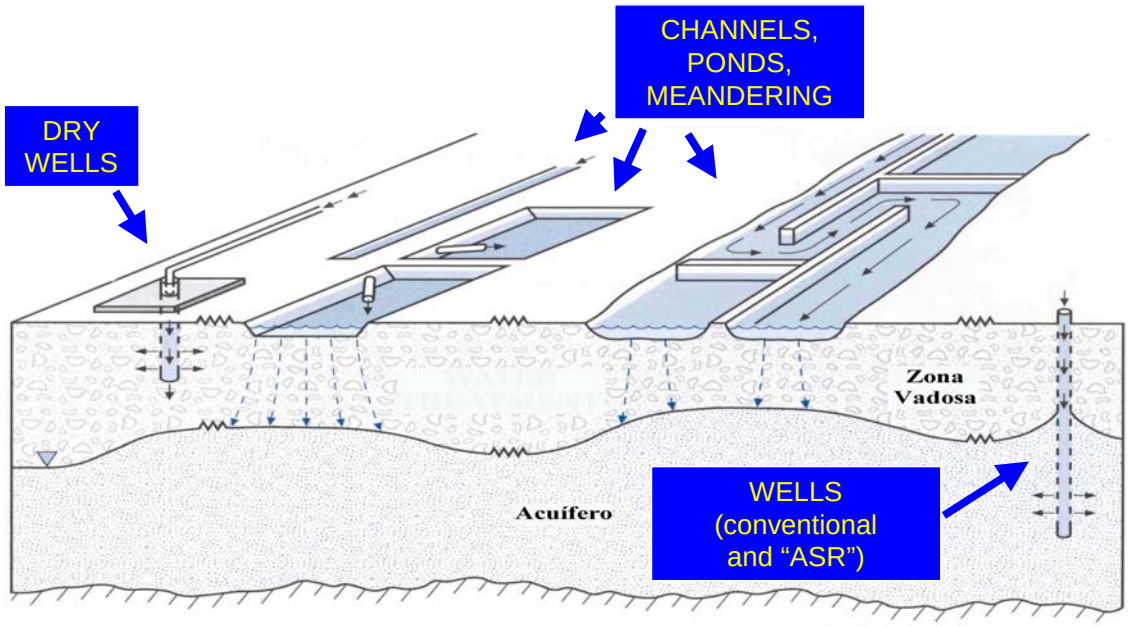


Figure 1.1: Surface, vadose zone and deep artificial recharge schemes (image conceded by Alfredo Pérez Paricio - personal notes)

Even if more properly classified as an induced recharge system, it is worth mentioning bank filtration as another broadly used recharge scheme. It consists in creating favourable conditions to natural infiltration, as pumping from wells relatively close to streams, rivers or lakes, to lower the water table and inducing a bigger entrance of surface water to the aquifer (Figure 1.2).

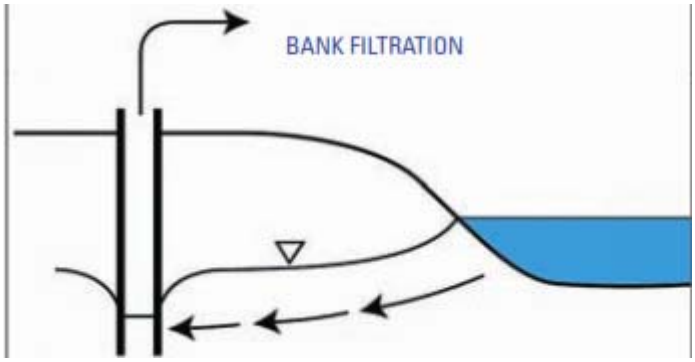


Figure 1.2: River bank filtration system (image from Gale I. (Editor), 2005)

The objective of artificial recharge is manifold: enhance groundwater resources by increasing short and long term underground storage of water for future use, stabilize groundwater levels to reduce land subsidence or seawater intrusion problems, use aquifers as natural water distribution systems, establish hydraulic control of contamination plumes, etc. Beside these applications, a great interest for this technique is related to the natural treatment provided to water by the processes occurring in the soil-aquifer system (filtration, sorption, mixing, redox reactions,

biodegradation, etc.), resulting in a decrease of suspended solids, pathogens, nitrogen, phosphates, metals, dissolved organic carbon, and even individual organic contaminants (Aronson et al., 1999 and references therein; Bouwer, 1991; Christensen et al., 2001 and references therein; Dror et al., 2004; Idelovitch et al., 2003; McCarty et al., 1981; Neuhauser et al., 2009; Ray (Editor) et al. 2003). This purifying capacity of the subsoil allowed to include, under specific constraints, waters of impaired quality (treated wastewater, stormwater runoff, and irrigation return flow) among the sources of recharge water beside the conventional ones (surplus of potable water, surface water from rivers and lakes, native groundwater, etc.). At present time, the issue is to understand whether emerging organic micropollutants can also be removed during artificial recharge. The advances in the analytical techniques achieved in the last two decades evidenced the ubiquitous presence of such compounds in surface, ground and drinking water, as well as in soils and sediments (Focazio et al., 2008; Kemper, 2008; Loos et al., 2009; Petrovic et al., 2004 and references therein). They include a variety of human/veterinary drugs, personal care products, hormones, illicit drugs, flame retardants, plasticizers, pesticides, surfactants as well as their transformation products. Their primary source is the discharge of effluents from wastewater treatment plants into surface water bodies, since conventional treatments are rarely efficient enough to remove these compounds completely (González et al., 2007; Johnson and Sumpter, 2001; Onesios et al., 2009; Petrovic et al., 2009 and references therein). They also proceed from solid waste disposal, spills and uncontrolled discharges from industries, spreading of manure and sewage sludge as organic fertilizer in agricultural soils, surface run-off, etc. In spite of their usually very low concentrations, in the range of ng/L to μg/L, a major concern is associated to potential chronic effects and synergic action of their mixtures on aquatic life and human health (Fent et al., 2006; Farré et al., 2008; Kumar et al., 2010). Therefore, it is not surprising that much work is being devoted to understanding their behaviour and fate in the environment so as to ensure their removal from water. Under this perspective, the passage of water through the soil-aquifer system during artificial recharge may represent an alternative or complementary treatment for their removal. As a general rule, the fate of organic pollutants within the aquifer depends on lithology, hydraulic and textural properties of the soil, temperature, physico-chemical properties of the specific compound, and microbial environment. Among all factors, the predominant redox state of the aquifer revealed to play a significant role for traditional organic pollutants (Aronson et al., 1999 and references therein; Bosma et al., 1996; Broholm and Arvin, 2000 and references therein; Christensen et al., 2001; Kao et al., 2003 and references therein). Yet, in the case of (emerging) organic micropollutants, the knowledge on a potential redox-dependent behaviour is still limited.

In this context, motivated by artificial recharge practices using Llobregat river water (Barcelona, Spain), the main objective of this thesis is to investigate on the potential effect of redox conditions on the fate of selected organic micropollutants, most of them being emerging contaminants, during artificial recharge of groundwater. The ultimate aim is to identify the most favourable conditions for their removal from water, for their following stimulation in the field test site.

The thesis is organized according to this general objective. The methodological approach used in the laboratory experiments is thoroughly described in Chapter 2 of the present document. Details on the protocols followed when assembling and disassembling the tests are provided in Appendix A. Results for some of the studied micropollutants are presented in Chapter 2, 3, 4, and Appendix D, which are based on papers submitted to international journals or in preparation. Plots and brief comments on the results for the remaining compounds, as well as all experimental data (general water chemistry, micropollutants) could be found in Appendix C. A summary of the information on the target pollutants (characteristics, analytical methods, etc.) and an overall sketch of the experiments performed have been included in Appendix B and C, respectively. Appendix E reports the work carried out in the field test site. Some numerical modeling of hydrochemistry evolution during the experiments was also carried out. Results from simulations are included in Chapter 2, while the code and original input files could be find in the complementary material (digital cd).

Chapter 2

Microcosm study on the fate of organic micropollutants in aquifer material under different anaerobic redox conditions

NOTE: the present chapter is based on the paper with the same title, by Barbieri, M., Carrera, J., Sànchez-Vila, X., Ayora, C., Cama, J., Köck-Schulmeyer, M., López de Alda, M., Barceló, D., Tobella Brunet, J., Hernández García, M.. Submitted.

2.1 Introduction

The ultimate motivation of this work is artificial recharge of aquifers. Artificial recharge is beneficial both in quantitative (augmentation of groundwater resources, long term underground storage, etc.) and qualitative terms (overall improvement of water quality during aquifer passage: decreasing of suspended solids, pathogens, nitrogen, phosphates, metals and dissolved organic carbon). The interest in this technique is also related to the capability of subsoil processes to partially or totally remove organic contaminants from water (Aronson et al., 1999 and references therein; Christensen et al., 2001 and references therein; Neuhauser et al., 2009). Nowadays a great scientific effort is dedicated to assess whether organic micropollutants could also be effectively removed (Barber et al., 2009; Díaz-Cruz and Barceló, 2008 and references therein; Heberer, 2007 and references therein; Hoppe-Jones et al., 2010). A number of such compounds are not eliminated by conventional water treatments (Gros et al., 2010; Onesios et al., 2009 and references therein; Petrovic et al., 2009 and references therein; Stackelberg et al., 2007). The passage of water

through the soil-aquifer system during artificial recharge may represent an alternative or complementary treatment for their removal.

As a general rule, the fate of organic pollutants within the aquifer depends on lithology, hydraulic and textural properties of the soil, temperature, physico-chemical properties of the specific compound, and microbial environment. Among all factors, the predominant redox state of the aquifer revealed to play a significant role (Aronson et al., 1999 and references therein; Bosma et al., 1996; Broholm and Arvin, 2000 and references therein; Christensen et al., 2001; Kao et al., 2003 and references therein). Since certain pollutants could be preferably removed under some particular redox conditions, such conditions could eventually be promoted in artificial recharge practices. Even more important, if different compounds are degraded under different redox environments, a water mass undergoing a sequence of redox states should have most of its initial contaminants eliminated.

Yet, in the case of organic micropollutants, the knowledge on a potential redox-dependent behaviour is still limited. Beside field evidences (Drewes et al., 2003; Heberer et al., 2008; Montgomery et al, 2003; Pavelic et al., 2005; Tubau et al., 2010; and references therein), laboratory tests under specific and controlled simplified conditions have been carried out. However, many of the experiments reported in literature adopted settings that are not representative or directly applicable to aquifer systems.

Specifically, aerobic/anoxic biodegradability and transformation mechanisms of organic micropollutants have been investigated in model systems for wastewater treatment. That is, laboratory experiments have been typically performed in wastewater matrices, and by using sludges from sewage treatment plants as adapted inocula (Clara et al., 2004; Quintana et al., 2005; Stasinakis et al., 2009; Zwiener et al., 2002). Their fate under aerobic/anaerobic or specific redox conditions have also been largely studied in aquatic environments. In these cases river-bed sediments rich in organic materials have been incubated with river water (Davis et al., 2006; Löffer et al., 2005; Radke et al., 2009) or with solutions/culture media containing specific electron acceptors (Bradley et al., 2001; Crawford et al., 1998; Lu et al., 2009; Somsamak et al., 2001). Some tests involved bacterial isolates, and have been carried out using standard silica sand or sintered materials as solid matrix for the colonization of the microorganisms (Crawford et al., 2000; Katz et al., 2001; Stucki et al., 1995). Finally, not only in the aforementioned studies but also when soil and aquifer material were included (Krueger et al., 1998; Schulz et al., 2008; Ying et al., 2008), the experiments have been often performed with concentrations of the target compounds from hundreds of μg/L to tens of mg/L.

The above works are indeed useful to demonstrate the susceptibility of specific micropollutants to microbial or abiotic transformation, to understand degradation pathways, and to identify intermediate or stable metabolites. However, the organic content of aquifer materials, which may influence sorption and partitioning behaviour of organic micropollutants, could be lower, the potential development of a sequence of redox states and the removal of micropollutants depends on the local native microorganisms, and target pollutants are found at concentrations some order of magnitudes lower.

We finally look at the quite limited laboratory experiments resembling real subsurface environments, a number of them related to managed aquifer recharge practices (Mansell and Drewes, 2004; Rauch et al., 2009; Hua et al., 2003; Baumgarten et al., 2011; Scheytt et al., 2004; Massmann et al., 2008). In such experiments, the fate of organic micropollutants has been usually assessed within the range of redox conditions developing naturally in the system and being representative of those actually occurring at field site, namely aerobic, anoxic (prevailing denitrifying) and seldom unspecified anaerobic conditions. The identification of potential abiotic processes by performing analogous sterile experiments was not always included in such studies.

Therefore, in the end, a wide range of organic micropollutants and the potential effect of various redox states on their fate in subsurface environments still remain to be investigated.

In this context, the aim of our work was to create and sustain diverse anaerobic redox conditions in systems involving natural aquifer material and settings potentially feasible in artificial recharge sites, so as to study the behaviour of organic micropollutants at realistic concentrations in such environments. The ultimate aim was to identify the most favourable conditions for their removal from water, for their following stimulation in a real field application. Results for the ubiquitous but still barely investigated β-blocker Atenolol will be presented in this chapter, whereas results for the remaining target compounds could be found in chapter 3, 4, and Appendix C and D.

Details on the experimental methodology, namely on the selection of the type/quantities of electron donors and acceptors used to stimulate the desired redox conditions, have been integrated. Limited information on the design criteria is usually provided in studies on the fate of organic pollutants adopting this approach (Bradley et al., 2001; Bosma et al., 1996; van der Zaan et al., 2009; Weiner et al., 1998; Ying et al., 2008). This hinders running analogous studies with some different setting (substrates, electron acceptors, durations, etc.).

The description of the microcosms hydrochemical evolution is also presented. Often this is not/poorly monitored or only incipiently reported in laboratory studies on the fate of organic contaminants, especially when focused on their transformation pathways, nor the actual occurrence

of the expected/stimulated redox condition is verified (Baumgarten et al., 2011; Bosma et al., 1996; Bradley et al., 2001; Gröning et al., 2007; Krueger et al., 1998; Rauch-Williams et al., 2009; Schulz et al., 2008; van der Zaan et al., 2009; Weiner et al., 1998; Ying et al., 2008). We conjecture that the assessment of the geochemical state and its quantitative modeling has to be included in this type of studies for a more complete interpretation of the experimental results, and for the potential subsequent design of real field applications.

2.2 Materials and methods

2.2.1 Sediments, water and micropollutants

The experimental set up included various sets of microcosms, each microcosm consisting of natural sediments and synthetic water spiked with a mixture of organic micropollutants.

Sediments were obtained from a test site for artificial recharge of groundwater located in Sant Vicenç dels Horts (Barcelona, Spain). The aquifer consists of quaternary alluvial sediments, mainly gravel and sand with a small fraction of lutites. Sediment samples for the experiments were collected prior to the starting up of the facilities, from a pit excavated in the bottom of the infiltration pond, namely from an oxic unsaturated horizon at about 1m depth. They were sieved through a 1mm grid to remove the coarse fraction, which was expected to be less active for surface and microbially mediated reactions. The sieved sediments were immediately used for assembling the microcosms or stored for a maximum of two days at 25ºC inside aluminum paper.

Chemical and mineralogical characteristics of the sediments used in the experiments are summarized in Table 2.1. X-ray diffraction (XRD) of powdered samples was used in an attempt to identify the minerals present in the sediment. Analysis was performed with a Bruker D-5005 diffractometer. Results were obtained using Cu radiation, with secondary Graphite monochromator. The analytical conditions were: θ /2 θ geometry, collecting data in the range (2θ) between 4° and 60° with a step scan of 0.05°, 3s per step measuring time. The evaluation of the spectra was made by using the Diffrac.Suite™ software and identification of chemical compounds by means of the PDF database Release 2001, Data Sets 1-51 plus 70-89.

Total Nitrogen, total Carbon and organic Carbon content were analyzed using an organic elemental analyzer with on line combustion-reduction-gas chromatography (TCD detector) model EA series 1108 (Thermo Fisher Scientific), set at conditions within the provider-recommended range. Data acquisition and calculations were done with the Eager 200 software (Thermo Fisher Scientific).

The grain size distribution was measured with the Laser diffraction particle-size analyser Coulter LS230 (Beckman Coulter Inc., Fullerton, CA, USA) with a Detection Limit of 0.04 μm. The content

in Mn and Fe(III) associated to oxide-hydroxides and oxides was obtained by sequential extraction (step 1 to 4 from Dold, 2003).

Table 2.1: Chemical and mineralogical characteristics of the sediments used in the experiments

minerals (XRD)	quartz, calcite, microcline, albite, dolomite, clinochlore, illite
Organic Carbon [%]	< 0.2
Nitrogen [%]	< 0.2
Total Carbon [%]	2.5
grain size	Maximum grain diameter: 1mm. Fraction with diameter < 4µm: 2 to 6 %
Mn and Fe(III) associated to oxide-hydroxides and oxides [mg/g air dried sediment]	Mn: 0.07; Fe (amorphous oxide-hydroxides): 0.19; Fe (crystalline oxides): 5.64

Water used for the preparation of all the experiments, called “common water” in the following, was artificially prepared to mimic the recharge water at the test site (from the Llobregat river water) except for the organic carbon content, which at this stage was set equal to zero. Its theoretical composition is shown in Table 2.2.

Table 2.2: Theoretical chemical composition of the water used for the preparation of all the experiments (“common water”).

Compounds and parameters	*[mg/L]*	*[mmol/L]*
Na	180	7.8
K	40	1.0
Ca	120	3.0
Mg	32	1.3
Cl	452	12.7
NO_3	10	0.2
SO_4	200	2.1
Alk [$CaCO_3$]	51	0.5
DOC [mgC_{org}/L]	0	0.0
COD [mgO_2/L]	0	0.0
NH_4	2	0.1
PO_4	2	0.02
O_2(aq)	7	0.2
pH (measured)	7.4	
Eh (measured)	250 mV	
T (measured)	25ºC	
E.C. [µS/cm] (measured)	1800	

The mixture of organic micropollutants used in all experiments included drugs (atenolol, carbamazepine, diclofenac, gemfibrozil, ibuprofen, sulfamethoxazole), pesticides (atrazine, simazine, terbuthylazine, prometryn, diuron, chlorphenvinfos, chlorpyrifos, diazinon), estrogens

(estrone, -estradiol), PAHs (naftalene, acenaphtene, fluorene, anthracene, fenanthrene, benz[a]anthracene, crysene, pyrene, fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a]anthracene, indeno[1,2,3-cd]pyrene and benzo[ghi]perylene), surfactant degradation products (4-tert-octylphenol, 4-nonylphenol), a phthalate (bis-diethylhexyl phthalate) and a biocide (triclosan). The selection of the compounds was based on the micropollutants occurrence in the Llobregat river (Céspedes et al., 2005; Muñoz et al., 2009; Rodriguez-Mozaz et al., 2004).

High purity (>96%) analytical standards of atenolol, carbamazepine, diclofenac, gemfibrozil, ibuprofen, sulfamethoxazole, simazine, diuron and estrone, and of the isotopic analogue atenolol *d7* used as surrogate standard for quantification of atenolol were supplied by Sigma–Aldrich. The standard containing the 16 PAHs at a concentration of 2000 mg L-1 in dichloromethane:benzene (1:1) as well as high purity (>96%) analytical standards of all the remaining compounds were purchased from AccuStandar. Individual stock solutions were prepared in methanol or in an appropriate solvent according to their properties. Working standard mixtures were then prepared at different concentrations by dilution of the individual stock solutions in methanol, and were used to prepare the spiking solution for the experiments (resulting concentration in the "initial water" described in § 2.2.4 was 10 µg/L for 4-octylphenol and 4-nonylphenol, and 1 µg/L for the rest of compounds) and to prepare the aqueous calibration standards (concentration range 1-1500 ng/L, surrogate standard 200 ng/L). Stock and working standard solutions were stored at -20 °C in the dark.

2.2.2 Biotic experiments - Creating sustainable redox conditions

A different anaerobic redox state was promoted in four different sets of batches by stimulating one specific step of the natural redox sequence for organic matter degradation (Table 2.3). To this end, easily degradable organic compounds were provided as electron donors and, depending on the target redox condition, NO_3, Mn(III/IV), Fe(III) or SO_4 was added as specific electron acceptor.

Table 2.3: Sequence of the overall redox reactions for the microorganisms mediated degradation of organic matter (i.e. Methanol and Acetate ions in the present experiments). Biomass growth is ignored in the stoichiometries.

		Redox potential	Energy yield
1a) $CH_3OH + 1.5\ O_2 \rightarrow HCO_3^- + H^+ + H_2O$ *1b)* $CH_3COO^- + 2O_2 + \rightarrow 2HCO_3^- + H^+$	*Aerobic respiration*		
2a) $CH_3OH + 1.2\ NO_3^- + 0.2\ H^+ \rightarrow HCO_3^- + 0.6\ N_2 + 1.6\ H_2O$ *2b)* $CH_3COO^- + 1.6\ NO_3^- + 0.6\ H^+ \rightarrow 2HCO_3^- + 0.8\ N_2 + 0.8\ H_2O$	*Nitrate reduction*	+	
3.1a) $CH_3OH + 3\ MnO_2(s) + 5\ H^+ \rightarrow HCO_3^- + 3\ Mn^{2+} + 4\ H_2O$ *3.1b)* $CH_3COO^- + 4\ MnO_2(s) + 7\ H^+ \rightarrow 2HCO_3^- + 4\ Mn^{2+} + 4\ H_2O$ *3.2a)* $CH_3OH + 6\ MnOOH(s) + 11\ H^+ \rightarrow HCO_3^- + 6\ Mn^{2+} + 10\ H_2O$ *3.2b)* $CH_3COO^- + 8\ MnOOH(s) + 15\ H^+ \rightarrow 2HCO_3^- + 8\ Mn^{2+} + 12\ H_2O$	*Mn oxide reduction*		increasing
4a) $CH_3OH + 6\ Fe(OH)_3(s) + 11\ H^+ \rightarrow HCO_3^- + 6\ Fe^{2+} + 16\ H_2O$ *4b)* $CH_3COO^- + 8\ Fe(OH)_3(s) + 15\ H^+ \rightarrow 2HCO_3^- + 8\ Fe^{2+} + 20\ H_2O$	*Fe ox/hydroxide reduction*		
5a) $CH_3OH + 0.75\ SO_4^{2-} \rightarrow HCO_3^- + 0.75\ HS^- + 0.25\ H^+ + H_2O$ *5b)* $CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^-$	*Sulphate reduction*	–	

Nitrate and sulphate were incorporated to the "common water" by dissolving magnesium nitrate hexahydrate and sodium sulphate, respectively. Mn(III/IV) and Fe(III) oxide-hydroxides were incorporated to the sediments as finely ground natural psilomelane and mixed ferrihydrite/goethite (1:10 in weight), respectively.

Sodium Acetate and the Methanol used as solvent in the micropollutants spiking solution were adopted as easily degradable substrates. They were incorporated to the "initial water" (§ 2.2.4) by dissolving anhydrous sodium acetate and when spiking the micropollutants mixture, respectively. In fact, the organic micropollutants introduced represented potential electron donors too, but their concentrations were so low that their effect on redox condition build-up was expected to be minimal.

The selection of the type of substrate was based on a revision of the existing literature and on preliminary scoping experiments (results not shown) regarding the degradation feasibility and rate for different organic compounds. Ideally, the selected substrate should promote the build-up of the desired redox conditions after a short lag-phase and form a small number of intermediate compounds (possibly being not fermentable) to facilitate assessment of the chemical evolution of the system.

The total amounts of organic substrate and controlling electron acceptor were selected so as to reach the desired redox state and to sustain it during a significant lapse of time. This implies on one

hand that the total amount of organic substrate had to be large enough to consume electron acceptors with reactions energetically more favourable than the target one.
On the other hand, for each potential selection of organic substrates the total amount of controlling electron acceptor initially available had to (slightly) exceed the stoichiometric quantity necessary for their complete mineralization. We used the stoichiometries in which biomass formation is not considered (see Table 2.3), since after the original sources of organic carbon have been depleted, dead cells could be recycled and degraded coupling with the reduction of some electron acceptor.
Further details on the selection of the initial amounts of electron donors and acceptors can be found in the Supporting Information at the end of this Chapter.
The definitive concentrations of the organic substrates (and the corresponding amount of target electron acceptor) to be initially available in each experiment could be established according to the degradation rates observed in the preliminary rough tests performed when selecting the type of substrates, where their potential toxicity toward microorganisms could be excluded too, and according to the desired duration of the experiments.
Namely, in the present tests the design initial concentration of Methanol was fixed by the quantity of spiking solution added to the "initial water", i.e. 2.7 mmol L^{-1}. Regarding acetate, according to the design constraints the selected initial concentrations exceeded natural levels in aquifer system. Still, they were inside the range of concentrations already used in injection experiments and bioremediation scenario in subsurface environments (Baker et al., 1999; Kerkhof et al., 2011), i.e. within a range applicable to a potential stimulation of some specific redox condition in artificial aquifer recharge field sites.

In a complementary set of batches called "Natural Conditions", with oxygen initially present in the system, neither additional electron acceptors were added to the "common water" or to the sediments, nor was Sodium Acetate added as electron donor to the "initial water". That is, no specific redox state was deliberately stimulated and the organic matter degradation reactions were expected to develop sequentially (Table 2.3, set "a" of reactions), until complete depletion of either the electron donors or acceptors available in the system. Also in this experiment the initial concentration of Methanol was 2.7 mmol L^{-1}, fixed by the quantity of spiking solutions added to the "initial water" during the assembling procedure.

The initial concentrations of electron donors and electron acceptors present in the five biotic experiments are shown in Table 2.4. Notice that in the NO_3-reducing experiment, due to some unidentified problem during the assembling procedure, some additional 2.9 mmol L^{-1} of

Methanol turned out to be present apart from the 2.7 mmol L^{-1} proceeding from the spiking solution, resulting in an actual initial Methanol concentration higher than expected.

Table 2.4: Initial analytical concentration of electron donors and acceptors in the five sets of microcosms.

	ELECTRON DONORS			ELECTRON ACCEPTORS				
Type of experiment	**Initial analytical DOC (mM)**	**contribution of the easily degradable organic substrates to the Initial analytical DOC [%]**		**Initial measured $O_{2(ac)}$ [mM]**	**Initial analytical NO_3 [mM]**	**Initial analytical SO_4 [mM]**	**Initial Mn(IV)**	**Initial Fe(III)**
NO_3 - reducing	9.7	CH_3COO^- CH_3OH	42.79 57.21	0.0	6.7	2.0	the amount originally present in the sediments	
Mn(IV) - reducing	6.7	CH_3COO^- CH_3OH	60.50 39.50	0.0	0.1	2.3	0.4 g of natural psilomelane added to the original sediment	the amount originally present in the sediments
Fe(III) - reducing	7.8	CH_3COO^- CH_3OH	68.17 31.83	0.0	0.1	2.3	the amount originally present in the sediments	0.95 g of natural mixed ferrihydrite/goethite (1:10 in weight) added to the original sediment
SO_4 - reducing	10.2	CH_3COO^- CH_3OH	75.38 24.62	0.0	0.1	5.3	the amount originally present in the sediments	
Natural conditions	2.5	CH_3COO^- CH_3OH	0.00 100.00	0.2	0.1	2.3	the amount originally present in the sediments	

2.2.3 Abiotic experiment

A sterile experiment was also conducted as common control reference for the biotic tests to identify potential abiotic processes affecting the micropollutants. The absence of biodegradation processes precludes the evolution of the redox sequence from evolving. This would render useless repeating the procedure described above to control the redox state.

The synthetic water used to prepare this experiment ("abiotic water") consisted in the previously described "common water" plus the same additional amounts of magnesium nitrate hexahydrate and sodium sulphate used respectively in the NO_3-reducing and SO_4-reducing experiments. Further on, during the assembling procedure (§ 2.2.4), the same amount of acetate used in the SO_4-reducing experiment was added to the sterile "initial water". The initial concentration of methanol was 2.7 mmol L^{-1}, fixed by the quantity of spiking solution added. Thus, in the end, the Abiotic experiment was characterized by the maximum NO_3, SO_4, Na, Mg and DOC concentration existing among the 5 biotic experiments.

2.2.4 Experimental procedure

2.2.4.1 Assembling the microcosms

The collected sediments were air dried at laboratory temperature (25ºC), homogenized in steel containers, and distributed in fractions of 120g (air-dry weight) into 0.3L glass bottles. The previously defined amounts of Mn(IV) or Fe(III) oxide-hydroxides powder were mixed with the

sediments of each bottle for Mn(IV)- and Fe(III)-reducing experiments, respectively. The 0.3L glass bottles were then placed inside a glove box under Argon atmosphere (maximum 0.1% of O_2).

The "common water" was prepared in a glass amber bottle. The previously defined amounts of NO_3 or SO_4 were added in the case of NO_3-reducing and SO_4-reducing experiments, respectively. The water was bubbled with Ar (purity ≥ 99.999%) during about 1 hour to remove all oxygen from water and bottle headspace. Afterwards, the bottle was closed with a screw-cap plus a PTFE protection seal and placed into the glovebox under Ar atmosphere, where the remaining part of the assembling procedure was performed.

The water was finalized by adding the predefined amount of Sodium Acetate and the spiking solution of micropollutants. After sampling the resulting "initial water" for chemical analyses, 0.24L of it were added to each one of the 0.3L glass bottles already containing the sediments. The assembling procedure was concluded by closing the bottles with screw-caps plus a PTFE protection seal, and gently shaking. A remaining headspace of about 15mL was left in each bottle.

The bottles were removed from the glove box and enveloped with aluminium foil to prevent photodegradation. Then, they were incubated under controlled temperature (25±2 ºC) and gently shaken few times during their lifetime (once every 2 days during the first week; once a week during the rest of the first month; then, once every 30 to 45 days) as well as the day before being sacrificed.

The "Natural Conditions" experiments were conducted without Ar bubbling or assembling within a glove box. Instead, dissolved oxygen was allowed in the water and oxygen gas was initially present in the headspace of the bottles.

In the case of the Abiotic experiment, prior to the beginning of the assembling procedure, the sediments and "abiotic water" were sterilized three times (once a day in three consecutive days) with autoclave at T=121 ºC and P = Patm + 1atm during 20 minutes; moreover, the glove box was sterilized with UV light before entering the material. As an additional precaution, 0.22 mmol L^{-1} of mercury chloride were added (as microbial poison) to the "initial water".

2.2.4.2 Disassembling the microcosms

Duplicate bottles were sacrificed at each sampling time according to predefined sampling schedules (Table 2.5). These had been defined according to the expected degradation rates of the organic substrates and micropollutants reported in the literature and those estimated in the preliminary scoping experiments (§ 2.2.2). Some sampling times were set equal for the different experiments, to facilitate comparisons.

One at a time, the two bottles were opened under Ar atmosphere, chemical parameters were measured, and aqueous samples for general chemistry and micropollutants analysis were collected and stored according with each laboratory recommendations.

Table 2.5: Sampling schedules (in days after assembling date) for each experiment.

	Sampling time [days]																
Type of experiment	*Initial water*	*duplicate batch sacrificing*															
NO_3-reducing	0	0.05	0.2	0.5	1.5	3	5		10		21						
Mn(IV)-reducing	0							7		14	25	42	63	91		194	
Fe(III)-reducing	0							7		14	24	42	63	91		199	
SO_4-reducing	0							7			18	36	65	89	133	215	
Natural conditions	0				1.1	3		7	10	15	26	42	62	89	135	192	
Abiotic	0	0.0	0.2	0.5	1.5	3	5		10		22	37	64	84	134	184	

The sterility of the Abiotic experiment was verified six times along its duration. An aliquot of water from devoted microcosms was spread on tryptic soy agar (TSA) plates and incubated in duplicate at 25ºC under aerobic conditions (during 1 week) and anaerobic conditions (during 2 weeks). None of the plates demonstrated microorganism growth.

Further details on the protocols followed to assemble and disassemble the experiments are provided in Appendix A of the present thesis.

2.2.5 Monitoring and analysis

Samples collected for analysing Cl^-, NO_3^-, NO_2^-, SO_4^{2-}, PO_4^{3-}, F^-, NH_4^+, DOC and COD (Chemical Oxygen Demand) were filtered through 0.45 µm PALL Acrodisc® Sterile Syringe Filters with Supor® membrane and frozen. Anions were analyzed by ion chromatography using a ICS-1000 instrument. The analytical error was estimated to be 14% for PO_4^{3-} and 13% for the remaining anions. NH_4^+ concentration was analyzed with a selective electrode Orion 9512. DOC was analyzed by 680 °C combustion catalytic oxidation/NDIR method using a TOC-V CSH instrument. The estimated analytical error was 20%. COD was analyzed by colorimetry with the spectrophotometer Spectroquant Nova 60.

Samples for the analysis of Fe and Mn, Ca, Mg, Na, K and minor elements were also filtered at 0.45 µm, acidified and stored at 4ºC. They were later analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES) using a Thermo Jarrel-Ash Iris Advantage HS instrument. Detection limits were 100 µg/L for K and Na, and 50 µg/L for the rest. The analytical

error was estimated below 3%. In the ICP-AES analyses, calibration with three laboratory sets of standards was performed every 10 samples, and regression coefficients of the calibration curves exceeded 0.999.

pH and temperature (Thermo Scientific 9157BN Triode pH electrode, refillable), Electrical Conductivity (Hanna Instruments, 76302W conductivity probe) and Dissolved Oxygen (Hanna Instruments, HI 76407/4 DO probe) were measured during the assembling/disassembling procedure with specific electrodes. Alkalinity was measured with a drop test kit Taylor K-1726, with a precision of 0.5 mmol L^{-1}.

Samples for analysis of Atenolol were filtered at 0.45 μm using WATERS Syringe filter with PTFE. Then, they were kept frozen until analysis, which was performed by using on-line solid phase extraction-liquid chromatography-tandem mass spectrometry. Briefly, water samples (10 mL) spiked with the isotopically labelled compound at a concentration of 200 ng/L, were extracted with the aid of an automated on-line SPE sample processor Prospekt-2 from Spark Holland (Emmen, The Netherlands) connected in series with the LC-MS/MS instrument. Sample preconcentration was performed by passing 5 mL of the sample through a previously conditioned (1 mL MeOH plus 1 mL HPLC water) Oasis HLB Prospekt™ cartridge (10×1 mm) from Waters (Mildford, MA, USA). After sample loading, the cartridge was washed with 1 mL of a 5% methanol water solution and further eluted with the chromatographic mobile phase. Chromatographic separation was performed with a Binary HPLC pump Model 1525 from Waters using a Purospher STAR RP-18e column (125x2 mm, 5 m particle diameter, from Merck, Darmstadt, Germany) and gradient elution with methanol and water as mobile phase. MS/MS detection was performed in the selected reaction monitoring (SRM) mode acquiring 2 SRM transitions per compound and 1 SRM transition per surrogate using a TQD triple-quadrupole mass spectrometer from Waters equipped with an electrospray interface. Quantitation was performed by the internal standard method using the corresponding deuterated compound as surrogate standard. Due to defective functioning (inaccurate sample volume acquisition) of the SPE processor, the first 3 results of the Mn(IV)-, Fe(III)- and SO_4-reducing experiments and the first 2 results of the “Natural Conditions” experiment could only be considered as semiquantitative.

2.2.6 Modeling

The hydrogeochemical evolution of the experiments was simulated by using CHEPROO (Bea et al., 2009), a Fortran 90 module using object-oriented concepts that simulates complex hydrobiogeochemical processes. The thermodynamic database used was that of EQ3NR code (Wolery, 1992).

The precipitation of calcite, magnesite, dolomite, siderite and amorphous iron sulphide was assumed to be controlled by kinetic. A simplified formulation was used to describe their reaction rate r [mol m^{-3} s^{-1}]:

$$r = k\sigma(1-\Omega) \quad [2.1]$$

where k is a rate constant [mol m^{-2} s^{-1}], σ is the reactive surface of the mineral [m^2 m^{-3}], and Ω is the saturation ratio [-].

The microbially mediated redox reactions for the organic substrates degradation (only the easily degradable substrates were considered, i.e. acetate and methanol) were described by kinetic rate laws based on Monod expressions:

$$r_i = k_i S \cdot \frac{TEA}{K_{i\_TEA} + TEA} \cdot \frac{K_{inhib\_I}}{K_{inhib\_I} + I} \quad [2.2]$$

where r_i is the rate of consumption of the substrate S [mol $L^{-1}s^{-1}$], k_i is the first order rate coefficient [s^{-1}], S is the substrate concentration [mol L^{-1}], TEA is the concentration of the particular Terminal Electron Acceptor [mol L^{-1}], K_{i_TEA} is the Monod half saturation constant with respect to TEA [mol L^{-1}], *I* is the concentration of an inhibiting substance (e.g. a competing TEA) [mol L^{-1}] and K_{inhib_I} is the inhibition constant [mol L^{-1}]. Multiple Monod and inhibition terms could be included in equation 2.2 if deemed necessary.

The code and the input files used in the simulations could be found in the digital support (cd) provided with the present thesis.

2.3. Results and discussion

2.3.1 General water chemistry

According to the reactions of Table 2.3, a general trend of decrease in DOC and increase in Alkalinity were expected in the biotic experiments. A simultaneous decrease in the concentration of the target dissolved electron acceptors was expected for the NO_3- and SO_4-reducing experiments, whereas an increase in the concentration of Fe(II) and Mn(II), products of the reduction of the target solid electron acceptors, was expected in the Mn- and Fe-reducing experiments. Details on the geochemical evolution (experimental datasets and simulations) of the biotic experiments are given below. The results from duplicate batches showed a satisfactory reproducibility at all sampling times. Actually, when plotting the measurements plus the error bars from each batch, there was always some overlap. Thus, the following graphics report the average of results and manual measurements from the duplicate bottles.

Regarding the Abiotic experiment, the hydrochemistry remained practically constant for the whole time as expected (results not shown).

2.3.1.1 NO_3-reducing experiments

Results from the NO_3-reducing experiments are shown in Figure 2.1. During the first 10 days, DOC decreased from 9.7 mmol L^{-1} to 1.5 mmol L^{-1}. Afterwards it remained practically constant. At day 10, the 6.7 mmol L^{-1} of nitrate initially present in the water have disappeared. Nitrite concentration began to increase after only some 12 hours, reaching a maximum at day 5 and becoming completely depleted by day 10. Very low concentrations of dissolved Manganese and Iron were detected after day 10, presumably from the dissolution and reduction of small quantities of the Mn and Fe oxides naturally present in the sediment. Sulphate remained constant during the whole experiment.

These observations suggest that nitrate reducing conditions were established within a short period (~ 0.5 days) of microbial adaptation and dominated the system during the first 10 days. The increase of nitrite, followed by its depletion, reflected the actual pathway for nitrate reduction, with nitrite being an intermediate product between nitrate and nitrogen. After day 10, a different more reducing condition was established.

The experiment was planned to guarantee complete depletion of organic carbon with excess of nitrate, allowing nitrate-reducing conditions to dominate during longer time. However, the actual initial nitrate and DOC concentrations (6.7 and 9.7 mmol L^{-1}, respectively) turned out to be different from their designed amounts (7.4 and 6.9 mmol L^{-1}, respectively) due to some unidentified problem during the assembling of the experiment. Consequently, nitrate and nitrite were completely depleted, while some organic carbon was still present after day 10.

The decrease of DOC (8.3 mmol L^{-1}) up to day 10 exceeded its expected stoichiometric removal (6.5 mmol L^{-1}) calculated by assuming that the only process that can change nitrate concentration was reduction coupled with organic matter oxidation, and according to reactions 2a and 2b of Table 2.3 in which biomass formation is not taken into account. This suggests that some organic carbon was used into microorganisms' growth. An estimation of such investment could be made by introducing an additional reaction. In fact, since carbon in bulk biomass has a redox state of 0, the formation of biomass require a partial oxidation in the case of Methanol (redox state of carbon = -2). Coupling it with the reduction of nitrate, the following stoichiometry could be written:

$CH_3OH + 0.4\ NO_3^- + 0.4\ H^+ \rightarrow CH_2O + 0.2\ N_2 + 1.2\ H_2O$ [reaction 2c]

where CH_2O has been used as simplified formula for biomass.

By using reactions 2a and 2b of Table 2.3, and reaction 2c, a conversion of 2.2 mmol L^{-1} of organic carbon into biomass could be finally estimated, i.e. about 27% of the total organic carbon consumption.

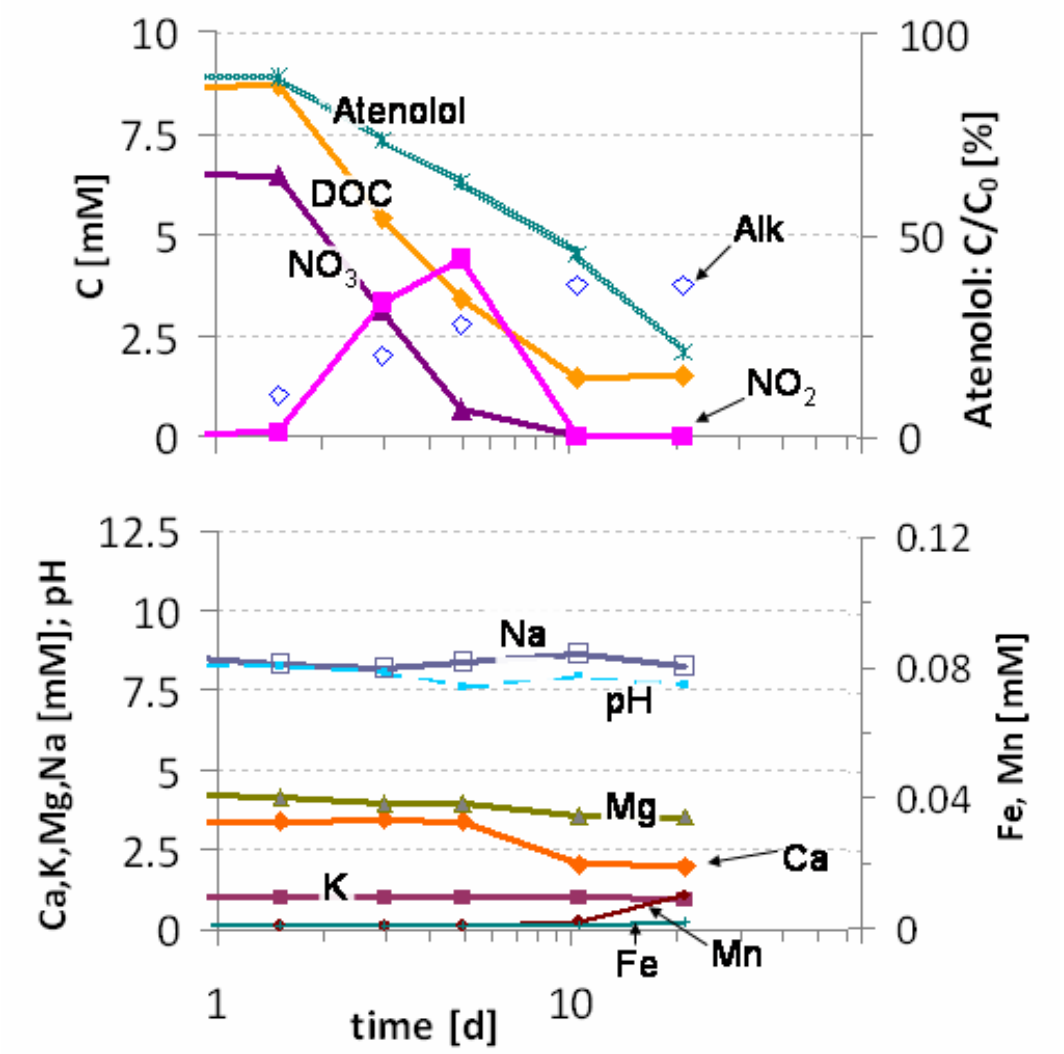


Figure 2.1: Chemical evolution with time in the NO3-reducing experiment

Alkalinity increased with time, but its final value at day 10 (3.75 mmol L^{-1}) was smaller than the expected one (7 mmol L^{-1}) calculated by taking into account the amount of organic substrate mineralized under the previous hypothesis. Part of this gap could be explained by the net reduction of Ca and Mg concentrations (0.9 mmol L^{-1} and 1.1 mmol L^{-1}, respectively), which suggests that precipitation of $CaCO_3$, $MgCO_3$ or mixed carbonates was limiting the actual increase in bicarbonate concentration. The Saturation Index (S.I.) of these minerals during the experiment supports this hypothesis. It ranged between 0.24 and 0.71 for calcite, and from 0.06 to 0.45 for magnesite. Throughout the chapter, S.I. values were calculated using the PHREEQC code with WATEQ

thermodynamic database (Parkhurst and Appelo, 1999). After the conclusion of the experiment, inspection of the sediment samples by SEM-EDS showed indeed small crystals of calcite and Mg-Ca carbonates on the surface of the sediment grains (Fig. 2.2A).

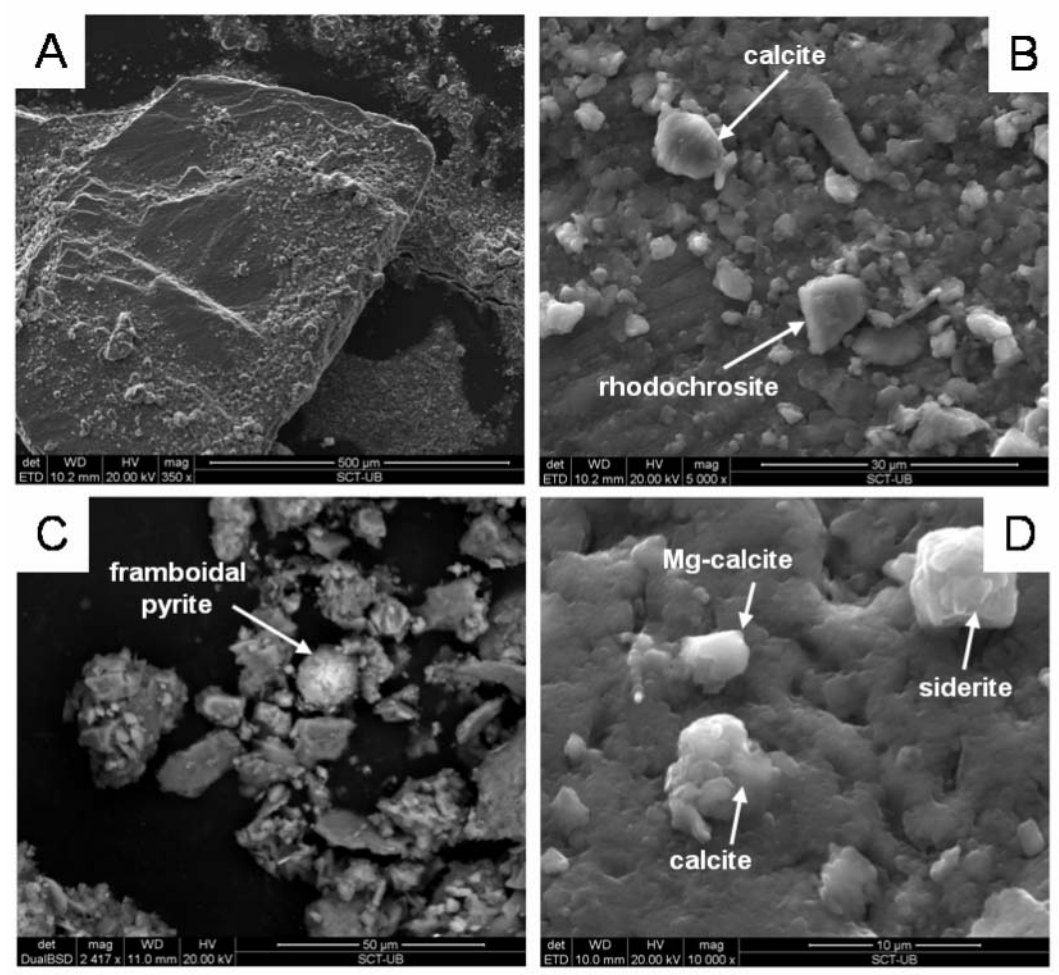


Figure 2.2: SEM images of sediment samples from the disassembled batches. A) Neo-formed carbonate grains on the cleavage surface of a feldspar crystal present in the sediment in the NO3-reducing experiment. B) precipitates of calcite and rhodochrosite in the Mn-reducing experiment. C) precipitates of Ca-, Mg-Ca- and Fe-carbonates in the Fe-reducing experiment. D) framboidal pyrite was occasionally observed in the Fe-reducing experiment, likely originated by the turnover of FeS previously precipitated

We next consider the equilibrium of the aqueous carbonate species with the gas in the headspace of the bottles. By day 10, about 0.3 mmol L^{-1} of inorganic carbon (representing the 7% of the total inorganic carbon inventory) have been transferred to the gas phase as $CO2_{(g)}$. Finally, taking into account the precision of Alkalinity measurements (±0.5 mmol L^{-1}), the overall inorganic carbon mass balance could be closed with an error of about 15%.

The simulations carried out with CHEPROO (Figure 2.3) support the feasibility of the previous hypotheses. The most important parameters used are reported in Table 2.6.

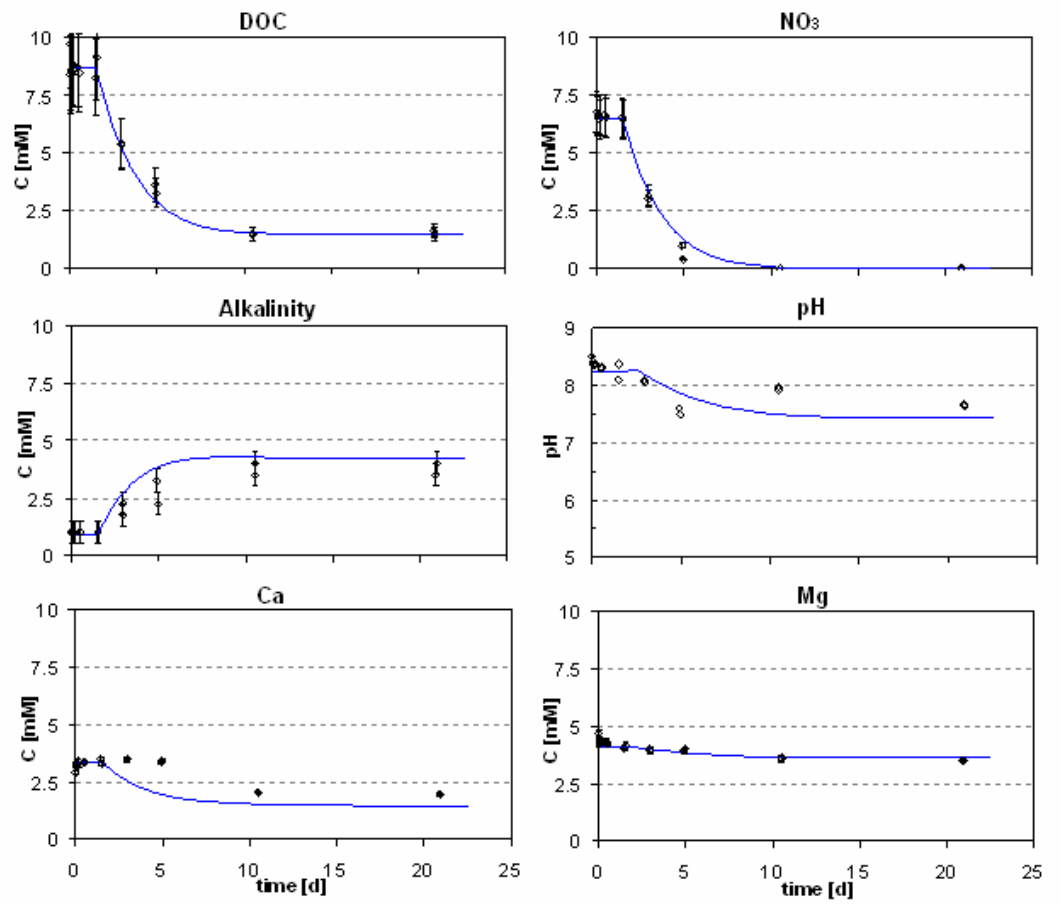


Figure 2.3: Chemical evolution with time in the NO3-reducing experiment: simulations (solid lines) versus experimental data (dots)

Table 2.6: Parameters used in the simulation of the NO3-reducing experiment

process	reactive rates constants		
	constant	used in the simulations	literature values
degradation of organic matter by NO3	first order rate coefficient k_{NO3} [s^{-1}]	6e-6[(a)]	1.2e-11[(b)] ; 7.5e-6[(c)] ; 1.16e-7[(d)]; 2e-5[(e)]
	half saturation constant K_{NO3} [mol L^{-1}]	8e-4[(a)]	1e-4[(b)] ; 3.6e-5[(c)] ; 8.1e-6[(d)] ; 8.1e-6[(e)]
calcite precipitation	$k\sigma$ [mol m^{-3} s^{-1}]	4.64e-7[(f)]	
magnesite precipitation		8e-8[(a)]	

(a) calibrated
(b) Brun and Engesgaard, 2002: substrate = CH_2O.
(c) Ojeda et al., 2008: substrate = CH_2O.
(d) Rolle et al., 2008: substrate = CH_2O.
(e) Watson et al., 2003: substrate = CH_3COOH.
(f) Inskeep and Bloom, 1986.

2.3.1.2 Mn(III/IV)-reducing experiments

Results from the Manganese reducing experiments are shown in Figure 2.4. DOC decreased with time from 6.7 to 1 mmol L^{-1}, starting after day 7 and reaching a significant removal rate after day 14. The small initial NO_3 (0.1 mmol L^{-1}) had already disappeared by day 7 (not shown), having only oxidized a small amount of DOC (a maximum of 0.2 mmol L^{-1}). Consistently with DOC decrease, dissolved Mn increased from day 7 reaching a concentration of 0.1 mmol L^{-1} at day 25, which is then maintained for the rest of the experiment. No Fe was detected and SO_4 concentration remained almost constant during the whole experiment.

Alkalinity increased slightly until day 25. Thereafter, up to day 42 it dropped down to a value that remained steady throughout the rest of the experiment. The net reduction of Ca and Mg concentrations (1.3 mmol L^{-1} and 1 mmol L^{-1} respectively) and a lower than expected increase of Mn concentration suggested precipitation of Mg-Ca carbonates and $MnCO_3$, limiting alkalinity and dissolved Mn. The computed S.I. with respect to calcite and rhodochrosite during the experiments (between -0.11 and 0.97 for calcite, and between 1.15 and 1.73 for rhodochrosite) supports this hypothesis. SEM-EDS examination of the sediments from the disassembled experiments showed the presence of small crystals of calcite, Mn-bearing carbonates, Mg-Ca carbonates, and rhodochrosite (Fig. 2.2B).

Regarding the low Mn concentration detected, aside from the fact that a fraction of the original DOC was invested in biomass growth implying a smaller total Mn^{2+} production than that corresponding to the complete mineralization of all substrates, an additional explanation could be some Mn^{2+} adsorption on the clay surfaces (von Gunten and Zobrist, 1993; Wang and Van Cappellen, 1996).

In summary, DOC and the redox sensitive species suggests that Mn-reducing conditions were established after ~ 1 week of microbial adaptation and were then maintained during the test. The slow dissolution of the natural source of Mn(III/IV) used in the experiment was likely representing a rate limiting factor for the Mn reduction. Since the exact identity of the Mn oxide-hydroxides added could not be confirmed, an unequivocal mass balance for C and Mn (according to reactions 3.1a to 3.2b of Table 2.3) could not be carried out and quantitative modeling was not performed.

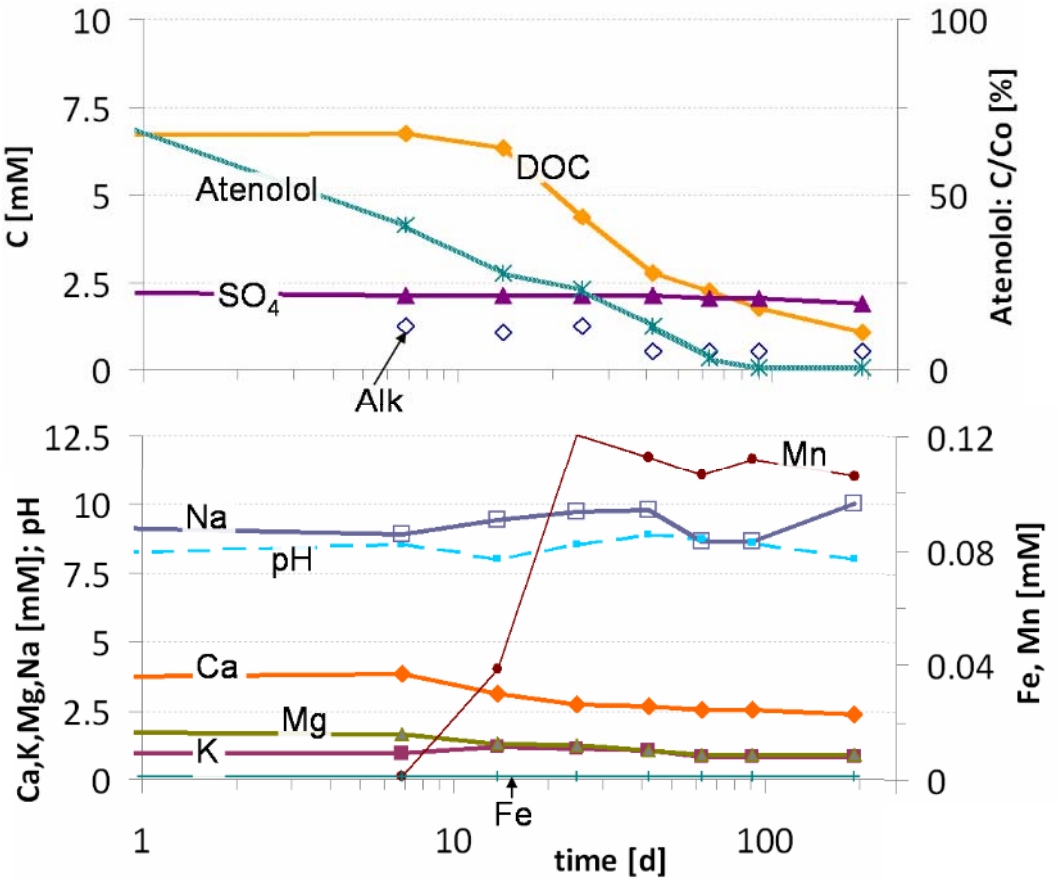


Figure 2.4: Chemical evolution with time in the Mn(III/IV)-reducing experiment

2.3.1.3 Fe(III)-reducing experiments

Results from the Iron reducing experiments are shown in Figure 2.5. A small decrease of DOC could be observed starting from day 7. By then, the small initial NO_3 (0.1 mmol L^{-1}) had already disappeared (not shown), having oxidized a small amount of DOC (a maximum of 0.2 mmol L^{-1}). Significant changes in water chemistry could be observed after day 14, but Fe was not detected until day 42, when the 2.3 mmol L^{-1} of initial SO_4 had been completely depleted. After day 42 the dissolved Fe (Fe(II) at the pH range of this experiment) increased slightly to about 0.06 mmol L^{-1}. DOC decreased with time from 7.8 to 3.2 mmol L^{-1} at day 42 and to complete depletion after day 91. Alkalinity increased from 1.2 to 3.0 mmol L^{-1} at day 42 and to 3.6 mmol L^{-1} thereafter. Ca and Mg concentrations decreased along the experiment: 1 and 0.6 mmol L^{-1} up to day 42, and 0.2 and 0 mmol L^{-1} after day 42, respectively. A very low concentration of dissolved Mn, never exceeding 0.01 mmol L^{-1}, was detected starting day 14. Probably it was produced by the reductive dissolution of some Mn mineral, naturally present in the sediments, causing a negligible effect on DOC concentration.

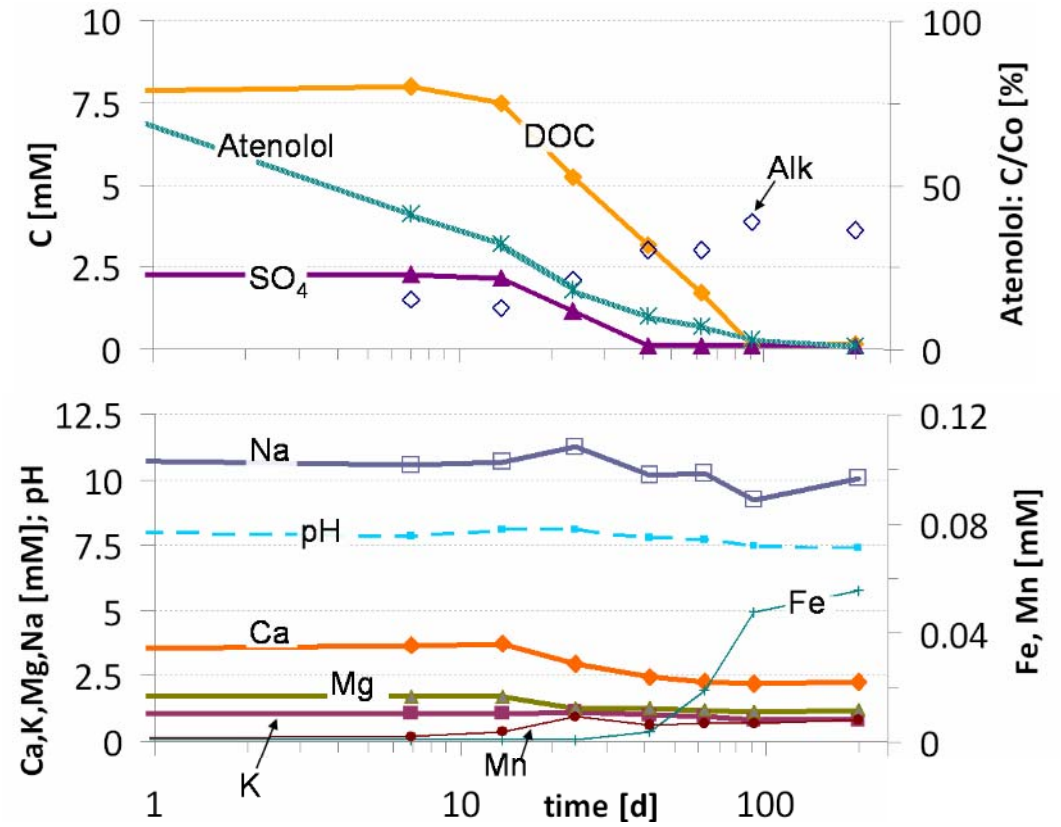


Figure 2.5: Chemical evolution with time in the Fe(III)-reducing experiment

The above observations suggests that, after approximately 1 week of microbial adaptation, both SO_4 and Fe(III) were reduced until day 42 causing FeS precipitation, whose low solubility prevented build up of dissolved Fe. This was confirmed by the dark colour of the sediments at disassembling. Occasionally some framboidal pyrite has been observed by SEM-EDS on the surface of sediment grains (Fig. 2.2C), likely generated by the ageing of precipitated FeS. Since dissolved Fe did not increase until SO_4 was exhausted, the rate of Fe(III)-reduction needs to be slower than SO_4-reduction. While this would contradict the sequence of Table 2.3, it was not entirely surprising since the Fe(III) source was a natural solid phase. Slow dissolution of this source may be the rate limiting mechanism for Fe reduction. This means that HS^- could be in part accumulated in solution. Concomitant Fe(III)- and SO_4-reduction and iron sulphide precipitation has already been observed in field and laboratory studies (Brown et al., 2000 and references therein; Ludvigsen et al., 1998; Jakobsen and Postma, 1999; Weiner et al., 1998). After day 42, SO_4 was exhausted and Fe(III) reducing conditions were likely to be dominating the system. In fact, concomitant occurrence of methanogenesis could not be excluded after day 42, favoured by the slow rate of Fe(III)-reduction. Coupling the two processes with siderite precipitation, this could represent another limiting factor for the increase of Fe(II) concentration. Similar scenarios have

been already reported in literature (Jakobsen and Cold, 2007). Additional potential explanations for the low Fe(II) concentration detected, aside from the fact that the investment of some DOC in biomass growth implies a smaller Fe(II) production than mineralization, could be some Fe(II) adsorption onto clay surfaces (Wang and Van Cappellen, 1996) and/or its incorporation with some remaining solid Fe(III) to form magnetite (mixed Fe(II)-Fe(III) oxide) (Broholm et al., 2000 and references therein; Brown et al., 2000; Lovley and Phillips, 1988).

Small crystals of calcite, Mg-Ca carbonates and siderite were observed on the surface of sediment grains (Fig. 2.2D), confirming that indeed precipitation of carbonates was limiting the increase of Alkalinity.

When modeling the experiment with CHEPROO, the best fits of experimental data were obtained under the hypothesis of organic matter being degraded during the first part of the experiment by SO_4 and, to a lesser extent, by Fe(III); then, after SO_4 depletion, concomitant Fe-reduction and methanogenesis were assumed to be responsible of the organic substrate consumption (Figure 2.6). The most important parameters used in the simulation, identified as simulation “A” in the following, are detailed in Table 2.7.

Figure 2.6: Chemical evolution with time in the Fe(III)-reducing experiment: simulation “A” (solid lines) versus experimental data (dots)

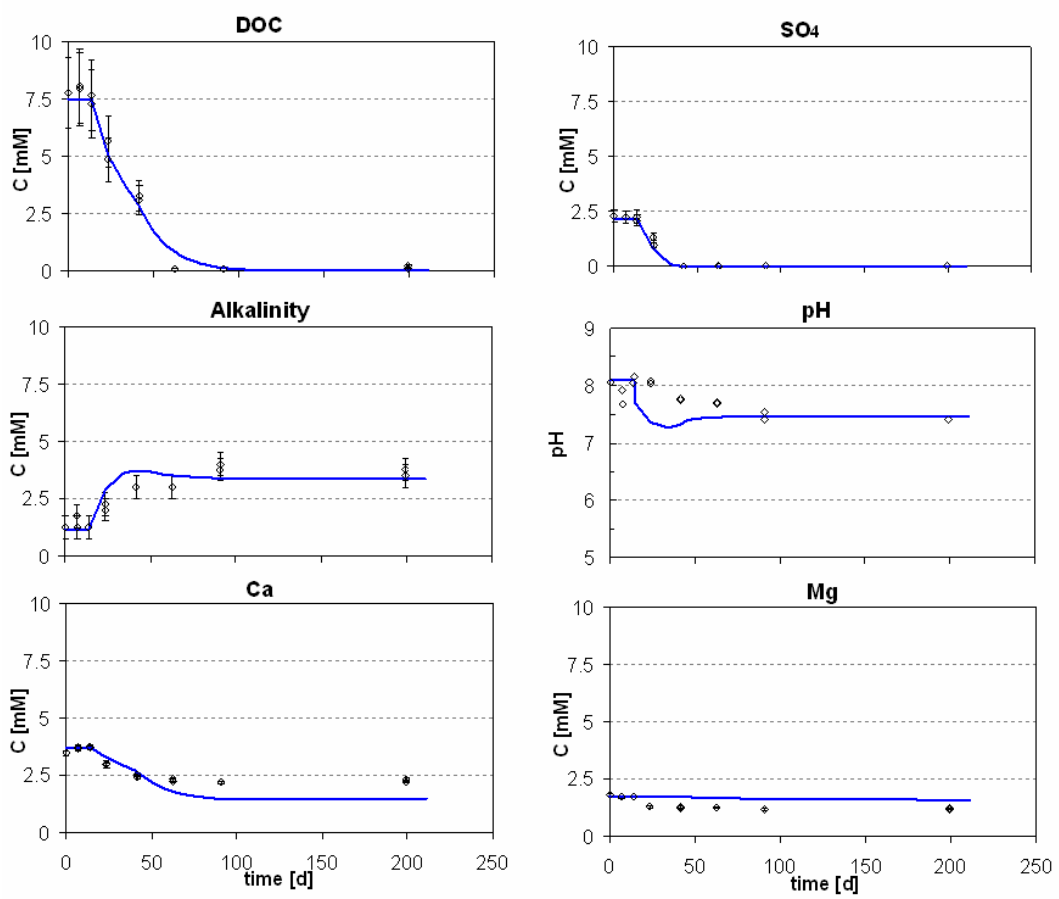


Table 2.7: Parameters used in the simulation "A" of the Fe(III)-reducing experiment.

process	reactive rates constants		
	constant	used in the simulations	literature values
degradation of organic matter by Fe(III)	first order rate coefficient $k_{Fe(OH)3}$ [s^{-1}]	5e-5[a]	1.2e-11[b] ; 1.27e-6[c]; 4.1e-7[d]
	half saturation constant $K_{Fe(OH)3}$ [mol L^{-1}]	5e-6[a]	8.1e-6[c]
	inhibition constant K_{inhib_SO4} [mol L^{-1}]	8e-6[a]	
degradation of organic matter by SO4	first order rate coefficient k_{SO4} [s^{-1}]	5e-7[a]	1.2e-11[b] ; 1.39e-6[c]; 1e-5[d]; 3e-6[e]
	half saturation constant K_{SO4} [mol L^{-1}]	1e-4[a]	1e-4[b] ; 8.1e-6[c] ; 1.6e-4[d] ; 5.9e-4[e]
	inhibition constant	-----	
methanogenesis	first order rate coefficient k_{CH4} [s^{-1}]	1.5e-7[a]	1.2e-11[b] ; 6.94e-6[c] ; 8e-7[d]; 2.2e-6[e]
	half saturation constant	-----	
	inhibition constant K_{inhib_SO4} [mol L^{-1}]	8e-6[a]	1e-7[b] ; 1.04e-3[c] ; 1.6e-5[d] ; 5.9e-4[e]
calcite precipitation	$k\sigma$ [mol m^{-3} s^{-1}]	4.64e-7[f]	
dolomite precipitation		7e-9[a]	
amorphous iron sulphide precipitation		8e-8[a]	
siderite precipitation		8e-8[a]	
ferrihydrite dissolution	equilibrium		

(a) calibrated
(b) Brun and Engesgaard, 2002: substrate = CH_2O.
(c) Rolle et al., 2008: substrate = CH_2O.
(d) Watson et al., 2003: substrate = CH_3COOH.
(e) Ojeda et al., 2008: substrate = CH_2O.
(f) Inskeep and Bloom, 1986.

Among the numberless combinations of hypotheses likely accounting for the complex geochemical evolution of the experiment, the simulations presented in this section were carried out without taking into account biomass growth during SO_4-reduction and methanogenesis.

The need of considering the occurrence of Fe(III)-reduction and methanogenesis beside SO_4-reduction was suggested by the bigger departure between model results and measurements (Figure 2.7) when considering the degradation of organic matter coupled with one of the following processes: SO_4-reduction (in this case biomass growth was included) (simulation "B"); SO_4-reduction and slow Fe(III)-reduction (simulation "C"); SO_4-reduction and fast Fe(III)-reduction (simulation "D"); SO_4-reduction and methanogenesis (inhibited by SO_4 presence) (simulation "E").

To be noted, moreover, that simulation “A” was obtained by considering inhibition of Fe(III)-reduction in the presence of SO_4. On the contrary, again, computed DOC and Alkalinity showed worst fits to experimental data (results not shown).

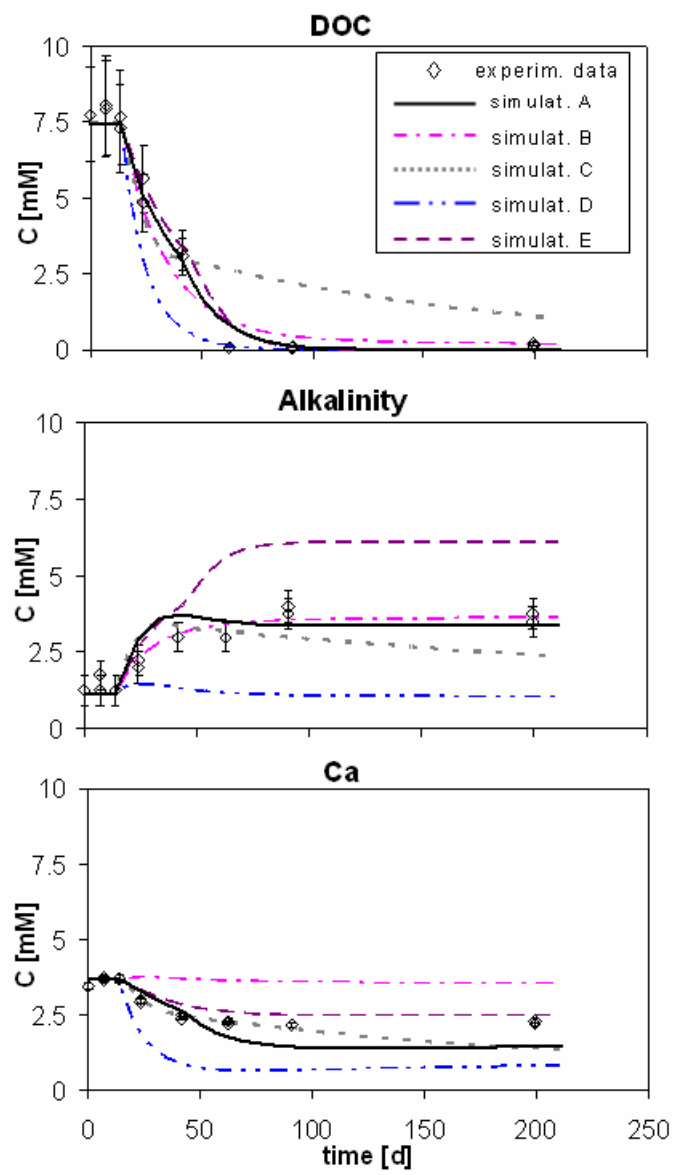


Figure 2.7: Chemical evolution with time in the Fe(III)-reducing experiment: simulations (lines) versus experimental data (dots). Namely, regarding simulations: (A) SO4-reduction, Fe(III)-reduction and methanogenesis (both the latter inhibited by SO4), (B) SO4-reduction (biomass growth included), (C) SO4-reduction and slow Fe(III)-reduction, (D) SO4-reduction and fast Fe(III)-reduction, (E) SO4-reduction and methanogenesis (inhibited by SO4).

2.3.1.4 SO_4-reducing experiments

Results from the sulphate reducing experiments are shown in Figure 2.8. A small decrease of DOC occurred prior to day 7, part of it (a maximum of 0.2 mmol L^{-1}) being associated with the depletion of the initial 0.1 mmol L^{-1} of NO_3 (not shown). Nevertheless, the significant decreases in both DOC and SO_4 could be observed between days 18 and 65. By then, the initial 10.2 mmol L^{-1} of DOC have been almost depleted (about 0.3 mmol L^{-1} remaining) and SO_4 concentration has decreased from the initial 5.3 mmol L^{-1} to 2.1 mmol L^{-1}. After day 65, SO_4 continued decreasing down to 1.3 mmol L^{-1} at the end of the experiment despite the fact that DOC had been already practically exhausted. In parallel, alkalinity increased continuously from the initial 1.5 mmol L^{-1} to 4.3 mmol L^{-1} at day 65, and up to 6.3 mmol L^{-1} by the end of the experiment. Ca and Mg concentrations decreased from 3.6 to 1.3 mmol L^{-1} and from 1.8 to 0.9 mmol L^{-1}, respectively. A very low concentration of Mn, never exceeding 0.01 mmol L^{-1}, was detected during the whole experiment, probably associated to dissolution or reduction of some Mn mineral naturally present in the sediments, this having a negligible effect on DOC.

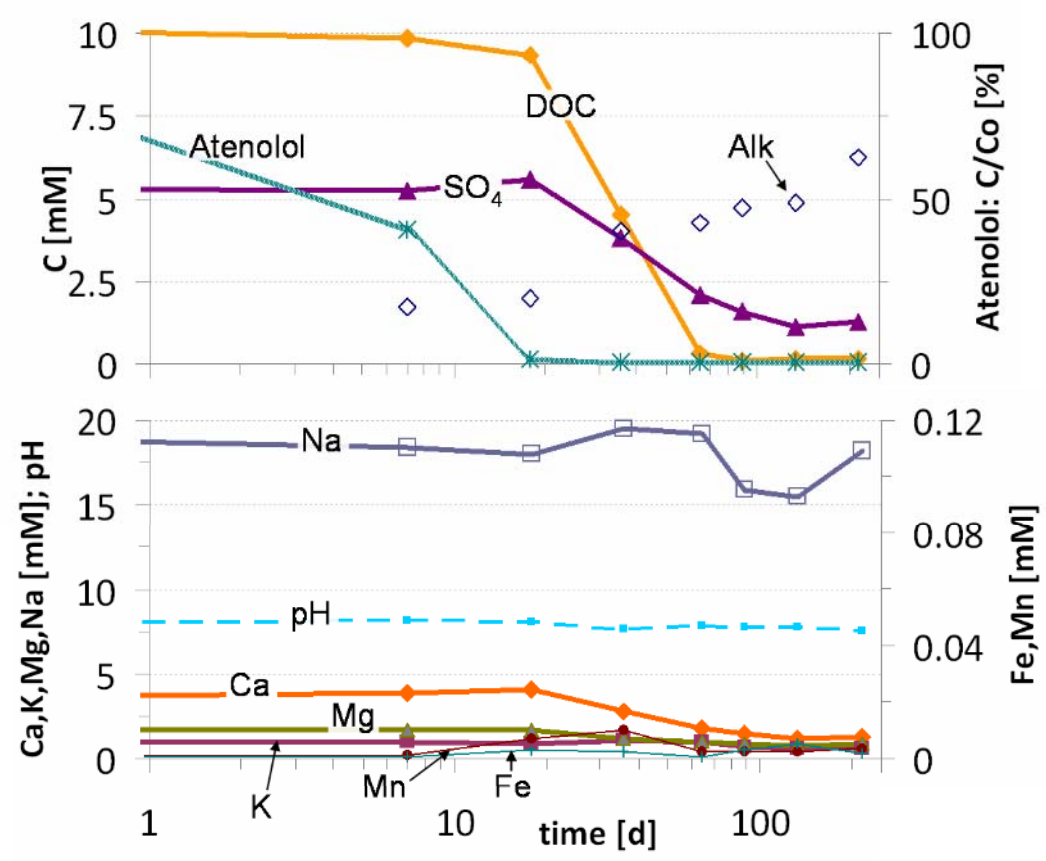


Figure 2.8: Chemical evolution with time in the SO4-reducing experiment

As explained in the case of Fe(III) and as result of SO_4-reduction, two alternative hypothesis might be formulated: (1) HS^- remained in solution, or (2) HS^- precipitated as FeS with

the Fe^{2+} produced by reduction of some of the Fe(III) oxides present in the original sediment. Hypothesis (1) was supported by a decrease in charge balance error for the water samples up to day 65. Hypothesis (2) was supported by the dark colour in the sediments after day 18.

Assuming hypothesis (1), by using the actual decrease in sulphate concentration (3.2 mmol L^{-1}) and DOC (9.9 mmol L^{-1}), the stoichiometries in Table 2.3 and the additional reactions:

$CH_3OH + 2\ Fe(OH)_3(s) + 4\ H^+ \rightarrow CH_2O + 2\ Fe^{2+} + 6\ H_2O$ [reaction 4c]

$CH_3OH + 0.25\ SO_4^{2-} + 0.25\ H^+ \rightarrow CH_2O + 0.25\ HS^- + H_2O$ [reaction 5c]

to take into account that in the case of Methanol the formation of biomass (simplified formula: CH_2O) require a partial oxidation too, it could be estimated that a total amount of 5.3 mmol L^{-1} DOC was mineralized up to day 65 while 4.6 mmol L^{-1} were inverted into microorganisms' growth (47%). Since sulphate still decreases and alkalinity increases after DOC is nearly exhausted, we concluded that biomass was reused (Alexander, 1999). Under this assumption and according with stoichiometry, to reduce the remaining 0.8 mmol L^{-1} SO_4, 0.17 mmol L^{-1} of the remaining DOC and some 1.5 mmol L^{-1} of accumulated biomass were further mineralized after day 65. Thus, the "net" investment of DOC into biomass during the whole experiment amounted to 3.1 mmol L^{-1}. Global balance of the experiment implies that 69% of organic carbon decay was associated to mineralization of the organic substrates coupled with sulphate reduction, and 31% was inverted into microorganisms' growth.

Assuming hypothesis (2), with some concomitant Fe(III) reduction occurring, a similar calculation could be made and the global balance resulted in 69 to 72% of organic carbon diminution associated to substrates mineralization coupled with sulphate reduction, a 7 to 10% coupled with Fe(III) reduction, and 21% of organic carbon inverted into microorganisms' growth.

Most likely, actual processes lied between these two extreme cases, implying that a fraction of the HS^- remained in solution as aqueous species while part of it was precipitated as FeS. In either case, SO_4-reduction coupled with organic matter degradation was the dominating process during the experiment. A microbial equilibration period or the enmasking of SO_4-reducing early stages by the analytical errors could explain the not significant changes characterizing water chemistry during the first 7 to 18 days of the experiment.

The total DOC mineralization estimated under both hypothesis (1) and (2) (6.9 and 7.9 mmol L^{-1}, respectively) were higher than the measured increase in alkalinity (4.8 mmol L^{-1}). Attributing the net reduction of Ca and Mg concentrations (2.3 mmol L^{-1} and 1 mmol L^{-1} respectively) to the precipitation of Mg and Ca carbonates, and taking into account the inorganic carbon present as gaseous phase in the headspace of the bottles as well as the precision of

alkalinity measurements, the expected alkalinity fitted quite well the measured value. The resulting error in the global carbon balance amounts to about 13% and 1% under hypothesis (1) and (2), respectively. Indeed, Mg and Ca carbonate crystals were observed on the sediment grain surfaces.

The results for the simulations of the experiment chemical evolution under an intermediate case between hypothesis (1) and (2) are reported in Figure 2.9. The most important parameters used are detailed in Table 2.8.

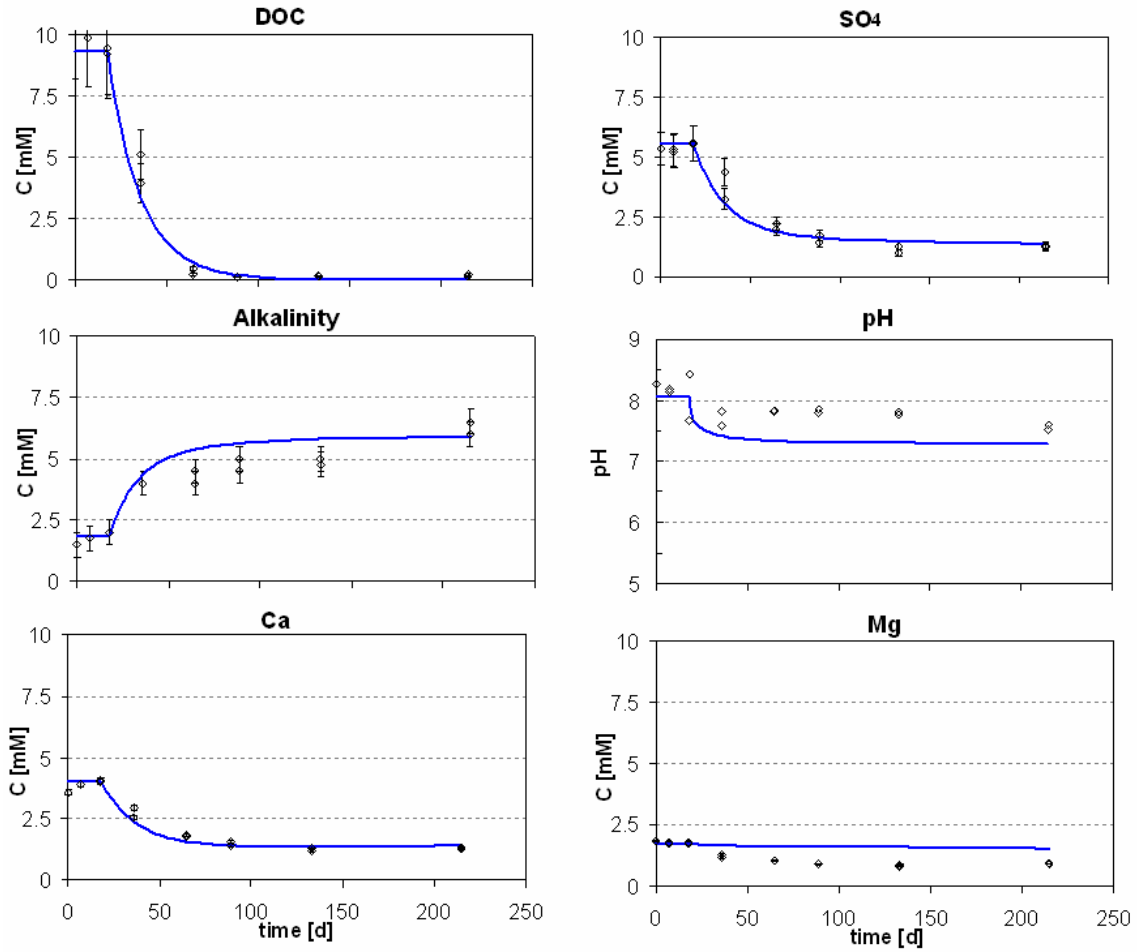


Figure 2.9: Chemical evolution with time in the SO4-reducing experiment: simulations (solid lines) versus experimental data (dots).

Table 2.8: Parameters used in the simulation of the SO4-reducing experiment

process	reactive rates constants		
	constant	used in the simulations	literature values
degradation of organic matter by Fe(III)	first order rate coefficient $k_{Fe(OH)3}$ [s^{-1}]	7e-6[(a)]	1.2e-11[(b)] ; 1.27e-6[(c)] ; 4.1e-7[(d)]
	half saturation constant $K_{Fe(OH)3}$ [mol L^{-1}]	5e-6[(a)]	8.1e-6[(c)]
	inhibition constant	-----	
degradation of organic matter by SO4	first order rate coefficient k_{SO4} [s^{-1}]	6e-7[(a)]	1.2e-11[(b)] ; 1.39e-6[(c)] ; 1e-5[(d)]; 3e-6[(e)]
	half saturation constant K_{SO4} [mol L^{-1}]	1e-4[(a)]	1e-4[(b)] ; 8.1e-6[(c)] ; 1.6e-4[(d)] ; 5.9e-4[(e)]
	inhibition constant	-----	
biomass "reutilization": degradation by SO4	first order rate coefficient k_{SO4} [s^{-1}]	1.5e-7[(a)]	
	half saturation constant K_{SO4} [mol L^{-1}]	1e-5[(a)]	
	inhibition constant K_{inhib_DOC} [mol L^{-1}]	8e-4[(a)]	
calcite precipitation	$k\sigma$ [mol m^{-3} s^{-1}]	4.64e-7[(f)]	
dolomite precipitation		7e-9[(a)]	
amorphous iron sulphide precipitation		8e-8[(a)]	
siderite precipitation		8e-8[(a)]	
ferrihydrite dissolution	equilibrium		

(a) calibrated
(b) Brun and Engesgaard, 2002: substrate = CH_2O.
(c) Rolle et al., 2008: substrate = CH_2O.
(d) Watson et al., 2003: substrate = CH_3COOH.
(e) Ojeda et al., 2008: substrate = CH_2O.
(f) Inskeep and Bloom, 1986.

2.3.1.5 "Natural Conditions" experiments

Results from the "Natural Conditions" experiments are shown in Figure 2.10. The initial 2.5 mmol L^{-1} DOC were almost completely depleted, starting from the very beginning of the experiment. The initial 0.2 mmol L^{-1} of dissolved O_2 (not shown) and 0.1 mmol L^{-1} of NO_3 were totally removed after 1 and 3 days, respectively. Dissolved Mn was observed at approximately day 10, reaching a maximum concentration of 0.03 mmol L^{-1} at day 62; then, Mn concentration decreased to about zero at the last sampling time of 192 days. Some dissolved Fe was detected between days 15 and 62, never exceeding 0.02 mmol L^{-1}. The initial 2.3 mmol L^{-1} SO_4 decreased with some fluctuations to 1.9 mmol L^{-1}, from day 15 to the end of the experiment. The alkalinity showed an overall increase during the experiment, from 0.5 to 2.3 mmol L^{-1}.

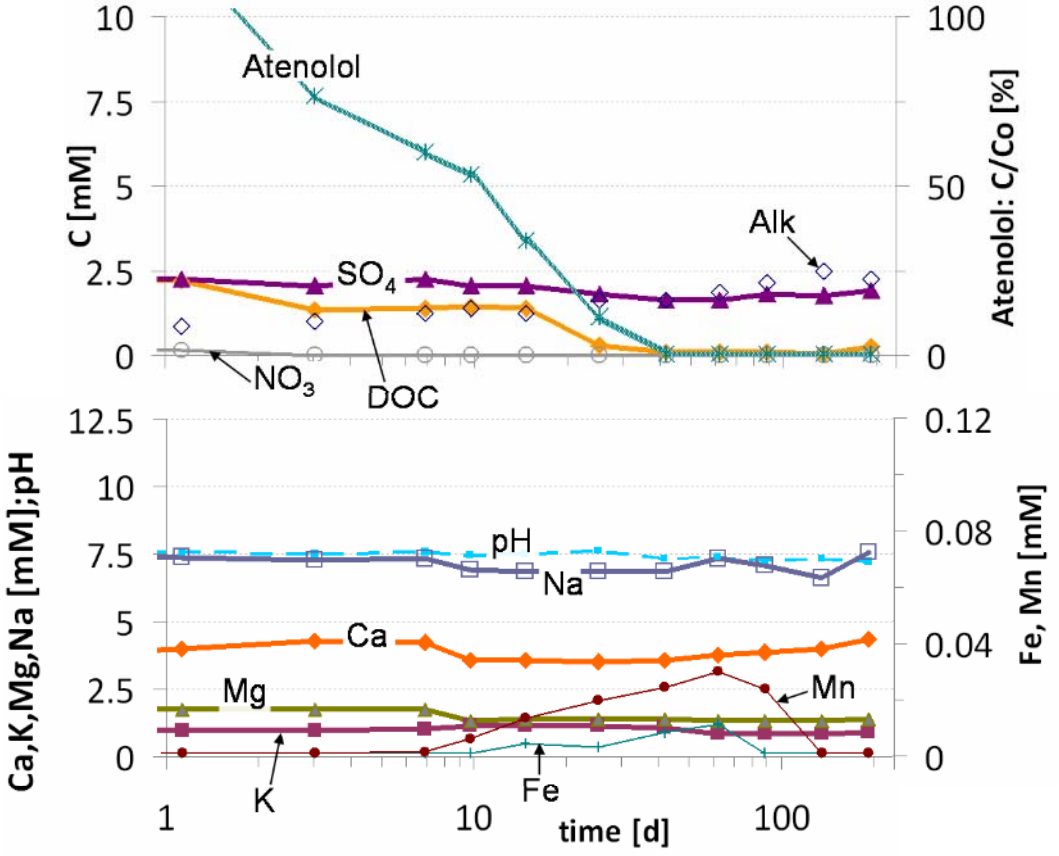


Figure 2.10: Chemical evolution with time in the experiment performed under natural conditions

To sum up, since no specific redox state was deliberately stimulated in the "Natural Conditions" experiment, the organic matter degradation reactions occurred in the expected sequence (Table 2.3, set "a" of reactions), until complete depletion of the specific electron acceptor (e.g. oxygen and nitrate) or, finally, of the electron donor. Aerobic degradation dominated the first day, and nitrate reduction appeared to control degradation until day 3. From there on, Fe and Mn-reducing conditions were found. These overlap with the SO_4-reduction, which occurred after day 15. The presence of small zones of dark colour in some of the retrieved solid suggested that some precipitation of iron sulphide occurred. Also the decrease in Mg (0.4 mmol L^{-1}), Mn (0.03 mmol L^{-1}) and Fe (0.02 mmol L^{-1}) suggested that some carbonate precipitates. The overall Ca increase of about 0.8 mmol L^{-1} suggested carbonate dissolution, even if its concentration has been fluctuating during the experiment. Small crystals of Mg-Ca carbonates and siderite have been observed on the surface of sediment grains.

2.3.2 Fate of Atenolol

As example of application of the described microcosm study to the fate of emerging organic micropollutants under different redox conditions, the results for the β-blocker Atenolol are presented. The temporal evolution of its average normalized concentration (with respect to each actual initial concentration C_0) for the 6 sets of experiments described above is shown in Figure 2.11. The error bars were calculated by taking into account the analytical errors and the difference between duplicate batches results. Concentrations are presented in relative terms, normalized as C/Co, in order to remove systematic errors from the analysis.

The behaviour of Atenolol was similar in the NO_3-reducing experiment than in the first 10 days of the "Natural Conditions". Little removal of Atenolol was observed until day 1.5. Then concentrations started to decrease, following almost the same trend in both experiments and reaching an overall removal of about 50% at day 10. In the Mn(IV)-, Fe(III)- and SO_4-reducing tests, the lack of intermediate sampling points hindered the identification of Atenolol behaviour during the first week. Nevertheless, also in these three set of experiments the same overall removal of Atenolol of about 50% could be observed at day 7.

After day 7-10, different evolutions of the concentration curves of Atenolol could be identified for each set of batches. At day 18, complete removal of Atenolol was reached in the SO_4-reducing experiment. In fact, through the evolution of the general hydrochemistry we could not confirm if during this period the target redox condition was still being established or had already developed. Under Mn-reducing condition, 90% of removal was reached at day 42, up to complete removal at day 91. Similarly, in the Fe(III)-reducing experiment, under actual mixed Fe-/SO_4-reducing conditions, about 90% was removed at day 42. Complete depletion occurred later on, almost at the end of the experiment, under the sole Fe-reducing or mixed Fe-reducing/methanogenetic conditions. In the "Natural Conditions" experiment, under some mixed Mn-/Fe-/SO_4-reducing conditions, complete removal of Atenolol was reached already at day 42.

Results for the Abiotic experiment evidenced that, within the first 5 days, the removal trend for Atenolol was the same observed in the NO_3-reducing and "Natural Conditions" experiment, determining an overall removal of about 50%. After day 5, taking into account the error bars, no additional removal could be observed.

Thus, the initial ~ 50% removal of Atenolol occurring within the first 5-10 days in all experiments could be attributed to some abiotic process, most likely sorption on the sediment grains. By then, some microbial processes were responsible of the remaining 50% removal of

Atenolol. Different evolutions could be observed depending on the experiment, i.e. mainly depending on the redox conditions dominating or being established in each system. The NO_3-reducing experiment was too short to be compared with the remaining tests. Yet, among the latter, qualitatively it could be assessed that the faster Atenolol biotic removal was observed in the SO_4-reducing experiment, under the most reducing (already established or still being established) condition up to that moment.

Aiming at a better characterization of Atenolol biotic removal, a number of samples from the different experiments were analysed looking for the potential presence of transformation products, specifically for Atenololic Acid. This compound was identified as product of Atenolol microbial hydrolysis in a study of Radjenovic et al., 2008. The expectation to find Atenololic Acid at least in the NO_3-reducing experiment was fostered by its occurrence in a similar experiment we carried out with much higher (1mg/L) initial Atenolol concentration (Barbieri et al., in preparation). Unfortunately, we found out that the analytical methodology used was not adequate to detect the potential presence of Atenololic Acid at concentrations in the ng/L order of magnitude (in the experiment the maximum attainable concentration was 1µg/L). Thus, its presence could not be confirmed due to analytical restraints.

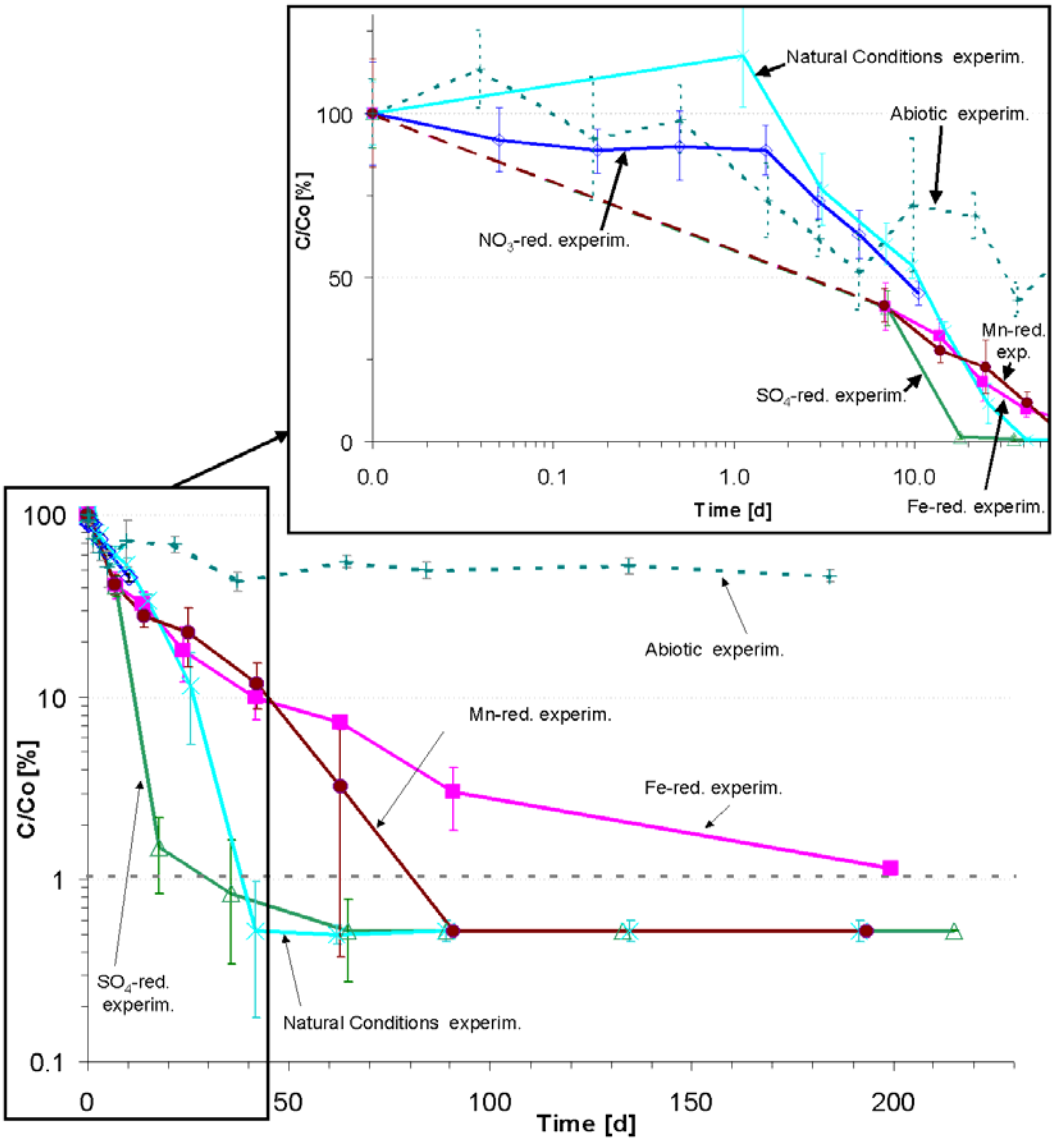


Figure 2.11: Temporal evolution of the Atenolol average normalized concentration in the different experiments.

2.4 Conclusions

The following concluding considerations and remarks can be made on the present study:

- The desired redox states have been quite successfully created and sustained in each set of experiments. The only exception was the Fe(III)-reducing experiment, where the Fe-reducing conditions were possibly dominant only after day 42 or mixed redox conditions characterized the whole experiment (mixed Fe-/SO_4-reducing up to day 42, and mixed Fe-reducing/methanogenetic after day 42). It is worth pointing that the use of natural sources of Mn and Fe is realistic, but complicates the development of controlled redox conditions. Natural sources are often quite crystalline, which slows down dissolution to the point of making it the rate limiting process.

- The assessment of the dominating redox states has been achieved by a thorough monitoring of water chemistry, focused on the redox-sensitive species but including major and minor ions too. Precipitation/dissolution of minerals as well as biomass production has to be taken into account for a correct interpretation of the main processes involved. Inspection of the sediments from the disassembled batch experiments through SEM-EDS has been fruitful used to confirm the occurrence of such processes.
- However, further improvements are required. Specifically, dissolved sulphide and methane should be analysed to better assess sulphate reducing conditions, especially in its early stages, and to check possible occurrence of methanogenesis. Additional desorption experiments could confirm Mn^{2+} and Fe^{2+} adsorption onto clay surfaces and/or exopolymeric substances (EPS). As general rule, whenever possible, the evaluation of the microbial state during the experiments (e.g.: identification of microbial communities, measurements of Hydrogen, etc.) would be also advisable as complementary tool for the identification of the prevailing redox state.
- Numerical modeling proved useful in confirming the concepts described above with literature kinetic rates. Matches between computations and observations could have been improved by varying the rates of carbonates precipitation, and by postulating likely occurring sorption onto biofilms. Departures between model results and measurements are small, but generally suggest an intricate coupling between biologic and inorganic processes.
- The sampling schedule has proven adequate for monitoring the temporal evolution of aqueous chemistry and micropollutant concentrations. Still, in the case of Mn-/Fe-/SO_4-reducing experiments some additional sampling point during the first week could have been useful to confirm Atenolol early removal trends.
- One of the aims of the study was to test systems representative of real aquifers and of conditions occurring either naturally or possibly being stimulated during managed artificial recharge operations. Such conditions may vary spatially and temporally along with recharge cycles and recharge water composition. Thus, the microbial communities naturally existing in the sediments used in the experiments, which were expected to carry out the biodegradation of organic matter and the removal of micropollutants, were not previously adapted to the redox conditions of interest. As a consequence, the first part of each experiment was characterized by a transition stage (of different duration) until the target redox state could be effectively established or observed. This hindered the interpretation of Atenolol results. Anyway, after a common removal for all experiments, which we associate to abiotic processes, different microbial removal trends were observed

for Atenolol, depending on the different sets of experiments, each one of them characterized by different redox conditions. This confirms that the redox state of the system could exert some influence on micropollutants behaviour. Even if neither the NO_3-reducing experiment was long enough to compare nitrate reducing conditions with the more reducing systems nor exact patterns could be isolated for each specific redox state, the faster Atenolol biotic removal rate was observed in the SO_4-reducing experiment, under the most reducing condition (while being established or in its early stage) up to that moment.

- Actually, correctly identifying of the actual biotic processes responsible for the removal of micropollutants (i.e. to distinguish biotransformation from biodegradation or even mineralization) requires the use of specific techniques, such as the use of isotopically labelled compounds and/or the identification of already known/new transformation products. In our study, we sought for Atenolol transformation product Atenololic Acid, but its presence could not be confirmed due to analytical restraints.
- Due to design constraints, the concentration of the easily degradable organic substrates used in the experiments were higher than those naturally present in aquifer systems or in most recharge waters, which likely affected the growth of the microbial communities present in the microcosms. Thus, regarding the micropollutant Atenolol the extrapolation of its biotic removal rates to natural subsurface environments would have to be faced carefully, being not straightforward. Still, the microcosm study proved the feasibility of specific redox environments to develop at test site and the capability of the local microorganisms to eliminate the target micropollutant, providing as well some overall removal pattern under the tested settings. In the end, such scenarios could eventually be promoted during artificial recharge at test site if less favourable removals of Atenolol are observed under the spontaneously occurring conditions.
- The removal of Atenolol reported in the literature varies between 0 and 60% in conventional wastewater treatments, improving up to 77% removal in advanced treatments such as Membrane Bioreactors (Gros et al., 2010; Radjenovic et al., 2009 and references therein). Thus, the overall complete removal observed in the experiments performed within this study suggests that the whole processes occurring in aquifers constitute a potentially efficient alternative water treatment for Atenolol. Depending on the redox state naturally occurring or possibly being deliberately stimulated in field applications, the time needed for a complete removal may be ensured by the large residence times in aquifers. Actually, the results from the “Natural Conditions” experiment, which better resemble the potential

conditions spontaneously occurring within the aquifer at Sant Vicenç test site during recharge, look very promising.

Supporting Information

Details on the selection of the initial amounts of electron donors and acceptors introduced in section "2.2.2 Biotic experiments - Creating sustainable redox conditions" are provided in the following.

The total amounts of organic substrate and controlling electron acceptor "i" (where i = 1 stands for NO_3, 2 for Mn(IV), 3 for Fe(III) or 4 for SO_4) to be initially available in the "i"-reducing experiment were selected so as to reach the desired redox state and to sustain it during a significant lapse of time.

This implies on one hand that the total amount of organic substrate had to be large enough to consume electron acceptors with reactions energetically more favourable than "i" (i.e. the ones above "i" in Table 2.3). Thus, all potential electron acceptors initially available in the system have to be quantified first. Then, the initial concentration n_{ij} (mmol L^{-1}) of substrate "j" (j=a for CH_3OH and j=b for CH_3COO^-, in the present experiments) in the i–th experiment must satisfy:

$$n_{ij} > \sum_k m_{kj} = \sum_k (Y_k / \upsilon_{kj}) \qquad [S2.1]$$

where m_{kj} (mmol L^{-1}) represents the stoichiometric amount of the j-th substrate potentially oxidized by the electron acceptors "k" (k=1,...,i-1) initially available in the system, Y_k (mmol L^{-1}) is the amount of "k" potential electron acceptor and υ_{kj} is its stoichiometric coefficient in the degradation reaction of the j-th substrate. In the case of solid phases (e.g. Mn and Fe oxides and hydroxides), the concentration Y_k can be estimated from their dissolution rate and the duration of the experiment. If the degradation of the j-th substrate could occur via different pathways, some of them possibly being of incomplete degradation, the υ_{kj} to be used in equation [S2.1] should be the smallest among those characterizing such stoichiometries, otherwise m_{kj} could be underestimated. For the same reason, if the potential formation of biomass (usually ignored, like in Table 2.3 for the present experiments) associated to the degradation of the j-th substrate by the electron acceptors "k" would require a partial oxidation, the υ_{kj} to be used in equation [S2.1] is that correspondent to such expense in "k" (e.g.: in the present experiments, according to reaction 2c specified in §2.3.1.1,

$\upsilon_{NO3\text{-}CH3OH}$ = 0.4). Finally, notice that equation [S2.1] implies that all electron acceptors are consumed by the "j" substrate. Therefore, this condition could be relaxed if the relative rates of degradation of all substrates are known.

On the other hand, for each potential selection of n_{ij} the total amount X_i of controlling electron acceptor "i" to be initially available had to (slightly) exceed the stoichiometric quantity necessary for the complete mineralization of such substrates. The quantity X_i (mmol L^{-1}) could be obtained by ensuring that it is at least sufficient to completely degrade all substrates "j", so:

$$X_i > \sum_j \upsilon_{kj} n_{ij} \qquad [S2.2]$$

where υ_{kj} is the stoichiometric coefficient of the i-th electron acceptor in the complete mineralization reaction of the j-th substrate. The use of υ_{kj} from the stoichiometries in which biomass formation is not considered (Table 2.3, for the present experiments) is convenient. In fact in the end, after the original sources of organic carbon have been depleted, dead cells could be recycled and degraded coupling with the reduction of some of the i-th electron acceptor.

The definitive concentrations n_{ij} (and the corresponding X_i) to be initially available in the "i" reducing experiment could be established according to the degradation rates observed in the preliminary rough tests performed when selecting the type of substrates, where their potential toxicity toward microorganisms could be excluded too, and according to the desired duration of the experiments.

Chapter 3

Effect of denitrifying conditions on the fate in aquifer material of the pharmaceuticals acetaminophen, diclofenac and sulfamethoxazole

NOTE: the present chapter is based on the paper with the same title, by Barbieri, M., Carrera, J., Sànchez-Vila, X., Ayora, C., Cama, J., Licha, T., Nödler, K., Köck-Schulmeyer, M., López de Alda, M., Osorio, V., Pérez, S., Barceló, D. In preparation.

3.1 Introduction

The last two decades have witnessed the ubiquitous emergence of pharmaceuticals in environmental matrices, i.e. surface water, ground water, soils and sediments (Focazio et al., 2008; Kemper, 2008; Loos et al., 2009; Petrovic et al., 2004 and references therein). Their primary source is the discharge of effluents from wastewater treatment plants (Gros et al., 2010; Onesios et al., 2009; Petrovic et al., 2009 and references therein). They also proceed from solid waste disposal, spills and uncontrolled discharges from industries, spreading of manure and sewage sludge as organic fertilizer in agricultural soils, surface run-off, etc. Their concentrations are usually very low. Still, they are a source of concern because of their potential chronic effects and synergic action of their mixtures on aquatic life and human health (Fent et al., 2006; Farré et al., 2008; Kumar et al., 2010).

As a result of this concern, significant efforts are being devoted to understanding the fate of pharmaceuticals in natural environments (Benotti and Brownawell, 2009; Holm et al., 1995; Hua et

al., 2003; Lam et al., 2004; Löffler et al., 2005; Packer et al., 2003; Yamamoto et al., 2009). Specially, intensive work is associated to managed aquifer recharge (Cordy et al., 2004; Díaz-Cruz and Barceló, 2008 and ref. therein; Hoppe et al., 2010 and ref. therein; Patterson et al., 2009; Ternes et al., 2007). Soil-aquifer processes have demonstrated to work as a natural treatment for the attenuation or complete removal of numerous contaminants, and the predominant redox conditions have proven to be an important controlling factor (Christensen et al., 2001). Nevertheless, knowledge about the behaviour of drugs in subsurface environment, their degradation pathways and the potential formation of transformation products is still limited.

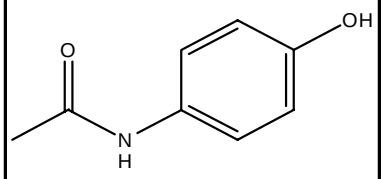
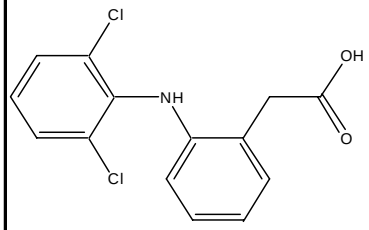
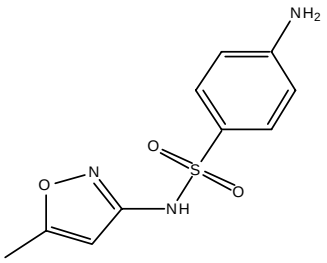
Our work is motivated by aquifer artificial recharge using Llobregat river water near Barcelona (Spain). We have investigated the fate of selected pharmaceuticals in aquifer material under different redox conditions. In the experiments under nitrate reducing conditions, acetaminophen, diclofenac and sulfamethoxazole exhibited an unreported and peculiar behaviour, with concentrations consistently dropping to near zero in the middle of the experiments, but recovering towards the end. In this paper we present these findings together with the hypothesis of a reversible effect under denitrifying conditions on the three compounds.

Acetaminophen (from now on APP), also known as Paracetamol, is the most heavily used over-the-counter analgesic in Europe. Diclofenac (DCF) is an important non-steroidal drug (NSAID) with anti-inflammatory, analgesic and antipyretic effects that is widely used for treatment of rheumatic diseases and for mild to moderate pain relief. Sulfamethoxazole (SMX) is a sulfonamide bacteriostatic antibiotic extensively used in both veterinary and human medicine. Their physicochemical characteristics are reported in Table 3.1. Recent monitoring reported the occurrence of these three drugs in the Llobregat river basin at concentrations in the ng/L range, with punctual maxima of some µg/L (Köch et al., 2011; Muñoz et al., 2009). The existing literature on their fate in subsurface environments describes sorption onto aquifer material to be not significant (Baumgarten et al., 2011; Lorphensri et al., 2007; Rauch et al., 2009). Field and laboratory studies on managed aquifer recharge (Heberer and Adam, 2004; Preuss et al., 2001; Rauch et al., 2009, and references therein; Scheytt et al., 2007; Ternes et al., 2007; Tiehm et al., 2010), covering a wide range of retention times and experiment duration, reported DCF to be quite efficiently eliminated under both aerobic (reported removals from 60% to 100%) and anaerobic conditions (removals from 40% to 70%). APP, which is far less studied in subsurface environment, was found to be efficiently removed during bank filtration and sewage effluent percolation through a sandy vadose zone (Godfrey et al., 2007; Vogel et al., 2005). Regarding SMX, however, mixed and sometimes contradictory results have been reported on its fate in soil-aquifer systems. Investigations on soil-aquifer-systems (Cordy et al., 2004) and natural attenuation in a

contaminated aquifer (Barber et al., 2009) described SMX as a persistent compound. Field data on bank filtration and aquifer recharge through ponds in Germany suggested SMX to be better degradable under strictly anaerobic (Schmidt et al., 2004) and anoxic conditions (Grünheid et al., 2005: 80% of SMX removed in 4 months retention time; Heberer et al., 2008: 99% removed in 1 month) than under aerobic ones (Grünheid et al., 2005: 53% removed in 50 days; Heberer et al., 2008: 52% removed in 1 month). On the contrary, in laboratory column and batch experiments related to the same recharge sites, the largest removals of SMX were obtained under aerobic conditions, ranging between 23% and 95% (depending on the experimental settings and duration), whereas those under comparable anoxic conditions varied between 0% and 65% (Baumgarten et al., 2011; Jekel et al., 2009).

In light of the findings from our experiments, we conjecture that in the case of DCF and SMX the wide range of reported removals as well as the apparent discrepancies in the literature could be explained, at least partially, by a reversible effect of denitrifying conditions on aromatic amines. Pereira et al. (2011) observed a chemical transformation of the aromatic amines aniline and sulfanilic acid in the presence of nitrite, and proposed some nitroaromatic compounds among the resulting products. On the other side, Heijman et al. (1995) provided evidence that nitroaromatic compounds could be reduced to the corresponding anilines in anaerobic aquifer columns. We hypothesize that in denitrifying environments, where nitrite is likely to occur as intermediate product between nitrate and nitrogen, aromatic amines could be temporarily and reversibly transformed into nitro derivatives by the action of nitrite, and that further on, they may return to the parent compounds when a different redox condition is established and/or nitrite disappears from the system. We also suspect a similar phenomenon to affect the amide APP.

Table 3.1: Physicochemical properties of the compounds of interest.

Compound	Structure	CAS number	$logK_{ow}$ (1)	pk_a (1)	Formula
Acetaminophen (APP)		103-90-2	0.34 ± 0.21	9.9 ± 0.1	$C_8H_9NO_2$
Diclofenac (DCF)		15307-86-5	4.06 ± 0.41	4.2 ± 0.1	$C_{14}H_{10}Cl_2NO_2$
Sulfamethoxazole (SMX)		723-46-6	0.89 ± 0.42	5.8 ± 0.5	$C_{10}H_{11}N_3O_3S$

(1) SciFinder predicted values

3.2. Materials and methods

The experimental set up was based on sets of microcosms containing natural sediments, synthetic water and organic pollutants, the latter at two markedly different individual initial concentrations, of 1 g/L in Experiment 1 and 1mg/L in Experiment 2. Both experiments included a biotic and an abiotic series to separate contaminant's biodegradation (both biotic mineralization and transformation included here) from sorption and other abiotic processes. Nitrate reducing conditions were stimulated in the biotic tests by adding easily degradable organic compounds as electron donors (sodium acetate and the methanol used as solvent in the pollutants' spiking solutions) and an excess of nitrate as specific electron acceptor.

3.2.1 Sediments, water and micropollutants

Sediments were obtained from a test site for artificial recharge of groundwater through surface ponds located in Sant Vicenç dels Horts (Barcelona, Spain). The aquifer consists of quaternary alluvial sediments, mainly gravel and sand with a small fraction of clays. Samples were collected prior to the start up of recharge, from an oxic unsaturated horizon at about 1m depth under the bottom of the infiltration pond. They were sieved to < 1mm and immediately used for assembling

the experiments, or stored before use for a maximum of two days at 25ºC inside aluminium foil. Their mineralogical and chemical characteristics are summarized in Table 3.2.

Table 3.2: Chemical and mineralogical characteristics of the sediments used in the experiments.

minerals (XRD)	quartz, calcite, microcline, albite, dolomite, clinochlore, illite
Organic Carbon [%]	< 0.2
Nitrogen [%]	< 0.2
Total Carbon [%]	2.5
grain size	Maximum grain diameter: 1mm. Fraction with diameter < 4µm: 2 to 6 %
Mn and Fe(III) associated to oxide-hydroxides and oxides [mg/g air dried sediment]	Mn: 0.07; Fe (amorphous oxide-hydroxides): 0.19; Fe (crystalline oxides): 5.64

Experiment water was artificially prepared to mimic recharge water (Llobregat river water) except for the organic carbon, which was initially null. Its theoretical composition is shown in Table 3.3.

Table 3.3: Chemical composition of the artificial water used in the experiments.

Compounds and parameters	***[mg/L]***	***[mmol/L]***
Na	180	7.8
K	40	1.0
Ca	120	3.0
Mg	32	1.3
Cl	452	12.7
NO_3	10	0.2
SO_4	200	2.1
Alk [$CaCO_3$]	51	0.5
DOC [mgC_{org}/L]	0	0.0
COD [mgO_2/L]	0	0.0
NH_4	2	0.1
PO_4	2	0.02
O_2(aq)	7	0.2
pH (measured)	7.4	
Eh (measured)	250 mV	
T (measured)	25ºC	
E.C. [µS/cm] (measured)	1800	

DCF and SMX were added to the water of “Experiment 1” with a spiking solution called “mixture 1” in the following. High purity (>96%) analytical standards of DCF, SMX and of their isotopic analogues (DCF *d4* and SMX *d4*) used as surrogate standards for quantification were supplied by Sigma–Aldrich. Individual stock solutions were prepared by dissolving each compound in methanol. Working standard mixtures were then prepared at different concentrations by dilution of the individual stock solutions in methanol. These were used to prepare “mixture 1” and the

aqueous calibration standards (concentration range 1-1500 ng/L, surrogate standard 200 ng/L). Stock and working standard solutions were stored at -20°C in the dark.

APP, DCF and SMX were added to the water of "Experiment 2" by the use of a spiking solution called "mixture 2" in the following. High purity (≥ 99%) analytical standards of APP, DCF and SMX, as well as ibuprofen-*d3* and paraxanthine-*d6* used as internal standards for quantification of APP and DCF, respectively, were supplied by Sigma Aldrich. SMX-$^{13}C_6$ used as internal standard for quantification of SMX was obtained from LCG Promochem. Individual stock solutions were prepared by dissolving each compound within methanol. Working standard mixtures were then prepared at different concentrations by dilution of the individual stock solutions in methanol. These were used as spiking solution ("mixture 2") and to prepare the aqueous calibration standards (concentration range 5-250 µg/L). Stock and working standard solutions were stored at -20°C in the dark.

Both spiking solutions included other organic pollutants that will not be discussed here (results could be found in Chapter 2 and 4, and Appendixes C and D). Specifically, "mixture 1" included also four more drugs (atenolol, carbamazepine, gemfibrozil, ibuprofen), pesticides (atrazine, simazine, terbuthylazine, prometryn, diuron, chlorphenvinfos, chlorpyrifos, diazinon), estrogens (estrone, -estradiol), PAHs (naftalene, acenaphtene, fluorene, anthracene, fenanthrene, benz[a]anthracene, crysene, pyrene, fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a]anthracene, indeno[1,2,3-cd]pyrene and benzo[ghi]perylene), surfactant degradation products (4-tert-octylphenol, 4-nonylphenol), a phthalate (bis-diethylhexyl phthalate) and a biocide (triclosan). "Mixture 2" included 23 more drugs: iopamidol, iomeprol, iohexol, iopromide, atenolol, metoprolol, sotalol, propranolol, famotidine, pantoprazole, erythromycin, roxithromycin, clarithromycin, carbamazepine, diazepam, primidone, clofibric acid, bezafibrate, gemfibrozil, phenazone, naproxene, ibuprofen, cetirizine, loratadine

3.2.2 Experimental procedure

The collected sediments were homogenized in steel containers and distributed in fractions of 120g (dry weight) into 0.3L glass bottles. The bottles were then placed inside a glove box under Argon atmosphere (maximum 0.1% of O_2).

Synthetic water (§ 3.2.1) was prepared in a glass amber bottle. NO_3^- concentration was increased by adding magnesium nitrate hexahydrate (increase of 3.63 mmol L^{-1} and 66 mmol L^{-1} for the waters of "Experiment 1" and "Experiment 2", respectively). The water was then purged with Ar (purity ≥ 99.999%) during about 1 hour to remove all oxygen from the solution and the bottle headspace. Afterwards, it was placed into the glovebox under Ar atmosphere, and 2.1 mmol L^{-1} of

anhydrous sodium acetate were added. Finally, the organic pollutants were added by spiking the different mixtures (1 or 2), resulting in the two initial waters used in the corresponding "Experiment 1" and "Experiment 2" (with resulting concentrations of 1 µg/L and 1 mg/L for each contaminant, respectively). The amount of methanol added as solvent in the spiking solutions should have been 2.7 mmol L^{-1} DOC in "Experiment 1", and 70 mmol L^{-1} DOC in "Experiment 2". As it turned out, due to some unidentified problem during the assembling procedure, the biotic series of "Experiment 1" contained 2.9 mmol L^{-1} of methanol additional to the 2.7 mmol L^{-1} coming from the spiking solution.

After sampling for chemical analyses, 0.24L of the prepared initial water was added to each 0.3L glass bottle already containing the sediments. The assembling procedure was concluded by closing the bottles with screw-caps plus a PTFE protection seal, and gently shaking. A remaining headspace of about 15mL was left in each bottle.

The microcosms were removed from the glove box and enveloped with aluminium foil to prevent photodegradation (Lam et al., 2004; Packer et al., 2003; Ryan et al., 2011; Trovó et al., 2009). Then, they were incubated under controlled temperature (25±2 ºC) and gently shaken few times during their lifetime (once every 2 days during the first week; once a week during the rest of the first month; then, once every 30 to 45 days) as well as the day before dismantlement.

Synthetic water and sediments for the abiotic series were sterilized prior to assembling. They were introduced three times (once a day in three consecutive days) into an autoclave at T=121 ºC and P = Patm+1 atm during 20 minutes. The glove box was sterilized with UV light before entering the material. As an additional precaution, 0.22 mmol L^{-1} of mercury chloride were added (as microbial poison) to the initial water.

Duplicate bottles were sacrificed according to pre-defined schedules (at day 0.05, 0.2, 0.5, 1.5, 3, 5, 10 and 21 for "Experiment 1"; at day 2, 5, 10, 15, 25, 41 and 87 for "Experiment 2"). One at a time, the two bottles were opened under Ar atmosphere, chemical parameters were measured, and aqueous samples for general chemistry and pollutants analysis were collected and stored according with each laboratory recommendations until analysis. Sterility of the abiotic series was verified six times in "Experiment 1" series and two times in "Experiment 2" series. To this end, an aliquot of microcosms water was spread on tryptic soy agar (TSA) plates and incubated in duplicate at 25ºC during 1 week under aerobic conditions and during 2 weeks under anaerobic conditions. None of the plates demonstrated microorganisms' growth.

3.2.3 Analytical methods

Biotic samples collected for analysing Cl^-, NO_3^-, NO_2^-, SO_4^{2-}, PO_4^{3-}, F^-, NH_4^+ and DOC (Dissolved Organic Carbon) and COD (Chemical Oxygen Demand) were filtered through 0.45 µm PALL Acrodisc® Sterile Syringe Filters with Supor® membrane and frozen. Anions were analyzed by ion chromatography using a ICS-1000 instrument. The analytical error was estimated to be 14% for PO_4^{3-} and 13% for the remaining anions. NH_4^+ concentration was analyzed with a selective electrode Orion 9512. DOC was analysed by 680 °C combustion catalytic oxidation/NDIR method using a TOC-V CSH instrument. The estimated analytical error was 20%. Biotic and abiotic samples for analysing COD (Chemical Oxygen Demand) were also filtered 0.45 µm, and were analyzed by colorimetry with the spectrophotometer Spectroquant Nova 60. Abiotic samples for Cl^-, NO_3^-, SO_4^{2-} and F^- were frozen and then analysed by using a Dionex DX-320 instrument with conductometric detection, a Dionex AS11-HC (2 x 250 mm) column and 23 mM KOH as eluent (isocratic separation at 30 °C). A flow rate of 0.38 mL min-1 was applied. Prior to chromatography, samples were filtered (Whatman Anotop 10 IC, 0.2 µm).

Samples for Fe and Mn, Ca, Mg, Na, K and minor elements were also filtered at 0.45 µm, acidified and stored at 4ºC. They were later analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES) using a Thermo Jarrel-Ash Iris Advantage HS instrument. Detection limits were 100 µg/L for K and Na, and 50 µg/L for the rest. The analytical error was estimated below 3%. In the ICP-AES analyses, calibration with three laboratory sets of standards was performed every 10 samples, and regression coefficients of the calibration curves exceeded 0.999.

During the assembling/disassembling procedure additional parameters were measured: pH and temperature (Thermo Scientific 9157BN Triode pH electrode, refillable), electrical conductivity (Hanna Instruments, 76302W conductivity probe), dissolved oxygen (Hanna Instruments, HI 76407/4 DO probe) and alkalinity (drop test kit Taylor K-1726, precision of 0.5 mmol L^{-1}).

Samples for analysis of DCF and SMX in “Experiment 1” were filtered at 0.45 µm using WATERS Syringe filter with PTFE. Then, they were kept frozen until analysis, performed by using on-line solid phase extraction-liquid chromatography-tandem mass spectrometry. Briefly, water samples (10 mL), spiked with a standard mixture of the isotopically labelled compounds at a concentration of 200 ng/L, were extracted with the aid of an automated on-line SPE sample processor Prospekt-2 from Spark Holland (Emmen, The Netherlands) connected in series with the LC-MS/MS instrument. Sample preconcentration was performed by passing 5 mL of the sample through a previously conditioned (1 mL Methanol plus 1 mL HPLC water) Oasis HLB Prospekt™ cartridge (10×1 mm) from Waters (Mildford, MA, USA). After sample loading, the cartridge was washed with 1 mL of a 5% methanol water solution and further eluted with the chromatographic

mobile phase. Chromatographic separation was performed with a Binary HPLC pump Model 1525 from Waters using a Purospher STAR RP-18e column (125x2 mm, 5 m particle diameter, from Merck, Darmstadt, Germany) and gradient elution with methanol and water as mobile phase. MS/MS detection was performed in the selected reaction monitoring (SRM) mode acquiring 2 SRM transitions per compound and 1 SRM transition per surrogate using a TQD triple-quadrupole mass spectrometer from Waters equipped with an electrospray interface. Quantitation was performed by the internal standard method using the corresponding deuterated compounds as surrogate standards.

Samples for analysis of APP, DCF and SMX in "Experiment 2" were kept frozen until analysis. Prior to analysis, samples were diluted (v/v) 1:2 (APP and DCF) and 1:4 (SMX) with aqueous 5 mM ammonium acetate solution, containing 4 % methanol. For analysis of APP and DCF, 100 ng/mL of paraxanthine-*d6* and ibuprofen-*d3* were used as internal standards, respectively. For SMX analysis 125 ng/mL SMX-$^{13}C_6$ was used. For additional matrix compensation, calibration standards were prepared in inorganic matrix according to 50 % of "Experiment 2" initial water concentration. Before analysis, all samples and standard solutions were centrifuged (Christ RVC 2-18, purchased from Fisher Scientific, Schwerte, Germany) for 30 min at room temperature. Analysis of APP and DCF was performed without preconcentration by an HPLC/MS-MS method according to Nödler et al., 2010. SMX analysis was performed by using the same instrumentation and eluents. However, the gradient was slightly different. Eluent A was 0.015 % formic acid + 5 % methanol. The elution started with 5 % B (methanol) followed by a gradient of 27 min to 65 % B. This was followed by a sharp gradient of 1 min to 95 % B, which was held for 5 min. After a gradient of 1 min to 5 % B the system was allowed to equilibrate for 11 min. Except for SMX all parameters of the mass spectrometer were as described in the literature (Nödler et al., 2010). For SMX, the quantifier and qualifier transitions in negative electrospray (-ESI) were 252 → 154 and 252 → 106, respectively. The respective collision energies were 13.5 and 17.5 V. The capillary voltage was –35 V.

3.3. Results and discussion

Oxidation by nitrate of the two main organic substrates occurs according to:

1) $CH_3OH + 1.2\ NO_3^- + 0.2\ H^+ \rightarrow HCO_3^- + 0.6\ N_2 + 1.6\ H_2O$

2) $CH_3COO^- + 1.6\ NO_3^- + 0.6\ H^+ \rightarrow 2HCO_3^- + 0.8\ N_2 + 0.8\ H_2O$

A decrease in DOC and an increase in alkalinity were expected in the biotic tests, together with a decrease in the concentration of the target electron acceptor nitrate.

Results for the general hydrochemistry from duplicate batches showed a satisfactory reproducibility at all sampling times. Actually, when plotting the data plus the error bars from each batch, there was always some overlap. Thus, the following graphics report the average of results and manual measurements from the duplicate bottles.

Regarding the abiotic tests, the hydrochemistry remained practically constant for the whole time as expected (results not shown).

The temporal evolution of the target organic pollutants is reported in terms of their average concentration (divided by each initial concentration C_0). Error bars were calculated by taking into account the analytical errors and the difference between duplicate batches results. Concentrations are presented in relative terms (C/C_0) in order to remove systematic errors from the analysis.

3.3.1 "Experiment 1" (pollutants at 1 µg/L individual initial concentration)

The evolution of hydrochemistry in the biotic series of "Experiment 1" is shown in Figure 3.1a. During the first 10 days, DOC decreased from 9.7 mmol L^{-1} to 1.5 mmol L^{-1} while alkalinity increased from 1 mmol L^{-1} to 3.75 mmol L^{-1}. Afterwards they remained practically constant. By day 10, the initial 6.7 mmol L^{-1} of nitrate had disappeared. Nitrite concentration began to increase after only some 12 hours, reaching a maximum at day 5 and becoming completely depleted at day 10. Very low concentrations of dissolved manganese and iron were detected after day 10 (results not shown), presumably from the dissolution and reduction of small quantities of the Mn and Fe oxides naturally present in the sediments. Sulphate remained constant and pH decreased with slight fluctuations from 8.5 to 7.7 during the whole experiment.

These observations suggest that nitrate reducing conditions where established within a short period (~ 0.5 days) of microbial adaptation and dominated the system during the first 10 days, while nitrate remained in the system. The increase of nitrite, followed by its depletion, reflected the actual pathway for denitrification, with nitrite being an intermediate product between nitrate and nitrogen. After day 10, a different more reducing condition was established.

To prevent loss of focus, the complete hydrochemical evolution and mass balance of the biotic test are not reported here (details in Barbieri et al., submitted). In summary, about 27% of the consumed organic carbon was estimated to be converted into biomass during the 10 days of nitrate reducing conditions. The remaining part was assumed to be mineralized and then precipitated as carbonates, transferred to the gas phase in the headspace of the bottles, or to remain is solution to

increase alkalinity. Under these assumptions, the overall inorganic carbon mass balance could be closed with an error of about 15%.

The time evolution of DCF and SMX in the biotic and abiotic tests is reported in Figures 3.1b and 3.1c, respectively. Overall, DCF was not removed after 10 days of the nitrate reducing conditions test. Its final normalized concentration in both the biotic as well as in the abiotic series remained around 100% (considering the error bars). Nevertheless, its concentration in the biotic series suffered a sudden drop at day 1.5, followed by total recovery by day 10.

SMX was affected by an overall biotic removal of about 20-30% during the 10 days of nitrate reducing conditions. Still, its concentration dropped significantly between days 1.5 and 10 of the biotic test, but rebounded afterwards. That is, the evolution of SMX was similar to that of DCF, but even more pronounced.

Looking at the behaviour of the two pharmaceuticals jointly with the evolution of hydrochemistry it can be observed that such unexpected process of drop and recovery of both DCF and SMX concentrations occurs only in the biotic series, concurrently and opposite to the evolution of nitrite.

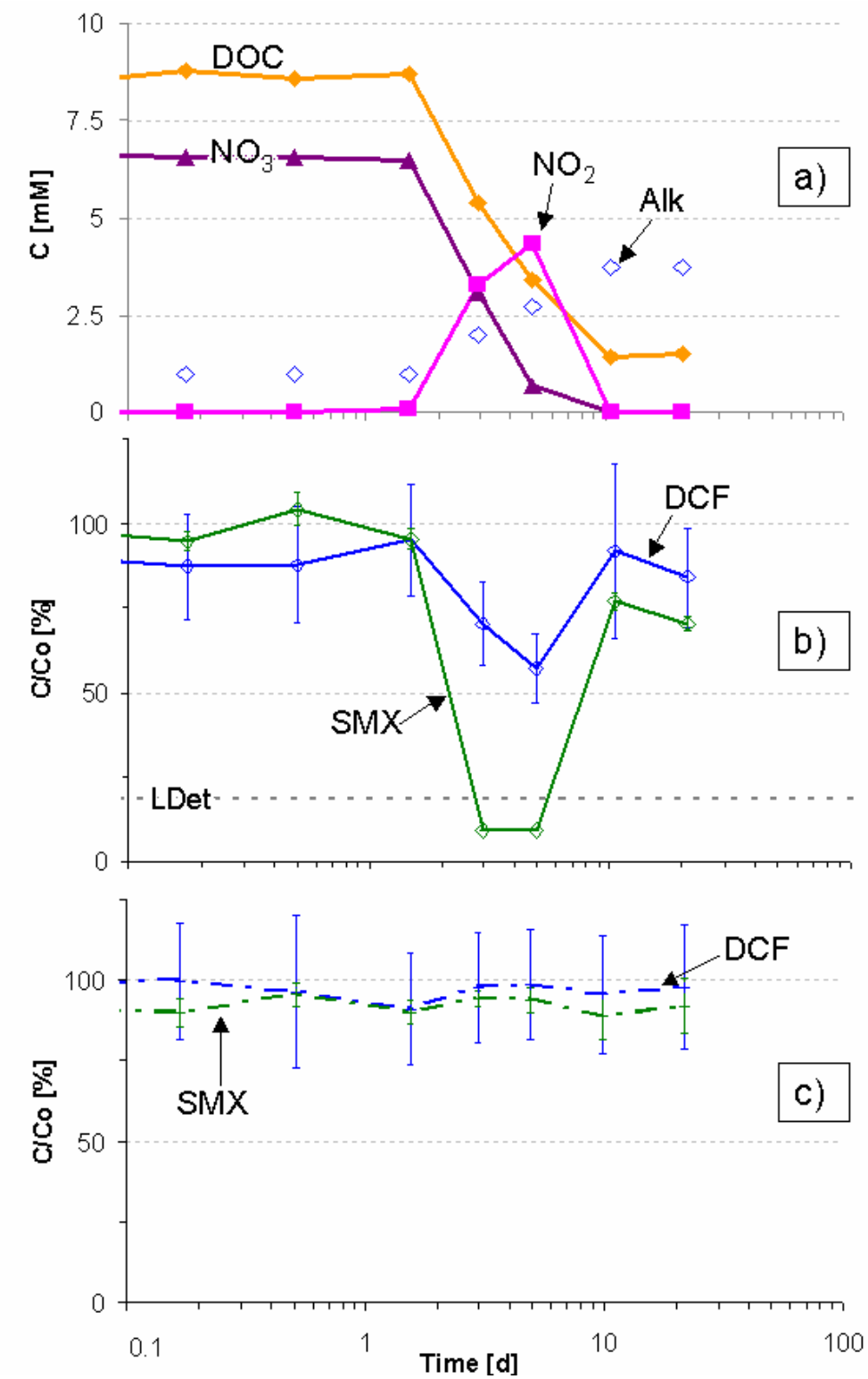


Figure 3.1: results for "Experiment 1". a) chemical evolution with time in the biotic test; b) evolution with time of the average normalized concentration (with respect to the initial value C_0) of DCF and SMX in the biotic test. "LDet" stays for Limit of Determination; c) idem in the abiotic test.

3.3.2 "Experiment 2" (pollutants at 1 mg/L individual initial concentration)

Results for the hydrochemistry of the biotic series of "Experiment 2" are shown in Figure 3.2a. Qualitatively, its evolution is consistent with that of the biotic series of "Experiment 1", though displaced in the time scale. DOC and nitrate decreased all experiment long, starting (appreciably) after day 5 and being still present at day 87 with final concentrations of 27.2 mmol L^{-1} and 7.5 mmol L^{-1}, respectively. Alkalinity increased continuously after day 2, from 0.8 mmol L^{-1} to 22 mmol L^{-1}. Nitrite concentration also began to increase at the same time reaching a maximum at day 41 and becoming completely depleted at day 87. Dissolved Manganese and Iron were not detected, sulphate remained constant, and pH ranged between 7.3 and 8.3 during the experiment (results not shown).

The above observations suggest that nitrate reducing conditions were established within approximately 2 days of microbial adaptation, and dominated the system during the rest of the test. The depletion of nitrite between day 41 and 87, when nitrate reduction was still occurring, could be likely explained by the process of nitrite reduction to nitrogen being faster than the production of nitrite from nitrate.

In this case, about the 10% of the organic carbon was estimated to be converted into biomass. Accounting for carbonates precipitation, transfer to the headspace of the bottles, and alkalinity build up, the overall inorganic carbon mass balance could be closed with an error of about 11%.

The evolution of APP, DCF and SMX in the biotic and abiotic series is reported in Figure 3.2b and 3.2c, respectively. After 87 days of nitrate reducing conditions, an overall removal of 50% could be observed for APP in both biotic and abiotic tests, which suggests that the main processes affecting this analgesic were abiotic. Yet, we cannot know the actual reaction that took place. It could be an unreported abiotic modification of the molecule structure, chemical hydrolysis or sorption to the sediments. Anyhow, it has to be pointed out that the biotic concentrations drop well below the abiotic ones between days 10 and 87, only recovering at the very end of the test.

On the whole, DCF was not or only slightly (10%) removed during the 87 days of both biotic and abiotic tests. Still, its concentration in the biotic set of microcosms suffered a sudden drop followed by total recovery between days 2 and 87.

SMX shows exactly the same non-monotonic behaviour in time when compared to DCF with the only exception that the overall biotic removal after 87 days was 47 ± 20 %.

Looking at the behaviour of the three pharmaceuticals jointly with the evolution of hydrochemistry it can be observed that, similar to "Experiment 1", the reversible process of drop

and rebound in APP, DCF and SMX concentrations occurs again only in the biotic series, concurrently and opposite to the evolution of nitrite.

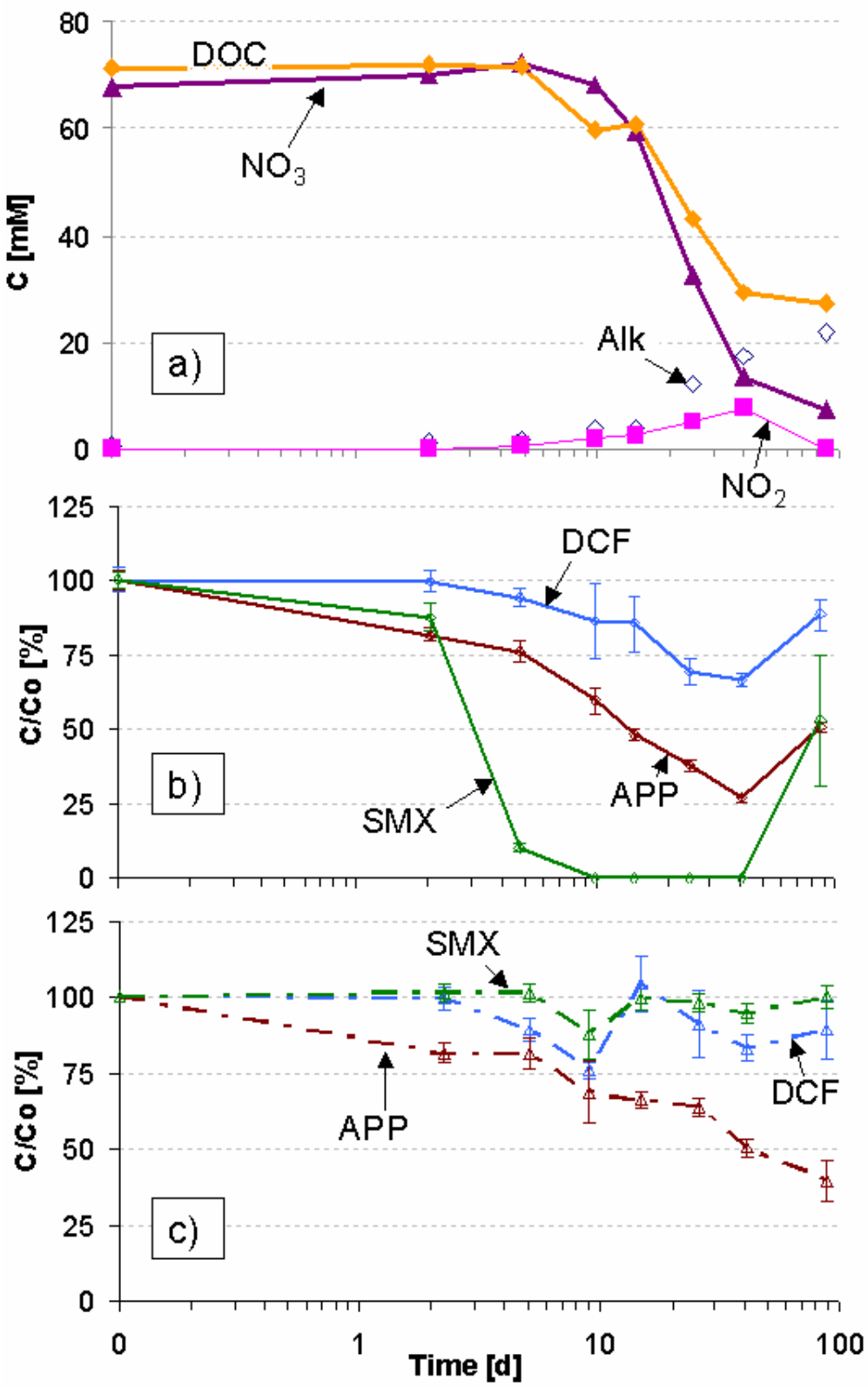
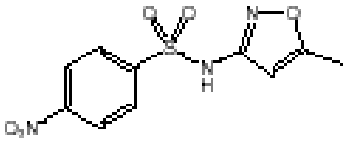


Figure 3.2: results for "Experiment 2". a) chemical evolution with time in the biotic test; b) evolution with time of the average normalized concentration (with respect to the initial value C_0) of APP, DCF and SMX in the biotic test; c) idem in the abiotic test.

3.3.3 Discussion upon the effect of denitrifying conditions on the target pharmaceuticals

A more thorough analysis of the water samples exhibited the presence of further compounds in the biotic series of both "Experiment 1" and "Experiment 2". These compounds emerged after day 0.5 and 2, respectively, representing possible candidates for DCF and SMX transformation products. Indeed, among them we could recognize the presence of Nitro-DCF (Osorio et al., in preparation) and 4-Nitro-SMX (Nödler et al., 2011). The nitro-derivative of DCF, Nitro-DCF was previously observed by Pérez and Barceló (2008) in aerated bioreactors loaded with activated sludge, and was identified as a product of microbial nitration at one of the aromatic rings. 4-Nitro-SMX was already observed by Naisbitt et al. (2002) in cell culture medium. Its characteristics and structure are reported in Table 3.4.

Table 3.4: Physicochemical properties of the transformation product 4-Nitro-SMX detected in the biotic series.

Compound (CAS)	Structure	pK_a [a,b]	Log K_{ow} [a]
4-nitro-SMX (29699-89-6)		5.65 ± 0.4	1.27 ± 0.41

[a] Scifinder predicted values. [b] pK_a of the secondary amine

As presented in Figure 3.3, the evolution of both Nitro-DCF and 4-Nitro-SMX concentrations developed almost opposite to that of their respective parent compounds, and matches very well that of nitrite. That is, their concentration increases with nitrite, while that of their parent compounds decreases. Further on, the two nitro derivatives become depleted when nitrite does, which coincides with the rebound of the parent compounds.

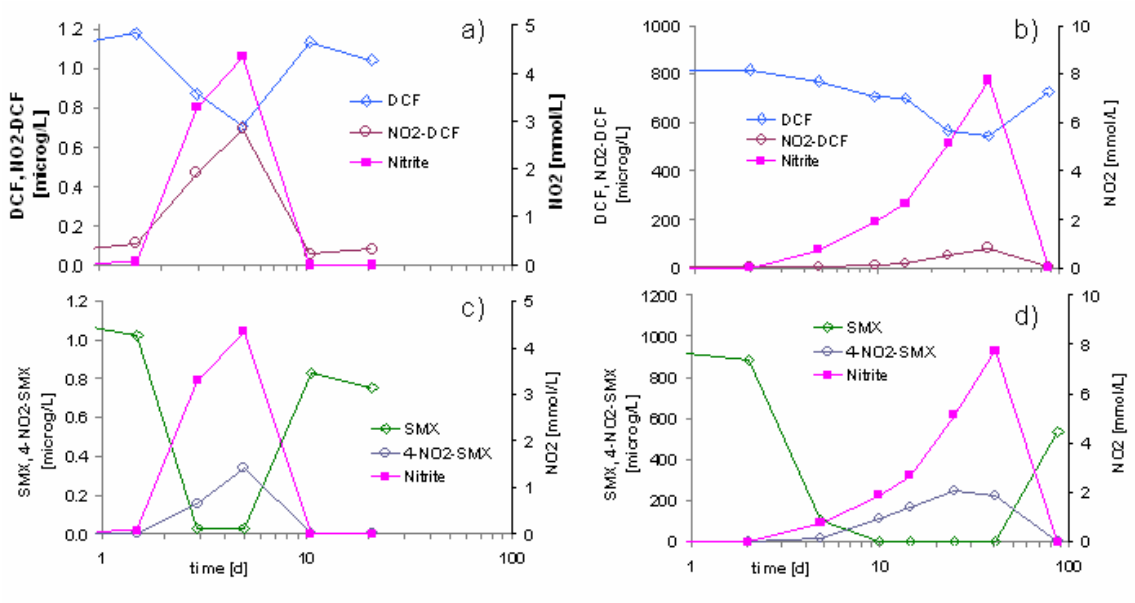


Figure 3.3: Evolution of DCF, Nitro-DCF (NO2-DCF), and nitrite in the biotic series of "Experiment 1" (plot "a)") and "Experiment 2" (plot "b"). Evolution of SMX, 4-Nitro-SMX (4-NO2-SMX), and nitrite in the biotic series of "Experiment 1" (plot "c)") and "Experiment 2" (plot "d").

The previous trends, observed in both "Experiment 1" and "Experiment 2", i.e. using individual initial concentration of the target drugs of 1 µg/L and 1 mg/L, respectively, suggest a transient and reversible nature in the formation of Nitro-DCF and 4-Nitro-SMX. In summary, we propose that the drop in concentration of SMX and DCF is at least partially caused by the formation of nitro products, which return to the parent compounds when the concentration of nitrite drops. The formation mechanism is driven by the action of nitrite on the amine attached to the ring, a process already observed by Chiron et al. (2010), Pereira et al. (2011), and Pérez and Barceló (2008). For the following retransformation mechanism, we propose 4-Nitro-SMX to be reduced back to their parent compounds. Looking at the chemical evolution of the tests, this process occurred starting from day 5 in "Experiment 1 and from day 42 in "Experiment 2, i.e. while nitrite is being progressively reduced to nitrogen. Heijman et al. (1995) already observed that nitroaromatic compounds could be reduced to the corresponding anilines in anaerobic aquifer columns. By means of complementary experiments we could finally confirm the feasibility of the proposed retransformation mechanism for the case of 4-Nitro-SMX back to SMX in "Experiment 2" (Nödler et al., 2011). For Nitro-DCF, the mechanism leading to the reappearance of its parent compound DCF remained unclear.

It is worth pointing out that the common feature among the two target pharmaceuticals DCF and SMX is the amine group attached directly to the ring. DCF, containing a secondary amine, exhibited only a partial reaction with nitrite while SMX, containing a primary amine, reacted totally.

Actually, Nitro-DCF and 4-Nitro-SMX were detected and quantified in the same water samples used for the analysis of their respective parent compounds, but after a further period of storage (freezing). Thus, the accuracy of the absolute concentrations reported in Figure 3.3 for the two transformation products could not be ensured. That is, a reliable mass balance for DCF, SMX and their respective nitro derivatives could not be calculated in our tests. Anyway, in the case of SMX in "Experiment 2", a part of the imbalance suggested by plot "d" in Figure 3.3 could be indeed explained by the detection of an additional transformation product, the Desamino-SMX (results not shown. Nödler et al., 2011). The presence of such compound in "Experiment 1" could not be confirmed due to analytical restraints. It has also to be pointed out that in both "Experiment 1" and "Experiment 2" the concentration of 4-Nitro-SMX was still increasing (from days 3 to 5, and from days 10 to 25, respectively) even if SMX was not detected anymore. This suggested the presence of some intermediate transformation product of SMX to 4-Nitro-SMX, which unfortunately we could not identify (Nödler et al., 2011).

Regarding the amide APP, we have not been able so far to detect and measure a nitro-derivative analogous to those detected for DCF and SMX. The process of drop and recovery of its concentration observed in the biotic series, also in this case concurrently and opposite to the evolution of nitrite, remain still unclear.

3.4. Conclusions

The following concluding considerations can be made on the present study:

- We have observed that the fate in aquifer material of the aromatic amines diclofenac and sulfamethoxazole, and of the amide acetaminophen could be temporarily and reversibly affected by denitrifying conditions. Namely, the concentration of the three drugs falls down when nitrite builds up, while later they rebound back up as nitrite reduces to nitrogen.
- Nitro-DCF and 4-Nitro-SMX, being respectively transformation products of DCF and SMX, were detected in the biotic series of both "Experiment 1" (DCF and SMX at initial concentration of 1 μg/L) and "Experiment 2" (DCF and SMX at initial concentration of 1 mg/L). The concentration of the two nitro-derivatives developed almost complementary to the measured decrease in the concentrations of their parent compounds, and correlated very well with the temporal evolution of the nitrite peak. Unfortunately, for APP we have not been able so far to detect and measure an analogous nitro-derivative.

- The action of nitrite on the aromatic amine was likely the responsible of the formation of the two detected nitro compounds. Data evidenced that secondary amines (i.e. DCF) could react only partially with nitrite, while primary amines (i.e. SMX) reacted totally.
- The reduction of 4-Nitro-SMX to its correspondent amino compound accounted, at least partially, for the reappearance of SMX between day 41and 87. The mechanism explaining the similar evolutions of DCF and Nitro-DCF remained unclear.
- There is a significant environmental implication of our work, at least related to aromatic amines: ignoring the observed feature of such compounds could induce experimenters to overestimate their actual elimination in field and laboratory studies. This may explain the inconsistencies on literature reports about their elimination (e.g. in the case of SMX). We guess that the observed reversible action of nitrite should have also to be taken into account when assessing the efficiency of wastewater treatment in removing organic compounds containing aromatic amines, since nitrification and denitrification processes occurs during the biological treatment.
- A thorough monitoring of the inorganic chemistry in field and laboratory studies is advisable to understand the fate of organic micropollutants, as proven by the relevant role played by nitrite in the fate of the analysed drugs.
- Identification and quantitative analysis of transformation products was needed to prove the process experienced by the DCF and SMX, and is also advisable when investigating the fate of organic micropollutants.

Chapter 4

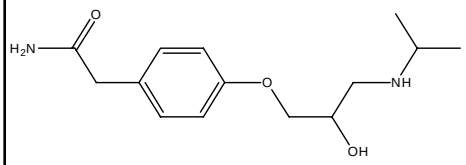
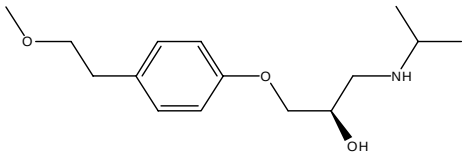
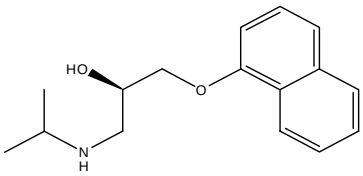
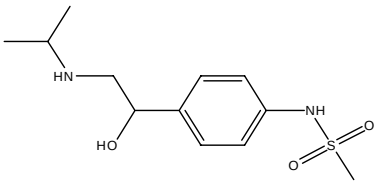
Fate of beta-blockers in aquifer material under nitrate reducing conditions

NOTE: the present chapter is based on the paper with the same title, by Barbieri, M., Carrera, J., Sànchez-Vila, X., Ayora, C., Cama, J., Licha, T., Nödler, K.. In preparation.

4.1 Introduction

Beta-blockers (β-adrenergic receptor antagonists) are a class of widely prescribed cardiovascular drugs, generally used for the treatment of hypertension, cardiac arrhythmias, cardio protection after heart attacks, and anxiety disorders. After excretion, substantial amounts of these drugs get into the wastewater and end up in sewage treatment plants. Since conventional wastewater treatments cannot remove them efficiently (Gabet-Giraud et al., 2010; Gros et al., 2010; Lin et al., 2009; Radjenovic et al., 2009), β-blockers are discharged into surface waters, where indeed they have been detected at concentrations ranging from ng/L to µg/L (Kasprzyk-Hordern et al., 2008; Martínez et al., 2010; Muñoz et al., 2008). The environmentally most relevant β-blockers are shown in Table 4.1, which also displays their physicochemical characteristics.

Table 4.1: Physicochemical properties of the target β-blockers.

Compound	Structure	CAS number	$logK_{ow}$ [(1)]	pk_a [(1)]	Formula
Atenolol		29122-68-7	0.1 ± 0.28	9.2 ± 0.4	$C_{14}H_{22}N_2O_3$
Metoprolol		37350-58-6	1.79 ± 0.4	9.2 ± 0.4	$C_{15}H_{25}NO_3$
Propranolol		525-66-6	3.48[(2)]	9.42[(2)]	$C_{16}H_{21}NO_2$
Sotalol		3930-20-9	0.32 ± 0.37	9.2 ± 0.4	$C_{12}H_{20}N_2O_3S$

(1) SciFinder predicted values
(2) from SRC database

Ecotoxicological studies reported β-blockers to affect the aquatic organisms, propranolol being the most harmful one (Fent et al., 2006; Küster et al., 2009). Several studies have thus been devoted to foster the understanding of their behaviour in aquatic-sediment systems. These studies focus on phototransformation, sorption to river sediments, quite rich in organic carbon, and aerobic biotransformation (Andreozzi et al., 2003; Liu and Williams, 2007; Ramil et al., 2010; Yamamoto et al., 2009). Yet, information on their fate in subsurface environments under reducing conditions is still lacking. Kybbey et al. (2007) studied the adsorption of nadolol, metoprolol and propranolol to a natural alluvial material, as well as to six individual mineral subcomponents of the sediments. Results from the batch experiments suggested compound hydrophobicity to be an important predictor of adsorption even to low carbon sorbents, with propranolol, the most hydrophobic compound studied, adsorbing to the greatest extent. At river bank filtration sites in Germany, Schmidt et al. (2007) reported removals of more than 70% for atenolol, metoprolol, bisoprolol and sotalol. Finally, Ternes et al. (2007) observed complete elimination of atenolol, celiprolol, metoprolol, propranolol, and sotalol during irrigation of an agricultural field with secondary treated sewage, and attributed the removal to biodegradation rather than to sorption.

In this context, motivated by artificial recharge of groundwater practices using Llobregat river water (Barcelona, Spain), and within a wider study on the potential effect of the redox state on organic micropollutants' fate (Barbieri et al., submitted), we studied the behaviour of the four β-blockers atenolol, metoprolol, propranolol and sotalol in aquifer material under nitrate reducing conditions. Results from the batch experiments performed are presented in the following.

4.2 Materials and methods

The set up was based on microcosms containing natural sediments, synthetic water and organic pollutants at individual initial concentrations of 1mg/L. The experiment included biotic and abiotic series, to separate contaminant's biodegradation (i.e. biotic mineralization or transformation) from sorption and other abiotic processes. Nitrate reducing conditions were stimulated in the biotic series by adding easily degradable organic compounds as electron donors (sodium acetate and the methanol used as solvent in the pollutants' spiking solutions) and an excess of nitrate as specific electron acceptor.

4.2.1 Materials and experimental procedure

Sediments were obtained from a test site for artificial recharge of groundwater through ponds located in Sant Vicenç dels Horts (Barcelona, Spain). The aquifer consists of quaternary alluvial sediments, mainly gravel and sand with a small fraction of clay. Samples were collected prior to the start of recharge operations, from an oxic unsaturated horizon at about 1m depth under the bottom of the infiltration pond. They were sieved to < 1mm and immediately used for assembling the experiments. Their mineralogical and chemical analysis revealed the presence of silicates, carbonates (calcite, dolomite), and some Mn(IV)/Mn(III) and Fe(III) oxides and oxide-hydroxides (associated content of Mn and Fe: 0.07 and 5.8 mg per g of air dried sediment, respectively). The fraction with grain size < 4μm was between 2 and 6% of the total. Their total carbon content was 2.5%, with an organic carbon and nitrogen content smaller than 0.2%.

Experiment water was artificially prepared based on the chemical composition of the recharge water (Llobregat river water) at the test site. Magnesium nitrate hexahydrate was used to add 66 mmol L^{-1} of NO_3^-. The resulting water was purged with argon during 1h to remove dissolved oxygen. Concentrations (mmol L^{-1}) of cations and anions were as follows: 7.8 (Na), 1.0 (K), 3.0 (Ca), 34.3 (Mg), 12.8 (Cl^-), 66.1 (NO_3^-), 2.1 (SO_4^{2-}), 0.5 (Alk), 0.1 (NH_4^+), and 0.02 (PO_4^{3-}). No dissolved organic carbon (DOC) was present in solution at this stage was set as null.

Atenolol, metoprolol, propranolol and sotalol were added to the water by the use of a spiking solution. Stock and working standard solutions were stored at -18 °C in the dark. Atenolol

and propranolol were purchased from Fagron (Barsbüttel, Germany), metoprolol and atenolol-D_7 were purchased from Sigma-Aldrich (Steinheim, Germany). Sotalol was obtained from a pharmaceutical preparation for intravenous injection from Carinopharm, Gronau, Germany (Carino Sotalol i.v. 40 mg). Atenololic acid was purchased from LGC Promochem (Wesel, Germany). Individual stock solutions were prepared by dissolving each compound in methanol. Working standard mixtures were then prepared at different concentrations by dilution of the individual stock solutions in methanol, to be used as spiking solution and to prepare the aqueous calibration standards Stock and working standard solutions were stored at -18 °C in the dark.

The spiking solution included several more drugs that will not be discussed here (results presented in Chapters 2 and 3, and Appendixes C and D): iopamidol, iomeprol, iohexol, iopromide, famotidine, pantoprazole, sulfamethoxazole, erythromycin, roxithromycin, clarithromycin, carbamazepine, diazepam, primidone, clofibric acid, bezafibrate, gemfibrozil, phenazone, naproxen, ibuprofen, diclofenac, cetirizine, loratadine, acetaminophen.

The assembling of the batches was carried out inside a glovebox, under Argon atmosphere. The “initial water” was obtained by adding 2.1mmol of anhydrous sodium acetate and 3mL of spiking solution per liter of the synthetic water described above, which yielded an initial DOC of 70mmol L^{-1} and a concentration of 1mg/L for each organic pollutant. After sampling the solution for chemical analyses, the microcosms were prepared by filling 0.3L glass bottles with 120g of air-dried and homogenized sediments, and 240mL of “initial water”. A remaining headspace of 15mL was left in each bottle. The assembling procedure was concluded by closing the bottles with screw-caps plus a PTFE protection seal, and gently shaking. The batches were removed from the glovebox, wrapped in aluminium foil to prevent photodegradation, and incubated at 25 ± 2 ºC. On a regular basis, they were gently shaken.

Synthetic water and sediments for the abiotic series were sterilized prior to assembling. They were introduced three times (once a day in three consecutive days) into an autoclave at T=121 ºC and P = Patm+1 atm during 20 minutes. The glove box was sterilized with UV light before introducing the materials. As an additional precaution, 0.22 mmol L^{-1} of mercury chloride were added (as microbial poison) to the “initial water”.

Duplicate bottles were sacrificed according to a pre-defined schedule (at days 2, 5, 10, 15, 25, 41 and 87). One at a time, the two bottles were opened under Ar atmosphere, chemical parameters were measured, and aqueous samples for general chemistry and pollutants analysis

were collected and stored according with each laboratory recommendations until analysis. The sterility of the abiotic series was verified two times along its duration. To this aim, an aliquot of water from devoted microcosms was spread on tryptic soy agar (TSA) plates and incubated in duplicate at 25ºC under aerobic conditions (during 1 week) and anaerobic conditions (during 2 weeks). None of the plates displayed microorganisms' growth.

4.2.2 Analytical methods

Biotic samples collected for analysing Cl^-, NO_3^-, NO_2^-, SO_4^{2-}, PO_4^{3-}, F^-, NH_4^+ and DOC (Dissolved Organic Carbon) and COD (Chemical Oxygen Demand) were filtered through 0.45 µm PALL Acrodisc® Sterile Syringe Filters with Supor® membrane and frozen. Anions were analyzed by ion chromatography using a ICS-1000 instrument. The analytical error was estimated to be 14% for PO_4^{3-} and 13% for the remaining anions. NH_4^+ concentration was analyzed with a selective electrode Orion 9512. DOC was analysed by 680 °C combustion catalytic oxidation/NDIR method using a TOC-V CSH instrument. The estimated analytical error was 20%. Biotic and abiotic samples for analysing COD (Chemical Oxygen Demand) were also filtered 0.45 µm, and were analyzed by colorimetry with the spectrophotometer Spectroquant Nova 60. Abiotic samples for Cl^-, NO_3^-, SO_4^{2-} and F^- were frozen and then analysed by using a Dionex DX-320 instrument with conductometric detection, a Dionex AS11-HC (2 x 250 mm) column and 23 mM KOH as eluent (isocratic separation at 30 °C). A flow rate of 0.38 mL min-1 was applied. Prior to chromatography, samples were filtered (Whatman Anotop 10 IC, 0.2 µm).

Samples for Fe and Mn, Ca, Mg, Na, K and minor elements were also filtered at 0.45 µm, acidified and stored at 4ºC. They were later analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES) using a Thermo Jarrel-Ash Iris Advantage HS instrument. Detection limits were 100 µg/L for K and Na, and 50 µg/L for the rest. The analytical error was estimated below 3%. In the ICP-AES analyses, calibration with three laboratory sets of standards was performed every 10 samples, and regression coefficients of the calibration curves exceeded 0.999.

pH and temperature (Thermo Scientific 9157BN Triode pH electrode, refillable), Electrical Conductivity (Hanna Instruments, 76302W conductivity probe), Dissolved Oxygen (Hanna Instruments, HI 76407/4 DO probe) and Alkalinity (drop test kit Taylor K-1726, precision of 0.5 mmol L^{-1}) were measured during the assembling/disassembling procedure.

Samples for analysis of β–blockers were kept frozen until analysis. Quantification of atenolol, metoprolol and sotalol was performed without preconcentration by an HPLC/ESI-MS-MS method as described by Nödler et al., 2010. Additionally, the individual MS-MS parameters of

atenololic acid and propranolol were included with this methodology and were as follows: For atenololic acid, the quantifier and qualifier transitions in positive electrospray (+ESI) were 268 → 145 and 268 → 191, respectively. The respective collision energies were -17.5 V and -12 V. The capillary voltage was set to 60 V. For propranolol, the quantifier and qualifier transitions in positive electrospray (+ESI) were 260 → 116 and 260 → 183, respectively. The applied collision energy was -8.5 V for both transitions and the capillary voltage was set to 55 V. Prior to analysis, samples were diluted (v/v) 1:2 with aqueous 5 mM ammonium acetate solution, containing 4 % methanol. 100 ng/mL of atenolol-D_7 was used as the internal standard. For additional matrix compensation, calibration standards were prepared in inorganic matrix according to 50 % of the experimental water concentration. Before analysis, all samples and standard solutions were centrifuged at 1500 rpm (Christ RVC 2-18, purchased from Fisher Scientific, Schwerte, Germany) for 30 min at room temperature. Six concentration levels (1 – 500 ng mL^{-1}) were used for the calibration and the correlation coefficients exceeded 0.99.

4.3 Discussion of results

4.3.1 General water chemistry

The chemical evolution of hydrochemistry in the biotic experiment is presented in Figure 4.1. Results from duplicate batches showed a satisfactory reproducibility at all sampling times. Actually, when plotting the data plus the error bars from each batch, there was always some overlap. Thus, the average of results from the duplicate bottles is reported in the figure.

DOC and nitrate decreased all experiment long, starting (appreciably) after day 5 and being still present at day 87 with final concentrations of 27.2 mmol L^{-1} and 7.5 mmol L^{-1}, respectively. Alkalinity increased continuously after day 2, from 0.8 mmol L^{-1} to 22 mmol L^{-1}. Nitrite concentration also began to increase after about 2 days reaching a maximum at day 41 and becoming completely depleted by day 87. Dissolved Manganese and Iron were almost not detected, sulphate remained constant throughout the experiment (results not shown), and pH ranged between 7.3 and 8.3.

The previous observations, consistent with the expected evolution of the redox sensitive species, suggest that nitrate reducing conditions were established within approximately 2 days of microbial adaptation, and dominated the system during the rest of the test. The occurrence of nitrite reflects the actual denitrification pathway, because nitrite is an intermediate product between nitrate and nitrogen. Its depletion between day 41 and 87, when nitrate reduction was still occurring, can be attributed to nitrite reduction to nitrogen being faster than the production of nitrite from nitrate, by the end of the experiment.

Quantitative consistency of the expected degradation processes was checked by a mass balance of electrons and major elements. We used the stoichiometries of organic matter (i.e., acetate and methanol) biodegradation in the presence of nitrate. We assumed that about 10% of the organic carbon is transformed into biomass and that in the case of methanol (redox state of carbon = -2) the formation of biomass requires a partial oxidation (bulk biomass has a redox state of 0). We estimated carbonates precipitation from the overall measured decrease of calcium and magnesium (3.0 mmol L^{-1} and 21.3 mmol L^{-1}, respectively, data not shown). We also took into account the inorganic carbon transferred to the headspace of the bottles, and the precision of Alkalinity measurements. With these assumptions, the overall inorganic carbon mass balance could be closed with an error of about 11%.

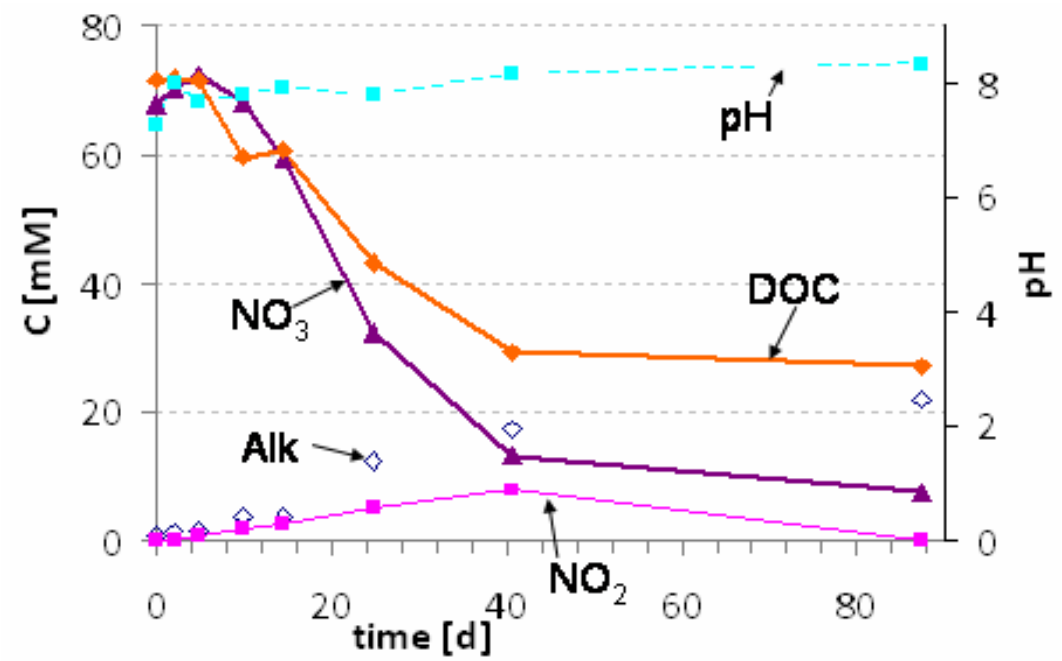


Figure 4.1: Chemical evolution with time in the biotic experiment.

Regarding the abiotic experiment, the hydrochemistry remained practically constant for the whole time as expected (results not shown).

4.3.2 Fate of the selected β-blockers

The temporal evolution of the average concentration of each drug is reported in the following Figures. Error bars were calculated by taking into account the analytical errors and the difference between duplicate batches results. Concentrations are presented in relative terms (C/C_0, where C_0 is the initial concentration) in order to remove systematic errors from the analysis.

4.3.2.1 Fate of atenolol (Figure 4.2).

The biotic and abiotic series display a similar trend during the first 5 to 10 days, which suggests that the initial disappearance of atenolol (an overall removal of about 14%) is caused by abiotic. Since Atenolol, with a pK_a of 9.2, is predominantly positively charged in the pH range of these experiments, such abiotic removal can be attributed to sorption affinity to the negative charges of clay minerals, originated by isomorphic substitution in the structure of the mineral.

No additional abiotic removal could be observed, taking into account the error bars, after day 10. That is, removal of atenolol must be caused by microbially mediated processes after day 10, when nitrate reducing conditions were already dominating the system. The arithmetic scale evolution of atenolol is virtually linear after day 5-10 (Fig. 2). This suggests a zero order kinetics. That is, atenolol biotic removal was controlled by factors other than NO_3 or atenolol concentration. A removal rate of 5.7 µg L^{-1} d^{-1} could be estimated. Only 35% of the initial atenolol was left by the end of the experiment (day 87).

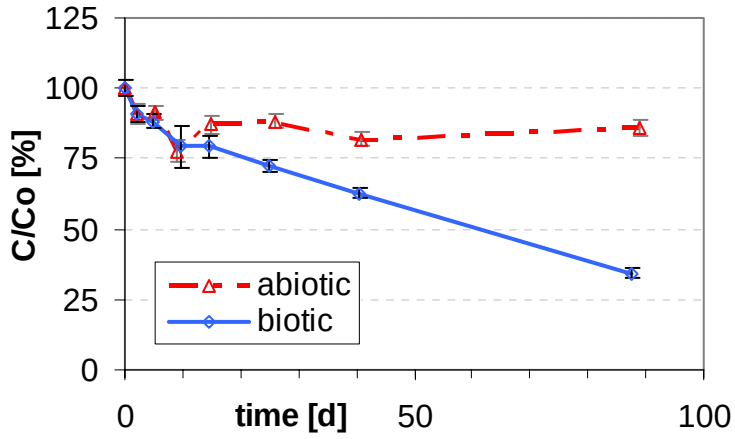


Figure 4.2: Evolution of atenolol average concentration (normalized to the initial concentration Co) during the biotic and abiotic experiments.

To get a better understanding of atenolol fate, all samples were analysed for atenololic acid, which was identified by Radjenovic et al. (2009) as a microbial transformation product of atenolol, generated by hydrolysis of its amide bond. Indeed, atenololic acid could be detected in our microcosms. The fact that it could only be found in water from the biotic series confirmed its microbial origin. The evolution of its concentration in the biotic experiment is shown in Figure 4.3. In an attempt to check the overall mass balance, we have also reported in the same graphic atenolol concentrations (biotic and, as reference, abiotic experiments) as well as the sum (named “SUM” in the following) for each sampling time of atenolol, atenololic acid and the amount of atenolol abiotically removed in the biotic experiment. In spite of some slight fluctuations, it could be

observed that the value of "SUM" remained almost equal to its initial value all time long Therefore, we can conclude that a small portion of atenolol molecules was sorbed, and the remaining part was biotransformed to atenololic acid. The likeliness of the process of amide hydrolysis, leading to the production of atenolol's corresponding carboxylic acid, was consistent with the findings of Helbling et al. (2010). In a study on several amide-containing compounds, they observed hydrolysis to be a preferential biotransformation pathway for primary amides, confirming atenolol to be hydrolyzed to atenololic acid. They also proposed a mechanism for such enzyme-catalyzed reaction, indicating amidases and proteolytic as the catalysts possibly involved. Such enzymes are ubiquitous in nature, and could obtain a quite high bioconversion yield by reactions involving C-N bond-containing substrates like amides (Fournand end Arnaud, 2001; Sharma et al., 2009).

The evolution of atenololic acid in Figure 4.3 exhibited a linear trend, similar and opposite to that of atenolol. A production rate of 5.5 µg L^{-1} d^{-1} could be estimated for the transformation product, which almost matches the rate of atenolol removal given above. In light of the microbial nature of the process linking the two compounds, the observed zero order kinetics, can be explained by an enzyme limited transformation of atenolol to atenololic acid, i.e. a biotransformation in which the enzyme concentration represented the rate limiting factor. Actually, the mass balance of atenololic acid may be complicated by other sources, such as degradation of metoprolol as discussed below.

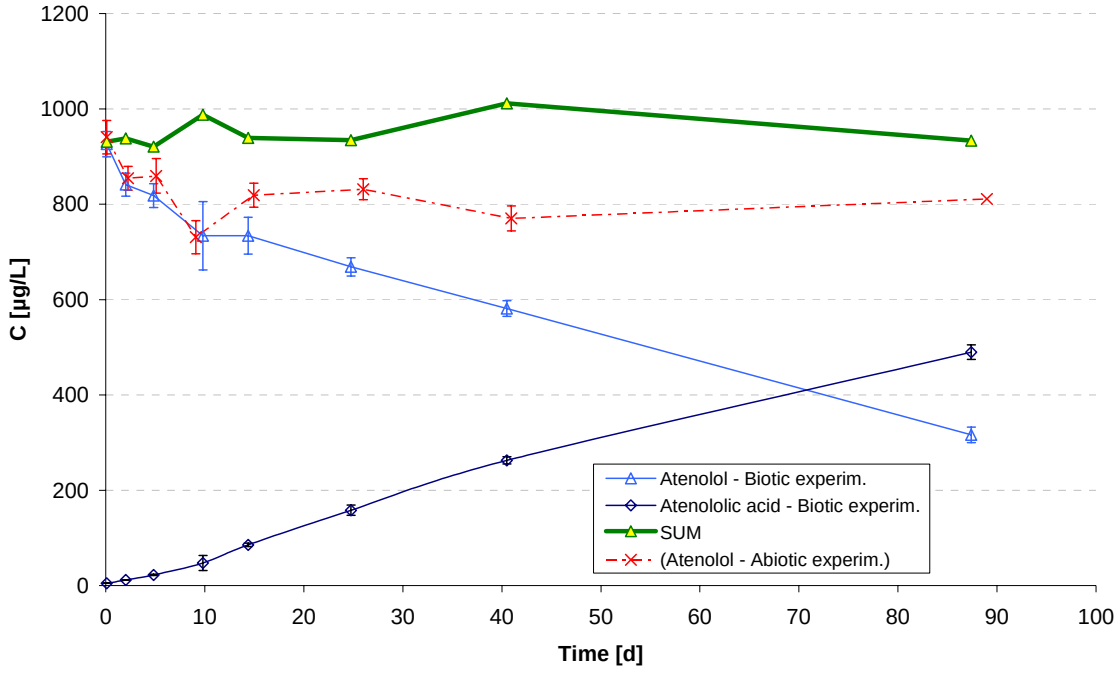


Figure 4.3: Evolution of atenolol and atenololic acid in the biotic series, atenolol in the abiotic series, and sum (for each sampling time) of atenolol, atenololic acid and the amount of atenolol abiotically removed in the biotic series.

4.3.2.2 Fate of metoprolol (Figure 4.4).

As in the case of atenolol, abiotic processes controlled metoprolol removal until day 5-10, with an overall removal of 15 to 20%. Later on, metoprolol concentration remained almost constant in the abiotic series, while it further decreased in the biotic one. By the end of the experiment, concentration of metoprolol had been reduced an additional 15 to 20%.

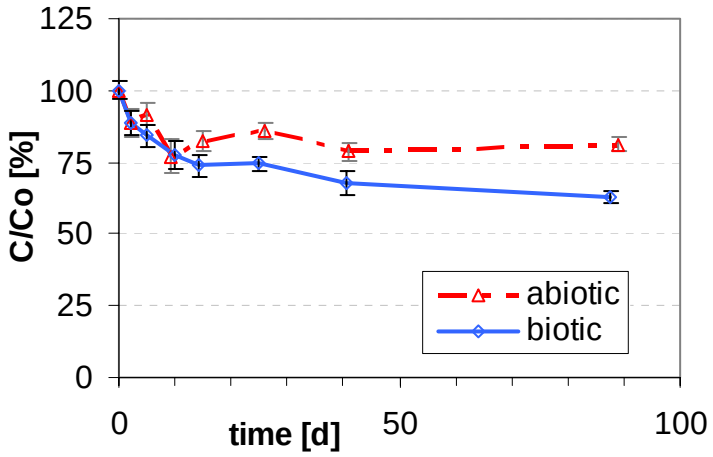


Figure 4.4: Evolution of metoprolol average concentration (normalized to the initial concentration Co) during the biotic and abiotic experiments.

The human and animal metabolite of metoprolol, metoprolol acid, displays the same structure of atenololic acid (Lennard, 1985 and references therein; Fang et al., 2004). One might conjecture that the observed atenoloic acid in our samples results from the biotransformation of not only atenolol, but also metoprolol. Nevertheless, to our knowledge this compound has not been demonstrated to be a bacterial transformation product of metoprolol. Moreover, the plot in Figure 4.5 shows that measured atenololic acid build-up is significantly smaller than the sum of atenolol and metoprolol removal. Therefore, under the previous conjecture the mass balance of the biotic experiment suggests that some additional undetected transformation product had to be formed from atenolol, metoprolol or even form atenololic acid, or that partial mineralization of some of these compounds had to occur.

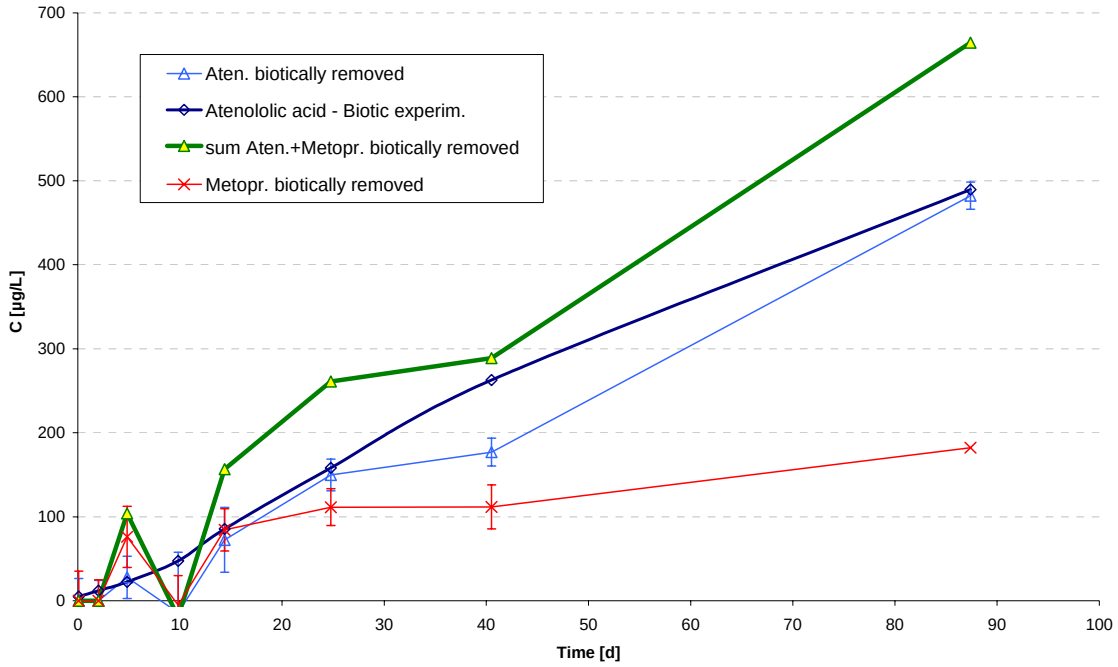


Figure 4.5: Evolution of atenololic acid and of the amounts of atenolol and metoprolol biotically removed (each compound individually and as sum, for each sampling time) in the biotic experiment.

4.3.2.3 Fate of propranolol (Figure 4.6)

The evolution of propranolol in the biotic and abiotic series presented almost the same trend all experiment long. This implies that abiotic processes dominated the removal of this compound. An overall abiotic removal of about 35% could be estimated at day 87. A very slight, but measurable, separation of the two concentration curves can be observed after day 15, resulting in an additional biotic removal for propranolol of about 10%.

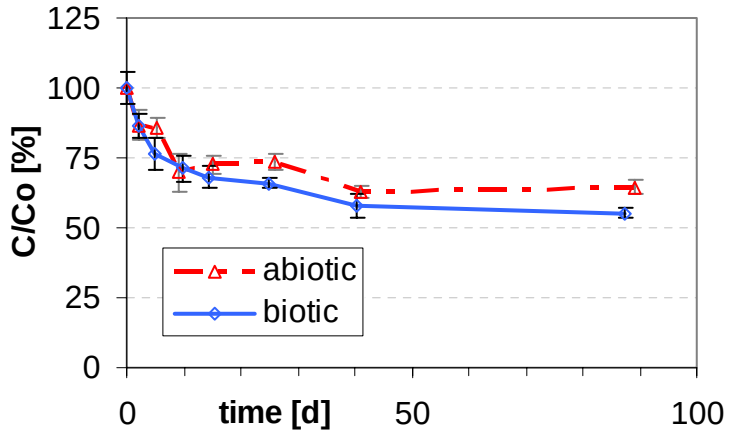


Figure 4.6: Evolution of propranolol average concentration (normalized to the initial concentration Co) during the biotic and abiotic experiments.

4.3.2.4 Fate of sotalol (Figure 4.7)

No abiotic removal could be observed for sotalol during the whole experiments. We find this surprising because, for the same reasons as atenolol, we expected some sorption onto clay. Therefore, we conjecture some unreported interference with the other three beta-blockers. On the other hand, some zero-order microbial processes seem to occur up to day 10 in the biotic experiment, leading to a removal of about 20 % with a rate of 2 µg L^{-1} d^{-1}. Further on, the concentration of sotalol in the biotic test remained almost constant.

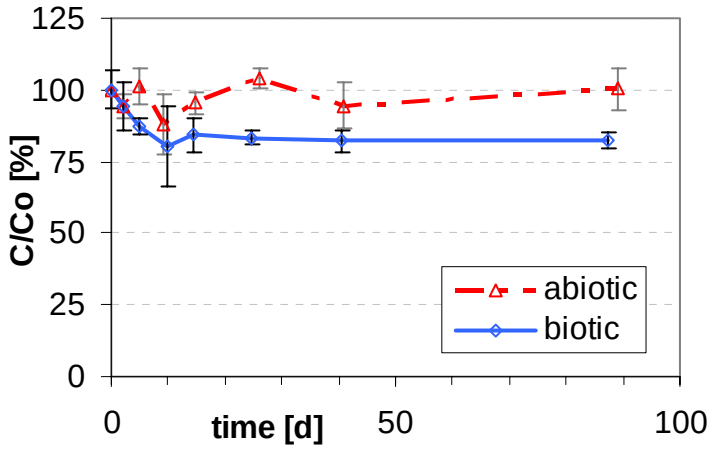


Figure 4.7: Evolution of sotalol average concentration (normalized to the initial concentration Co) during the biotic and abiotic experiments.

4.4 Conclusions

The four target beta-blockers display similar structures but somewhat different behaviour in saturated aquifer sediments under nitrate reducing conditions.

Some abiotic processes, probably sorption to clay minerals, were responsible of 14%, 15-20% and 35% of atenolol, metoprolol and propranolol removal, respectively. These processes dominated the first 5-10 days of the 87 days of experiment in the case of atenolol and metoprolol, whereas occurred almost all time long for propranolol. All the three compounds exhibited also a biotic removal. At day 87, additional 50% of atenolol has disappeared. The detection of atenololic acid in the biotic samples suggested atenolol to be biotransformed during the experiment by hydrolysis of its amide bond, according to the mechanism proposed by Radjenovic et al. (2009). The temporal evolution of the concentration of both compounds reflected zero order kinetics, likely indicating an enzyme limited biotransformation of atenolol into atenololic acid. By the end of the experiment, metoprolol and propranolol have experienced additional 15-20% and 10% of biotic removal,

respectively. Somehow differently, no abiotic removal was observed for sotalol, exhibiting only a microbial removal of about 20% during the first 10 days of experiment.

The average removal reported for the four target -blockers in conventional wastewater treatments plants ranges from 58 to 80% for atenolol, 20 to 40% for metoprolol, 20 to 60% for propranolol, and 20 to 50% for sotalol (Gabet et al., 2010; Gros et al., 2010; Lin et al., 2009; Radjenovic et al., 2009). Thus, the overall removals observed in the experiments presented in this paper (i.e., approximately 65% removal for atenolol, 30% to 40% for metoprolol, 45% for propranolol, and 20% for sotalol) suggest that the processes occurring in aquifers potentially constitute an alternative water treatment for the studied beta-blockers, which could lead to at least comparable or even higher removal efficiency of conventional wastewater treatment plants. The longer time needed to reach such removals may be ensured by the large residence times in aquifers. Further investigation is needed to confirm these findings at lower concentrations of the target pollutants.

Chapter 5

General conclusions

This chapter summarizes the main concluding considerations arisen from the present thesis.

- The design criteria and the methodology used in the batch experiments resulted to be adequate to objectives. The desired redox states have been quite successfully created and sustained in each set of experiments by the addition of sufficient quantities of electron donors and acceptors. It is worth pointing that the use of natural sources of Mn and Fe in the Mn- and Fe-reducing experiments was realistic, but complicated the development of controlled redox conditions. Natural sources are often quite crystalline, which slows down dissolution to the point of making it the rate limiting process. In the case of the Fe(III)-reducing experiment, for instance, concomitant Fe- and SO_4-reduction occurred during the first part of the test as well as mixed Fe-reducing/methanogenetic conditions dominated after SO_4 depletion.
- The assessment of the dominating redox states has been achieved by a thorough monitoring of water chemistry, focused on the redox-sensitive species but including major and minor ions too. Precipitation/dissolution of minerals as well as biomass production has to be taken into account for a correct interpretation of the main processes involved. Inspection of

the sediments from the disassembled batch experiments through SEM-EDS has been fruitful used to confirm the occurrence of such processes.

- However, further improvements are required. Specifically, dissolved sulphide and methane should be analysed to better assess sulphate reducing conditions, especially in its early stages, and to check possible occurrence of methanogenesis. Additional desorption experiments could confirm Mn^{2+} and Fe^{2+} adsorption onto clay surfaces and/or exopolymeric substances (EPS). As general rule, whenever possible, the evaluation of the microbial state during the experiments (e.g.: identification of microbial communities, measurements of Hydrogen, etc.) would be also advisable as complementary tool for the identification of the prevailing redox state.
- Numerical modeling proved useful in confirming the concepts described above with literature kinetic rates. Matches between computations and observations could have been improved by varying the rates of carbonates precipitation, and by postulating likely occurring sorption onto biofilms. Departures between model results and measurements are small, but generally suggest an intricate coupling between biologic and inorganic processes.
- The sampling schedule has proven adequate for monitoring the temporal evolution of aqueous chemistry and micropollutant concentrations. Still, in the case of Mn-/Fe-/SO_4-reducing experiments some additional sampling point during the first week could have been useful to confirm early removal trends for some of the target contaminants.
- One of the aims of the study was to test systems representative of real aquifers and of conditions occurring either naturally or possibly being stimulated during managed artificial recharge operations. Such conditions may vary spatially and temporally along with recharge cycles and recharge water composition. Thus, the microbial communities naturally existing in the sediments used in the experiments, which were expected to carry out the biodegradation of organic matter and the removal of micropollutants, were not previously adapted to the redox conditions of interest. As a consequence, the first part of each experiment was characterized by a transition stage (of different duration) until the target redox state could be effectively established or observed. This hindered the interpretation of results and redox effect for some of the studied micropollutants (e.g. atenolol).
- Due to design constraints, the concentration of the easily degradable organic substrates used in the experiments were higher than those naturally present in aquifer systems or in most recharge waters, which likely affected the growth of the microbial communities present in the microcosms. Thus, the extrapolation of the observed micropollutants results to natural

subsurface environments would have to be faced carefully, being not straightforward. Still, the microcosm study proved the feasibility of specific redox environments to develop at test site and the capability of the local microorganisms to eliminate the target micropollutants, providing as well some overall removal pattern under the tested settings. In the end, such scenarios could eventually be promoted during artificial recharge at test site if less favourable removals of the pollutants of interest are observed under the spontaneously occurring conditions.

- Results for micropollutants exhibited that some of them indeed presented a different behaviour from experiment to experiment, i.e. depending on the predominant redox conditions. This confirmed that the redox state of the system could exert an influence on organic micropollutants fate. Even if neither the NO_3-reducing experiment was long enough to compare nitrate reducing conditions with the more reducing systems nor exact patterns could be isolated for micropollutants behaviour under each specific redox state, the higher and often faster elimination of the target compounds was observed in the SO_4-reducing or in the Natural Conditions (dominated by mixed Mn-Fe-SO4-reducing conditions) experiments, under the most reducing condition.
- Some other micropollutants, differently, were removed by biotic processes but seemed to be not redox sensitive. Other ones, finally, only experienced an abiotic removal (possibly due to sorption to sediments, chemical hydrolysis, etc.) or exhibited a recalcitrant behaviour.
- A reversible unreported phenomenon could be identified for the drugs diclofenac, sulfamethoxazole and acetaminophen under denitrifying conditions, in both experiments at low (1µg/L) and high (1mg/L) pollutants initial concentrations. There could be a significant environmental implication of this finding, at least for aromatic amines like diclofenac and sulfamethoxazole: ignoring the observed feature could induce experimenters to overestimate their actual elimination in field and laboratory studies. This may explain some inconsistencies on literature reports about their removal (e.g. in the case of sulfamethoxazole). The relevant role played by nitrite in the previous phenomenon confirmed that a thorough monitoring of the inorganic chemistry in field and laboratory studies is advisable to understand the fate of organic micropollutants. We guess that the observed reversible action of nitrite should have also to be taken into account when assessing the efficiency of wastewater treatment in removing organic compounds containing aromatic amines, since nitrification and denitrification processes occurs during the biological treatment.

- The experiments included biotic and abiotic series to separate contaminant's biodegradation (i.e. biotic mineralization or transformation) from sorption and other abiotic processes. Actually, correctly identifying of the actual biotic processes responsible for the removal of micropollutants requires the use of specific techniques, such as the use of isotopically labelled compounds and/or the identification of already known/new transformation products. In our study, identification and quantitative analysis of transformation products (namely, atenololic acid, nitro-diclofenac and 4-nitro-sulfamethoxazole) allowed to prove the process experienced by atenolol (experiments at 1mg/L initial concentration), diclofenac and sulfamethoxazole. The use of such techniques is also advisable when investigating the fate of organic micropollutants.
- An experiment at higher pollutants concentration (1mg/L each compound) was also carried out under nitrate reducing conditions, to check the representativeness of studies at concentrations easier to be tested and analysed. Nevertheless the correspondent NO3-reducing experiment at low micropollutants concentrations (1μg/L each compound) was finally too short to allow comparison. Anyway, results from the high concentrations experiment provided patterns of behaviour in aquifer material under nitrate reducing conditions for 26 pharmaceuticals. Analogous (e.g. for iodinated contrast media) or, on the opposite, quite different (e.g. for beta blockers) evolutions could be observed for drugs of the same class and characterized by similar properties.
- The ultimate aim of the experiments carried out was to identify the most favourable redox conditions for the removal of the target micropollutants from water, for their potential following stimulation in artificial recharge field sites. For the compounds presented in the main chapters of the present thesis (i.e. atenolol and 3 more beta-blockers, diclofenac, sulfamethoxazole, and acetaminophen) as well as for some of those included in the appendix, the overall removals yielded during the experiments were comparable or even higher than those reported for conventional water treatment plants. This suggests that the whole processes occurring in aquifers constitute a potentially efficient alternative water treatment for a number of organic (even emerging) micropollutants. Depending on the redox state naturally occurring or possibly being deliberately stimulated in field applications, the time needed for a complete removal may be ensured by the large residence times in aquifers.

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APPENDIX A

Laboratory protocols for the batch experiments

The protocols for assembling and disassembling the different types of experiments are detailed below. The assembling and disassembling procedures are carried out under controlled temperature (T = 25ºC).

To facilitate the reading, in the following we will refer to the batch experiments with micropollutants at 1 µg/L individual concentrations presented in this thesis as applied example.

A1. ASSEMBLING PROTOCOL

The Steps from 0 to 3 of the following procedure are common to the assembling of both types of biotic and abiotic experiments. The remaining specific parts are detailed in separate paragraphs (A1.1 and A1.2).

Step 0. Material Cleaning

Accurate cleaning (3 rinsing with MQ water, 3 with methanol and 3 with acetone) of Ni glass bottles of volume Vbottle (in the present study, Vbottle = 0.3L) as well as of all the material to be used during assembling (for glass material: 10 rinsing with MQ water, 3 with methanol and 3 with acetone). The quantity of bottles Ni depends on the experiment "i" to be assembled (Table A1).

Table A1. Number of batch assembled in each experiment

Experiment (i)	*Number of bottles (Ni)*
NO3-reducing experiment	*14*
Mn-reducing experiment	*14*
Fe-reducing experiment	*14*
SO4-reducing experiment	*14*
Natural condition experiment	*22*
abiotic part1	*19*
abiotic-part2	*13*

Step 1. Sediment sampling and sieving

In the field: sediment sampling and sieving through d_{sieve} = 1mm sieve. The quantity of sediment to be sampled depends on the number of microcosms Ni to be assembled and on the quantity of sediment Qsedim to be set in each microcosm (in the present study, Qsedim = 120g). The sieved sediment is packed using aluminium paper and stored at 25ºC during a maximum of 1 day before Step 2 or at 4ºC for a longer storage (Fig. A1).

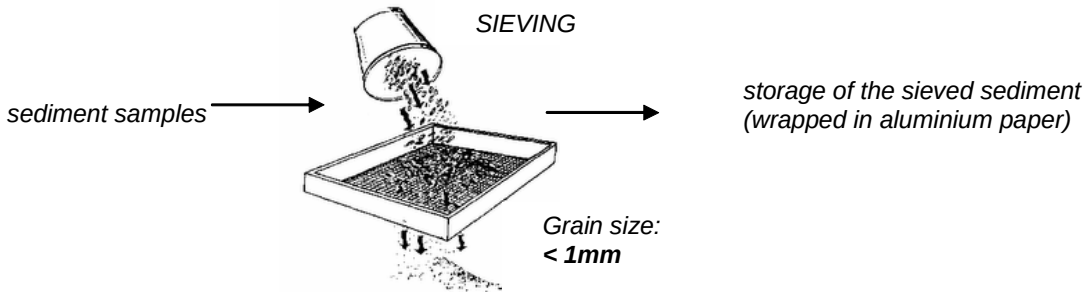


Figure A1. Sieving and storage of sediments

Step 2. Preparation of the sediment

The sediment is homogenized in steel containers and distributed into the Ni bottles (Qsedim into each bottle of volume Vbottle). The present Step has to be performed:

- the day before assembling, in the case of the biotic experiments;
- three days before assembling, in the case of abiotic experiments.

Step 3. Preparation of the "common water"

One or maximum two days before assembling the experiments: preparation of *Xi* Litres of the synthetic "common water". The volume *Xi* depends on the experiment "i" to be assembled, taking into account that each batch will finally contain a volume Vwater of water (in the present study, Vwater = 0.24L). After preparation, the "common water" is stored at 4 ºC in an amber bottle of adequate volume closed with screw-cap plus a PTFE protection seal. The composition of the synthetic water has to be representative of a test site composition (Table 2.2 included in chapter 2 of the present thesis)

A1.1 BIOTIC EXPERIMENTS

Step 4. Adding electron acceptor

Additional quantities of electron acceptor are added to the Synthetic Basic Water or to the sediment, depending on the set "i" of experiments:

4a) In the case of NO_3- and SO_4-reducing experiments, an additional quantity of nitrate or sulphate is respectively dissolved into the "common water". That is, in our example additional 450mg/L (7.27mol/L) of nitrate were added in the case of NO3-reducing experiment and 330mg/L (7.27mol/L) of sulphate were added in the case of SO4-reducing experiment.

4b) In the case of Mn(III/IV) and Fe(III)-reducing experiments, an additional quantity of Mn(III/IV) or Fe(III) oxide/hydroxides is respectively added to the sediment in form of powder. That is, in our example additional 0.4g of finely ground natural psilomelane and 0.95g of mixed ferrihydrite/goethite were respectively added into each 0.3L glass bottle (already containing 120g of sediment).

4c) In the case of the Natural Condition experiments, no additional electron acceptor is added.

Step 5. Purging the "common water" (only for anaerobic experiments)

The "common water" has to be bubbled with Argon until $O_{2(ac)}$ ~ 0mg/L and no Oxygen gas is present in the bottle headspace (about 1h, in the present study). Then, closing the bottle containing now X_i Litres of anaerobic synthetic water.

Step 6. Preparing the glove box (only for anaerobic experiments)

Put:

- the N_i glass bottles (containing the sediments and, in case of Mn- and Fe-reducing experiments, also the additional oxide-hydroxides).
- all the material to be used for completing the assembling and to sample and measure parameters in the water.
- the bottle(s) containing the target micropollutants spiking solution(s).
- the amber bottle containing the *Xi* Litres of anaerobic synthetic water.

into the anaerobic glove box (under Argon atmosphere, pressure = atmospheric pressure, residual $O_{2(gas)}$ < 0.1%).

Step 7. Preparation of the "Initial water"

Preparation of X_i Litres of the "INITIAL WATER *i* " according to the following steps (Fig A2):

a) Open the big bottle containing the *X*i Litres of the (anaerobic, in the case of anaerobic experiments) “common water”.
b) Add the selected quantity of easy degradable organic substrates. The quantity q_i of reactive to be added has been selected in the design phase, too, and depends on the type of experiment “i" (§2.2.2 of the present thesis). Close the bottle and shake to ensure a good mixing. Then, open again the bottle.
c) Put 1Liter of the solution in an amber glass flask. Using precision glass syringe or pipette, add an aliquot of the micropollutants spiking solution(a) into the flask and shake adequately to ensure a good mixing. The magnitude of the aliquot depends on the quantity *Xi* of “initial water” to be prepared and the target concentration for the micropollutants.
d) Pour the content of the 1L flask into the big amber bottle. Close the big bottle and shake to ensure a good mixing. Then, open again the bottle. The X_i Litres of “INITIAL WATER *i* ” are ready.

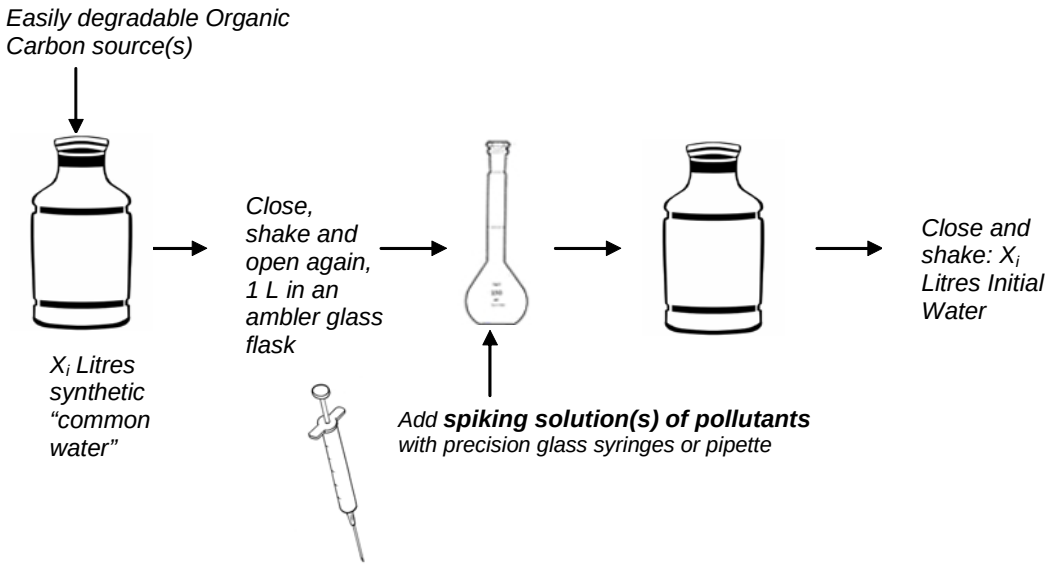


Figure A2. Preparation of the initial water

Step 8. Sampling the Initial Water

Sampling of the “INITIAL WATER *i*” for its posterior analysis (major and minor components; micropollutants) and for immediate parameters measurement.
The aliquots of water to be sampled for each type of analysis as well as the characteristics of the sampling material (syringe, filters if filtering is requested, sampling bottles, etc.) are previously accorded with the specialist laboratories performing the analysis.

The compounds and parameters monitored in the present study are detailed in §2.2.5 of the thesis. As example of the possible complexity of samples preparation, the procedure followed in our case is illustrated in the Figure A3.

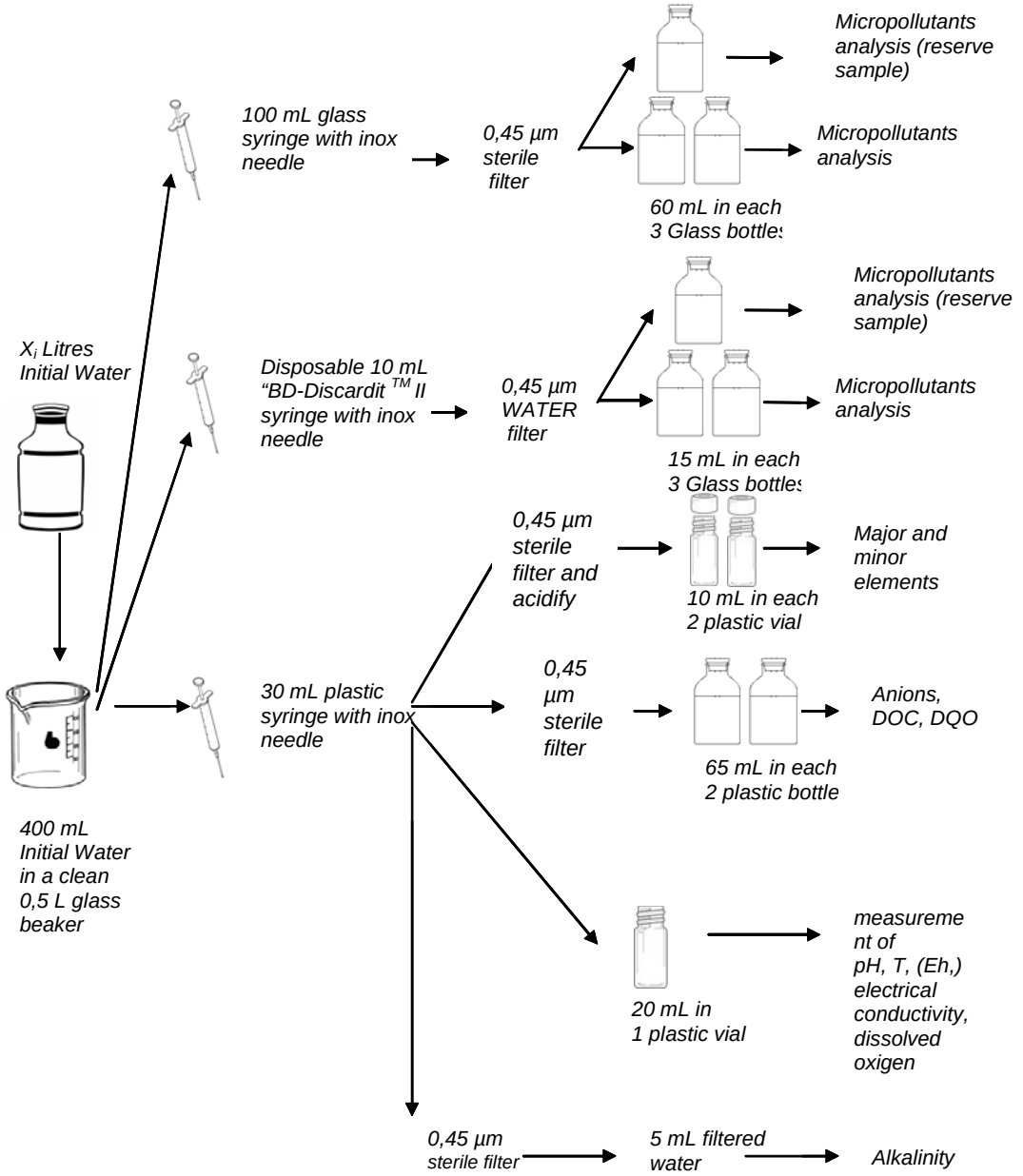


Figure A3. Sampling the "initial water" - procedure followed in the present study.

Step 9. Preparation of the N_i microcosms

a) using a graduated beaker, put the previously designed quantity Vwater (in the present study: Vwater = 0.24L) of the "INITIAL WATER *i* " into each one of the N_i bottles (already containing sediments and, in case of Mn- and Fe-reducing experiments, the additional oxide-hydroxides too).

b) close the N_i bottles with screw-cap plus a PTFE protection seal and gently mix each of them. A remaining headspace of Vgas = 15mL characterize each microcosm. The N_i batch are assembled (Fig A.4).

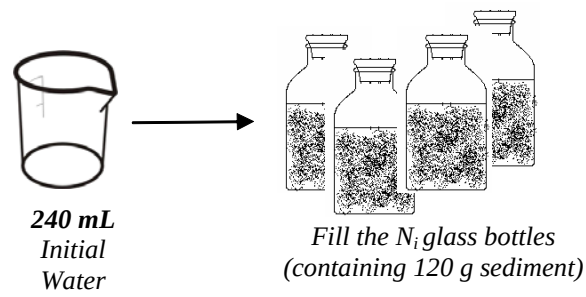


Figure A4. Preparation of the N_i microcosms

Step 10. Opening the glove box (only for anaerobic experiments)
Open the glove box and retire the microcosms, the samples and all the material used.

Step 11. Microcosms and samples storage
Wrap the N_i batches with aluminium foil, in order to guarantee dark conditions and prevent photodegradation. Then, leave them at T=25ºC until their scheduled sacrifice date. In fact, they have to be gently shaken few times during their lifetime (e.g. once every 2 days during the first week; once a week during the rest of the first month; then, once every 30 to 45 days) as well as the day before being sacrificed.
Carry the samples to the specific laboratories for immediate analysis or store them according to each laboratory recommendations (refrigerator or freezer).

Step 12. Cleaning
Accurate cleaning (with MQ water, methanol and acetone) of all the material used (as in Step 0).

NOTE:

- In the present study,the "Natural Conditions" experiments were conducted without including the Steps 5 and 6. That is, dissolved oxygen was allowed in the water and oxygen gas was initially present in the headspace of the bottles

A1.2 ABIOTIC EXPERIMENTS

Step 4. Reproducing the water composition of the biotic experiments
The same additional quantities of NO3 and SO4 used in Step 4 of §A1.1 are provided to the "common water.

Step 5. Sterilizing the soil and the "common water"
The N_i glass bottles (each one already containing the quantity Qsedijm. of sediment) are sterilized three times by autoclave at T=121 ºC and P = Patm+1 atm during 20 minutes. The three cycles must be separated by at least 24 hours the one from the other.
For the "common water", one autoclave cycle is enough.

Step 6. Purging the sterilized "common water"
Bubbling the sterilized "common water" with Argon until $O_{2(ac)}$ ~ 0mg/L and no Oxygen gas is present in the bottle headspace (about 1 hour, in the present study). Then, closing the bottle containing now X_i Litres of anaerobic and sterile "common water". This step procedure is performed inside a horizontal flow cabinet. All the material used has been previously sterilized. The Argon is passed through a sterile filter of 0.1 µm filter while bubbling.

Step 7. Preparing the glove box
The day before assembling the experiments, put:
- the N_i sterilized glass bottles;
- all the material to be used for completing the assembling and to sample and measure parameters in the water.
- the amber bottle containing the *Xi* Litres of anaerobic sterilized "common water"

into the anaerobic glove box (under Argon atmosphere, pressure = atmospheric pressure, residual Oxygen gas < 0.1%).
Leave an Ultra Violet light switched on inside the glove box during one night.

Step 8. Preparation of the "Initial water"
Switch off the UV light. Enter the bottle containing the micropollutants spiking solution(s) into the glove-box, maintaining the Argon atmosphere (pressure = atmospheric pressure, residual Oxygen gas < 0.1%). Prepare the X_i Litres of the "INITIAL WATER *i* " according to the following steps (Fig A.2):

a) open the big bottle containing the *X*i Litres of the Synthetic Basic Water.
b) add the same type of organic substrate added in Step 7b of §A1.1. Close the bottle and shake to ensure a good mixing. Then, open again the bottle.
c) add the quantity q_{poison} of the "poison" selected during the design of the experiments. In the present study, 60 mg of $HgCl_2$ has been added for each one of the *Xi* Litres of Initial Water being prepared. Close the bottle and shake to ensure a good mixing. Then, open again the bottle.
d) same as step7c of §A1.1
e) same as step7d of §A1.1

Step 9. Sampling the Initial Water
Same as step 8 of §A1.1. To avoid accidents when sampling the Initial Water, containing a "poison" which can has adverse effect also on the personal carrying out the assembling of the microcosms, no needle are used coupled with the syringes: the tip of the syringe is directly submerged into the water to be sampled.

Step 10. Preparation of the N_i microcosms
Same as step 9a, 9b of §A1.1.

Step 11. Opening the glove box
Open the glove box and retire the microcosms, the samples and all the material used, with special care for the material that has been in contact with the "poison".

Step 12. Microcosms and samples storage
Same as step 11 of §A1.1

Step 13. Cleaning
Accurate cleaning (with MQ water, methanol and acetone) of all the material used. Special health security measures have to be taken when cleaning the material that has been in contact with the "poison". The solid and liquid wastes produced must be disposed in the adequate way.

A2. DISASSEMBLING PROTOCOL

A2.1 BIOTIC EXPERIMENTS

According to a defined schedule, all the N_i microcosms belonging to each experiment "i" is sacrificed. Each microcosm is disassembled jointly with its duplicate. The disassembling procedure consists of the following steps:

Step 0. Microcosms preparation

About 24 hours before the disassembling, gently mix the 2 microcosms to be disassembled.

Step 1. Anaerobic conditions preparation

Put all the disassembling material (syringes, sampling bottles, etc.), the measurements equipments and the 2 microcosms to be disassembled into the anaerobic glove box (under Argon atmosphere, pressure = atmospheric pressure, residual Oxygen gas < 0.1%).

Step 2. Disassembling of the 1st of the 2 microcosms

a) throw away the aluminium foil covering the bottle. Open the bottle;

b) water sampling for posterior analysis (major and minor components; micropollutants) and for immediate parameters measurement.

The aliquots of water to be sampled for each type of analysis as well as the characteristics of the sampling material (syringe, filters if filtering is requested, sampling bottles, etc.) are previously accorded with the specialist laboratories performing the analysis. The sampling procedure followed in the present study is illustrated in the Figure A5. It is similar to that described in Step 8 of §A1.1 but, due to the limited quantity of water available in the batches, a smaller number of samples are collected.

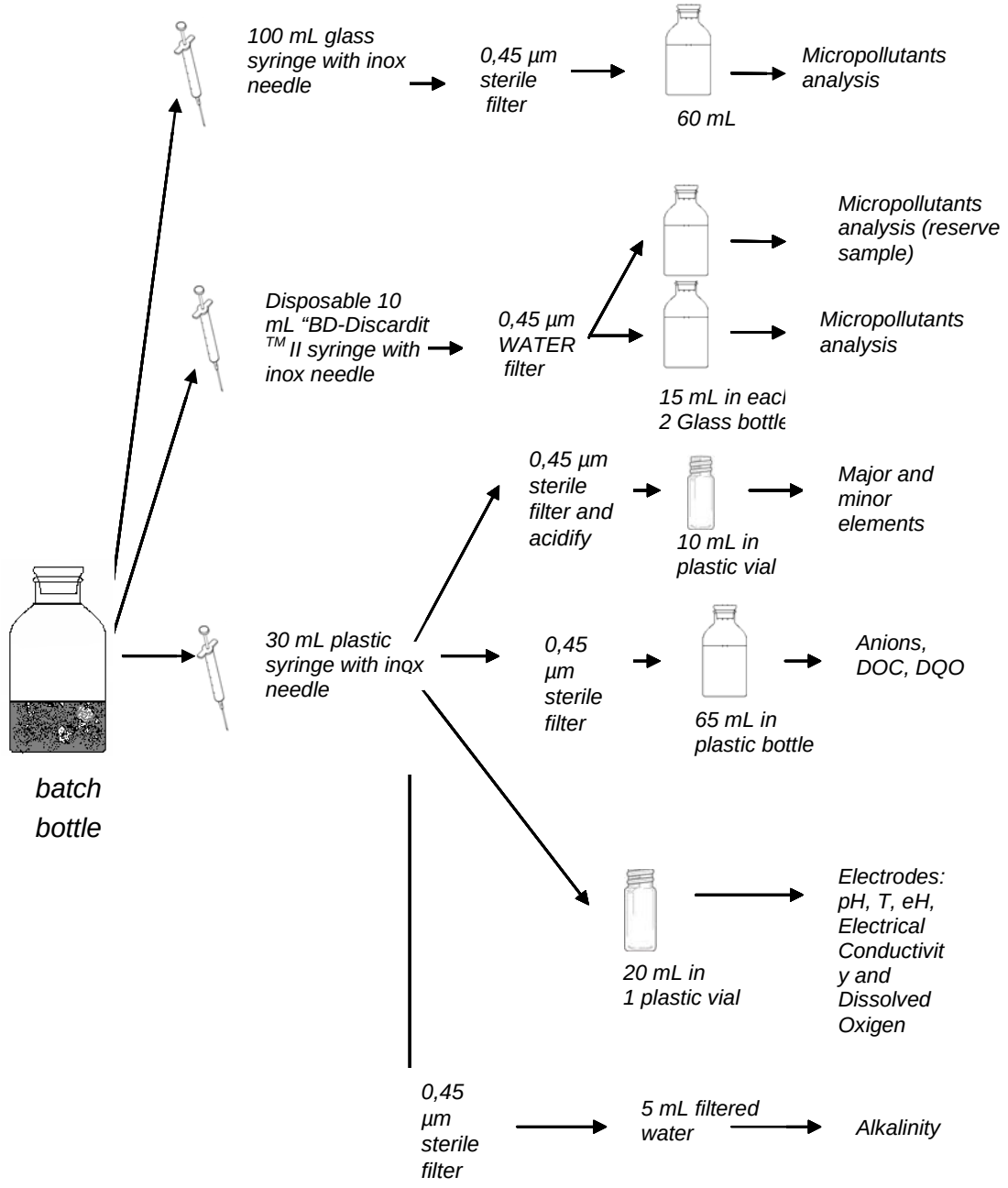


Figure A5. Sampling the microcosms - procedure followed in the present study.

Step 3. Disassembling of the 2nd of the 2 microcosms

Disassembling the 2nd of the 2 microcosms by repeating the procedure detailed in the Step 2.

Step 4. Opening the glove box

Open the glove box, retire the samples and all the material used.

Step 5. Microcosms and samples storage

Carry the samples to the specific laboratories for immediate analysis or store them according to each laboratory recommendations (refrigerator or freezer).

Store the disassembled microcosm (now containing only sediments) into the laboratory for possible future analysis of sediment fraction.

Step 6. Cleaning
Same as step 0 of §A1.

A2.2 ABIOTIC EXPERIMENTS

According to a defined schedule, all the N_i microcosms belonging to the *"i"* abiotic experiments are sacrificed. The disassembling of n_i microcosms is devoted to the monitoring of the chemistry of the system and micropollutants behaviour. The disassembling of the remaining m_i microcosms (where $n_i + m_i = N_i$) is devoted to check if abiotic conditions are actually being maintained during the experiments. Each microcosm is disassembled jointly with its duplicate.
In the case of the abiotic experiment performed in this study:

- m_i = 3 for abiotic-part1 experiment
- m_i = 3 for abiotic-part2 experiment
- the m_i microcosms devoted to microbiological control have not been sacrificed in duplicate

The disassembling procedure consists of the following steps:

A2.2.1 Disassembling of the n_i microcosms

The procedure is the same detailed in §A2.1. Moreover, to avoid accidents when sampling the water of the microcosm (Step 2, point "b)"), containing a "poison" which can has adverse effect also on the personal carrying out the assembling of the microcosms, no needle are used coupled with the syringes: the tip of the syringe is directly submerged into the water to be sampled.
Special health security measures have to be taken when cleaning the material (Step 6) that has been in contact with the "poison". The solid and liquid wastes produced must be disposed in the adequate way.

A2.2.2 Disassembling of the m_i microcosms:

In the present study, to check the efficiency of the sterilization process, 100 µL of the samples was spread on TSA plates *(Trypticase soy agar,* a rich growth medium) and incubated in duplicate at 25ºC during one in aerobic and two weeks in anaerobic conditions, respectively. The following steps describe, thus, how to perform such procedure. If another microbiological technique has been selected during the design phase, the following procedure is not valid and has to be adequately modified. Note that, has specified before, in our case the m_i microcosms were not sacrificed in duplicate.

Step 0. Microcosm preparation
About 24 hours before the disassembling, gently mix the microcosm to be disassembled.

Step 1. Disassembling of the microcosm
The microcosm and material are placed in the horizontal flow cabinet. Then:

a) throw away the aluminium foil covering the bottle. Open the bottle.

b) using an automatic sterile pipette, spread 100 µl of the liquid phase of the microcosm on a TSA plate, using a digralski spreader. Repeat this procedure so many times as designed. In the present study, 4 plates are prepared: 2 for duplicate aerobic incubation and 2 for duplicate anaerobic incubation (Figure A6).

c) close the bottle

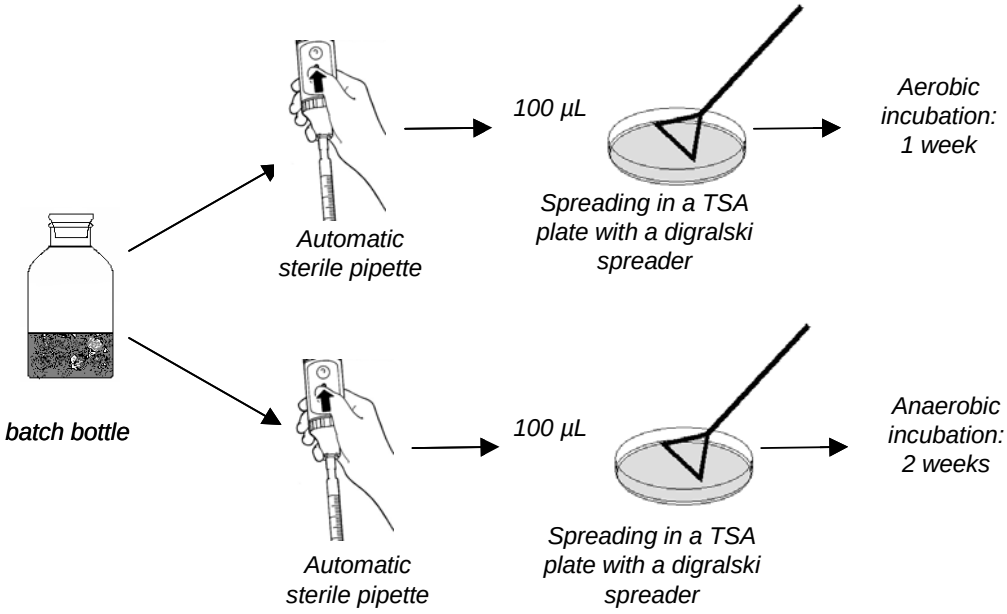


Figure A6. Microbiological growth control procedure (done in duplicate)

Step 2. Incubation of the plates

The TSA plates with the sample inoculum are incubated at 25º, in aerobic and anaerobic conditions. The anaerobic conditions are generated by using na anaerobiose kit (Anaerocult, Merck).

Step 3. Microcosm and samples storage

Remove the microcosm, the plates and all the material used from the sterile chamber. Adequately clean the chamber.

Store the disassembled microcosm (containing soil and water) into the laboratory for possible future sediment fraction or biological analysis.

Step 5. Cleaning

Same as step 13 of §A1.2: accurate cleaning (with MQ water, methanol and acetone) of all the material used. Special health security measures have to be taken when cleaning the material that have been in contact with the "poison". The solid and liquid wastes produced must be disposed in the adequate way.

Step 6. Control of the plates

Plates are incubated one (for aerobic) or two (for anaerobic) weeks. After these periods, the abiotic conditions are checked.

NOTE to all the Assembling/Disassembling Protocols:

The composition of the "common water", the grainsize of the soil tested and the diameter d_{sieve} of the sieve (if sieving the sediment is necessary), Ni, Vbottle, Qsediment , Xi_i, Vwater , Vgas, the type and quantity q_i of easy degradable organic substrate, the quantity of additional electron acceptors, the quantity $q_{substrate}$, the type of solid electron acceptor, the type and quantity q_{poison} of "poison", the method for checking a potential biological activity, etc … are selected when designing the experiments. Such selection does not necessarily coincide with the selection done for the experiments presented in the present thesis and used here as example.

APPENDIX B

Summary of the information on the target pollutants

- list, characteristics, chemicals, analytical methods -

B1. Batch experiments with micropollutants at 1 µg/L individual initial concentration

The mixture of organic micropollutants used in this group of experiments was actually yielded by the use of two different spiking solutions ("A" and "B" in the following), each one provided by a different laboratory. Namely:

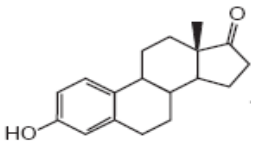
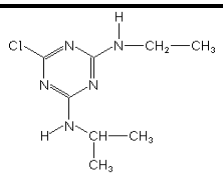
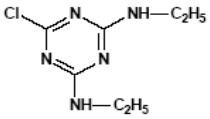
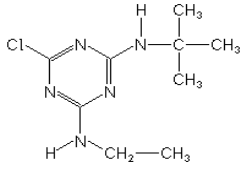
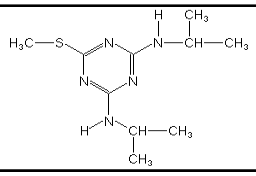
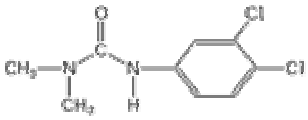
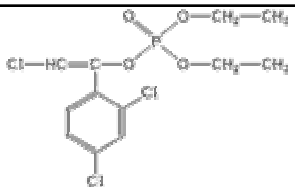
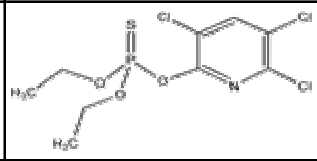
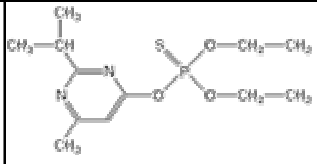
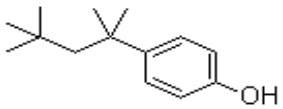
- spiking solution "A" was provided by the Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA), CSIC - Barcelona, Spain.
- spiking solution "B" was provided by the laboratory LABAQUA – Alicante, Spain.

B1.1 List and characteristics of the target micropollutants

The compounds included in spiking solution "A" are pharmaceuticals (atenolol, carbamazepine, diclofenac, gemfibrozil, ibuprofen, sulfamethoxazole), pesticides (diuron, simazine) and an estrogen (estrone). Their main characteristics are reported in Table B1 and Table B2. It is worth to be mentioned that the six pharmaceuticals have been also studied in the batch experiments at 1mg/L individual concentration (§ B2).

The compounds included in spiking solution "B" are pesticides (atrazine, terbuthylazine, prometryn, chlorphenvinfos, chlorpyrifos, diazinon), an estrogen (β-estradiol), PAHs (naftalene, acenaphtene, fluorene, anthracene, fenanthrene, benz[a]anthracene, crysene, pyrene, fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a] anthracene, indeno[1,2,3-cd]pyrene and benzo[ghi]perylene), surfactant degradation products (4-tert-octylphenol, 4-nonylphenol), a phthalate (bis-diethylhexyl phthalate) and a biocide (triclosan). Nevertheless, due to unexpected incompatibility with the experimental material (i.e., filters used for sampling), unsuitable storage conditions for some compound, and inconsistency in the analytical determinations, only the results for atrazine, terbuthylazine, prometryn, chlorphenvinfos and diazinon could be considered reliable in all experiments. For chlorpyriphos and 4-tert-octylphenol (4-t-OP), results were reliable in the NO3-reducing and the abiotic experiments. Only these seven compounds have been thus included in Table B1 and in the discussion of results in § C3.

Table B1: Main characteristics of the pesticides, the estrogen and the surfactant degradation product included in the experiments at 1μg/L individual concentration. The properties of the target drugs could be found in Table B2

Substance	Category	sub-category	Compound	structure	CAS number	logKow	pka	formula
ESTROGEN			**Estrone**		53-16-7	3.43		$C_{18}H_{22}O_2$
PESTICIDES	Herbicide	Triazine (Chlorotriazine)	**Atrazine**		19-12-24-9	2.61	1.7	$C_8H_{14}ClN_5$
			Simazine		122-34-9	2.18	1.62	$C_7H_{12}ClN_5$
			Terbuthylazine		005915-41-3	3.21	2	$C_9H_{16}ClN_5$
		Triazine (Methylthiotriazine)	**Prometryne**		7287-19-6	3.51	4.1	$C_{10}H_{19}N_5S$
		Phenil ureas	**Diuron** or DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea)		330-54-1	2.68		$C_9H_{10}Cl_2N_2O$
	Insecticide	Organophosphates	**Chlorfphenvinphos**		470-90-6	3.81		$C_{12}H_{14}Cl_3O_4P$
			Chlorpyrifos		002921-88-2	4.96		$C_9H_{11}Cl_3NO_3PS$
			Diazinon		000333-41-5	3.81		$C_{12}H_{21}N_2O_3PS$
SURFACTANT DEGRADATION PRODUCT			**4-tert-Octylphenol (4-t-OP)**		000140-66-9	4.12		$C_{14}H_{22}O$

B1.2 Chemicals

Regarding spiking solution “A”:

high purity (>96%) analytical standards atenolol, carbamazepine, diclofenac, gemfibrozil, ibuprofen, sulfamethoxazole, diuron, simazine and estrone, and of their isotopic analogues atenolol *d7*, carbamazepine *d10*, diclofenac *d4*, ibuprofen *d3*, sulfamethoxazole *d4*, diuron *d6*, simazine *d10* and estrone *d4* used as surrogate standards for quantification were supplied by Sigma–Aldrich. Individual stock solutions were prepared in methanol with a concentration of 1 mg/mL. Working standard mixtures were then prepared at different concentrations by dilution of the individual stock solutions in methanol, and were used to prepare the spiking solution for the experiments (resulting concentration in the “initial water” described in § 2.2.4 was 1 µg/L for each compounds) and the aqueous calibration standards (concentration range 1-1500 ng/L, surrogate standards 200 ng/L). Stock and working standard solutions were stored at -20 °C in the dark.

Regarding spiking solution “B”:

the standard containing the 16 PAHs at a concentration of 2000 mg L^{-1} in dichloromethane:benzene (1:1) as well as high purity (>96%) analytical standards of all the remaining compounds were purchased from AccuStandar. Individual stock solutions were prepared in an appropriate solvent according to the properties of each compound. PCB-30 and atrazine-d5 were used as surrogate and internal standard, respectively. A standard mixture was prepared in methanol at a concentration of 10 mg/L for each compound (100 mg/L for 4-t-OP and 4-NP), and was used as spiking solution for the batches (resulting concentration in the “initial water” described in § 2.2.4 was 10 µg/L for 4-octylphenol and 4-nonylphenol and 1 µg/L for the rest of compounds) and for preparation of the aqueous calibration standards (concentration range 1-1000 ng/L, surrogate standards 200 ng/L).

B1.3 Analytical methods

Regarding spiking solution “A”:

the analytical method used for the compounds included in spiking solution “A” was the same described for atenolol in §2.2.5 of the present thesis.

Regarding spiking solution “B”:

micropollutants are analyzed using stir bar sorptive extraction- thermal desorption-gas chromatography mass spectrometry (SBSE-TD-GC-MS). Samples are daily prepared using either Milli-Q for calibration or samples. The optimized extraction is performed with 20 mm long x 0.5 mm film thickness PDMS commercial stir bars (Twister®) supplied by Gerstel (Mülheim a/d Ruhr, Germany). The SBSE procedure consists of a 50 mL water sample with 20% NaCl added, extracted with a 20 mm long (0.5 mm thickness film) stir bar at 900 rpm for 14 hours at room temperature. Sodium chloride by Merck (Darmstadt, Germany) is used to increase the ionic strength of samples. The GC-MS is carried out with an Agilent 6890/5973 system (Agilent Technologies, Palo Alto, CA, USA) equipped with a commercial thermal desorption unit, TDS-2 (Gerstel), connected to a programmed-temperature vaporisation (PTV) injector CIS-4 plus (Gerstel) by a heated transfer line. The TDS-2 plus is equipped with a TDSA autosampler (Gerstel) able to handle the program for 20 coated stir bars. The analyses are carried out using an HP-5 MS column (30 m x 0.25 mm I.D x 0.25 µm film thickness of 5% phenyl, 95% poly-dimethylsiloxane). After SBSE, the twister desorption are carried out at 280ºC for 6 minutes under a helium flow of 75 mL·min^{-1} in the splitless mode while maintaining a temperature of 20ºC in the PTV injector of the GC-MS system. Subsequently, the programmable temperature vaporisation system (PTV) is ramped to a final temperature of 280ºC and the analytes are transferred to the GC column. The column is programmed for 70ºC for 2 minutes, ramped at 30ºC/min to 200ºC, held for 1 minute, and

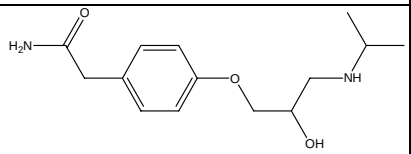
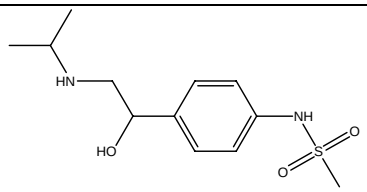
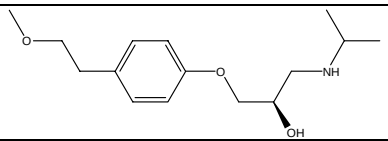
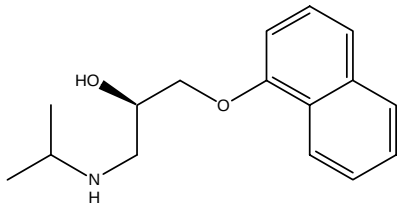
finally increased 3ºC/min to 280ºC and held for 2 minutes. MS using selected ion monitoring is selected, analyzing the area of a characteristic ion for each compound for quantification. PCB-30 is spiked into each sample at 0.2 µg/L as a surrogate. Atrazine-D5 is used as an internal standard for the quantification of atrazine.

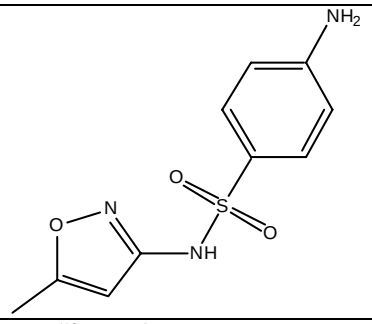
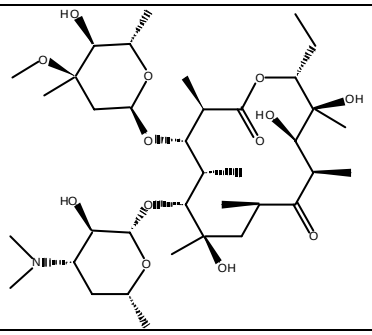
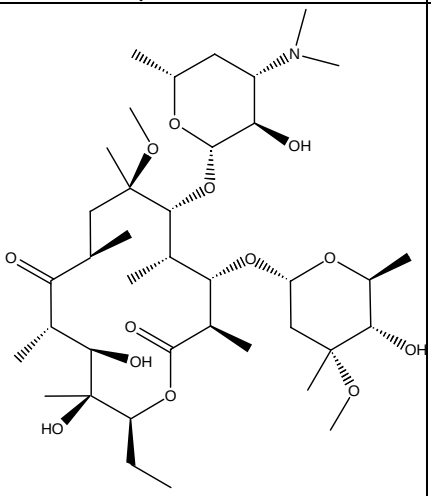
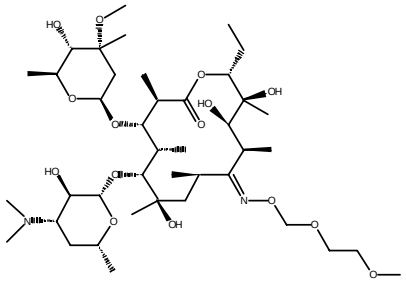
B2 Batch experiments with pollutants at 1 mg/L individual initial concentration

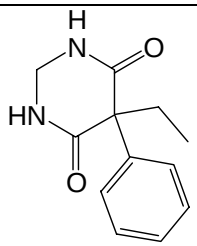
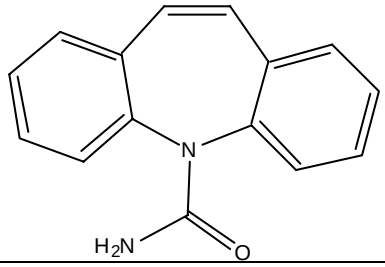
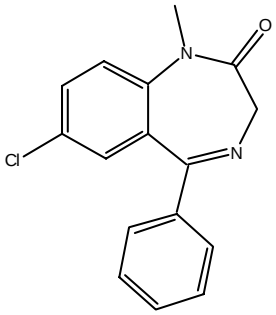
B2.1 List and characteristics of the target pollutants

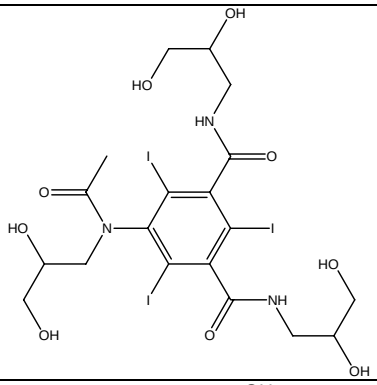
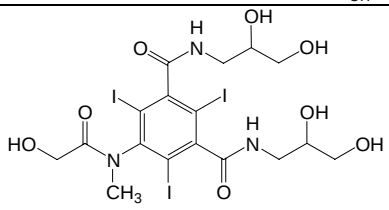
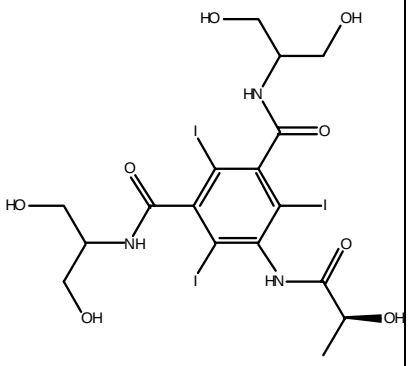
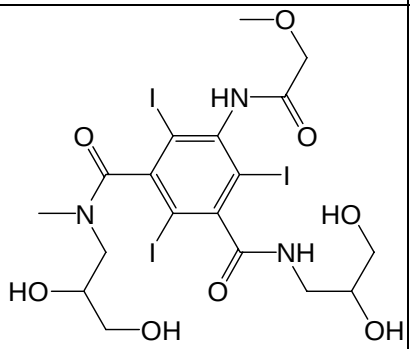
The mixture of organic pollutants included 24 drugs: atenolol, metoprolol, propranolol, sotalol, clarithromycin, erythromycin, roxithromycin, sulfamethoxazole, carbamazepine, diazepam, primidone, iohexol, iomeprol, iopamidol, iopromide, bezafibrate, clofibric acid, gemfibrozil, diclofenac, ibuprofen, naproxene, phenazone, famotidine, pantoprazole, cetirizine, loratadine, acetaminophen. Some of their main characteristics are reported in Table B2.

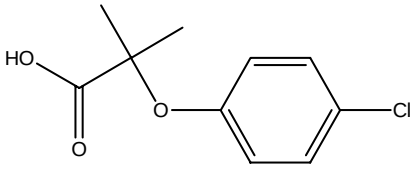
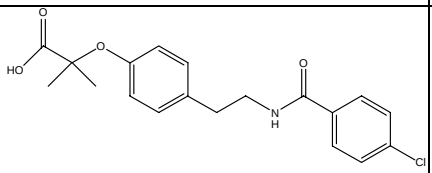
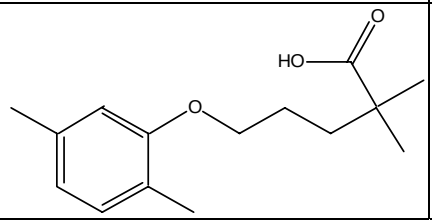
Table B2: Main characteristics of the micropollutants included in the experiments at 1mg/L individual concentration ([a]: Scifinder predicted values)

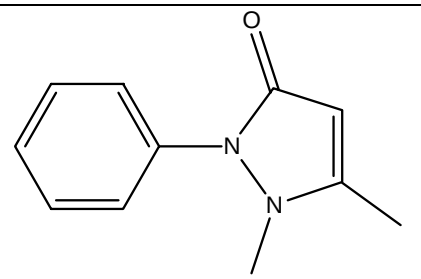
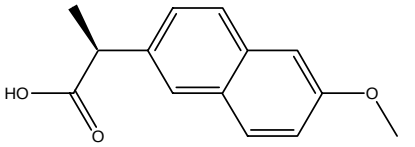
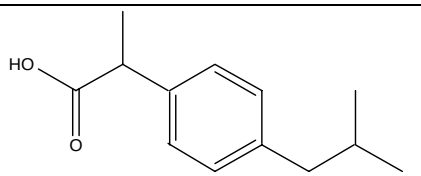
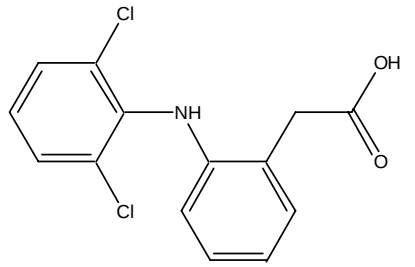
Category	Sub-category	Compound	Structure	log Kow[a]	pka[a]	Formula
Antihypertensive agents	B-blocker	Atenolol		0.1	9.2	$C_{14}H_{22}N_2O_3$
		Sotalol		0.32	9.2	$C_{12}H_{20}N_2O_3S$
		Metoprolol		1.79	9.2	$C_{15}H_{25}NO_3$
		Propranolol		3.37	9.42	$C_{16}H_{21}NO_2$

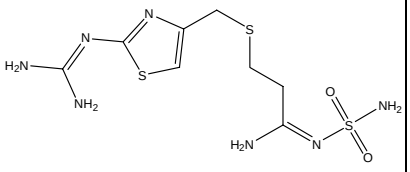
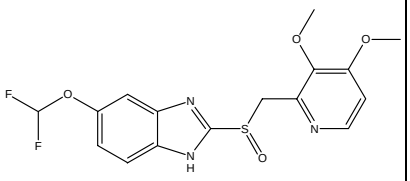
Antibiotics	**Sulfonamide**	**Sulfamethoxazole**		0.89	5.8	$C_{10}H_{11}N_3O_3S$
	Macrolide	**Erythromycin**		2.83	8.2 (basic pKa)	$C_{37}H_{67}NO_{13}$
		Clarithromycin		3.16	8.2 (basic pKa)	$C_{38}H_{69}NO_{13}$
		Roxithromycin		2.75	8.8 (basic pKa)	$C_{41}H_{76}N_2O_{15}$

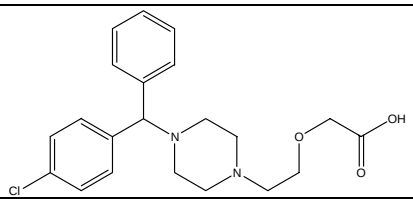
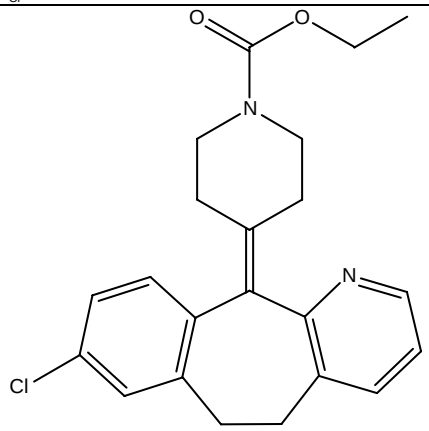
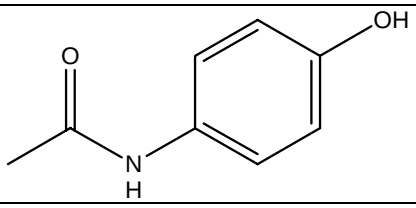
Neuro-active compounds /Anticonvulsants, sedative)	**Pyrimidinediones**	**Primidone**		0.4	12.3	$C_{12}H_{14}N_2O_2$
	Carboxamide	**Carbamazepine**		2.67	13.9	$C_{15}H_{12}N_2O$
	Benzodiazepine	**Diazepam**		2.96		$C_{16}H_{13}ClN_2O$

Contrast media	**Iodinated (for enhancing X-ray based imaging methods)**	**Iohexol**		-4.16	11.4	$C_{19}H_{26}I_3N_3O_9$
		Iomeprol		-3.08	11.4	$C_{17}H_{22}I_3N_3O_8$
		Iopamidol		-2.09	10.9	$C_{17}H_{22}I_3N_3O_8$
		Iopromide		-2.95	10.6	$C_{18}H_{24}I_3N_3O_8$

Lipid regulator		**Clofibric acid**		2.72	3.2	$C_{10}H_{11}ClO_3$
		Bezafibrate (acid)		3.46	3.3	$C_{19}H_{20}ClNO_4$
		Gemfibrozil		4.39	4.8	$C_{15}H_{22}O_3$

Anti-inflammatory	non-steroidal anti-inflammatory drug (NSAID)	**Phenazone**		0.27		$C_{11}H_{12}N_2O$
		Naproxen		3	4.8	$C_{14}H_{14}O_3$
		Ibuprofen		3.72	4.4	$C_{13}H_{18}O_2$
		Diclofenac		4.06	4.2	$C_{14}H_{10}Cl_2NO_2$

Ulcer treatment compounds	**H2-receptor antagonists (H2RA)**	**Famotidine**			6.8	$C_8H_{15}N_7O_2S_3$
	Proton pump inhibitors (PPI)	**Pantoprazole**		1.69	7.7	$C_{16}H_{15}F_2N_3O_4S$

Antihistamines		**Cetirizine**		2.17	2.9	$C_{21}H_{25}ClN_2O_3$
		Loratadine		5.94		$C_{22}H_{23}ClN_2O_2$
Analgesic		**Paracetamol (Acetaminophen)**		0.34	9.9	$C_8H_9NO_2$

B2.2 Chemicals and analytical method

Details on the materials and the analytical method used could be found in the correspondent sections of Chapters 3 and 4, and in Nödler et al. (2010). Only Famotidine is not included in the previous references, so the specifications are reported here:

Supplier: Sigma Aldrich (Steinheim, Germany)

+ESI, capillary voltage: 30 V, Quantifier: 338-->189 (collision energy: -14 V), Qualifier: 338->259 (collision energy: -6 V). The atenolol *d7* was used as the internal standard.

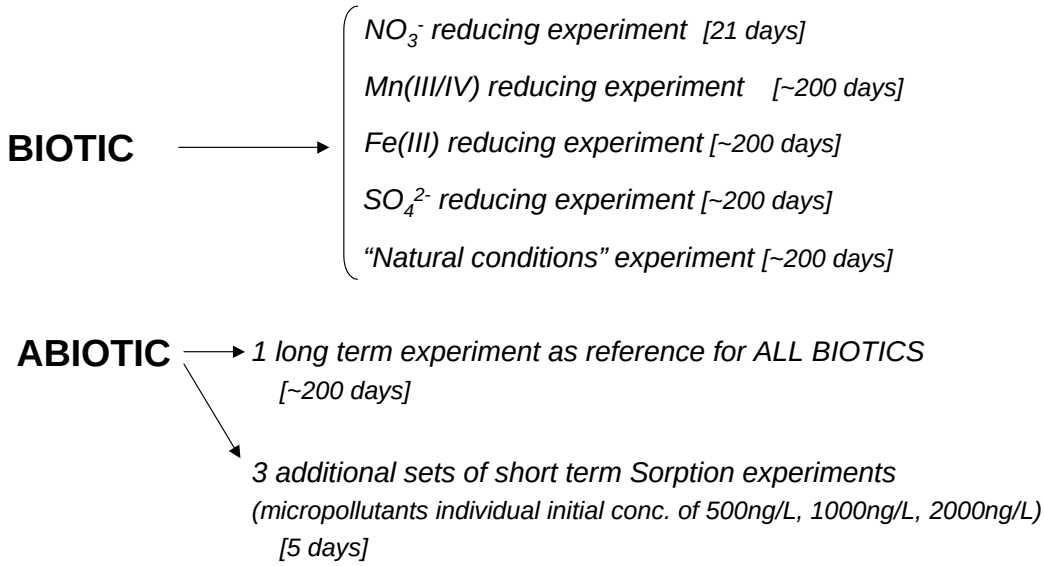
The calibration range of all compounds was 1 – 500 ng mL^{-1}

APPENDIX C

Experimental data from the batch experiments

C1. Summary of the experiments performed

C1.1 Sketch of the batch experiments performed with micropollutants at 1 µg/L individual initial concentration



In fact, for practical reasons (i.e. number of bottles to be assembled, space inside the glovebox, liters of water to be prepared, etc.) the abiotic long term experiment (referred also simply as "abiotic" experiment in the following) has been carried out by means of 2 separate but analogous (i.e., same theoretical compostion of the "initial water") sets of batches. The first one (abiotic-part 1, samples labelled "NO3-AB" in the following tables) had a duration of 22 days. The second one (abiotic-part 2, samples labelled "SO4-AB-BIS" in the following tables) lasted 184 days but the bottles were sacrified starting from day 37.

Results from the Sorption experiments are not included in the following since they do not provide additional relevant information. In the end, for the three tested concentrations they almost confirmed the behaviour observed during the first 5 days of the long term abiotic experiment. Possibly, moreover, their duration was too short to catch the abiotic processes actually affecting some of the target compounds. In fact, as could be seen in §C3, in the long term abiotic experiment some pollutant started to disappear after several days of experiment, and some other was continually removed even up to 180 days of test.
The Sorption experiment consisted of set of batches with and without sediments, in order to identify if the removal of the pollutants was generated by chemical processes (e.g. hydrolysis) or was related to the presence of sediments (e.g. adsorption processes, etc.). During the 5 tested days, usually the evolution of concentrations in both types of batches was very similar, indicating that the dominant processes, where some process occurred, were mostly related to the presence of the sediments.
A number data from the Sorption experiments was also not reliable.

C1.2 Sketch of the batch experiments performed with pollutants at 1 mg/L individual initial concentration

BIOTIC ⟶ *NO_3^- reducing experiment* *[88 days]*

ABIOTIC ⟶ *1 long term experiment as reference for the BIOTIC experiment* *[88 days]*

C2. Experimental data - General water chemistry

The following tables and figures report the averages of results and manual measurements from the duplicate batches or, for the initial time t = 0, from duplicate samples of the “initial water”.

C2.1 Tables and figures of experimental data for the batch experiments with micropollutants at 1 µg/L individual initial concentration

TABLE C1: Averages of measured parameters and concentrations for the NO3-reducing and Mn-reducing experiments (labels “NO3-G3-…” and “Mn-G2-…”, respectively) - Batches with micropollutants at 1µg/L individual initial concentration

		average of measured parameters		Caverage [mmol/L]												
sample name	***average time [d]***	***pH***	***C.E. [mS/cm]***	***Alk***	***DOC***	***NO_3^-***	***NO_2^-***	***SO_4^{2-}***	***Ca***	***K***	***Mg***	***Na***	***Fe***	***Mn***	***P***	***S***
NO3-G3-INITIAL average	0	8.5	3.20	1.0	9.7	6.7	0.001	2.0	2.9	1.0	4.7	9.1	0.001	0.001	0.019	2.0
NO3-G3-1 average	0.05	8.4	3.05	1.0	8.5	6.6	0.001	2.0	3.2	1.0	4.3	8.7	0.001	0.001	0.011	2.0
NO3-G3-2 average	0.2	8.4	3.10	1.0	8.8	6.6	0.001	2.0	3.3	1.0	4.3	8.8	0.001	0.001	0.009	2.0
NO3-G3-3 average	0.5	8.3	3.10	1.0	8.6	6.6	0.007	2.0	3.3	1.0	4.3	8.7	0.001	0.001	0.009	2.0
NO3-G3-4 average	1.5	8.2	2.95	1.0	8.7	6.5	0.1	2.0	3.4	1.0	4.1	8.3	0.001	0.001	0.006	2.0
NO3-G3-5 average	3	8.1	3.30	2.0	5.4	3.1	3.3	2.0	3.4	1.0	4.0	8.2	0.001	0.001	0.003	2.0
NO3-G3-6 average	5	7.6	2.75	2.7	0.0	0.7	4.3	2.0	3.4	1.0	4.0	8.4	0.001	0.001	0.003	2.0
NO3-G3-7 average	10	8.0	2.25	3.7	1.5	0.008	0.001	2.0	2.0	1.0	3.6	8.6	0.001	0.002	0.003	2.0
NO3-G3-8 average	21	7.6	2.48	3.7	1.5	0.008	0.001	2.0	1.9	0.9	3.5	8.2	0.002	0.010	0.003	2.0
Mn-G2-INITIAL	0	7.9	2.50	1.0	6.7	0.1	0.001	2.3	3.7	1.0	1.8	9.4	0.001	0.001	0.022	2.2
Mn-G2-1 average	7	8.6	2.68	1.2	6.8	0.008	0.001	2.1	3.8	1.0	1.6	8.9	0.001	0.001	0.003	2.1
Mn-G2-2 average	14	8.0	2.50	1.1	6.3	0.008	0.001	2.1	3.1	1.2	1.3	9.4	0.001	0.038	0.003	1.9
Mn-G2-3 average	25	8.5	2.15	1.2	4.3	0.008	0.001	2.1	2.7	1.1	1.2	9.7	0.001	0.121	0.003	1.9
Mn-G2-4 average	42	8.9	1.90	0.5	2.8	0.008	0.001	2.1	2.6	1.1	1.1	9.8	0.001	0.113	0.003	1.9
Mn-G2-5 average	63	8.7	1.95	0.5	2.3	0.008	0.001	2.0	2.5	0.8	0.9	8.6	0.001	0.106	0.003	2.0
Mn-G2-6 average	91	8.6	2.25	0.5	1.8	0.008	0.001	2.0	2.5	0.8	0.9	8.7	0.001	0.112	0.003	2.0
Mn-G2-7 average	194	8.0	2.05	0.5	1.0	0.008	0.001	1.9	2.4	0.8	0.9	10.0	0.001	0.106	0.003	2.0

TABLE C2: Averages of measured parameters and concentrations for the Fe-reducing, SO4-reducing and Natural Conditions experiments (labels "Fe-G2-…", "SO4-G2-…" and BL-G2-…", respectively) - Batches with micropollutants at 1µg/L individual initial concentration

		average of measured parameters		Caverage [mmol/L]													
sample name	***average time [d]***	***pH***	***C.E. [mS/cm]***	***Alk***	***DOC***	***NO_3^-***	***NO_2^-***	***SO_4^{2-}***	***Ca***	***K***	***Mg***	***Na***	***Fe***	***Mn***	***P***	***S***
Fe-G2-INICIAL	0	8.1	3.20	1.2	7.8	0.1	0.001	2.3	3.5	1.1	1.8	10.8	0.001	0.001	0.017	2.1
Fe-G2-1 average	7	7.8	2.35	1.5	8.0	0.008	0.001	2.3	3.7	1.0	1.7	10.5	0.001	0.002	0.003	2.1
Fe-G2-2 average	14	8.1	3.80	1.2	7.5	0.008	0.001	2.2	3.7	1.0	1.7	10.7	0.001	0.004	0.003	2.1
Fe-G2-3 average	24	8.1	2.20	2.1	5.2	0.008	0.001	1.1	3.0	1.1	1.3	11.2	0.001	0.009	0.003	1.1
Fe-G2-4 average	42	7.8	2.15	3.0	3.2	0.008	0.001	0.1	2.4	1.0	1.2	10.2	0.004	0.006	0.003	0.0
Fe-G2-5 average	63	7.7	2.00	3.0	1.7	0.008	0.001	0.1	2.3	1.0	1.2	10.2	0.019	0.006	0.003	0.0
Fe-G2-6 average	91	7.5	3.40	3.9	0.1	0.008	0.001	0.1	2.2	0.8	1.2	9.2	0.047	0.007	0.003	0.0
Fe-G2-7 average	199	7.4	2.15	3.6	0.2	0.008	0.001	0.1	2.3	0.8	1.2	10.0	0.055	0.008	0.003	0.0
SO4-G2-INICIAL	0	7.9	2.70	1.5	10.2	0.1	0.001	5.3	3.5	1.1	1.8	19.1	0.001	0.001	0.017	5.7
SO4-G2-1 average	*7*	8.2	4.00	1.7	9.8	0.008	0.001	5.2	3.9	1.0	1.7	18.5	0.001	0.001	0.003	5.7
SO4-G2-2 average	*18*	8.1	3.20	2.0	9.3	0.008	0.001	5.6	4.0	0.9	1.7	18.0	0.003	0.007	0.003	5.7
SO4-G2-3 average	*36*	7.7	3.15	4.0	4.5	0.008	0.001	3.8	2.7	1.1	1.2	19.5	0.002	0.010	0.003	3.6
SO4-G2-4 average	65	7.8	2.50	4.2	0.3	0.008	0.001	2.1	1.8	1.0	1.0	19.2	0.001	0.002	0.003	2.6
SO4-G2-5 average	89	7.8	2.40	4.7	0.1	0.008	0.001	1.6	1.5	0.7	0.9	16.0	0.003	0.002	0.003	1.5
SO4-G2-6 average	133	7.8	2.40	4.9	0.1	0.008	0.001	1.1	1.2	0.6	0.8	15.5	0.005	0.003	0.003	1.2
SO4-G2-7 average	215	7.6	2.50	6.2	0.2	0.008	0.001	1.3	1.3	0.6	0.9	18.3	0.002	0.004	0.003	1.3
BL-G2-INICIAL average	0	7.5	2.28	0.5	2.5	0.1	0.001	2.3	3.5	1.0	1.7	7.6	0.001	0.001	0.018	2.1
BL-G2-1 average	*1.1*	7.5	2.15	0.9	2.2	0.128	0.007	2.3	4.0	1.0	1.7	7.4	0.001	0.001	0.003	2.1
BL-G2-2 average	*3*	7.5	2.50	1.0	1.3	0.008	0.014	2.0	4.3	1.0	1.7	7.3	0.001	0.001	0.003	2.1
BL-G2-3 bis	7	7.5	2.45	1.2	1.4	0.008	0.001	2.3	4.2	1.0	1.7	7.3	0.001	0.002	0.003	2.1
BL-G2-4 average	10	7.5	2.30	1.4	1.4	0.008	0.001	2.1	3.5	1.1	1.3	6.9	0.001	0.006	0.003	1.9
BL-G2-5 average	15	7.5	2.25	1.2	1.4	0.008	0.001	2.0	3.5	1.1	1.4	6.9	0.005	0.014	0.003	2.0
BL-G2-6 average	26	7.6	2.10	1.6	0.3	0.008	0.001	1.8	3.5	1.1	1.4	6.8	0.004	0.020	0.003	1.6
BL-G2-7 average	42	7.3	1.90	1.6	0.1	0.008	0.001	1.6	3.6	1.1	1.4	6.8	0.009	0.025	0.003	1.4
BL-G2-8 average	62	7.4	1.95	1.9	0.1	0.008	0.001	1.6	3.7	0.9	1.3	7.3	0.012	0.030	0.003	1.7
BL-G2-9 average	89	7.3	2.35	2.1	0.1	0.008	0.001	1.8	3.9	0.9	1.3	7.1	0.001	0.024	0.003	1.8
BL-G2-10 average	135	7.3	2.20	2.5	0.0	0.008	0.001	1.7	4.0	0.8	1.3	6.6	0.001	0.001	0.003	1.8
BL-G2-11 average	192	7.2	2.00	2.2	0.3	0.008	0.001	1.9	4.3	0.9	1.4	7.5	0.001	0.001	0.003	2.0

TABLE C3: Averages of measured parameters and concentrations for the abiotic long term experiment (labels “NO3-AB-…” for abiotic-part1 and “SO4-AB-BIS-…” for abiotic-part2) - Batches with micropollutants at 1µg/L individual initial concentration

NOTE: Due to some incompatibility with the Hg present in the samples, the absolute values of the Chemical Oxygen Demand (DQO in the following) concentrations were neither reliable nor comparable between the two series “NO3-AB” (abiotic-part 1) and “SO4-AB-BIS” (abiotic-part 2). Thus, they were expressed as relative concentrations C/Co [%], each one with respect to its correspondent initial Co.

		average of		Caverage [mmol/L]													
sample name	***average time [d]***	***pH***	***C.E. [mS/cm]***	***Alk***	***DQO***	***DQO [C/Co %]***	***NO_3^-***	***NO_2^-***	***SO_4^{2-}***	***Ca***	***K***	***Mg***	***Na***	***Fe***	***Mn***	***P***	***S***
NO3-AB-INICIAL average	0.0	7.7	5.40	1.7	25.3	100	8.0	0.004	5.7	3.0	0.8	4.9	17.7	0.001	0.001	0.017	5.6
NO3-AB-1 average	0.04	7.8	3.50	1.5	24.2	96	8.6	0.004	5.8	3.5	0.9	4.6	18.1	0.001	0.001	0.013	5.5
NO3-AB-2 average	0.2	7.8	3.30	1.7	25.0	99	8.7	0.004	5.8	3.9	0.8	4.4	17.9	0.001	0.001	0.010	5.6
NO3-AB-3	0.5	7.7	3.30	1.6	24.8	98	8.6	0.004	5.9	3.7	0.9	4.4	17.7	0.001	0.001	0.009	5.4
NO3-AB-4 average	1.5	7.4	3.40	1.6	25.3	100	8.7	0.004	5.8	4.1	0.9	4.2	18.2	0.001	0.001	0.006	5.5
NO3-AB-5 average	3	7.4	3.50	1.6	22.6	89	8.5	0.004	6.4	4.1	1.0	4.3	18.7	0.001	0.001	0.005	5.5
NO3-AB-6 average	5	7.5	3.50	1.5	22.3	88	8.5	0.005	6.4	4.5	0.8	3.9	16.2	0.001	0.001	0.003	5.3
NO3-AB-7 average	10	8.1	3.35	1.6	24.0	95	8.5	0.004	6.5	4.4	0.8	4.0	16.7	0.001	0.001	0.003	5.3
NO3-AB-8 average	22	8.4	3.40	1.5	24.6	97	8.5	0.004	6.4	4.8	0.8	3.7	18.4	0.001	0.001	0.001	5.5
SO4-AB-BIS-INIC. aver.	0	7.4	3.70	1.2	15.5	100	7.4	0.004	5.6	2.9	1.0	4.4	20.0	0.001	0.001	0.015	5.4
SO4-AB-BIS-1 average	37	7.9	3.50	1.4	13.5	87	7.4	0.004	5.3	4.4	0.9	3.6	20.3	0.001	0.001	0.001	5.4
SO4-AB-BIS-2 average	64	7.5	3.40	1.7	13.8	89	7.2	0.004	5.1	4.6	0.8	3.6	20.3	0.001	0.001	0.001	5.5
SO4-AB-BIS-3 average	84	7.9	3.45	2.2	13.4	87	7.5	0.004	5.5	4.6	0.9	3.6	18.9	0.001	0.001	0.001	5.4
SO4-AB-BIS-4 average	134	6.7	not measured	2.0	14.3	93	7.5	0.004	5.4	4.8	0.9	3.8	24.6	0.001	0.001	0.001	5.7
SO4-AB-BIS-5 average	184	6.0	not measured	2.0	15.5	100	-----	0.004	5.1	4.7	0.9	3.8	24.6	0.001	0.001	0.000	5.7

FIGURE C1: Chemical evolution with time in the NO3-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration
Presented in §2.3.1.1 of the present thesis (Figure 2.1)

FIGURE C2: Chemical evolution with time in the Mn-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration
Presented in §2.3.1.2 of the present thesis (Figure 2.4)

FIGURE C3: Chemical evolution with time in the Fe-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration
Presented in §2.3.1.3 of the present thesis (Figure 2.5)

FIGURE C4: Chemical evolution with time in the SO4-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration
Presented in §2.3.1.4 of the present thesis (Figure 2.8)

FIGURE C5: Chemical evolution with time in the Natural Conditions experiment - Batches with micropollutants at 1µg/L individual initial concentration
Presented in §2.3.1.5 of the present thesis (Figure 2.10)

FIGURE C6: Chemical evolution (redox sensitive species) with time in the abiotic long term experiment - Batches with micropollutants at 1µg/L individual initial concentration

NOTE: Due probably to the presence of Hg in the samples, the fluctuations of DQO, NO3, SO4 concentrations, and to a less extent that of Alk, are quite wide. The error bars (calculated according to eq. C1 of §C3) has been thus included in the plot confirming that, as expected, the previous redox sensitive species remained almost constant during the experiment. The stability of SO4 concentration was also confirmed by the evolution of the elemental sulfur (S).

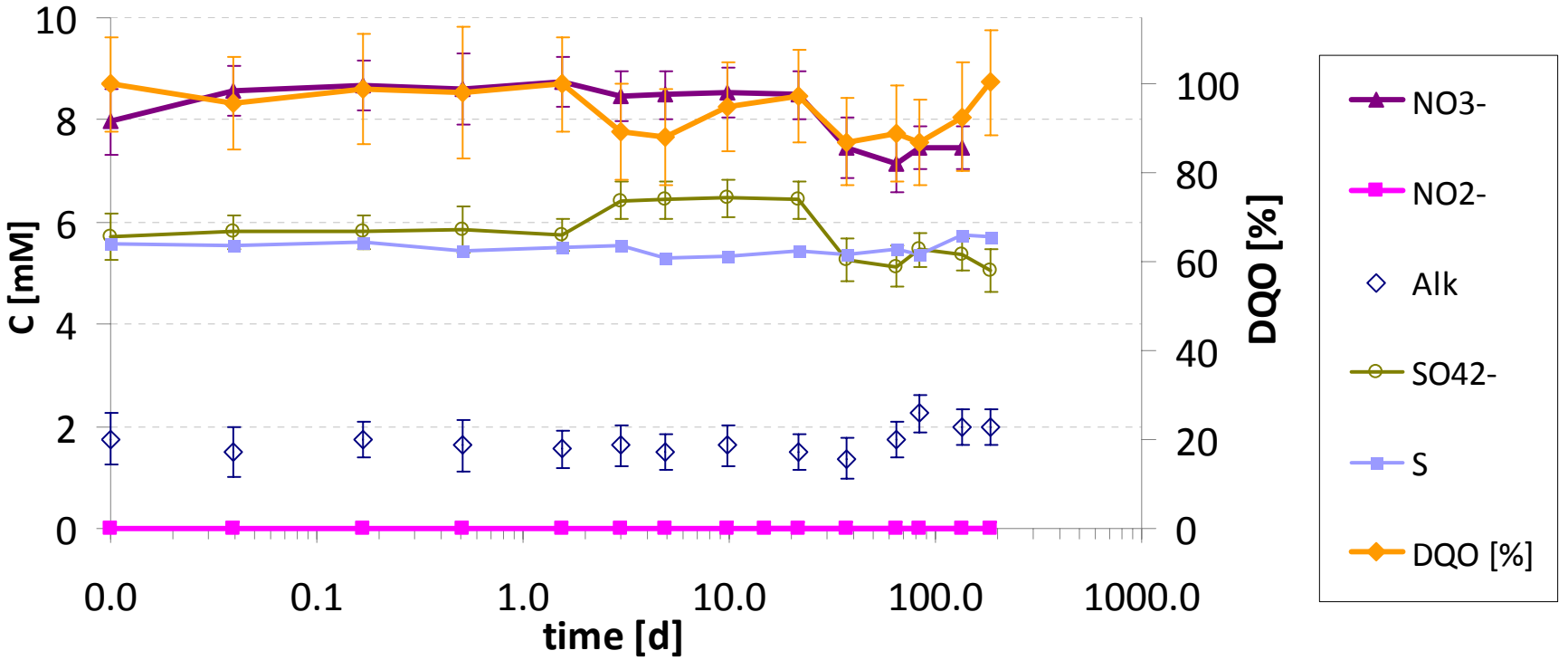
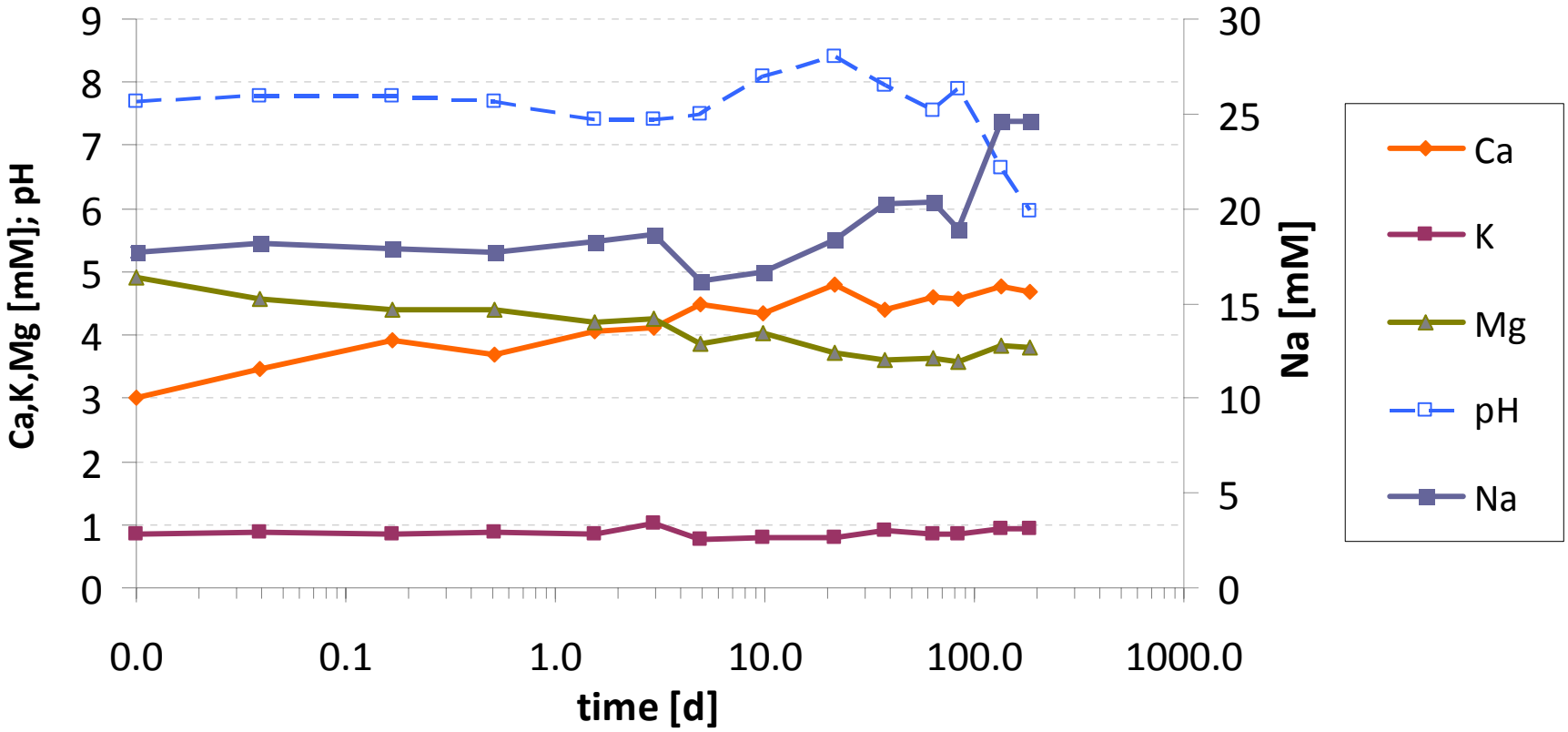


FIGURE C7: Chemical evolution (pH and cations) with time in the abiotic long term experiment - Batches with micropollutants at 1µg/L individual initial concentration



C2.2 Tables and figures of experimental data for the batch experiments with pollutants at 1 mg/L individual initial concentration

TABLE C4: Averages of measured parameters and concentrations for the NO3-reducing experiment (labels “NO3-GAB-…”) - Batches with pollutants at 1mg/L individual initial concentration

		average of measured parameters		Caverage [mmol/L]												
sample name	***average time [d]***	***pH***	***C.E. [mS/cm]***	***Alk***	***DOC***	***NO_3^-***	***NO_2^-***	***SO_4^{2-}***	***Ca***	***K***	***Mg***	***Na***	***Fe***	***Mn***	***P***	***S***
NO3-GAB-INICIAL	0	7.3	7.40	0.7	71.5	67.7	0.0	1.9	3.1	1.2	49.9	11.1	0.001	0.001	0.017	2.0
NO3-GAB-1 average	2	8.0	7.45	1.2	71.9	70.2	0.01	1.8	4.9	1.3	46.6	10.5	0.001	0.001	0.006	2.0
NO3-GAB-2 average	5	7.6	8.30	1.7	71.7	72.3	0.7	2.1	5.0	1.3	46.4	10.5	0.001	0.001	0.003	2.0
NO3-GAB-3 average	10	7.8	7.90	3.7	59.7	68.0	1.9	2.0	4.9	1.3	45.7	10.4	0.001	0.002	0.003	2.0
NO3-GAB-4 average	14	7.9	7.35	3.9	60.7	59.5	2.7	2.0	4.4	1.3	45.6	10.4	0.001	0.002	0.003	2.0
NO3-GAB-5 average	25	7.8	6.25	12.4	43.0	32.3	5.1	2.0	2.6	1.2	43.4	10.3	0.001	0.001	0.003	2.0
NO3-GAB-6 average	40	8.2	5.50	17.5	29.4	13.4	7.8	1.7	0.8	1.4	17.0	10.4	0.001	0.001	0.003	1.9
NO3-GAB-7 average	87	8.3	4.55	22.0	27.2	7.5	0.01	2.1	0.1	1.2	28.5	8.9	0.001	0.001	0.003	2.0

TABLE C5: Averages of measured parameters and concentrations for the abiotic experiment (labels "NO3-GAB-…") - Batches with pollutants at 1mg/L individual initial concentration

NOTE: NO3 and SO4 concentrations seem to increase during the experiment. In fact their concentrations in the last samples (i.e. NO3-GAB-7 average) are equal to the expected "initial" concentration. This phenomenum occurs also to chloride, which is supposed to be conservative. That is, the NO3, SO4 and Cl- suffered like a derive of their concentrations, probably being affected by the presence of Hg in the samples. They were thus considered unreliable. Yet, the almost coincidence of their final value (i.e. NO3-GAB-7 average) with the theoretical initial concentration suggests that they actually remained constant during the experiment. The stability of SO4 concentration is also confirmed by the evolution of the elemental sulfur (S).

		average of measured parameters		Caverage [mmol/L]													
sample name	***average time [d]***	***pH***	***C.E. [mS/cm]***	***Alk***	***DQO***	***NO_3^-***	***NO_2^-***	***SO_4^{2-}***	***Cl^-***	***Ca***	***K***	***Mg***	***Na***	***Fe***	***Mn***	***P***	***S***
NO3-GAB-AB-INICIAL aver.	0	7.8	7.50	1.5	83.3	52.6	0.004	1.6	10.3	2.6	0.9	28.2	11.6	0.001	0.001	0.014	1.8
NO3-GAB-AB-1 average	2	7.7	7.20	1.1	83.8	54.8	0.004	1.7	10.7	4.0	1.0	26.3	11.1	0.001	0.001	0.007	1.8
NO3-GAB-AB-2 average	5	7.6	7.35	1.4	94.3	-----	0.004	-----	-----	4.3	1.0	26.1	11.2	0.001	0.001	0.006	1.8
NO3-GAB-AB-3 average	10	7.7	7.40	1.5	89.1	58.5	0.004	1.8	11.4	4.7	1.0	25.5	11.4	0.001	0.001	0.004	1.8
NO3-GAB-AB-4	14	7.7	7.60	1.5	70.9	60.2	0.004	1.4	11.8	5.0	1.0	25.3	11.4	0.001	0.001	0.002	1.8
NO3-GAB-AB-5 average	25	7.3	7.15	1.1	92.4	58.7	0.004	1.8	11.5	5.1	1.0	25.5	11.3	0.001	0.001	0.001	1.8
NO3-GAB-AB-6 average	40	7.4	7.50	1.2	85.3	63.8	0.005	2.0	12.4	5.2	1.0	25.1	11.0	0.001	0.001	0.001	1.8
NO3-GAB-AB-7 average	87	8.0	7.35	1.1	99.6	66.8	0.004	2.1	13.0	5.2	1.0	25.2	11.4	0.001	0.001	0.001	1.8

FIGURE C8: Chemical evolution (redox sensitive species) with time in the abiotic experiment - Batches with pollutants at 1mg/L individual initial concentration

NOTE: The error bars (calculated according to eq. C1 of §C3) has been included in the plot to justify the fluctuations of DQO and Alk. The stability of SO4 concentration is confirmed by the evolution of the elemental sulfur (S). As expected, the redox sensitive species remained almost constant during the experiment.

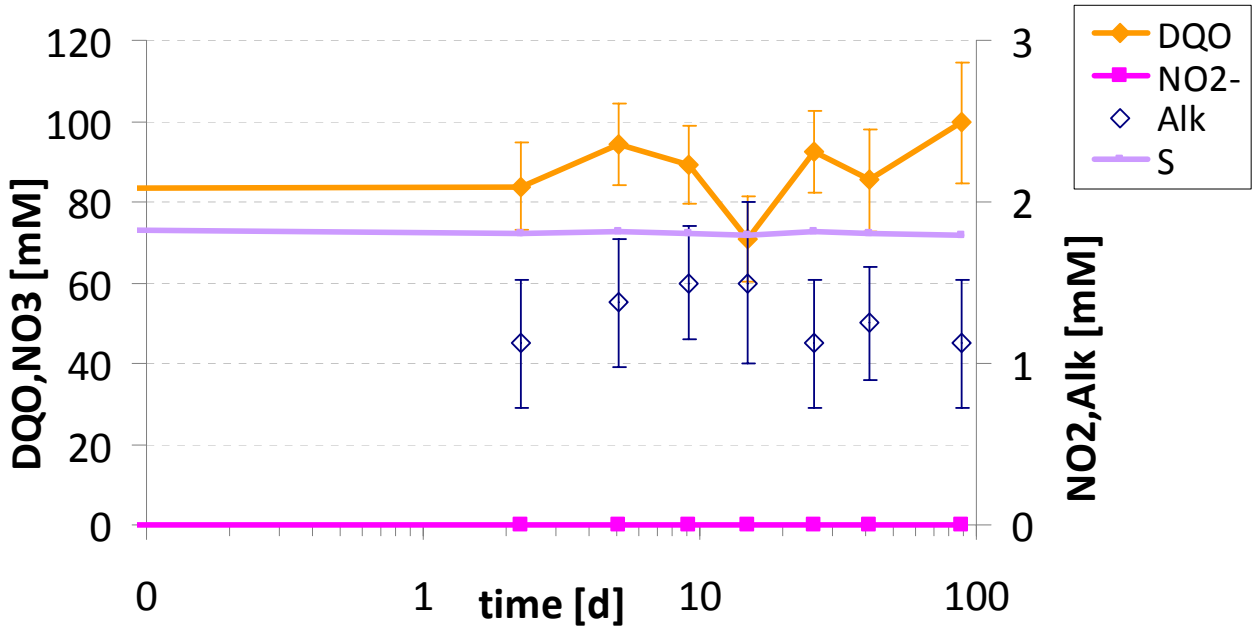
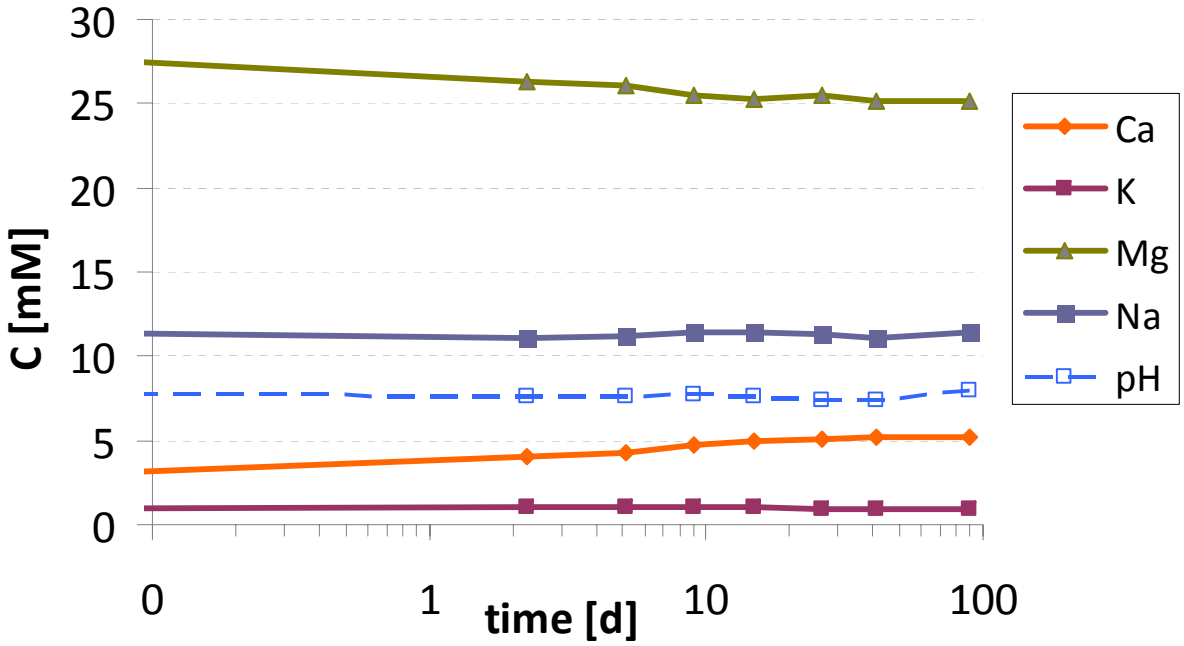


FIGURE C9: Chemical evolution (ph and cations) with time in the abiotic experiment - Batches with pollutants at 1mg/L individual initial concentration



C3. Experimental data - Micropollutants

The raw data from water samples analysis are provided in the following tables. The average concentrations (indicated as $C_{average}$ or simply C), calculated for each sampling time by using results from duplicate batches or, for the initial time t = 0, by using results from duplicate samples of the "initial water", are also reported. The error σ associated to the averages has been determined by taking into account the difference between duplicate batches results and the analytical errors (relative standard deviation provided by the specific laboratories). Namely, in more general terms, the error σ associated to the average concentration $C_{average}$ of N concentrations Ci has been calculated as:

$$\sigma = \sqrt{\sigma_A^{\,2} + \sigma_B^{\,2}} \qquad \text{[eq. C1]}$$

where:

$\sigma_A^2 = [\Sigma_N (C - Ci)^2] / (N - 1)$

$\sigma_B^2 = (RSD/100 * C)^2 / N$

and with: $C = Caverage = [\Sigma_N Ci\ ] / N$

RSD = relative standard deviation of the analytical method (provided by the specific laboratories)

In the plots, the temporal evolution of the target compounds is presented in terms of their average concentrations and the correpondent error bar. Actually, they are expressed in relative terms (divided by the initial concentration Co of the correspondent experiment, i.e. $C_{average}/Co$ or simply C/Co, and σ/Co) in order to remove systematic errors from the analysis. The values of such calculated normalized averages and their correspondent normalized error are also reported in the tables below.

Legend for the following tables:

n.d. = not detected or not fulfilling confirmation criteria of the analytical method.
When calculating the average concentrations, "n.d." has been substituted by a value of LOD/2, where LOD is the Limit Of Detection provided by the specific laboratory for each compound and each set of analyses performed.

- - - - - = not reliable data

< LDet = below the Limit of Determination (LDet).
When calculating the average concentrations, "< LDet" has been substituted by a value of LDet/2, where LDet were provided for each compound by the specific laboratory performing the analysis.

<u>xxx</u> = concentration not reliable in absolute terms (µg/L) but reliable when transformed in relative concentration (C/Co %)

"reserve": indicates additioonal reserve samples stored while assembling/disassembling for possible posterior analysis (in case of any problem with the original samples).

C3.1 Tables of experimental data for the batch experiments with micropollutants at 1 µg/L individual initial concentration

In the following, data for the compounds included in the spiking solutions "A" and "B", i.e. analysed by different methods (§B1), are presented in separate tables. The relative standard deviation (RSD) used for the calculation of the errors according to eq. C1 are reported in Table C6 and C7. It has to be pointed out that, as commented also in §2.2.5, due to defective functioning (inaccurate sample volume acquisition) of the SPE processor in one of the sets of analysis, some of the results for micropollutants from spiking solution "A" could be considered only as semiquantitative. For these data, reported in *italics* in the following tables, a relative standard deviation of RSD_1*3 has been considered, where RSD_1 is the relative standard deviation associated to that set of analysis.

TABLE C6: Relative standard deviation associated to the analysis of the compounds of spiking solution "A" – Batch experiments with micropollutants at 1µg/L individual initial concentration

	Relative Standard Deviation (RSD)	
compound	for the data affected by defective functioning of the processor: RSD_1*3 [%]	for the remaining data: RSD_2 [%]
Atenolol	16.5	10.5
Carbamazepine	12.3	19.5
Diclofenac	11.7	24.7
Gemfibrozil	10.2	13.0
Ibuprofen	18.0	12.4
Sulfamethoxazole	69.9	3.8
Estrone	13.8	27.9
Diuron	47.1	21.3
Simazine	23.1	3.3

TABLE C7: Relative standard deviation associated to the analysis of the compounds of spiking solution "B" – Batch experiments with micropollutants at 1µg/L individual initial concentration

compound	**Relative Standard Deviation (RSD)**
Atrazine	15.5
Terbuthylazine	16.5
Chlorphenvinfos	15.5
Chlorpyrifos	15.0
Diazinon	14.5
Prometryn	15.0
4-t-OP	17.5

TABLE C8: Analytical concentrations (Ci) of the micropollutants of spiking solution "A" in the NO3-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Ci [ng/L]								
time [d]	sample name	Diuron	Simazine	Estrone	Diclofenac	Ibuprofen	Carbamazepine	Gemfibrozil	Atenolol	Sulfamethoxazole
0.0	NO3-G3-INITIAL n°1	868	1356	875	1231	915	1130	948	983	1001
0.0	NO3-G3-INITIAL n°2	1050	1626	1077	1245	1180	1417	970	1197	1142
0.05	NO3-G3-1	-------	-------	-------	-------	-------	-------	-------	-------	-------
0.05	NO3-G3-1bis	1107	1640	872	1192	1090	1378	952	1001	1176
0.05	NO3-G3-1 reserve	1089	-------	866	1080	1016	-------	828	-------	942
0.05	NO3-G3-1bis reserve	957	-------	1026	1079	1010	-------	767	-------	1035
0.2	NO3-G3-2	909	1495	898	1111	1091	1292	881	956	1002
0.2	NO3-G3-2bis	973	1536	729	1053	1033	1343	929	973	1028
0.5	NO3-G3-3	1055	1743	759	1158	1184	1522	969	1045	1146
0.5	NO3-G3-3bis	978	1497	917	1017	1270	1345	932	918	1086
1.5	NO3-G3-4	1100	1571	832	1174	1113	1391	969	939	1034
1.5	NO3-G3-4bis	1019	1441	811	1183	1136	1562	954	996	1012
2.9	NO3-G3-5	1020	1521	111	885	1106	1249	1054	808	n.d.
3.0	NO3-G3-5bis	936	1479	141	857	1170	1243	1038	793	n.d.
4.9	NO3-G3-6	927	1438	131	696	1035	1126	1087	731	n.d.
5.0	NO3-G3-6bis	791	1285	64	718	1106	1064	1011	644	n.d.
10.5	NO3-G3-7	774	1367	624	958	748	1085	1004	505	814
10.5	NO3-G3-7bis	762	1430	655	1314	943	1080	1107	483	837
20.9	NO3-G3-8	788	1315	557	1045	1102	1052	839	222	762
20.9	NO3-G3-8bis	730	1315	557	1039	832	994	803	236	747

TABLE C9: Analytical concentrations (Ci) of the micropollutants of spiking solution "A" in the Mn-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Ci [ng/L]								
time [d]	sample name	Diuron	Simazine	Estrone	Diclofenac	Ibuprofen	Carbamazepine	Gemfibrozil	Atenolol	Sulfamethoxazole
0	*Mn-G2-INITIAL n°1*	-------	*1002*	-------	-------	-------	*858*	-------	*1646*	-------
0	Mn-G2-INITIAL n°2	1091	1755	1124	1102	997	1409	718	1718	1023
7	*Mn-G2-1*	*811*	*870*	*598*	*1251*	*909*	*1260*	*987*	*701*	-------
7	*Mn-G2-1bis*	*635*	*816*	*355*	*958*	*678*	*1180*	*643*	*664*	-------
14	*Mn-G2-2*	*696*	*969*	*345*	*1016*	*767*	*1064*	*710*	*436*	-------
14	*Mn-G2-2bis*	*702*	*813*	*439*	*1085*	*794*	*1214*	*812*	*474*	-------
25	*Mn-G2-3*	*819*	*1198*	*455*	*1349*	*947*	*1785*	*874*	*463*	-------
25	*Mn-G2-3bis*	*647*	*675*	*430*	*1221*	*955*	*973*	*937*	*285*	-------
42	Mn-G2-4	750	1698	527	1027	960	1390	1102	165	815
42	Mn-G2-4bis	734	1765	808	1059	1106	1540	1065	243	759
63	Mn-G2-5	733	1885	732	1156	1081	1620	939	3	762
63	Mn-G2-5bis	696	1696	515	1013	1074	1358	879	109	746
91	Mn-G2-6	603	1568	446	1028	1092	1372	1271	n.d.	685
91	Mn-G2-6bis	732	1752	518	1093	1328	1412	908	n.d.	590
194	Mn-G2-7	549	1596	280	1067	1070	1327	989	n.d.	507
194	Mn-G2-7bis	672	1477	589	1096	1137	1195	1341	n.d.	548

TABLE C10: Analytical concentrations (Ci) of the micropollutants of spiking solution "A" in the Fe-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Ci [ng/L]								
time [d]	sample name	Diuron	Simazine	Estrone	Diclofenac	Ibuprofen	Carbamazepine	Gemfibrozil	Atenolol	Sulfamethoxazole
0	*Fe-G2-INICIAL n°1*	*852*	*717*	*955*	*1082*	*1133*	*907*	*998*	*1140*	*3511*
0	Fe-G2-INICIAL nº2	1021	900	834	1071	1104	1071	790	1095	1001
7	*Fe-G2-1*	*437*	*365*	*770*	*880*	*1155*	*496*	*960*	*492*	-------
7	*Fe-G2-1bis*	*429*	*332*	*821*	*860*	*1172*	*482*	*1047*	*410*	-------
14	*Fe-G2-2*	*485*	*431*	*809*	*875*	*1057*	*584*	*939*	*378*	-------
14	*Fe-G2-2bis*	*409*	*359*	*632*	*732*	*944*	*507*	*858*	*327*	-------
24	*Fe-G2-3*	*301*	*147*	*583*	*893*	*1229*	*370*	*1017*	*155*	-------
24	*Fe-G2-3bis*	*340*	*142*	*608*	*897*	*1140*	*412*	*1106*	*243*	-------
42	Fe-G2-4	600	403	454	1003	1122	860	1044	91	67
42	Fe-G2-4bis	627	500	453	1079	1146	935	1176	126	61
63	Fe-G2-5	548	695	414	955	1125	920	1104	81	-------
63	Fe-G2-5bis	537	602	412	949	1126	869	1019	78	-------
91	Fe-G2-6	468	607	312	935	1173	792	1246	24	-------
91	Fe-G2-6bis	460	556	288	950	1029	812	1064	42	-------
199	Fe-G2-7	434	483	275	906	1070	790	1143	13	-------
199	Fe-G2-7bis	405	470	193	888	1025	710	867	12	n.d.

TABLE C11: Analytical concentrations (Ci) of the micropollutants of spiking solution "A" in the SO4-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Ci [ng/L]								
time [d]	sample name	Diuron	Simazine	Estrone	Diclofenac	Ibuprofen	Carbamazepine	Gemfibrozil	Atenolol	Sulfamethoxazole
0	*SO4-G2-INICIAL nº1*	*308*	*196*	*606*	*1199*	*1329*	*484*	*1077*	*778*	*4185*
0	SO4-G2-INICIAL nº2	1014	912	838	1110	1330	1177	719	1162	1017
7	*SO4-G2-1*	*420*	*379*	*697*	*833*	*951*	*550*	*843*	*454*	-------
7	*SO4-G2-1bis*	*682*	*655*	*752*	*930*	*1035*	*853*	*926*	*491*	-------
18	*SO4-G2-2*	*557*	*629*	*491*	*855*	*908*	*774*	*830*	*12*	-------
18	*SO4-G2-2bis*	*500*	*591*	*664*	*913*	*1015*	*788*	*935*	*23*	-------
36	*SO4-G2-3*	*647*	*613*	*431*	*1012*	*1200*	*934*	*1000*	*16*	-------
36	*SO4-G2-3bis*	*406*	*245*	*337*	*740*	*878*	*539*	*764*	*n.d.*	-------
65	SO4-G2-4	453	22	332	950	1103	775	764	4	28
65	SO4-G2-4bis	474	21	382	910	1078	801	692	n.d.	44
89	SO4-G2-5	425	593	309	1015	1009	818	796	n.d.	27
89	SO4-G2-5bis	414	553	295	1003	977	744	802	n.d.	n.d.
133	SO4-G2-6	389	447	240	940	983	765	816	n.d.	n.d.
133	SO4-G2-6bis	403	525	70	745	741	705	727	n.d.	n.d.
215	SO4-G2-7	360	464	28	758	90	710	812	n.d.	n.d.
215	SO4-G2-7bis	394	535	87	779	565	828	817	n.d.	n.d.

TABLE C12: Analytical concentrations (Ci) of the micropollutants of spiking solution "A" in the Natural Conditions experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Ci [ng/L]								
time [d]	**sample name**	**Diuron**	**Simazine**	**Estrone**	**Diclofenac**	**Ibuprofen**	**Carbamazepine**	**Gemfibrozil**	**Atenolol**	**Sulfamethoxazole**
0	*BL-G2-INICIAL nº1*	-------	-------	-------	-------	-------	-------	-------	-------	-------
0	*BL-G2-INICIAL nº2*	-------	-------	-------	-------	-------	-------	-------	-------	-------
0	BL-G2-INICIAL nº1 reserve	1131	1696	831	1090	1197	1228	823	1112	1061
0	BL-G2-INICIAL nº2 reserve	1044	1496	790	1049	1043	1056	940	1020	983
1.1	*BL-G2-1*	*901*	*1209*	*607*	*1332*	*923*	*908*	*845*	*1197*	-------
1.1	*BL-G2-1bis*	*849*	*1405*	*587*	*1394*	*994*	*1038*	*898*	*1311*	-------
3	*BL-G2-2*	*693*	*989*	*451*	*1049*	*766*	*750*	*728*	*866*	-------
3	*BL-G2-2bis*	*736*	*961*	*597*	*1163*	*868*	*819*	*806*	*771*	-------
7	*BL-G2-3*	-------	-------	-------	-------	-------	-------	-------	-------	-------
7	BL-G2-3bis	854	1532	588	1033	1247	1018	832	641	900
10	BL-G2-4	793	1272	682	932	1123	908	1056	570	745
10	BL-G2-4bis	861	1514	703	1018	1205	1111	928	571	774
15	BL-G2-5	719	1261	649	1018	991	914	1085	370	643
15	BL-G2-5bis	712	1412	600	972	965	1032	1002	348	724
26	BL-G2-6	726	1002	506	976	997	871	892	78	262
26	BL-G2-6bis	647	810	518	982	922	911	1114	168	220
42	BL-G2-7	670	1047	494	977	972	947	931	7	114
42	BL-G2-7bis	529	965	441	876	1031	822	937	n.d.	72
62	BL-G2-8	536	1207	314	960	1109	938	897	5	88
62	BL-G2-8bis	456	1127	275	883	1098	823	1032	6	72
89	BL-G2-9	461	1140	37	845	283	832	970	< LDet	< LDet
89	BL-G2-9bis	435	1106	87	900	854	799	1022	n.d.	< LDet
135	BL-G2-10	419	1005	n.d.	883	n.d.	780	716	< LDet	< LDet
135	BL-G2-10bis	337	986	< LDet	780	n.d.	769	747	n.d.	< LDet
192	BL-G2-11	302	837	n.d.	444	n.d.	768	264	< LDet	< LDet
192	BL-G2-11bis	325	940	< LDet	487	n.d.	697	356	n.d.	n.d.

TABLE C13: Analytical concentrations (Ci) of the micropollutants of spiking solution “A” in the abiotic-part1 experiment - Batches with micropollutants at 1µg/L individual initial concentration

NOTE 1: results of samples 6 to 8bis for diclofenac, gemfibrozil and sulfamethoxazole where analysed in a separate set of analysis, which reference “inicial water” sample was “SO4-AB-BIS-inicial reserve” reported in Table C14. The relative average concentrations C/Co and the correspondent error bars were further calculated (Table C32 and C33) by using the latter as Co.

NOTE 2: “*” means that the sample analysed was the "reserve" sample, except for the case of Gemfibrozil and Sulfamethoxazole

		Ci [ng/L]								
time [d]	sample name	Diuron	Simazine	Estrone	Diclofenac	Ibuprofen	Carbamazepine	Gemfibrozil	Atenolol	Sulfamethoxazole
0	NO3-AB-INICIAL n°1		-------		960	-------		1084	-------	917
0	NO3-AB-INICIAL n°2				1002			1016		996
0	NO3-AB-INICIAL reserve	1524	1084	880	------	846	1590	------	776	------
0.04	NO3-AB-1*		--------		933	--------		1480	--------	982
0.04	NO3-AB-1bis*	1368	1377	856	1005	1022	1963	1530	880	750
0.2	NO3-AB-2*	1608	1455	1007	1015	975	1999	1896	811	836
0.2	NO3-AB-2bis*	1259	1105	1009	940	816	1595	2480	620	885
0.5	NO3-AB-3*	1793	1391	793	945	888	1919	1497	761	913
no duplicate batch for time = 0.5d										
1.5	NO3-AB-4*	1371	1105	561	937	794	1562	1589	625	879
1.6	NO3-AB-4bis*	956	1025	624	848	866	1560	1372	514	843
3	NO3-AB-5*	1772	1361	744	963	869	2065	1129	497	895
3	NO3-AB-5bis*	1065	1441	650	958	815	1887	1140	463	907
5	NO3-AB-6	1023	1284	433	1057	930	2262	1152	340	830
5	NO3-AB-6bis	1054	1388	686	1060	1018	2467	1256	462	868
10	NO3-AB-7	1063	1196	521	1083	852	1959	1250	665	844
10	NO3-AB-7bis	1225	1384	499	967	737	2593	1036	450	760
22	NO3-AB-8	1248	1503	498	1119	647	2444	1227	558	886
22	NO3-AB-8bis	1368	1623	596	981	904	2049	1104	507	779

TABLE C14: Analytical concentrations (Ci) of the micropollutants of spiking solution “A” in the abiotic-part2 experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Ci [ng/L]								
time [d]	sample name	Diuron	Simazine	Estrone	Diclofenac	Ibuprofen	Carbamazepine	Gemfibrozil	Atenolol	Sulfamethoxazole
0	SO4-AB-BIS-INICIAL reserva	1188	925	1059	1074	1262	1158	1000	1203	906
37	SO4-AB-BIS-1 reserva	660	1048	392	989	828	1137	1167	552	745
37	SO4-AB-BIS-1bis reserva	688	1105	368	1055	742	1436	1160	487	790
64	SO4-AB-BIS-2 reserva	700	1218	292	1024	1002	1231	1200	672	982
64	SO4-AB-BIS-2bis reserva	649	1079	285	999	872	1201	1251	660	952
84	SO4-AB-BIS-3 reserva	690	1030	226	984	954	1031	1005	632	915
84	SO4-AB-BIS-3bis reserva	611	940	235	1060	847	998	1007	568	851
134	SO4-AB-BIS-4	639	1062	200	1090	972	1071	1178	608	987
134	SO4-AB-BIS-4bis	690	1026	234	1073	926	1109	1122	661	946
184	SO4-AB-BIS-5	676	921	164	1059	943	1008	938	559	958
184	SO4-AB-BIS-5bis	640	940	122	980	874	951	869	550	908

TABLE C15: Analytical concentrations (Ci) of the micropollutants of spiking solution “B” in the NO3-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Ci [ng/L]						
time [d]	sample name	Chlorphenvinfos	Chlorpyrifos	Diazinon	Prometryn	Atrazine	Terbuthylazine	4-t-OP
0.0	NO3-G3-INITIAL n°1	538	528	903	1007	515	617	7446
0.0	NO3-G3-INITIAL n°2	628	585	894	815	636	709	7571
0.05	NO3-G3-1	649	625	858	793	759	864	7551
0.05	NO3-G3-1bis	477	482	653	793	860	790	7416
0.2	NO3-G3-2	559	524	894	834	704	729	7400
0.2	NO3-G3-2bis	508	480	876	813	553	422	6550
0.5	NO3-G3-3	631	581	884	725	742	626	6187
0.5	NO3-G3-3bis	606	584	1170	632	474	572	7609
1.5	NO3-G3-4	513	467	628	718	740	760	7468
1.5	NO3-G3-4bis	492	465	785	708	975	991	6363
2.9	NO3-G3-5	451	411	605	604	678	652	4695
3.0	NO3-G3-5bis	438	431	715	806	645	706	3878
4.9	NO3-G3-6	464	421	431	739	603	636	5351
5.0	NO3-G3-6bis	510	481	709	824	797	505	4748
10.5	NO3-G3-7	403	379	625	756	781	626	3764
10.5	NO3-G3-7bis	414	383	634	661	769	632	3766
20.9	NO3-G3-8	201	188	499	499	774	440	4169
20.9	NO3-G3-8bis	284	260	593	563	602	603	4360

TABLE C16: Analytical concentrations (Ci) of the micropollutants of spiking solution “B” in the Mn-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Ci [ng/L]						
time [d]	sample name	Chlorphenvinfos	Chlorpyrifos	Diazinon	Prometryn	Atrazine	Terbuthylazine	4-t-OP
0	Mn-G2-INITIAL	1044	580	1084	878	825	1098	9608
7	Mn-G2-1	611	639	1022	945	821	972	8496
7	Mn-G2-1bis	551	515	943	906	937	906	4502
14	Mn-G2-2	392	414	965	1024	676	1011	8658
14	Mn-G2-2bis	623	589	1287	1383	919	803	6519
25	Mn-G2-3	372	384	980	778	683	970	3480
25	Mn-G2-3bis	434	383	852	765	922	1018	2293
42	Mn-G2-4	260	265	997	705	749	944	2110
42	Mn-G2-4bis	314	364	1142	842	1069	913	1909
63	Mn-G2-5	110	458	187	509	473	606	2964
63	Mn-G2-5bis	156	572	470	1005	723	1154	4143
91	Mn-G2-6	74	95	719	514	646	945	661
91	Mn-G2-6bis	81	79	763	844	590	993	554
194	Mn-G2-7	24	34	411	651	273	498	889
194	Mn-G2-7bis	39	40	530	557	436	578	764

TABLE C17: Analytical concentrations (Ci) of the micropollutants of spiking solution "B" in the Fe-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Ci [ng/L]						
time [d]	sample name	Chlorphenvinfos	Chlorpyrifos	Diazinon	Prometryn	Atrazine	Terbuthylazine	4-t-OP
0	Fe-G2-INICIAL n°1	866	860	5208	1088	1054	999	9984
0	Fe-G2-INICIAL nº2	836	882	2226	1189	983	930	8696
7	Fe-G2-1	499	491	3237	874	623	853	1928
7	Fe-G2-1bis	741	716	3663	1196	1014	979	9163
14	Fe-G2-2	836	850	3641	1217	827	901	9686
14	Fe-G2-2bis	694	733	3574	1213	1126	988	9154
24	Fe-G2-3	467	460	380	983	112	70	4021
24	Fe-G2-3bis	461	452	200	1006	54	41	3690
42	Fe-G2-4	283	284	740	1015	440	276	1115
42	Fe-G2-4bis	339	335	1045	975	426	375	2977
63	Fe-G2-5	317	326	952	1043	816	640	3045
63	Fe-G2-5bis	276	291	1114	1064	893	638	3057
91	Fe-G2-6	118	530	91	803	1050	787	3815
91	Fe-G2-6bis	143	555	259	559	400	899	2187
199	Fe-G2-7	92	98	588	932	672	551	3903
199	Fe-G2-7bis	96	98	500	886	945	629	3247

TABLE C18: Analytical concentrations (Ci) of the micropollutants of spiking solution "B" in the SO4-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Ci [ng/L]						
time [d]	sample name	Chlorphenvinfos	Chlorpyrifos	Diazinon	Prometryn	Atrazine	Terbuthylazine	4-t-OP
0	SO4-G2-INICIAL nº1	-------	-------	-------	-------	-------	-------	-------
0	SO4-G2-INICIAL nº2	805	341	1399	987	323	748	11181
7	SO4-G2-1	-------	-------	-------	-------	-------	-------	-------
7	SO4-G2-1bis	-------	-------	-------	-------	-------	-------	-------
18	SO4-G2-2	451	451	1126	1006	1299	1149	1153
18	SO4-G2-2bis	442	421	1246	1086	889	1041	690
36	SO4-G2-3	288	286	829	852	424	124	178
36	SO4-G2-3bis	278	335	228	989	133	1	1551
65	SO4-G2-4	132	154	135	829	129	below calibr.	820
65	SO4-G2-4bis	98	106	221	919	165	61	765
89	SO4-G2-5	127	388	-------	879	705	612	4644
89	SO4-G2-5bis	87	322	-------	862	415	327	4789
133	SO4-G2-6	136	144	375	1063	368	254	1395
133	SO4-G2-6bis	127	130	301	914	423	290	505
215	SO4-G2-7	76	78	496	941	646	575	171
215	SO4-G2-7bis	79	81	416	890	910	660	270

TABLE C19: Analytical concentrations (Ci) of the micropollutants of spiking solution "B" in the Natural Conditions experiment - Batches with micropollutants at 1µg/L individual initial concentration

time [d]	sample name	Ci [ng/L]						
		Chlorphenvinfos	Chlorpyrifos	Diazinon	Prometryn	Atrazine	Terbuthylazine	4-t-OP
0	BL-G2-INICIAL nº1	1054	493	1472	960	1167	1292	10449
0	BL-G2-INICIAL nº2	1012	1018	-------	1250	824	1069	9737
1.1	BL-G2-1	704	687	1332	965	1269	1032	2143
1.1	BL-G2-1bis	481	485	1291	867	833	943	518
3	BL-G2-2	308	374	1106	817	779	903	1238
3	BL-G2-2bis	210	252	930	703	1005	811	3193
7	BL-G2-3	543	570	1162	972	987	1195	682
7	BL-G2-3bis	509	516	1084	844	1062	839	1021
10	BL-G2-4	464	659	662	1227	921	386	2483
10	BL-G2-4bis	214	245	316	below calibr.	758	495	412
15	BL-G2-5	514	328	815	903	684	671	1788
15	BL-G2-5bis	444	408	897	899	863	774	1721
26	BL-G2-6	320	301	357	826	225	91	1274
26	BL-G2-6bis	227	345	254	699	227	86	312
42	BL-G2-7	328	307	754	874	946	644	2429
42	BL-G2-7bis	189	278	982	899	783	637	1328
62	BL-G2-8	161	694	347	667	665	654	5221
62	BL-G2-8bis	136	699	373	677	505	591	5355
89	BL-G2-9	138	139	642	908	785	759	1642
89	BL-G2-9bis	191	194	704	885	1019	959	1037
135	BL-G2-10	124	129	448	676	724	594	1189
135	BL-G2-10bis	95	99	454	694	660	615	940
192	BL-G2-11	51	51	308	718	711	542	932
192	BL-G2-11bis	47	62	368	601	365	481	863

TABLE C20: Analytical concentrations (Ci) of the micropollutants of spiking solution "B" in the abiotic-part1 experiment - Batches with micropollutants at 1µg/L individual initial concentration

time [d]	sample name	Ci [ng/L]						
		Chlorphenvinfos	Chlorpyrifos	Diazinon	Prometryn	Atrazine	Terbuthylazine	4-t-OP
0	NO3-AB-INICIAL n°1	857	654	931	910	1157	1139	10367
0	NO3-AB-INICIAL n°2	1153	512	961	1011	992	1016	7542
0.04	NO3-AB-1*	1112	460	684	1182	775	1044	10340
0.04	NO3-AB-1bis*	1163	409	691	1031	817	1099	10250
0.2	NO3-AB-2*	-------	-------	-------	-------	-------	-------	-------
0.2	NO3-AB-2bis*	1085	575	608	1171	910	809	6560
0.5	NO3-AB-3*	1062	408	621	1088	967	1107	7082
duplicate batch for time = 0.								
1.5	NO3-AB-4*	1193	642	754	1152	1178	1124	6305
1.6	NO3-AB-4bis*	1196	207	659	1169	899	1134	6665
3	NO3-AB-5*	1189	206	394	1117	1043	1139	8416
3	NO3-AB-5bis*	1006	213	300	1194	1036	1155	7018
5	NO3-AB-6	854	182	308	1187	1156	1087	7068
5	NO3-AB-6bis	1081	*79*	316	1001	1122	1054	6489
10	NO3-AB-7	670	624	267	824	834	1035	6696
10	NO3-AB-7bis	627	597	317	1007	848	1248	6988
22	NO3-AB-8	506	336	104	998	656	1052	5440
22	NO3-AB-8bis	434	301	107	913	717	997	5248

TABLE C21: Analytical concentrations (Ci) of the micropollutants of spiking solution “B” in the abiotic-part2 experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Ci [ng/L]						
time [d]	sample name	Chlorphenvinfos	Chlorpyrifos	Diazinon	Prometryn	Atrazine	Terbuthylazine	4-t-OP
0	SO4-AB-BIS-INIC. nº1	-------	-------	-------	-------	-------	-------	-------
0	SO4-AB-BIS-INIC. nº2	892	947	329	1019	882	918	7576
37	SO4-AB-BIS-1	788	720	232	1225	895	873	4261
37	SO4-AB-BIS-1bis	724	692	68	1079	1085	957	3504
64	SO4-AB-BIS-2	669	671	94	1265	1856	1267	3280
64	SO4-AB-BIS-2bis	628	809	154	1069	787	1127	2128
84	SO4-AB-BIS-3	652	896	82	1083	1142	1071	2298
84	SO4-AB-BIS-3bis	656	604	128	1136	1126	1535	2459
134	SO4-AB-BIS-4	383	396	468	1231	865	714	6009
134	SO4-AB-BIS-4bis	335	222	511	1286	1128	831	3023
184	SO4-AB-BIS-5	421	475	431	1248	958	720	5882
184	SO4-AB-BIS-5bis	432	416	421	1240	904	753	5587

TABLE C22: Averages (Caverage [ng/L]) and normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution “A” in the NO3-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Caverage [ng/L] and Caverage/Co [%]																	
		Diuron		Simazine		Estrone		Diclofenac		Ibuprofen		Carbamazepine		Gemfibrozil		Atenolol		Sulfamethoxazole	
average time [d]	sample name	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]
0	NO3-G3-INITIAL average	959	100	1491	100	976	100	1238	100	1047	100	1274	100	959	100	1090	100	1071	100
0.05	NO3-G3-1 average	1051	110	1640	110	921	94	1117	90	1038	99	1378	108	849	89	1001	92	1051	98
0.2	NO3-G3-2 average	941	98	1516	102	814	83	1082	87	1062	101	1318	103	905	94	965	88	1015	95
0.5	NO3-G3-3 average	1017	106	1620	109	838	86	1088	88	1227	117	1433	113	951	99	982	90	1116	104
1.5	NO3-G3-4 average	1060	110	1506	101	822	84	1178	95	1124	107	1477	116	961	100	967	89	1023	95
3	NO3-G3-5 average	978	102	1500	101	126	13	871	70	1138	109	1246	98	1046	109	800	73	25	2
5	NO3-G3-6 average	859	90	1361	91	98	10	707	57	1071	102	1095	86	1049	109	687	63	25	2
10	NO3-G3-7 average	768	80	1399	94	639	66	1136	92	845	81	1082	85	1056	110	494	45	825	77
21	NO3-G3-8 average	759	79	1315	88	557	57	1042	84	967	92	1023	80	821	86	229	21	755	70

TABLE C23: Normalized errors (σ/Co [%]) associated to the normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution “A” in the NO3-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

	error (±) for Caverage/Co [%]								
name	Diuron	Simazine	Estrone	Diclofenac	Ibuprofen	Carbamazepine	Gemfibrozil	Atenolol	Sulfamethoxazole
NO3-G3-INITIAL average	20	13	25	17	20	21	9	16	10
NO3-G3-1 average	16	4	18	14	8	21	12	10	11
NO3-G3-2 average	16	3	21	16	10	15	9	7	3
NO3-G3-3 average	17	12	20	17	12	18	10	11	5
NO3-G3-4 average	18	7	17	17	10	19	9	8	3
NO3-G3-5 average	17	3	3	12	10	13	10	6	0
NO3-G3-6 average	17	8	5	10	10	12	12	7	0
NO3-G3-7 average	12	4	13	26	15	12	13	4	3
NO3-G3-8 average	13	2	11	15	20	12	8	2	2

TABLE C24: Averages (Caverage [ng/L]) and normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution "A" in the Mn-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Caverage [ng/L] and Caverage/Co [%]																							
		Diuron		Simazine				Estrone		Diclofenac		Ibuprofen		Carbamazepine				Gemfibrozil		Atenolol				Sulfamethoxazole	
average time [d]	name	[ng/L]	[%]	part 1 [ng/L]	part 1 [%]	part 2 [ng/L]	part 2 [%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	part 1 [ng/L]	part 1 [%]	part 2 [ng/L]	part 2 [%]	[ng/L]	[%]	part 1 [ng/L]	part 1 [%]	part 2 [ng/L]	part 2 [%]	[ng/L]	[%]
0	Mn-G2-INITIAL	1091	100	1002	100	1755	100	1124	100	1102	100	997	100	858	100	1409	100	718	100	1646	100	1718	100	1023	100
7	*Mn-G2-1 average*	*723*	*66*	*843*	*84*	-	-	*476*	*42*	*1104*	*100*	*793*	*80*	*1220*	*142*	-	-	*815*	*114*	*682*	*41*	-	-	-------	-------
14	*Mn-G2-2 average*	*699*	*64*	*891*	*89*	-	-	*392*	*35*	*1051*	*95*	*781*	*78*	*1139*	*133*	-	-	*761*	*106*	*455*	*28*	-	-	-------	-------
25	*Mn-G2-3 average*	*733*	*67*	*937*	*93*	-	-	*442*	*39*	*1285*	*117*	*951*	*95*	*1379*	*161*	-	-	*905*	*126*	*374*	*23*	-	-	-------	-------
42	Mn-G2-4 average	742	68	-	-	1731	99	667	59	1043	95	1033	104	-	-	1465	104	1084	151	-	-	204	12	787	77
63	Mn-G2-5 average	714	65	-	-	1791	102	624	56	1084	98	1078	108	-	-	1489	106	909	127	-	-	56	3	754	74
91	Mn-G2-6 average	668	61	-	-	1660	95	482	43	1061	96	1210	121	-	-	1392	99	1089	152	-	-	3	0.2	637	62
194	Mn-G2-7 average	610	56	-	-	1537	88	435	39	1082	98	1104	111	-	-	1261	90	1165	162	-	-	3	0.2	527	52

TABLE C25: Normalized errors (σ/Co [%]) associated to the normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution "A" in the Mn-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

	error (±) for Caverage/Co [%]								
name	Diuron	Simazine	Estrone	Diclofenac	Ibuprofen	Carbamazepine	Gemfibrozil	Atenolol	Sulfamethoxazole
Mn-G2-INITIAL	21	23	28	25	12	32	13	17	4
Mn-G2-1 average	*25*	*14*	*16*	*21*	*19*	*14*	*35*	*5*	-------
Mn-G2-2 average	*21*	*18*	*7*	*9*	*10*	*17*	*13*	*4*	-------
Mn-G2-3 average	*25*	*40*	*4*	*13*	*12*	*68*	*11*	*8*	-------
Mn-G2-4 average	10	4	21	17	14	16	14	3	4
Mn-G2-5 average	10	8	18	19	9	20	13	4	2
Mn-G2-6 average	12	8	10	17	20	14	38	0.01	7
Mn-G2-7 average	12	5	21	17	11	14	38	0.01	3

TABLE C26: Averages (Caverage [ng/L]) and normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution "A" in the Fe-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Caverage [ng/L] and Caverage/Co [%]																	
		Diuron		Simazine		Estrone		Diclofenac		Ibuprofen		Carbamazepine		Gemfibrozil		Atenolol		Sulfamethoxazole	
average time [d]	name	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]
0	Fe-G2-INICIAL nº2	1021	100	900	100	834	100	1071	100	1104	100	1071	100	790	100	1095	100	1001	100
7	*Fe-G2-1 average*	*433*	*42*	*349*	*39*	*795*	*95*	*870*	*81*	*1164*	*105*	*489*	*46*	*1003*	*127*	*451*	*41*	-------	-------
14	*Fe-G2-2 average*	*447*	*44*	*395*	*44*	*721*	*86*	*803*	*75*	*1000*	*91*	*545*	*51*	*898*	*114*	*353*	*32*	-------	-------
24	*Fe-G2-3 average*	*320*	*31*	*145*	*16*	*596*	*71*	*895*	*84*	*1185*	*107*	*391*	*37*	*1061*	*134*	*199*	*18*	-------	-------
42	Fe-G2-4 average	613	60	452	50	454	54	1041	97	1134	103	897	84	1110	141	109	10	64	6
63	Fe-G2-5 average	542	53	649	72	413	49	952	89	1125	102	894	84	1062	134	79	7	-------	-------
91	Fe-G2-6 average	464	45	581	65	300	36	943	88	1101	100	802	75	1155	146	33	3	-------	-------
199	Fe-G2-7 average	419	41	476	53	234	28	897	84	1048	95	750	70	1005	127	13	1	3	0.3

TABLE C27: Normalized errors (σ/Co [%]) associated to the normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution "A" in the Fe-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

	error (±) for Caverage/Co [%]								
name	Diuron	Simazine	Estrone	Diclofenac	Ibuprofen	Carbamazepine	Gemfibrozil	Atenolol	Sulfamethoxazole
Fe-G2-INICIAL nº2	21	3	28	25	12	20	13	11	4
Fe-G2-1 average	*14*	*7*	*10*	*7*	*13*	*4*	*12*	*7*	-------
Fe-G2-2 average	*16*	*9*	*17*	*11*	*14*	*7*	*11*	*5*	-------
Fe-G2-3 average	*11*	*3*	*7*	*7*	*15*	*4*	*13*	*6*	-------
Fe-G2-4 average	9	8	11	18	9	13	18	2	0.5
Fe-G2-5 average	8	7	10	16	9	12	15	1	-------
Fe-G2-6 average	7	4	7	15	13	10	21	1	-------
Fe-G2-7 average	6	2	9	15	9	11	27	0.1	0.01

TABLE C28: Averages (Caverage [ng/L]) and normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution "A" in the SO4-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Caverage [ng/L] and Caverage/Co [%]																	
		Diuron		Simazine		Estrone		Diclofenac		Ibuprofen		Carbamazepine		Gemfibrozil		Atenolol		Sulfamethoxazole	
average time [d]	name	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]
0	SO4-G2-INICIAL nº2	1014	100	912	100	838	100	1110	100	1330	100	1177	100	719	100	1162	100	1017	100
7	*SO4-G2-1 average*	*551*	*54*	*517*	*57*	*724*	*86*	*882*	*79*	*993*	*75*	*701*	*60*	*885*	*123*	*473*	*41*	-------	-------
18	*SO4-G2-2 average*	*528*	*52*	*610*	*67*	*578*	*69*	*884*	*80*	*962*	*72*	*781*	*66*	*883*	*123*	*18*	*2*	-------	-------
36	*SO4-G2-3 average*	*527*	*52*	*429*	*47*	*384*	*46*	*876*	*79*	*1039*	*78*	*736*	*63*	*882*	*123*	*10*	*1*	-------	-------
65	SO4-G2-4 average	463	46	22	2	357	43	930	84	1090	82	788	67	728	101	2	0.2	36	4
89	SO4-G2-5 average	419	41	573	63	302	36	1009	91	993	75	781	66	799	111	0.3	0.03	15	1
133	SO4-G2-6 average	396	39	486	53	155	19	843	76	862	65	735	62	772	107	0.3	0.03	3	0.3
215	SO4-G2-7 average	377	37	500	55	57	7	768	69	328	25	769	65	814	113	0.3	0.03	3	0.3

TABLE C29: Normalized errors (σ/Co [%]) associated to the normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution "A" in the SO4-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

	error (±) for Caverage/Co [%]								
name	Diuron	Simazine	Estrone	Diclofenac	Ibuprofen	Carbamazepine	Gemfibrozil	Atenolol	Sulfamethoxazole
SO4-G2-INICIAL nº2	21	3	28	25	12	20	13	11	4
SO4-G2-1 average	*26*	*23*	*10*	*9*	*10*	*19*	*12*	*5*	-------
SO4-G2-2 average	*18*	*11*	*16*	*8*	*11*	*6*	*14*	*1*	-------
SO4-G2-3 average	*24*	*30*	*9*	*18*	*20*	*24*	*25*	*1*	-------
SO4-G2-4 average	7	0	9	15	7	9	12	0	1.1
SO4-G2-5 average	6	3	7	16	7	10	10	0	1.7
SO4-G2-6 average	6	6	15	18	14	9	13	0	0.0
SO4-G2-7 average	6	6	5	12	25	11	10	0.0	0.01

TABLE C30: Averages (Caverage [ng/L]) and normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution "A" in the Natural Conditions experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Caverage [ng/L] and Caverage/Co [%]																	
		Diuron		Simazine		Estrone		Diclofenac		Ibuprofen		Carbamazepine		Gemfibrozil		Atenolol		Sulfamethoxazole	
average time [d]	name	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]
0	BL-G2-INICIAL average	1088	100	1596	100	810	100	1070	100	1120	100	1142	100	881	100	1066	100	1022	100
1.1	*BL-G2-1 average*	*875*	*80*	*1307*	*82*	*597*	*74*	*1363*	*127*	*958*	*86*	*973*	*85*	*872*	*99*	*1254*	*118*	-------	-------
3	*BL-G2-2 average*	*715*	*66*	*975*	*61*	*524*	*65*	*1106*	*103*	*817*	*73*	*785*	*69*	*767*	*87*	*818*	*77*	-------	-------
7	BL-G2-3 bis	854	78	1532	96	588	73	1033	97	1247	111	1018	89	832	94	641	60	900	88
10	BL-G2-4 average	827	76	1393	87	693	85	975	91	1164	104	1010	88	992	113	571	54	760	74
15	BL-G2-5 average	715	66	1337	84	625	77	995	93	978	87	973	85	1043	118	359	34	683	67
26	BL-G2-6 average	687	63	906	57	512	63	979	92	959	86	891	78	1003	114	123	12	241	24
42	BL-G2-7 average	599	55	1006	63	468	58	927	87	1001	89	885	77	934	106	4	0.4	93	9
62	BL-G2-8 average	496	46	1167	73	294	36	922	86	1103	99	880	77	964	109	5	1	80	8
89	BL-G2-9 average	448	41	1123	70	62	8	872	82	569	51	815	71	996	113	1	0.1	31	3
135	BL-G2-10 average	378	35	996	62	1	0.1	832	78	3	0	775	68	731	83	1	0.1	31	3
192	BL-G2-11 average	313	29	889	56	1	0.1	466	44	3	0	733	64	310	35	1	0.1	21	2

TABLE C31: Normalized errors (σ/Co [%]) associated to the normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution "A" in the Natural Conditions experiment - Batches with micropollutants at 1µg/L individual initial concentration

	error (±) for Caverage/Co [%]								
name	Diuron	Simazine	Estrone	Diclofenac	Ibuprofen	Carbamazepine	Gemfibrozil	Atenolol	Sulfamethoxazole
BL-G2-INICIAL average	16	9	20	18	13	17	13	10	6
BL-G2-1 average	*27*	*16*	*7*	*11*	*12*	*11*	*8*	*16*	-------
BL-G2-2 average	*22*	*10*	*14*	*11*	*11*	*7*	*9*	*11*	-------
BL-G2-3 bis	17	3	20	24	14	17	12	6	3
BL-G2-4 average	12	11	17	17	10	18	15	4	3
BL-G2-5 average	10	7	16	17	8	14	13	3	6
BL-G2-6 average	11	9	13	16	9	11	21	6	3
BL-G2-7 average	12	4	12	17	9	13	10	0.4	3
BL-G2-8 average	9	4	8	16	9	13	15	0.1	1
BL-G2-9 average	6	2	5	15	36	10	11	0.1	0.1
BL-G2-10 average	8	2	0.1	15	0.03	9	8	0.1	0.1
BL-G2-11 average	5	5	0.1	8	0.03	10	8	0.1	1

TABLE C32: Averages (Caverage [ng/L]) and normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution "A" in the abiotic-part1 experiment - Batches with micropollutants at 1µg/L individual initial concentration

NOTE 1: results of samples 6 to 8bis for diclofenac, gemfibrozil and sulfamethoxazole where analysed in a separate set of analysis, which reference "inicial water" sample was "SO4-AB-BIS-inicial reserve" reported in Table C34. The relative average concentrations C/Co and the correspondent error bars are calculated by using the latter as Co.

		Caverage [ng/L] and Caverage/Co [%]																	
		Diuron		Simazine		Estrone		Diclofenac		Ibuprofen		Carbamazepine		Gemfibrozil		Atenolol		Sulfamethoxazole	
average time [d]	name	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]
0	NO3-AB-INICIAL	1524	100	1084	100	880	100	981	100	846	100	1590	100	1050	100	776	100	957	100
0.04	NO3-AB-1 average	1368	90	1377	127	856	97	969	99	1022	121	1963	123	1505	143	880	113	866	91
0.2	NO3-AB-2 average	1433	94	1280	118	1008	115	978	100	895	106	1797	113	2188	208	716	92	860	90
0.5	NO3-AB-3	1793	118	1391	128	793	90	945	96	888	105	1919	121	1497	143	761	98	913	95
1.5	NO3-AB-4 average	1164	76	1065	98	593	67	893	91	830	98	1561	98	1480	141	570	73	861	90
3	NO3-AB-5 average	1419	93	1401	129	697	79	960	98	842	100	1976	124	1134	108	480	62	901	94
5	NO3-AB-6 average	1039	68	1336	123	559	64	1058	99	974	115	2365	149	1204	120	401	52	849	94
10	NO3-AB-7 average	1144	75	1290	119	510	58	1025	95	794	94	2276	143	1143	114	558	72	802	89
22	NO3-AB-8 average	1308	86	1563	144	547	62	1050	98	775	92	2246	141	1165	117	532	69	832	92

TABLE C33: Normalized errors (σ/Co [%]) associated to the normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution "A" in the abiotic-part1 experiment - Batches with micropollutants at 1µg/L individual initial concentration

	error (±) for Caverage/Co [%]								
name	Diuron	Simazine	Estrone	Diclofenac	Ibuprofen	Carbamazepine	Gemfibrozil	Atenolol	Sulfamethoxazole
NO3-AB-INICIAL	21	3	28	18	12	20	10	11	6
NO3-AB-1 average	19	4	27	18	15	24	14	12	17
NO3-AB-2 average	21	23	23	18	16	24	44	19	4
NO3-AB-3	25	4	25	24	13	24	19	10	4
NO3-AB-4 average	22	6	14	17	11	14	20	11	4
NO3-AB-5 average	36	6	17	17	10	19	10	6	3
NO3-AB-6 average	10	7	24	17	12	22	13	12	4
NO3-AB-7 average	14	13	12	18	13	34	18	20	7
NO3-AB-8 average	14	9	15	19	23	26	14	7	9

TABLE C34: Averages (Caverage [ng/L]) and normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution "A" in the abiotic-part2 experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Caverage [ng/L] and Caverage/Co [%]																	
		Diuron		Simazine		Estrone		Diclofenac		Ibuprofen		Carbamazepine		Gemfibrozil		Atenolol		Sulfamethoxazole	
average time [d]	name	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]
0	SO4-AB-BIS-INICIAL	1188	100	925	100	1059	100	1074	100	1262	100	1158	100	1000	100	1203	100	906	100
37	SO4-AB-BIS-1 average	674	57	1076	116	380	36	1022	95	785	62	1287	111	1163	116	520	43	768	85
64	SO4-AB-BIS-2 average	674	57	1148	124	289	27	1011	94	937	74	1216	105	1225	123	666	55	967	107
84	SO4-AB-BIS-3 average	650	55	985	106	231	22	1022	95	900	71	1014	88	1006	101	600	50	883	97
134	SO4-AB-BIS-4 average	664	56	1044	113	217	21	1081	101	949	75	1090	94	1150	115	634	53	967	107
184	SO4-AB-BIS-5 average	658	55	930	101	143	13	1020	95	909	72	980	85	904	90	554	46	933	103

TABLE C35: Normalized errors (σ/Co [%]) associated to the normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution "A" in the abiotic-part2 experiment - Batches with micropollutants at 1µg/L individual initial concentration

	error (±) for Caverage/Co [%]								
name	Diuron	Simazine	Estrone	Diclofenac	Ibuprofen	Carbamazepine	Gemfibrozil	Atenolol	Sulfamethoxazole
SO4-AB-BIS-INICIAL	21	3	28	25	12	20	13	11	4
SO4-AB-BIS-1 average	9	5	7	17	7	24	11	5	4
SO4-AB-BIS-2 average	9	11	5	17	10	15	12	4	4
SO4-AB-BIS-3 average	10	7	4	17	9	12	9	5	6
SO4-AB-BIS-4 average	9	4	5	18	7	13	11	5	4.3
SO4-AB-BIS-5 average	9	3	4	17	7	12	10	3	9

TABLE C36: Averages (Caverage [ng/L]) and normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution "B" in the NO3-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Chlorphenvinfos		Chlorpyrifos		Diazinon		Prometryn		Atrazine		Terbuthylazine		4-t-OP	
average time [d]	name	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]
0	NO3-G3-INITIAL average	583	100	557	100	899	100	911	100	576	100	663	100	7509	100
0.05	NO3-G3-1 average	563	97	554	99	756	84	793	87	810	141	827	125	7484	100
0.2	NO3-G3-2 average	534	92	502	90	885	98	824	90	629	109	576	87	6975	93
0.5	NO3-G3-3 average	619	106	583	105	1027	114	679	74	608	106	599	90	6898	92
1.5	NO3-G3-4 average	503	86	466	84	707	79	713	78	858	149	876	132	6916	92
3	NO3-G3-5 average	445	76	421	76	660	73	705	77	662	115	679	102	4287	57
5	NO3-G3-6 average	487	84	451	81	570	63	782	86	700	122	571	86	5050	67
10	NO3-G3-7 average	409	70	381	68	630	70	709	78	775	135	629	95	3765	50
21	NO3-G3-8 average	243	42	224	40	546	61	531	58	688	120	522	79	4265	57

TABLE C37: Normalized errors (σ/Co [%]) associated to the normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution "B" in the NO3-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

	error (±) for Caverage/Co [%]						
name	Chlorphenvinfos	Chlorpyrifos	Diazinon	Prometryn	Atrazine	Terbuthylazine	4-t-OP
NO3-G3-INITIAL average	15	13	10	18	18	15	12
NO3-G3-1 average	23	21	18	9	20	17	12
NO3-G3-2 average	12	11	10	10	22	34	14
NO3-G3-3 average	12	11	25	11	35	12	18
NO3-G3-4 average	10	9	15	8	33	29	15
NO3-G3-5 average	9	8	11	18	13	13	10
NO3-G3-6 average	11	11	23	11	27	17	10
NO3-G3-7 average	8	7	7	11	15	11	6
NO3-G3-8 average	11	10	10	8	25	20	7

TABLE C38: Averages (Caverage [ng/L]) and normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution “B” in the Mn-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Chlorphenvinfos		Chlorpyrifos		Diazinon		Prometryn		Atrazine		Terbuthylazine		4-t-OP	
average time [d]	name	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]
0	Mn-G2-INITIAL	1044	100	580	100	1084	100	878	100	825	100	1098	100	9608	100
7	Mn-G2-1 average	581	56	577	99	983	91	925	105	879	107	939	85	6499	68
14	Mn-G2-2 average	508	49	501	86	1126	104	1203	137	797	97	907	83	7588	79
25	Mn-G2-3 average	403	39	383	66	916	85	772	88	803	97	994	91	2887	30
42	Mn-G2-4 average	287	28	314	54	1069	99	774	88	909	110	928	85	2009	21
63	Mn-G2-5 average	133	13	515	89	329	30	757	86	598	72	880	80	3554	37
91	Mn-G2-6 average	77	7	87	15	741	68	679	77	618	75	969	88	608	6
194	Mn-G2-7 average	31	3	37	6	470	43	604	69	355	43	538	49	826	9

TABLE C39: Normalized errors (σ/Co [%]) associated to the normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution “B” in the Mn-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

	error (±) for Caverage/Co [%]						
name	Chlorphenvinfos	Chlorpyrifos	Diazinon	Prometryn	Atrazine	Terbuthylazine	4-t-OP
Mn-G2-INITIAL	16	15	15	15	16	17	18
Mn-G2-1 average	7	19	11	12	15	11	31
Mn-G2-2 average	17	23	24	32	23	17	19
Mn-G2-3 average	6	7	12	9	23	11	9
Mn-G2-4 average	5	13	14	14	30	10	3
Mn-G2-5 average	3	17	19	41	23	37	10
Mn-G2-6 average	1	3	8	28	10	11	1
Mn-G2-7 average	1	1	9	11	15	8	1

TABLE C40: Averages (Caverage [ng/L]) and normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution “B” in the Fe-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Chlorphenvinfos		Chlorpyrifos		Diazinon		Prometryn		Atrazine		Terbuthylazine		4-t-OP	
average time [d]	name	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]
0	Fe-G2-INICIAL average	851	100	871	100	3717	100	1138	100	1018	100	965	100	9340	100
7	Fe-G2-1 average	620	73	604	69	3450	93	1035	91	819	80	916	95	5545	59
14	Fe-G2-2 average	765	90	791	91	3608	97	1215	107	977	96	944	98	9420	101
24	Fe-G2-3 average	464	55	456	52	290	8	994	87	83	8	56	6	3855	41
42	Fe-G2-4 average	311	37	310	36	893	24	995	87	433	42	326	34	2046	22
63	Fe-G2-5 average	297	35	308	35	1033	28	1053	93	854	84	639	66	3051	33
91	Fe-G2-6 average	131	15	543	62	175	5	681	60	725	71	843	87	3001	32
199	Fe-G2-7 average	94	11	98	11	544	15	909	80	809	79	590	61	3575	38

TABLE C41: Normalized errors (σ/Co [%]) associated to the normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution “B” in the Fe-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

	error (±) for Caverage/Co [%]						
name	Chlorphenvinfos	Chlorpyrifos	Diazinon	Prometryn	Atrazine	Terbuthylazine	4-t-OP
Fe-G2-INICIAL average	11	11	58	12	12	13	16
Fe-G2-1 average	22	20	12	22	29	14	55
Fe-G2-2 average	15	14	10	11	23	13	13
Fe-G2-3 average	6	6	4	9	4	2	6
Fe-G2-4 average	6	6	6	10	5	8	14
Fe-G2-5 average	5	5	4	10	11	8	4
Fe-G2-6 average	3	7	3	16	46	13	13
Fe-G2-7 average	1	1	2	9	21	9	7

TABLE C42: Averages (Caverage [ng/L]) and normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution “B” in the SO4-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Chlorphenvinfos		Chlorpyrifos		Diazinon		Prometryn		Atrazine		Terbuthylazine		4-t-OP	
average time [d]	name	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]
0	SO4-G2-INICIAL nº2	805	100	341	100	1399	100	987	100	323	100	748	100	11181	100
7	SO4-G2-1 average	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------
18	SO4-G2-2 average	447	56	436	128	1186	85	1046	106	1094	339	1095	146	922	8
36	SO4-G2-3 average	283	35	311	91	529	38	920	93	279	86	63	8	865	8
65	SO4-G2-4 average	115	14	130	38	178	13	874	89	147	45	61	8	792	7
89	SO4-G2-5 average	107	13	355	104			871	88	560	173	470	63	4717	42
133	SO4-G2-6 average	131	16	137	40	338	24	988	100	396	123	272	36	950	8
215	SO4-G2-7 average	77	10	79	23	456	33	916	93	778	241	618	83	220	2

TABLE C43: Normalized errors (σ/Co [%]) associated to the normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution “B” in the SO4-reducing experiment - Batches with micropollutants at 1µg/L individual initial concentration

	error (±) for Caverage/Co [%]						
name	Chlorphenvinfos	Chlorpyrifos	Diazinon	Prometryn	Atrazine	Terbuthylazine	4-t-OP
SO4-G2-INICIAL nº2	16	15	15	15	16	17	18
SO4-G2-1 average							
SO4-G2-2 average	6	15	11	13	97	20	3
SO4-G2-3 average	4	14	31	14	64	12	9
SO4-G2-4 average	3	11	5	11	9	1	1
SO4-G2-5 average	4	18		9	66	28	5
SO4-G2-6 average	2	5	4	15	18	5	6
SO4-G2-7 average	1	3	5	11	63	13	1

TABLE C44: Averages (Caverage [ng/L]) and normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution "B" in the Natural Conditions experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Chlorphenvinfos		Chlorpyrifos		Diazinon		Prometryn		Atrazine		Terbuthylazine		4-t-OP	
average time [d]	name	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]
0	BL-G2-INICIAL average	1033	100	755	100	1472	100	1105	100	996	100	1180	100	10093	100
1.1	BL-G2-1 average	592	57	586	78	1312	89	916	83	1051	106	987	84	1330	13
3	BL-G2-2 average	259	25	313	41	1018	69	760	69	892	90	857	73	2215	22
7	BL-G2-3 bis	526	51	543	72	1123	76	908	82	1024	103	1017	86	851	8
10	BL-G2-4 average	339	33	452	60	489	33	1227	111	840	84	440	37	1447	14
15	BL-G2-5 average	479	46	368	49	856	58	901	82	773	78	723	61	1754	17
26	BL-G2-6 average	273	26	323	43	305	21	763	69	226	23	88	7	793	8
42	BL-G2-7 average	258	25	292	39	868	59	886	80	865	87	641	54	1879	19
62	BL-G2-8 average	149	14	697	92	360	24	672	61	585	59	623	53	5288	52
89	BL-G2-9 average	164	16	166	22	673	46	897	81	902	91	859	73	1339	13
135	BL-G2-10 average	109	11	114	15	451	31	685	62	692	69	604	51	1064	11
192	BL-G2-11 average	49	5	56	7	338	23	660	60	538	54	511	43	898	9

TABLE C45: Normalized errors (σ/Co [%]) associated to the normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution "B" in the Natural Conditions experiment - Batches with micropollutants at 1µg/L individual initial concentration

	error (±) for Caverage/Co [%]						
name	Chlorphenvinfos	Chlorpyrifos	Diazinon	Prometryn	Atrazine	Terbuthylazine	4-t-OP
BL-G2-INICIAL average	11	50	15	21	27	18	13
BL-G2-1 average	17	21	9	11	33	11	12
BL-G2-2 average	7	12	11	10	19	10	14
BL-G2-3 bis	6	9	9	12	12	24	3
BL-G2-4 average	18	39	17	17	15	8	15
BL-G2-5 average	7	9	7	9	15	9	2
BL-G2-6 average	7	6	5	11	2	1	7
BL-G2-7 average	10	5	13	9	15	6	8
BL-G2-8 average	2	10	3	6	13	7	7
BL-G2-9 average	4	6	6	9	19	15	5
BL-G2-10 average	2	3	3	7	9	6	2
BL-G2-11 average	1	1	4	10	25	6	1

TABLE C46: Averages (Caverage [ng/L]) and normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution “B” in the abiotic-part1 experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Chlorphenvinfos		Chlorpyrifos		Diazinon		Prometryn		Atrazine		Terbuthylazine		4-t-OP	
average time [d]	name	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]
0	NO3-AB-INICIAL average	1005	100	583	100	946	100	961	100	1075	100	1078	100	8954	100
0.04	NO3-AB-1 average	1137	113	434	75	687	73	1106	115	796	74	1071	99	10295	115
0.2	NO3-AB-2 average	1085	108	575	99	608	64	1171	122	910	85	809	75	6560	73
0.5	NO3-AB-3	1062	106	408	70	621	66	1088	113	967	90	1107	103	7082	79
1.5	NO3-AB-4 average	1195	119	424	73	707	75	1161	121	1038	97	1129	105	6485	72
3	NO3-AB-5 average	1097	109	210	36	347	37	1155	120	1040	97	1147	106	7717	86
5	NO3-AB-6 average	967	96	130	22	312	33	1094	114	1139	106	1070	99	6778	76
10	NO3-AB-7 average	648	65	610	105	292	31	916	95	841	78	1142	106	6842	76
22	NO3-AB-8 average	470	47	319	55	106	11	956	99	686	64	1025	95	5344	60

TABLE C47: Normalized errors (σ/Co [%]) associated to the normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution “B” in the abiotic-part1 experiment - Batches with micropollutants at 1µg/L individual initial concentration

	error (±) for Caverage/Co [%]						
name	Chlorphenvinfos	Chlorpyrifos	Diazinon	Prometryn	Atrazine	Terbuthylazine	4-t-OP
NO3-AB-INICIAL average	24	20	11	13	15	14	26
NO3-AB-1 average	13	10	7	17	9	12	14
NO3-AB-2 average	17	15	9	18	13	12	13
NO3-AB-3	16	11	10	17	14	17	14
NO3-AB-4 average	13	53	10	13	21	12	9
NO3-AB-5 average	18	4	8	14	11	12	15
NO3-AB-6 average	19	13	3	18	12	12	10
NO3-AB-7 average	8	12	5	17	9	19	10
NO3-AB-8 average	7	7	1	12	8	12	8

TABLE C48: Averages (Caverage [ng/L]) and normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution “B” in the abiotic-part2 experiment - Batches with micropollutants at 1µg/L individual initial concentration

		Chlorphenvinfos		Chlorpyrifos		Diazinon		Prometryn		Atrazine		Terbuthylazine		4-t-OP	
average time [d]	name	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]	[ng/L]	[%]
0	SO4-AB-BIS-INICIAL nº2	892	100	947	100	329	100	1019	100	882	100	918	100	7576	100
37	SO4-AB-BIS-1 average	756	85	706	75	150	46	1152	113	990	112	915	100	3883	51
64	SO4-AB-BIS-2 average	649	73	740	78	124	38	1167	115	1322	150	1197	130	2704	36
84	SO4-AB-BIS-3 average	654	73	750	79	105	32	1109	109	1134	129	1303	142	2379	31
134	SO4-AB-BIS-4 average	359	40	309	33	490	149	1259	124	997	113	773	84	4516	60
184	SO4-AB-BIS-5 average	427	48	446	47	426	130	1244	122	931	106	737	80	5735	76

TABLE C49: Normalized errors (σ/Co [%]) associated to the normalized averages (Caverage/Co [%]) of concentrations for micropollutants of spiking solution “B” in the abiotic-part2 experiment - Batches with micropollutants at 1µg/L individual initial concentration

	error (±) for Caverage/Co [%]						
name	Chlorphenvinfos	Chlorpyrifos	Diazinon	Prometryn	Atrazine	Terbuthylazine	4-t-OP
SO4-AB-BIS-INICIAL nº2	16	15	15	15	16	17	18
SO4-AB-BIS-1 average	11	8	36	16	20	13	9
SO4-AB-BIS-2 average	9	13	13	18	87	19	12
SO4-AB-BIS-3 average	8	23	10	12	14	39	4
SO4-AB-BIS-4 average	6	13	18	14	24	13	29
SO4-AB-BIS-5 average	5	7	13	13	12	10	10

C3.2 Tables of experimental data for the batch experiments with pollutants at 1 mg/L individual initial concentration

TABLE C50: Analytical pollutants concentrations (Ci) in the NO3-reducing experiment - Batches with pollutants at 1mg/L individual initial concentration

Compound	Ci [µg/L]															
	GAB initial nº1	GAB initia nº2	GAB 1	GAB 1 bis	GAB 2	GAB 2 bis	GAB 3	GAB 3 bis	GAB 4	GAB 4 bis	GAB 5	GAB 5 bis	GAB 6	GAB 6 bis	GAB 7	GAB 7 bis
Acetaminophen	781	760	634	628	573	604	483	435	368	381	280	302	215	200	386	396
Atenolol	929	923	844	838	825	811	783	685	711	757	667	670	583	580	326	307
Bezafibrate	899	872	936	895	861	847	914	773	807	917	814	847	805	839	847	796
Carbamazepine	856	862	818	820	817	809	810	742	800	811	822	823	750	744	735	756
Cetirizine	750	760	713	710	719	703	634	614	658	658	737	702	646	650	695	685
Clarithromycin	849	836	485	476	471	468	306	277	306	307	287	270	309	279	266	280
Clofibric acid	987	942	1027	985	936	933	1002	833	881	1021	885	919	957	980	1002	945
Diazepam	857	854	821	789	810	775	781	699	762	770	783	777	785	776	758	756
Diclofenac	840	804	833	806	782	764	780	636	652	757	548	590	544	551	753	701
Erythromycin A	674	668	479	448	436	438	341	293	315	339	297	299	273	270	213	208
Famotidine	100	98	83	82	77	75	48	36	34	39	10	9	8	10	20	20
Gemfibrozil	1019	987	1010	1050	958	897	1038	853	928	1055	967	1011	1023	986	1061	1022
Ibuprofen	1026	1023	985	1012	1002	960	1007	870	956	1003	978	1002	1022	1023	1030	1006
Iohexol	1040	963	1002	1079	933	968	1019	894	1033	1012	924	941	585	933	980	1012
Iomeprol	1039	1036	965	1022	939	1001	941	895	1036	1052	967	961	1063	955	1065	969
Iopamidol	1164	1135	1187	1122	1111	1107	1032	1017	1178	1115	911	947	1123	1132	1216	1229
Iopromide	936	909	953	888	895	1024	878	769	886	913	825	881	720	999	1038	990
Loratadine	507	486	268	393	371	396	282	168	323	270	146	156	137	133	166	162
Metoprolol	1007	987	910	863	859	820	802	743	714	760	736	751	700	647	638	619
Naproxen	956	923	955	921	888	870	896	800	820	889	816	813	850	852	830	815
Pantoprazole	486	465	433	446	337	338	207	217	173	175	65	61	15	13	< Ldet	< Ldet
Phenazone	783	815	788	783	777	758	784	668	743	771	742	777	796	800	774	766
Primidone	112	115	102	109	107	96	109	96	105	105	104	97	96	87	94	95
Propranolol	1060	990	861	914	824	743	761	697	675	722	677	677	618	561	574	560
Roxithromycin	928	934	579	547	543	549	373	301	360	368	330	325	329	315	301	309
Sotalol	967	890	822	927	804	822	838	656	747	816	769	776	783	743	757	771
Sulfamethoxazole	1012	1010	860	914	98	115	< Ldet	< Ldet	< Ldet	< Ldet	< Ldet	< Ldet	< Ldet	< Ldet	378	692
Day	0	0	2	2	5	5	10	10	14	14	25	25	40	41	87	87

TABLE C51: Analytical pollutants concentrations (Ci) in the abiotic experiment - Batches with pollutants at 1mg/L individual initial concentration

Compound	Ci [µg/L]															
	GAB-AB-initial nº1	*GAB-AB-initial nº2*	GAB-AB-1	GAB-AB-1 bis	GAB-AB-2	GAB-AB-2 bis	GAB-AB-3	GAB-AB-3 bis	GAB-AB-4	*no duplicate batch for time = 14d*	GAB-AB-5	GAB-AB-5 bis	GAB-AB-6	GAB-AB-6 bis	GAB-AB-7	GAB-AB-7 bis
Acetaminophen	*741*	*715*	873	837	820	889	644	793	692		685	651	509	546	368	462
Atenolol	*733*	*613*	873	836	863	856	710	752	819		825	837	772	768	820	802
Bezafibrate	*706*	*732*	916	890	1114	1029	875	1086	929		1269	1008	1103	1121	1058	1225
Carbamazepine	*725*	*635*	928	923	929	948	798	855	917		931	920	877	889	922	931
Cetirizine	*638*	*603*	799	738	765	759	561	661	635		652	599	571	565	548	571
Clarithromycin	*736*	*600*	561	335	382	397	233	304	205		173	186	152	142	145	158
Clofibric acid	*725*	*690*	934	914	1093	1035	852	1036	958		1198	976	997	1032	995	1113
Diazepam	*768*	*643*	984	945	943	965	768	858	906		907	929	856	871	908	883
Diclofenac	*916*	*653*	1271	1233	1101	1147	941	965	1312		1048	1236	1019	1078	1207	1041
Erythromycin A	*711*	*584*	698	575	569	575	391	464	385		332	338	276	266	230	225
Famotidine	-------	-------	-------	-------	-------	-------	-------	-------	-------		-------	-------	-------	-------	-------	-------
Gemfibrozil	*872*	*619*	1255	1147	1036	1105	882	894	1254		971	1096	950	979	1089	896
Ibuprofen	*726*	*594*	965	958	968	936	782	833	987		991	981	948	936	982	914
Iohexol	*762*	*737*	948	887	1008	1116	873	817	1021		1045	1034	908	983	992	1076
Iomeprol	*896*	*660*	1180	1060	1117	1142	1065	822	1147		1122	1085	1271	984	1226	947
Iopamidol	*937*	*592*	1177	1000	977	1005	846	759	961		980	811	911	905	1066	946
Iopromide	*693*	*525*	869	898	901	988	812	830	910		924	901	968	979	935	1109
Loratadine	*474*	*522*	578	488	557	526	422	448	426		416	389	375	350	336	358
Metoprolol	*693*	*599*	832	779	812	851	661	734	747		767	787	700	728	730	743
Naproxen	*692*	*611*	925	895	955	960	775	896	920		1005	954	937	927	958	1016
Pantoprazole	-------	-------	-------	-------	-------	-------	-------	-------	-------		-------	-------	-------	-------	-------	-------
Phenazone	*754*	*575*	848	787	850	802	684	730	847		886	846	876	840	954	917
Primidone	*63*	*58*	66	66	113	116	100	108	123		127	122	123	124	125	133
Propranolol	*705*	*647*	807	745	750	784	584	665	650		647	670	555	570	565	590
Roxithromycin	*709*	*593*	608	389	413	437	268	329	249		193	210	169	160	160	174
Sotalol	*598*	*567*	725	689	732	789	606	716	717		771	791	671	749	716	789
Sulfamethoxazole	*672*	*564*	928	920	924	926	753	852	907		900	894	857	875	899	926
Day	0	0	2	2	5	5	10	10	14	14	25	25	40	41	87	87

TABLE C52: Averages (Caverage [µg/L]) and correspondent errors (σ [µg/L]) for pollutants concentrations in the NO3-reducing experiment - Batches with pollutants at 1mg/L individual initial concentration

Compound	Caverage and correspondent error [µg/L]															
	GAB initial average	+/-	GAB 1 - average	+/-	GAB 2 - average	+/-	GAB 3 - average	+/-	GAB 4 - average	+/-	GAB 5 - average	+/-	GAB 6 - average	+/-	GAB 7 - average	+/-
Acetaminophen	771	26	631	18	589	28	459	36	374	14	291	18	207	13	391	13
Atenolol	926	27	841	24	818	25	734	72	734	39	669	19	581	17	316	16
Bezafibrate	886	31	916	39	854	26	844	103	862	81	830	33	822	34	821	43
Carbamazepine	859	25	819	23	813	24	776	53	805	24	822	23	747	22	746	26
Cetirizine	755	23	712	20	711	23	624	22	658	19	720	32	648	18	690	21
Clarithromycin	842	25	481	15	469	13	292	22	306	9	278	15	294	23	273	12
Clofibric acid	964	42	1006	41	935	27	918	122	951	102	902	35	968	32	974	49
Diazepam	855	24	805	32	793	33	740	62	766	22	780	23	780	23	757	21
Diclofenac	822	34	819	30	773	25	708	104	704	77	569	34	547	16	727	42
Erythromycin A	671	19	463	25	437	12	317	35	327	19	298	9	271	8	210	7
Famotidine	99	3	82	3	76	2	42	8	36	4	9	0	9	2	20	1
Gemfibrozil	1003	37	1030	41	927	51	946	133	992	95	989	42	1004	39	1042	40
Ibuprofen	1024	29	998	34	981	41	938	100	980	43	990	33	1022	29	1018	33
Iohexol	1002	61	1041	62	950	37	956	93	1022	32	933	29	759	248	996	36
Iomeprol	1038	29	993	50	970	51	918	42	1044	32	964	28	1009	81	1017	74
Iopamidol	1150	38	1155	56	1109	32	1024	31	1146	55	929	37	1127	33	1223	36
Iopromide	922	32	921	52	960	96	823	81	900	32	853	46	859	199	1014	44
Loratadine	497	20	331	89	383	21	225	81	297	38	151	9	135	5	164	5
Metoprolol	997	31	886	42	839	36	773	47	737	38	743	24	674	42	628	22
Naproxen	940	35	938	36	879	28	848	72	854	55	814	23	851	24	822	26
Pantoprazole	476	20	440	16	338	10	212	9	174	5	63	4	14	1	2	0
Phenazone	799	32	786	22	768	25	726	85	757	29	759	33	798	23	770	23
Primidone	114	4	106	6	101	8	102	10	105	3	100	5	92	7	94	3
Propranolol	1025	58	887	45	784	61	729	50	699	38	677	19	590	44	567	19
Roxithromycin	931	27	563	28	546	16	337	51	364	12	327	10	322	14	305	10
Sotalol	928	60	875	78	813	26	747	130	782	54	772	22	763	36	764	24
Sulfamethoxazole	1011	29	887	46	106	12	2	3	2	3	2	3	2	3	535	222
Day	0		2		5		10		14		25		41		87	

TABLE C53: Averages (Caverage [µg/L]) and correspondent errors (σ [µg/L]) for pollutants concentrations in the abiotic experiment - Batches with pollutants at 1mg/L individual initial concentration

	Caverage and correspondent error [µg/L]																
Compound	***GAB-AB-initial average***	***+/-***	**GAB-AB-initial average CALCULATED**	**+/-**	**GAB-AB-1 average**	**+/-**	**GAB-AB-2 average**	**+/-**	**GAB-AB-3 average**	**+/-**	**GAB-AB 4**	**+/-**	**GAB-AB-5 average**	**+/-**	**GAB-AB-6 average**	**+/-**	**GAB-AB-7 average**
Acetaminophen	*728*	*28*	1045		855	35	855	54	718	107	692	28	668	31	528	30	415
Atenolol	*673*	*87*	941		855	35	859	25	731	36	819	33	831	25	770	22	811
Bezafibrate	*719*	*28*	873		903	31	1072	68	980	152	929	37	1139	187	1112	34	1141
Carbamazepine	*680*	*66*	970		925	26	939	30	826	47	917	37	926	27	883	26	926
Cetirizine	*620*	*31*	816		769	48	762	22	611	73	635	25	625	41	568	17	560
Clarithromycin	*668*	*98*	785		448	161	390	15	268	51	205	8	179	11	147	8	152
Clofibric acid	*707*	*32*	886		924	30	1064	51	944	133	958	38	1087	160	1015	38	1054
Diazepam	*705*	*90*	1025		964	38	954	31	813	68	906	36	918	30	863	26	895
Diclofenac	*784*	*187*	1256		1252	44	1124	46	953	32	1312	52	1142	137	1048	51	1124
Erythromycin A	*647*	*92*	922		637	89	572	17	428	53	385	15	335	10	271	11	228
Famotidine	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------
Gemfibrozil	*745*	*180*	1170		1201	84	1070	57	888	26	1254	50	1033	93	964	34	993
Ibuprofen	*660*	*95*	987		961	28	952	35	807	43	987	39	986	29	942	28	948
Iohexol	*749*	*27*	883		917	50	1062	82	845	47	1021	41	1040	31	945	59	1034
Iomeprol	*778*	*168*	1170		1120	91	1130	37	943	174	1147	46	1103	41	1128	205	1086
Iopamidol	*764*	*245*	1084		1088	129	991	34	802	65	961	38	896	122	908	26	1006
Iopromide	*609*	*120*	885		883	32	944	67	821	26	910	36	912	31	974	29	1022
Loratadine	*498*	*37*	801		533	65	541	26	435	22	426	17	402	22	363	21	347
Metoprolol	*646*	*69*	906		805	44	832	36	698	55	747	30	777	26	714	28	737
Naproxen	*651*	*60*	911		910	33	958	27	835	88	920	37	980	46	932	27	987
Pantoprazole	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------
Phenazone	*665*	*128*	831		817	48	826	42	707	38	847	34	866	37	858	35	935
Primidone	*61*	*4*	71		66	2	114	4	104	7	123	5	125	5	124	4	129
Propranolol	*676*	*45*	896		776	49	767	33	625	60	650	26	659	25	562	19	578
Roxithromycin	*651*	*84*	824		499	155	425	20	299	44	249	10	201	13	164	8	167
Sotalol	*582*	*28*	750		707	33	760	45	661	80	717	29	781	27	710	59	753
Sulfamethoxazole	*618*	*78*	911		924	27	925	26	802	74	907	36	897	26	866	28	912
Day	*0*		0		2		5		9		15		26		41		89

NOTE: For all compounds, the actual “initial” average concentration is < the expected 1000 µg/L and < of concentration at time t=2d. Thus, we’ve considered the actual “intial” data and its averages not reliable. We have thenSince we needed a Co to calculate the C/Co for all sampling times, we’ve estimated it based on the data of the biotic NO3-reducing experiment. We supposed that in both abiotic and biotic experiments all processes occurring for t<2d were not microbially driven (i.e. these abiotic processes are occurring in both the experiments so: "GAB 1-prom. %" ABIOTIC = "GAB 1-prom. %" BIOTIC). Then, we could calculate "GAB-AB-INITIAL average CALCULATED" [µg/L] = "GAB-AB-1aver." [µg/L] * "GAB INITIAL-aver." [%] / "GAB 1-aver. [%]

TABLE C54: Normalized averages (Caverage/Co [%]) for pollutants concentrations in the NO3-reducing experiment - Batches with pollutants at 1mg/L individual initial concentration

	Caverage/Co [%]							
Compound	**GAB initial average**	**GAB 1 - average**	**GAB 2 - average**	**GAB 3 - average**	**GAB 4 - average**	**GAB 5 - average**	**GAB 6 - average**	**GAB 7 - average**
Acetaminophen	100	82	76	60	49	38	27	51
Atenolol	100	91	88	79	79	72	63	34
Bezafibrate	100	103	96	95	97	94	93	93
Carbamazepine	100	95	95	90	94	96	87	87
Cetirizine	100	94	94	83	87	95	86	91
Clarithromycin	100	57	56	35	36	33	35	32
Clofibric acid	100	104	97	95	99	94	100	101
Diazepam	100	94	93	87	90	91	91	89
Diclofenac	100	100	94	86	86	69	67	88
Erythromycin A	100	69	65	47	49	44	40	31
Famotidine	100	83	77	42	37	9	9	20
Gemfibrozil	100	103	92	94	99	99	100	104
Ibuprofen	100	97	96	92	96	97	100	99
Iohexol	100	104	95	95	102	93	76	99
Iomeprol	100	96	93	88	101	93	97	98
Iopamidol	100	100	96	89	100	81	98	106
Iopromide	100	100	104	89	98	93	93	110
Loratadine	100	67	77	45	60	30	27	33
Metoprolol	100	89	84	78	74	75	68	63
Naproxen	100	100	94	90	91	87	91	87
Pantoprazole	100	92	71	44	37	13	3	0.4
Phenazone	100	98	96	91	95	95	100	96
Primidone	100	93	89	90	92	88	81	83
Propranolol	100	87	76	71	68	66	58	55
Roxithromycin	100	60	59	36	39	35	35	33
Sotalol	100	94	88	80	84	83	82	82
Sulfamethoxazole	100	88	10	0.2	0.2	0.2	0.2	53
Day	0	2	5	10	14	25	41	87

TABLE C55: Normalized errors (σ/Co [%]) associated to the normalized average concentrations (Caverage/Co [%]) of pollutants in the NO3-reducing experiment - Batches with pollutants at 1mg/L individual initial concentration

	error (±) for Caverage/Co [%]							
Compound	**GAB initial average**	**GAB 1 - average**	**GAB 2 - average**	**GAB 3 - average**	**GAB 4 - average**	**GAB 5 - average**	**GAB 6 - average**	**GAB 7 - average**
Acetaminophen	3.4	2.4	3.6	4.7	1.8	2.3	1.6	1.7
Atenolol	2.9	2.6	2.7	7.8	4.2	2.1	1.8	1.7
Bezafibrate	3.6	4.4	2.9	11.6	9.2	3.7	3.8	4.8
Carbamazepine	2.9	2.7	2.8	6.1	2.8	2.7	2.5	3.0
Cetirizine	3.0	2.7	3.1	3.0	2.5	4.2	2.4	2.7
Clarithromycin	3.0	1.8	1.6	2.6	1.0	1.7	2.7	1.4
Clofibric acid	4.3	4.3	2.8	12.7	10.6	3.6	3.3	5.1
Diazepam	2.8	3.7	3.9	7.2	2.6	2.6	2.7	2.5
Diclofenac	4.2	3.7	3.1	12.6	9.4	4.2	2.0	5.2
Erythromycin A	2.9	3.8	1.9	5.3	2.9	1.3	1.2	1.0
Famotidine	3.4	2.7	2.4	8.2	4.1	0.4	2.0	0.6
Gemfibrozil	3.7	4.1	5.0	13.3	9.4	4.2	3.8	4.0
Ibuprofen	2.8	3.3	4.0	9.7	4.2	3.2	2.8	3.2
Iohexol	6.1	6.2	3.7	9.3	3.2	2.9	24.7	3.6
Iomeprol	2.8	4.8	4.9	4.0	3.1	2.7	7.8	7.2
Iopamidol	3.3	4.9	2.7	2.7	4.8	3.2	2.8	3.1
Iopromide	3.5	5.7	10.4	8.8	3.5	5.0	21.5	4.8
Loratadine	4.1	18.0	4.2	16.3	7.7	1.7	1.0	1.1
Metoprolol	3.1	4.2	3.6	4.7	3.8	2.4	4.2	2.2
Naproxen	3.8	3.8	3.0	7.6	5.9	2.5	2.6	2.7
Pantoprazole	4.2	3.3	2.0	1.9	1.1	0.8	0.3	0.01
Phenazone	4.0	2.8	3.2	10.6	3.6	4.1	2.8	2.8
Primidone	3.4	5.1	7.3	8.5	2.6	4.6	5.8	2.4
Propranolol	5.6	4.4	6.0	4.9	3.7	1.9	4.3	1.8
Roxithromycin	2.9	3.0	1.7	5.5	1.3	1.1	1.5	1.1
Sotalol	6.5	8.4	2.8	14.0	5.8	2.4	3.8	2.6
Sulfamethoxazole	2.8	4.6	1.2	0.01	0.01	0.01	0.01	22.0

TABLE C56: Normalized averages (Caverage/Co [%]) for pollutants concentrations in the abiotic experiment - Batches with pollutants at 1mg/L individual initial concentration

	Caverage/Co [%]							
Compound	**GAB-AB-initial-CALCUL. average**	**GAB-AB-1 average**	**GAB-AB-2 average**	**GAB-AB-3 average**	**GAB-AB-4**	**GAB-AB-5 average**	**GAB-AB-6 average**	**GAB-AB-7 average**
Acetaminophen	100	82	82	69	66	64	51	40
Atenolol	100	91	91	78	87	88	82	86
Bezafibrate	100	103	123	112	106	130	127	131
Carbamazepine	100	95	97	85	95	95	91	95
Cetirizine	100	94	93	75	78	77	70	69
Clarithromycin	100	57	50	34	26	23	19	19
Clofibric acid	100	104	120	106	108	123	114	119
Diazepam	100	94	93	79	88	90	84	87
Diclofenac	100	100	90	76	105	91	83	90
Erythromycin A	100	69	62	46	42	36	29	25
Famotidine	-------	-------	-------	-------	-------	-------	-------	-------
Gemfibrozil	100	103	92	76	107	88	82	85
Ibuprofen	100	97	96	82	100	100	95	96
Iohexol	100	104	120	96	116	118	107	117
Iomeprol	100	96	97	81	98	94	96	93
Iopamidol	100	100	91	74	89	83	84	93
Iopromide	100	100	107	93	103	103	110	115
Loratadine	100	67	68	54	53	50	45	43
Metoprolol	100	89	92	77	82	86	79	81
Naproxen	100	100	105	92	101	108	102	108
Pantoprazole	-------	-------	-------	-------	-------	-------	-------	-------
Phenazone	100	98	99	85	102	104	103	113
Primidone	100	93	162	147	174	177	175	183
Propranolol	100	87	86	70	73	74	63	65
Roxithromycin	100	60	52	36	30	24	20	20
Sotalol	100	94	101	88	96	104	95	100
Sulfamethoxazole	100	101	101	88.0	99.5	98.5	95.0	100
Day	0	2	5	9	15	26	41	89

TABLE C57: Normalized errors (σ/Co [%]) associated to the normalized average concentrations (Caverage/Co [%]) of pollutants in the abiotic experiment - Batches with pollutants at 1mg/L individual initial concentration

	error (±) for Caverage/Co [%]							
Compound	**GAB-AB-initial-CALCUL. average**	**GAB-AB-1 average**	**GAB-AB-2 average**	**GAB-AB-3 average**	**GAB-AB-4**	**GAB-AB-5 average**	**GAB-AB-6 average**	**GAB-AB-7 average**
Acetaminophen		3.4	5.2	10.3	2.7	2.9	2.9	6.5
Atenolol		3.7	2.6	3.9	3.5	2.7	2.3	2.8
Bezafibrate		3.6	7.7	17.4	4.3	21.4	3.9	14.0
Carbamazepine		2.7	3.1	4.8	3.8	2.8	2.7	2.8
Cetirizine		5.9	2.7	9.0	3.1	5.1	2.0	2.7
Clarithromycin		20.5	2.0	6.5	1.0	1.3	1.0	1.3
Clofibric acid		3.4	5.8	15.0	4.3	18.1	4.3	10.0
Diazepam		3.8	3.0	6.6	3.5	2.9	2.6	3.0
Diclofenac		3.5	3.6	2.5	4.2	10.9	4.1	9.7
Erythromycin A		9.6	1.8	5.8	1.7	1.1	1.2	0.8
Famotidine	-------	-------	-------	-------	-------	-------	-------	-------
Gemfibrozil		7.1	4.9	2.3	4.3	7.9	2.9	11.9
Ibuprofen		2.8	3.6	4.3	4.0	2.9	2.8	5.6
Iohexol		5.7	9.3	5.3	4.6	3.5	6.7	7.5
Iomeprol		7.8	3.1	14.9	3.9	3.5	17.5	17.1
Iopamidol		11.9	3.1	6.0	3.5	11.3	2.4	8.2
Iopromide		3.6	7.6	3.0	4.1	3.5	3.2	14.3
Loratadine		8.2	3.3	2.8	2.1	2.8	2.6	2.3
Metoprolol		4.9	4.0	6.1	3.3	2.9	3.1	2.5
Naproxen		3.6	3.0	9.7	4.0	5.0	3.0	5.4
Pantoprazole	-------	-------	-------	-------	-------	-------	-------	-------
Phenazone		5.8	5.0	4.6	4.1	4.5	4.2	4.5
Primidone		2.6	5.2	9.3	6.9	7.2	5.0	9.5
Propranolol		5.5	3.6	6.7	2.9	2.8	2.1	2.7
Roxithromycin		18.8	2.5	5.3	1.2	1.6	1.0	1.3
Sotalol		4.4	6.0	10.7	3.8	3.5	7.8	7.5
Sulfamethoxazole		2.9	2.9	8.08	3.98	2.82	3.06	3.5
Day	0	2	5	9	15	26	41	89

C3.3 Figures and comments on results for the batch experiments with micropollutants at 1 µg/L individual initial concentration

It is worth to be mentioned again that due to defective functioning (inaccurate sample volume acquisition) of the SPE processor in one of the sets of analysis, some of the results for micropollutants from spiking solution "A" could be considered only as semiquantitative. Namely, they are the first 3 results of the Mn-, Fe- and SO_4-reducing experiments and the first 2 results of the "Natural Conditions" experiment (they have been marked adecuately in the previos tables reporting the experimental data). For these data, a multiplicative factor of 3 has been applied to the RSD when calculating the error bars (§C3.1). Still, the interpretation of micropollutants behaviour corresponding to those samples has to be faced carefully.

C3.3.1 Atenolol

(included in spiking solution "A")

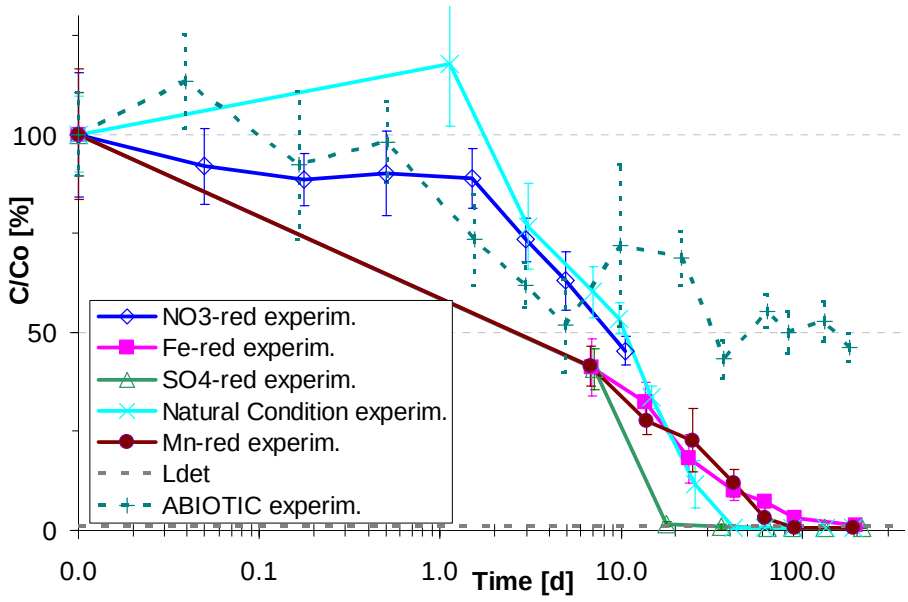


FIGURE C10: Evolution with time of atenolol average normalized concentration (C/Co [%]) in all the experiments (biotics and abiotic) with micropollutants at 1 µg/L individual initial concentration

Results for atenolol in the batch experiments with micropollutants at 1 µg/L individual initial concentration are presented in Chapter 2.

C3.3.2 Carbamazepine
(included in spiking solution “A”)

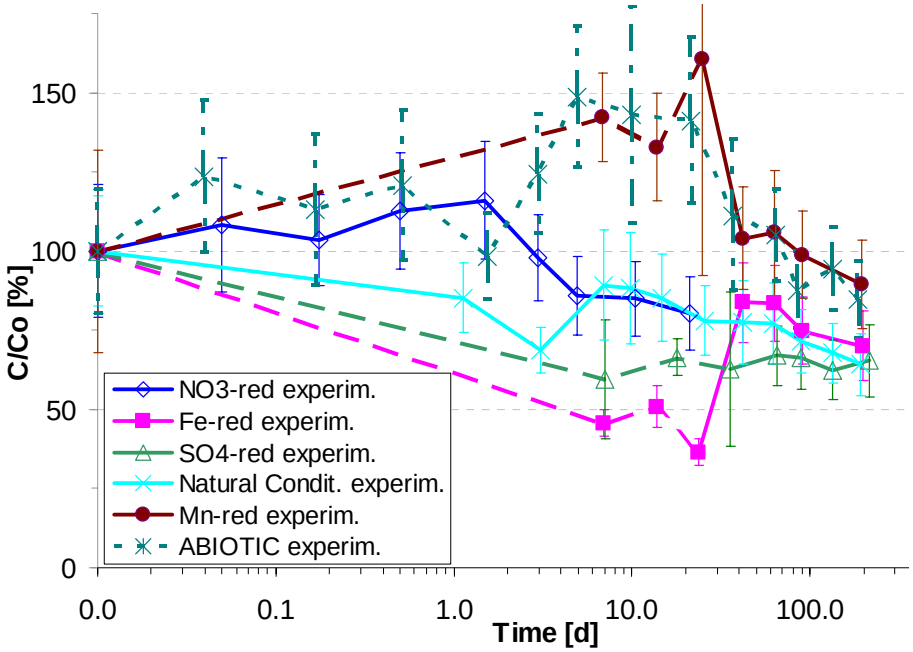


FIGURE C11: Evolution with time of carbamazepine average normalized concentration (C/Co [%]) in all the experiments (biotics and abiotic) with micropollutants at 1 µg/L individual initial concentration

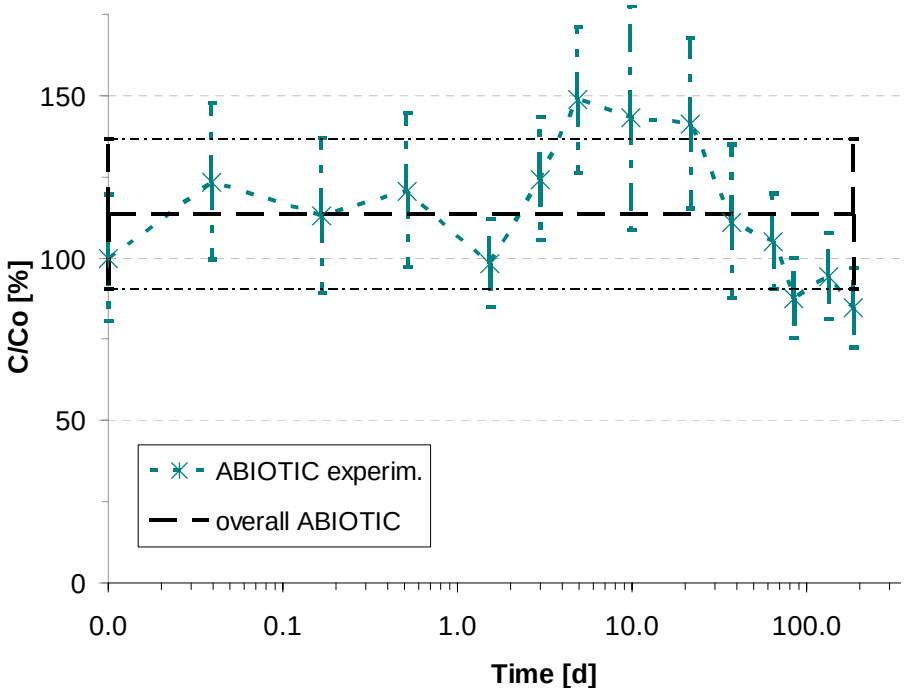


FIGURE C12: Evolution with time of carbamazepine average normalized concentration in the abiotic experiment (line “abiotic experim.”) and plot of the calculated “overall abiotic” concentration (described in the following)

COMMENTS to Figure C12:
Looking at the temporal evolution of carbamazepine average normalized concentration (line “abiotic experim.”) and taking into account its error bars, the individual C/Co are almost fluctuating around a same value. An “overall abiotic” concentration has been thus calculated as average of all individual analytical concentrations Ci and has been also plotted. It amounted to 113 ± 23 %. To determine its error bar, eq.C1 of §C3 has been used. In spite of the large fluctuations of the line ““abiotic experim.” and the numerous points exceeding the initial concentration Co (i.e., 100%), the calculated “overall abiotic” suggest that carbamazepine was not removed during the whole abiotic experiment. To simplify the comparison with the biotic experiments, the line “overall abiotic” (and its correspondent error bar) has been included in the following graphics instead of line “abiotic experim.”.

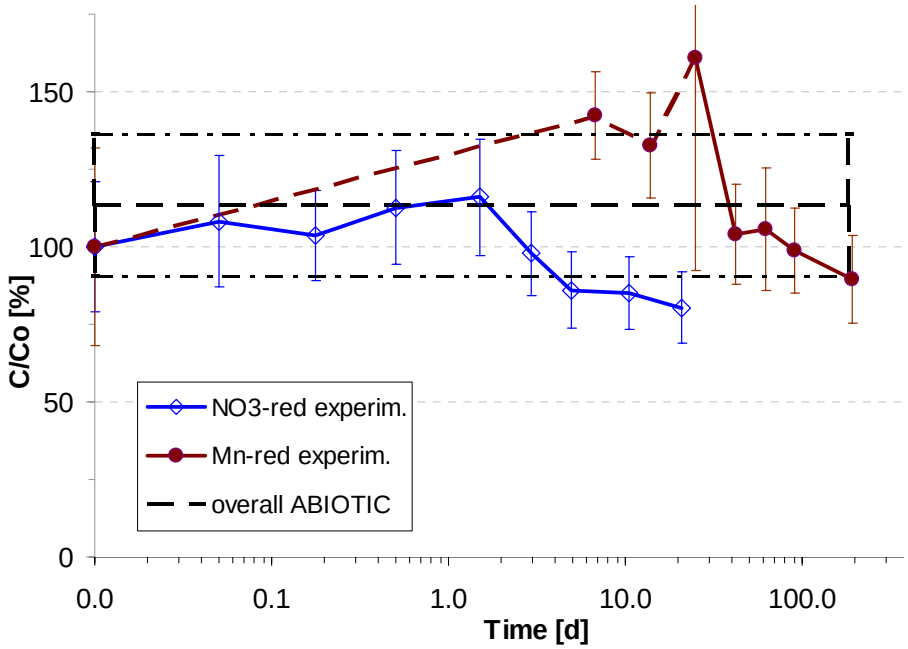


FIGURE C13: Temporal evolution of carbamazepine average normalized concentration (C/Co [%]) in the NO3- and Mn-reducing experiments versus the “overall abiotic” concentration

COMMENTS to Figure C13:
No biotic removal could be observed during the NO3- and Mn-reducing experiments.

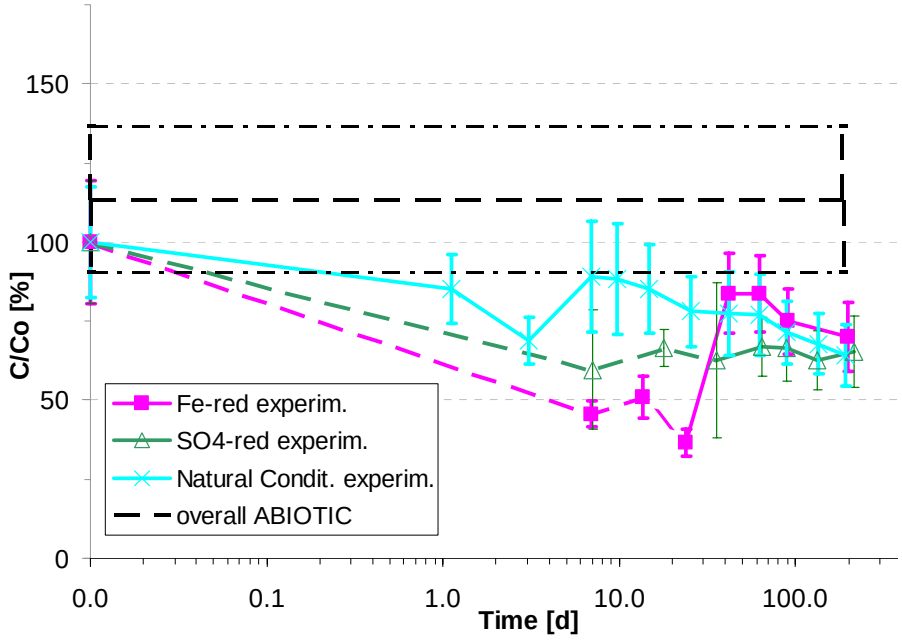


FIGURE C14: Temporal evolution of carbamazepine average normalized concentration (C/Co [%]) in the Fe-reducing, SO4-reducing and Natural Conditions experiments versus the "overall abiotic" concentration

COMMENTS to Figure C14:
After day 135, by taking into account the error bars, a biotic removal of 4 and 9 % could be estimated in the Natural Conditions and SO4-reducing experiments, respectively. No biotic removal could be observed in the Fe-reducing experiment.

C3.3.3 Diclofenac
(included in spiking solution "A")

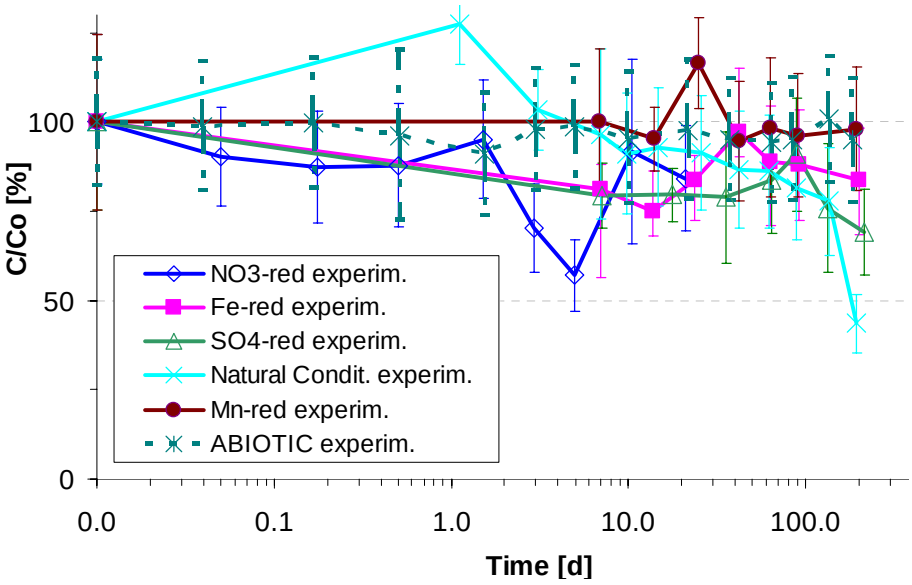


FIGURE C15: Evolution with time of diclofenac average normalized concentration (C/Co [%]) in all the experiments (biotics and abiotic) with micropollutants at 1 µg/L individual initial concentration

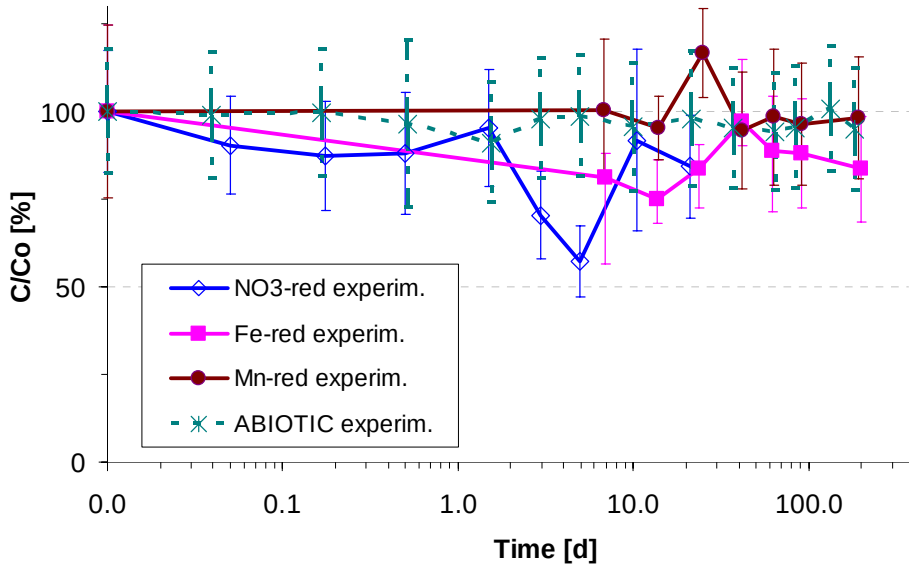


FIGURE C16: Temporal evolution of diclofenac average normalized concentration (C/Co [%]) in the NO3-, Mn- and Fe-reducing experiments versus the evolution in the abiotic experiment

COMMENTS to Figure C16:
No overall (neither biotic nor abiotic) removal in the NO3-, Mn-, and Fe-reducing experiment.
Details on the fate of diclofenac in the NO3-reducing experiment are presented in Chapter 3.

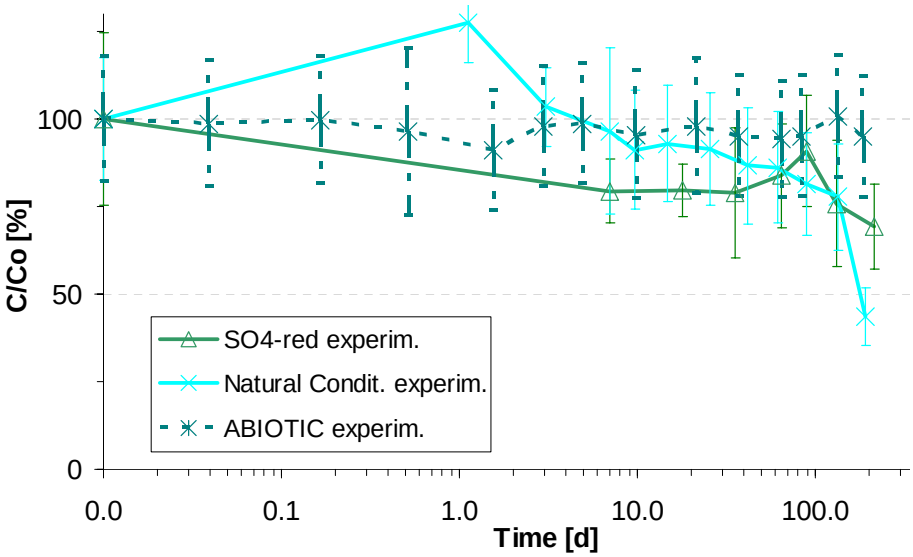


FIGURE C17: Temporal evolution of diclofenac average normalized concentration (C/Co [%]) in the SO4-reducing and Natural Conditions experiments versus the evolution in the abiotic experiment

COMMENTS to Figure C17:
No overall (neither biotic nor abiotic) removal in the SO4-reducing experiment.
After day 89, Diclofenac is biotically removed in the Natural Conditions experiments. Considering the error bars, an overall removal of about 30% could be estimated by the end of the experiment.

C3.3.4 Gemfibrozil
(included in spiking solution "A")

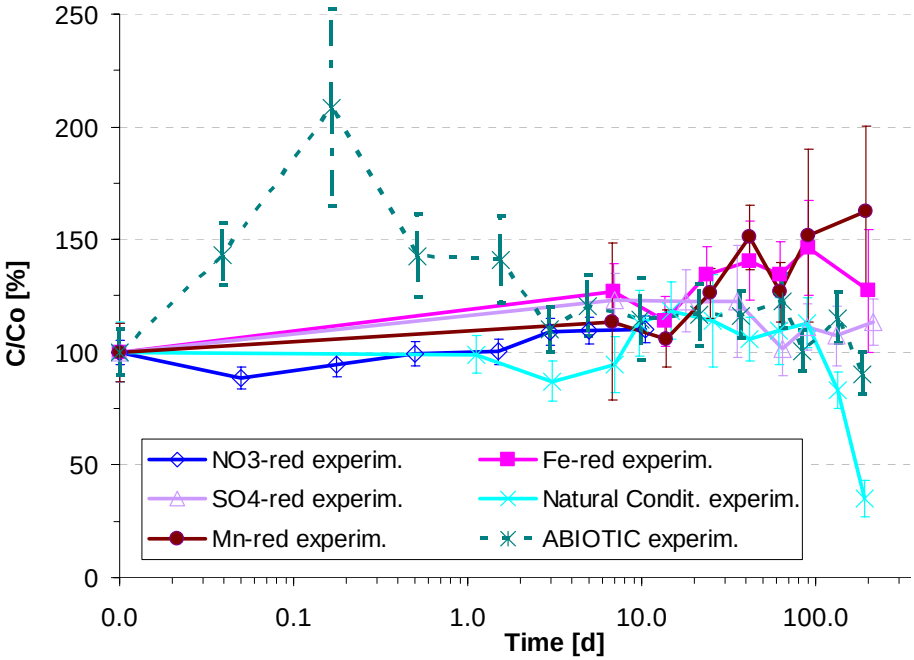


FIGURE C18: Evolution with time of gemfibrozil average normalized concentration (C/Co [%]) in all the experiments (biotics and abiotic) with micropollutants at 1 µg/L individual initial concentration

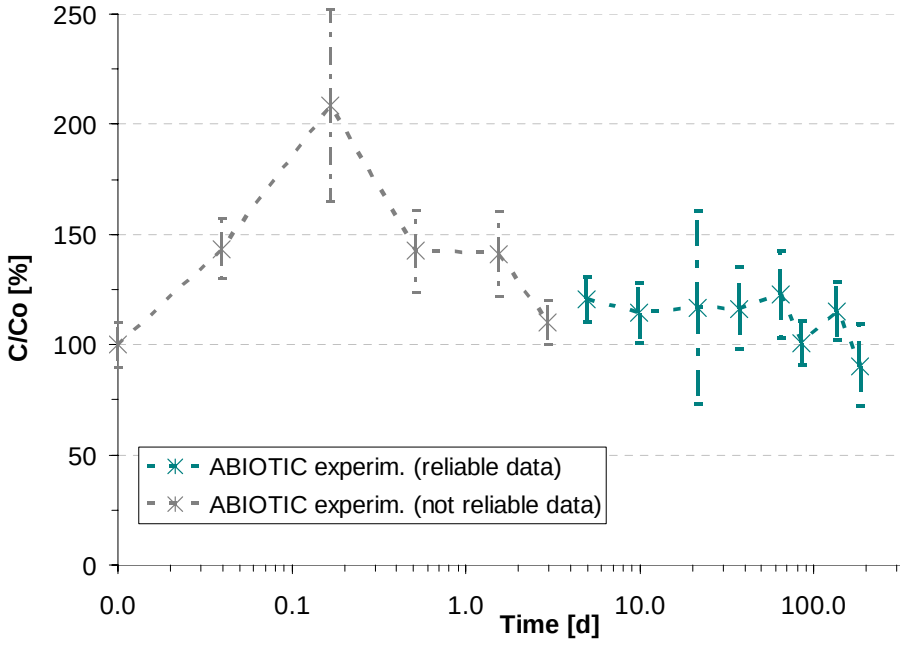


FIGURE C19: Evolution with time of gemfibrozil average normalized concentration in the abiotic experiment.

COMMENTS to Figure C19:
Some of the results of the abiotic experiment are extremely high and seem to be not reliable. They will not be considered in the following. To be consitent, all data (grey points in the plot) from that

same set of analysis will not be used. The remaining data proceed from a different set of analysis, in which a sample of the "initial water" was included as reference. This allowed calculating their respective normalized average concentrations (C/Co). No overall abiotic removal could be observed by the end of the experiment.

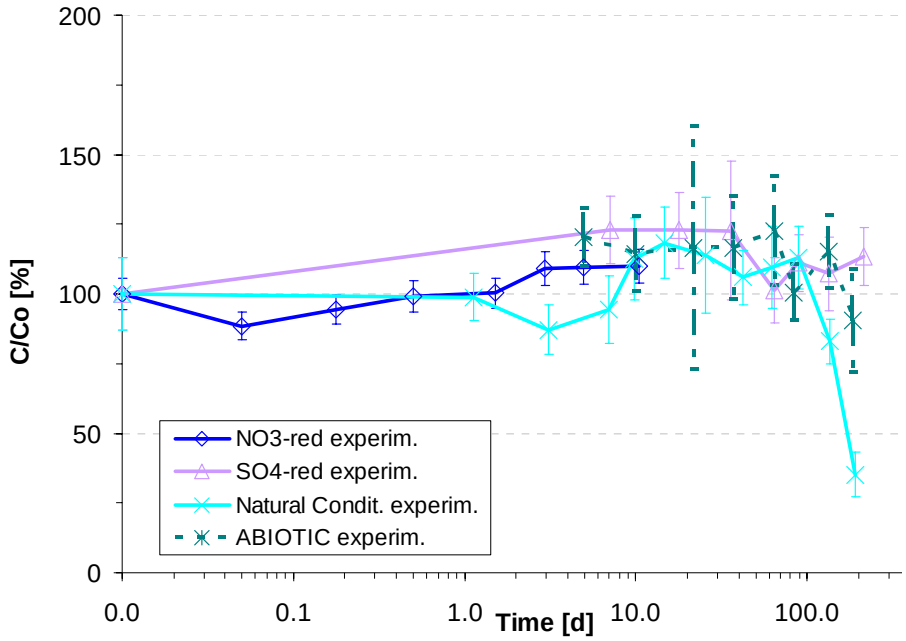


FIGURE C20: Temporal evolution of gemfibrozil average normalized concentration (C/Co [%]) in the NO3-reducing, SO4-reducing and Natural Conditions experiments versus the evolution in the abiotic experiment

COMMENTS to Figure C20:
No removal in the NO3- and SO4-reducing experiment.
After day 135, Gemfibrozil is biotically removed in the Natural Conditions experiments. Considering the error bars, an overall removal of about 40% could be estimated by the end of the experiment.

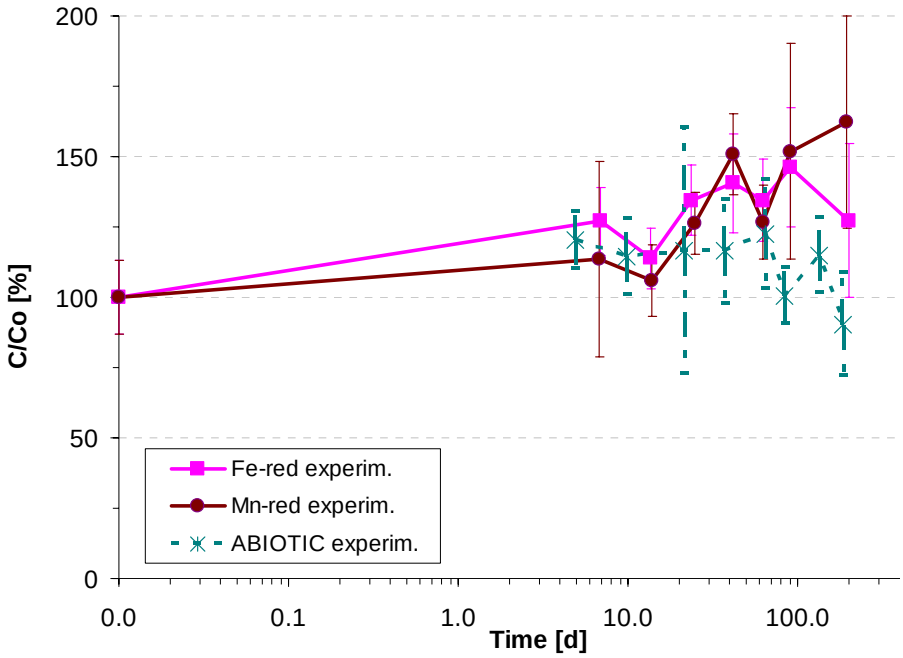


FIGURE C21: Temporal evolution of gemfibrozil average normalized concentration (C/Co [%]) in the Mn- and Fe-reducing experiments versus the evolution in the abiotic experiment

COMMENTS to Figure C21:
The high relative concentration reached by Gemfibrozil in the Mn- and Fe-reducing experiment are most likely only an effect of the respective initial concentrations Co being quite lower than the expected 1000ng/L and of the following concentrations (which are in the range of 1000ng/L). Possible explication: some problem during the sampling of the initial samples or during the analysis. Even considering the theoretical Co = 1000ng/L, no overall removal could be observed during both the Mn- and Fe-reducing experiment (plot not reported).

C3.3.5 Ibuprofen
(included in spiking solution “A”)

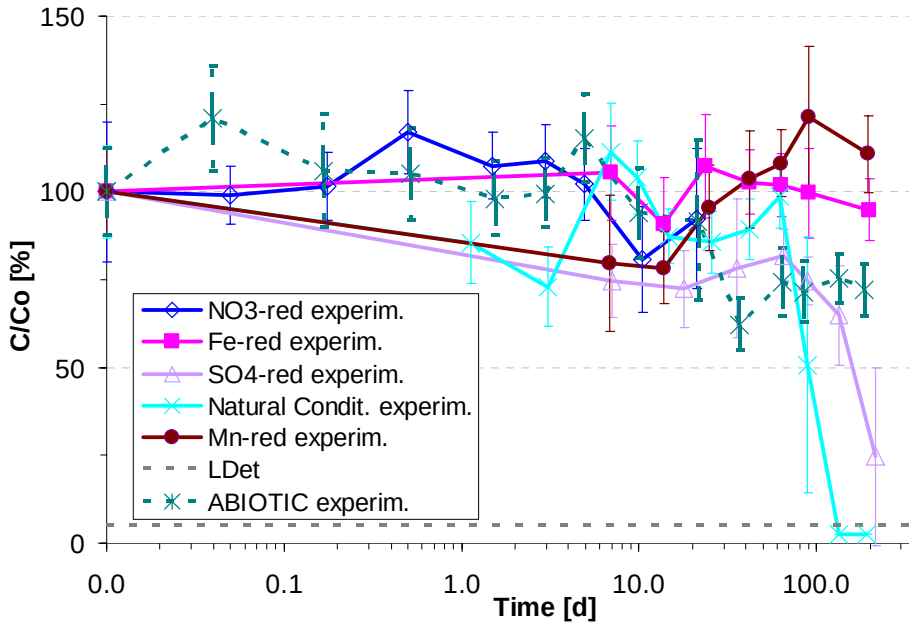


FIGURE C22: Evolution with time of ibuprofen average normalized concentration (C/Co [%]) in all the experiments (biotics and abiotic) with micropollutants at 1 µg/L individual initial concentration

COMMENTS to Figure C22:
The evolution of the abiotic experiment seems to be not coherent with the biotic ones. In fact, the plot show that some abiotic process is ocuring in this experiment, but not in the biotic ones (where biotic+abiotic processes are expected to occur). Thus, it will not be used in the following.

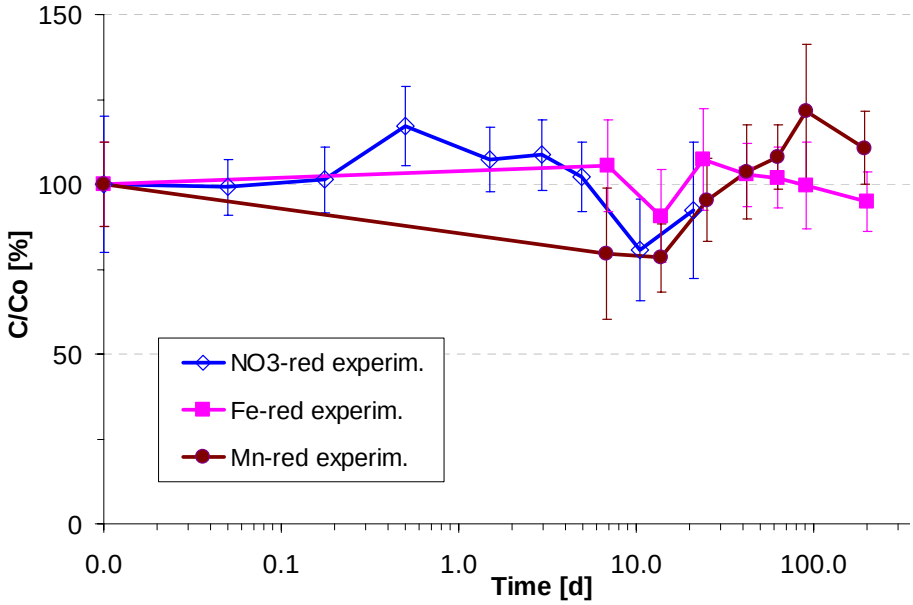


FIGURE C23: Temporal evolution of ibuprofen average normalized concentration (C/Co [%]) in the NO3-, Mn- and Fe-reducing experiments

COMMENTS to Figure C23:
No overall removal in the NO3-, Mn-, and Fe-reducing experiments.

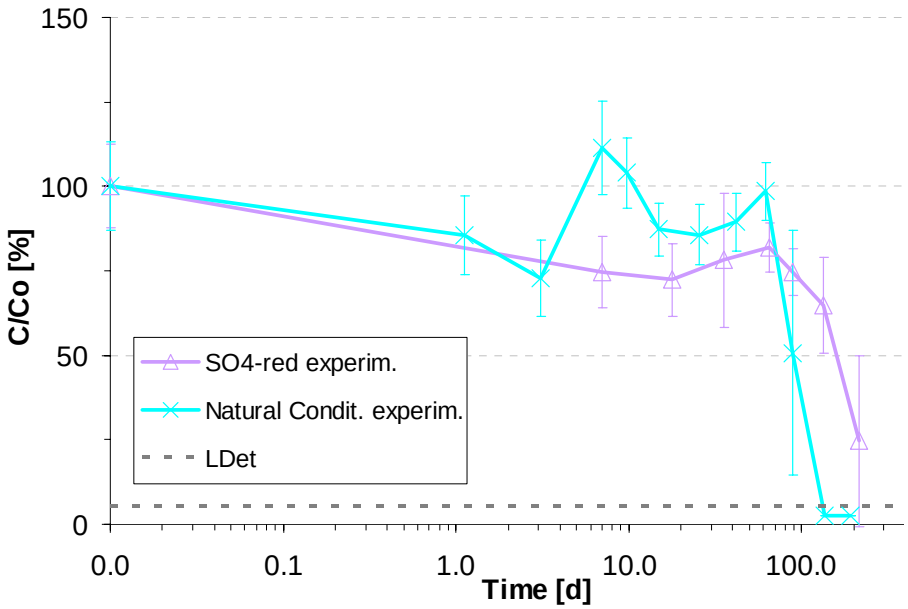


FIGURE C24: Temporal evolution of ibuprofen average normalized concentration (C/Co [%]) in the SO4-reducing and Natural Conditions experiments

COMMENTS to Figure C24:
Ibuprofen was completely removed by day 135 in the Natural Conditions experiments. An overall removal ranging between 50% and 100% was yielded in the SO4-reducing experiment by the 215. Due to the unreliability of results from the abiotic experiment, it could not be distinguished the nature of the processes involved (biotic or abiotic?). The fluctuations and the wide error bars enable also to identify the beginning of such processes.

C3.3.6 Sulfamethoxazole
(included in spiking solution “A”)

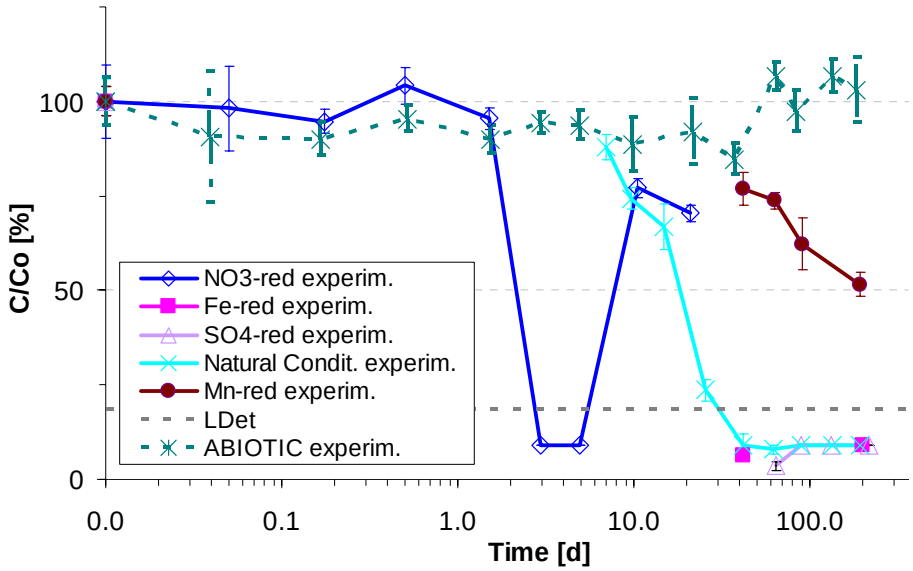


FIGURE C25: Evolution with time of sulfamethoxazole average normalized concentration (C/Co [%]) in all the experiments (biotics and abiotic) with micropollutants at 1 µg/L individual initial concentration

COMMENTS to Figure C25:
Details on the fate of sulfamethoxazole in the NO3-red. experiment are presented in Chapter 3. Its short duration, anyway, does not allow the comparison with the rest of experiments in the long term. Even if the concentration curves are not complete because of the unreliability of numerous results (analytical problems), a biotic removal could be observed in all the remaining biotic tests. By day 42, sulfamethoxazole has completely disappeared in the Fe-, SO4- reducing and Natural Conditions experiments. In the Mn-red. experiment an overall biotic removal of about 50% could be observed by day 194.

C3.3.7 Estrone
(included in spiking solution “A”)

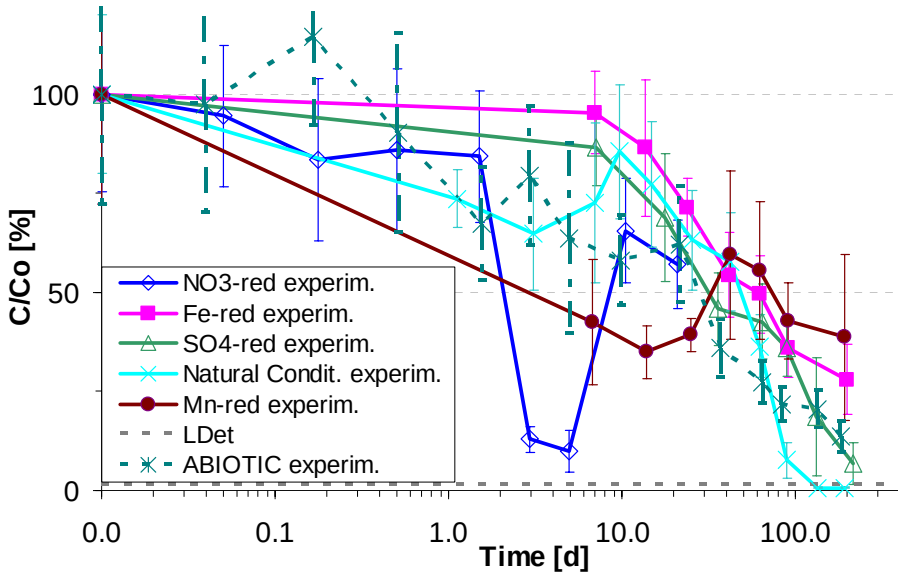


FIGURE C26: Evolution with time of estrone average normalized concentration (C/Co [%]) in all the experiments (biotics and abiotic) with micropollutants at 1 µg/L individual initial concentration

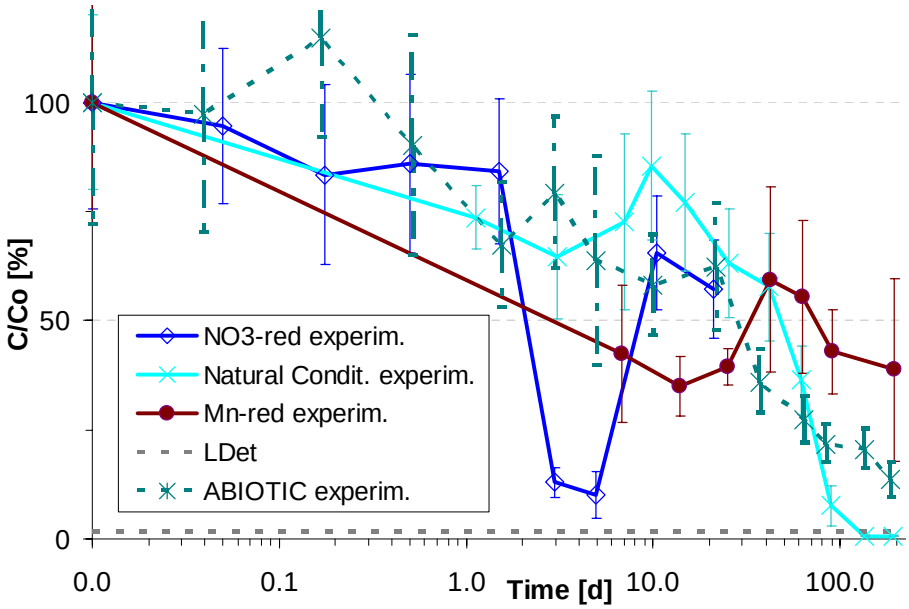


FIGURE C27: Temporal evolution of estrone average normalized concentration (C/Co [%]) in the NO3-reducing, Mn-reducing and Natural Conditions experiments versus the evolution in the abiotic experiment

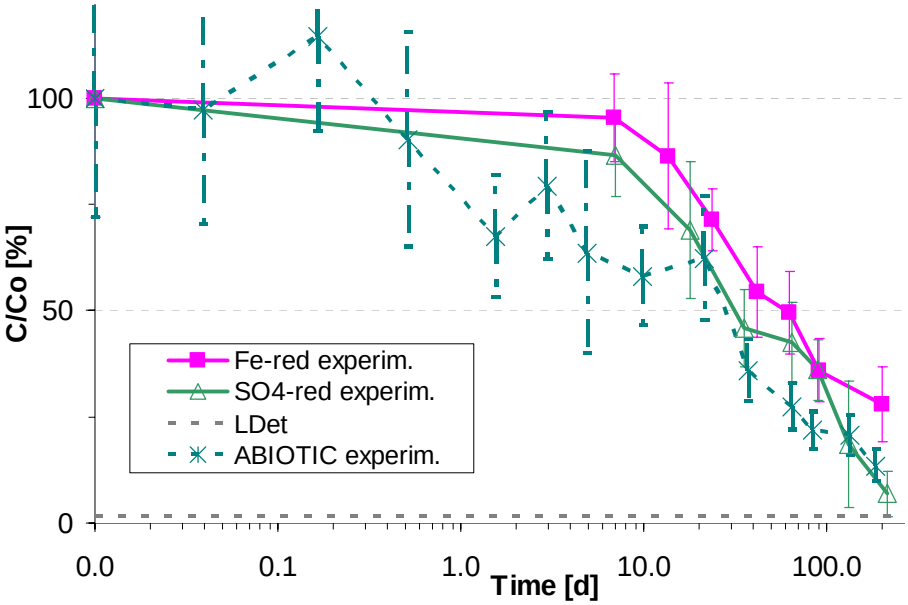


FIGURE C28: Temporal evolution of estrone average normalized concentration (C/Co [%]) in the Fe- and SO4- reducing experiments versus the evolution in the abiotic experiment

COMMENTS to Figure C26, C27 and C28:
The overall evolution of estrone concentrations in all experiment is almost similar, including that in the abiotic test. This suggests that some continuous abiotic process is responsible of the removal of the target micropollutant experiments long. The overall removal after about 200 day reached the 70 to 90%. Only in the case of the Natural Conditions experiment, some additional biotic process likely occurred after day 64, and complete removal is yielded at day 135.
The peculiar disappearence and furher rebound of concentrations during the NO3-reducing experiment, similar to that described for the amine containing compounds diclofenac and sulfamethoxazole in chapter 3, could not be explanined.

C3.3.8 Atrazine
(included in spiking solution “B”)

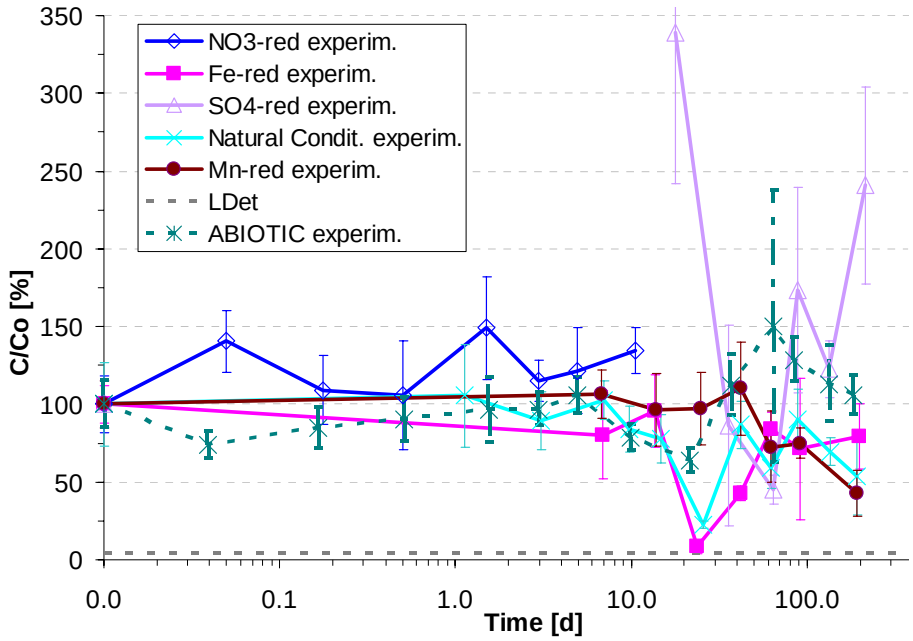


FIGURE C29: Evolution with time of atrazine average normalized concentration (C/Co [%]) in all the experiments (biotics and abiotic) with micropollutants at 1 µg/L individual initial concentration

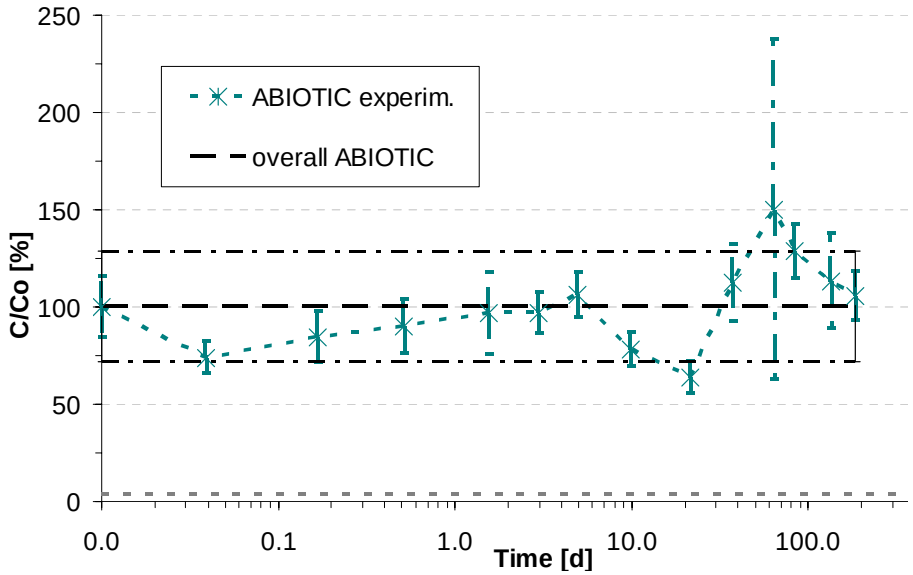


FIGURE C30: Evolution with time of atrazine average normalized concentration in the abiotic experiment and plot of the calculated “overall abiotic” concentration.

COMMENTS to Figure C30:
No abiotic removal could be observed for atrazine. To simplify the comparison with the biotic experiments, an “overall abiotic” concentration was calculated as described for carbamazepine (§3.3.2), amounting to 100 ± 29%.

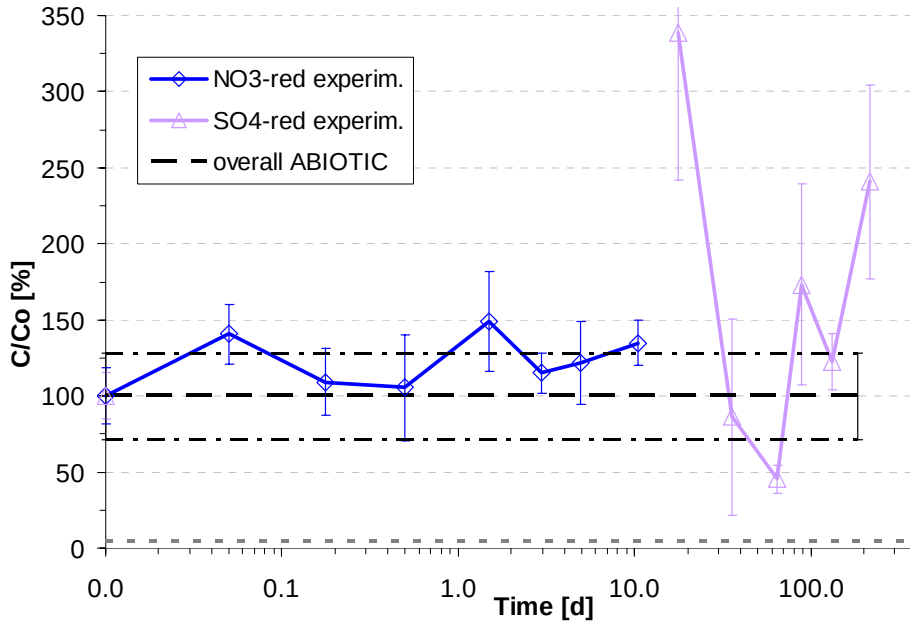


FIGURE C31: Temporal evolution of atrazine average normalized concentration (C/Co [%]) in the NO3- and SO4-reducing experiments versus the "overall abiotic" concentration

COMMENTS to Figure C31:
No biotic removal could be identified in the NO3-reducing experiment.
Regarding the SO4-reducing experiment, almost all concentrations are above 100%. This is likely an effect of atrazine initial concentrations Co being significantly lower (around 320 ng/L) than the expected 1000ng/L and of the following concentrations, and affecting the calculation of the normalized concentrations. In spite of it, some biotic reversible procees seem to affect atrazine between day 17 and 215 of the SO4-red. experiment. Unfortunately, we could not identify it.

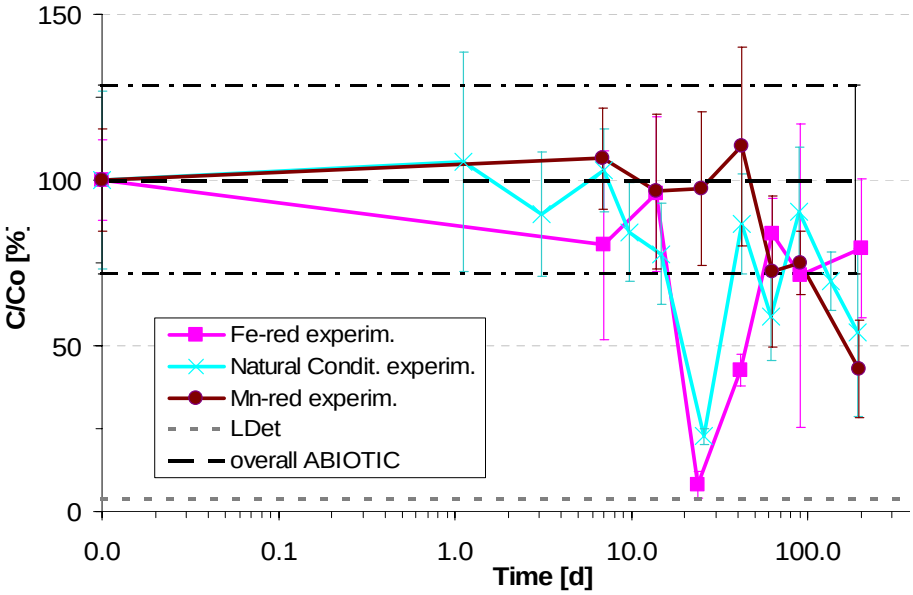


FIGURE C32: Temporal evolution of atrazine average normalized concentration (C/Co [%]) in the Mn-reducing, Fe-reducing and Natural Conditions experiments versus the "overall abiotic" concentration

COMMENTS to Figure C32:
Taking into account the error bars, no overall removal was observed in the Mn-reducing, Fe-reducing and Natural Conditions experiments. Nevertheless, similar to the case of SO4-reducing experiment but shifted in the time scale, a sudden drop followed by recuperation of atrazine concentration could be observed between days 14 to 65-89.

C3.3.9 Simazine
(included in spiking solution "A")

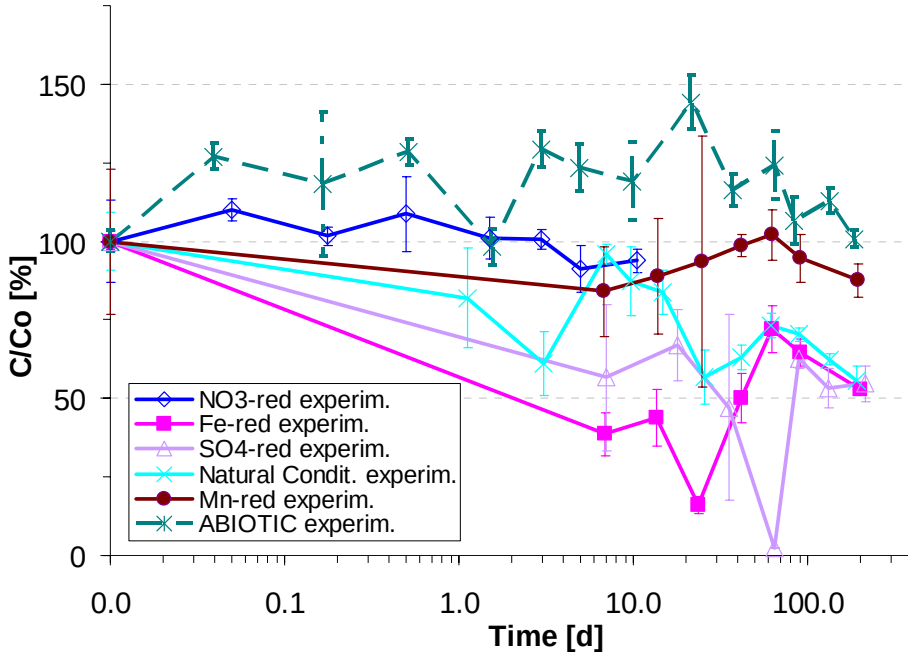


FIGURE C33: Evolution with time of simazine average normalized concentration (C/Co [%]) in all the experiments (biotics and abiotic) with micropollutants at 1 µg/L individual initial concentration

COMMENTS to Figure C33:
Almost all concentrations in the abiotic experiment are well above 100%. Even considering the error bars, concentrations do not solape the one with the others so as to allow the calculation and use of an "overall abiotic". Thus, the abiotic data are not considered as reference for the biotic experiments in the followig.

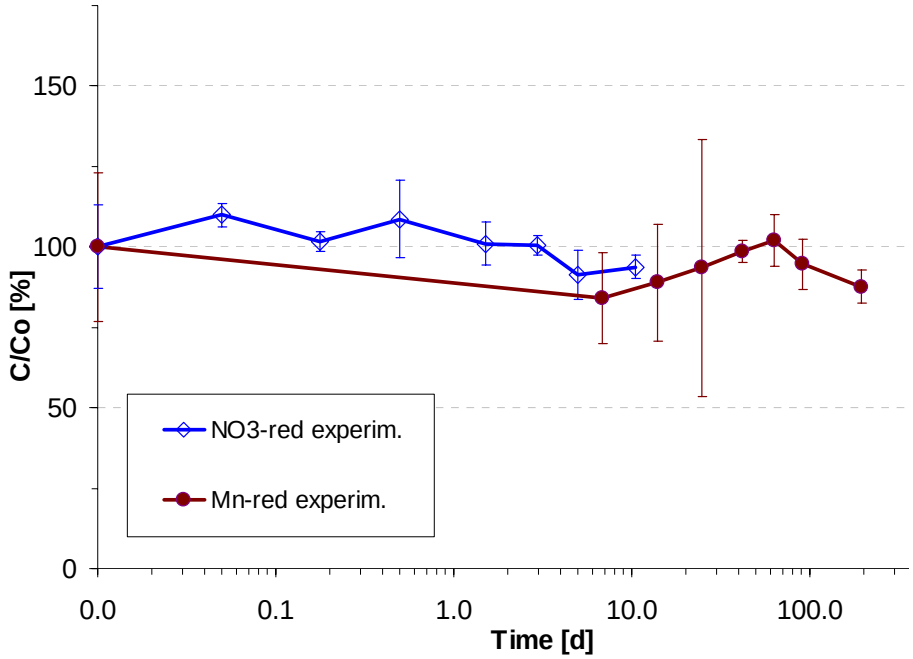


FIGURE C34: Temporal evolution of simazine average normalized concentration (C/Co [%]) in the NO3- and Mn- reducing experiments

COMMENTS to Figure C34:
No removal could be observed in the NO3- and Mn-reducing experimest.

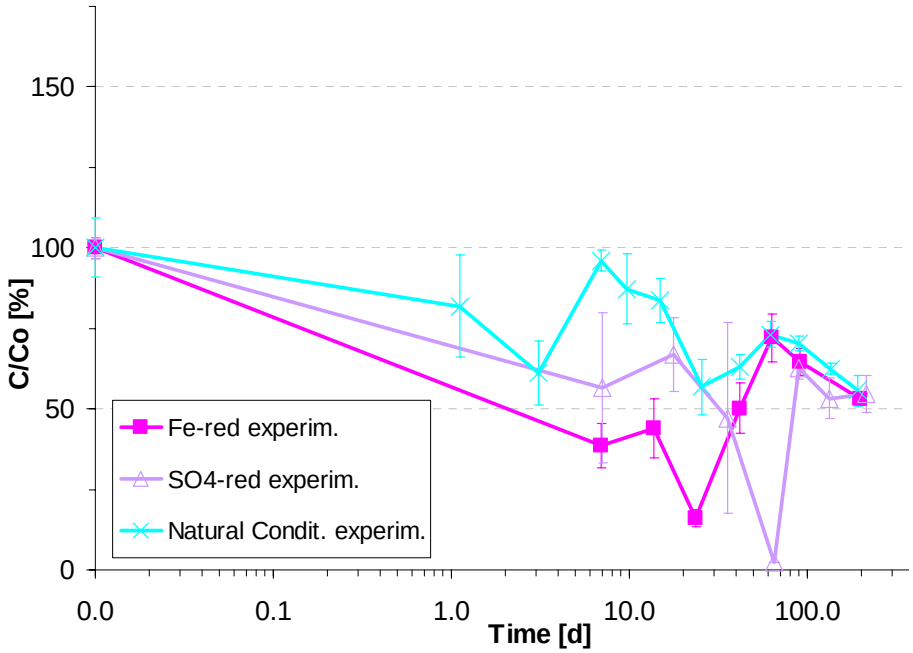


FIGURE C35: Temporal evolution of simazine average normalized concentration (C/Co [%]) in the Fe-reducing, SO4-reducing and Natural Conditions experiments

COMMENTS to Figure C35:
In the case of Fe-, SO4-red and Natural Conditions experiments two main points could be commented:

- similar to the previously described case of atrazine, some partially reversible process seem to affect simazine between day 10 and 87, resulting in a drop followd by at least a partial recovery of concentrations
- an overall removal of 40-50% was reached around day 200. Due to the unreliability of the reference abiotic experiment, the nature of the process involved (biotic or abiotic?) could not be distinguished.

C3.3.10 Terbuthylazine
(included in spiking solution “B”)

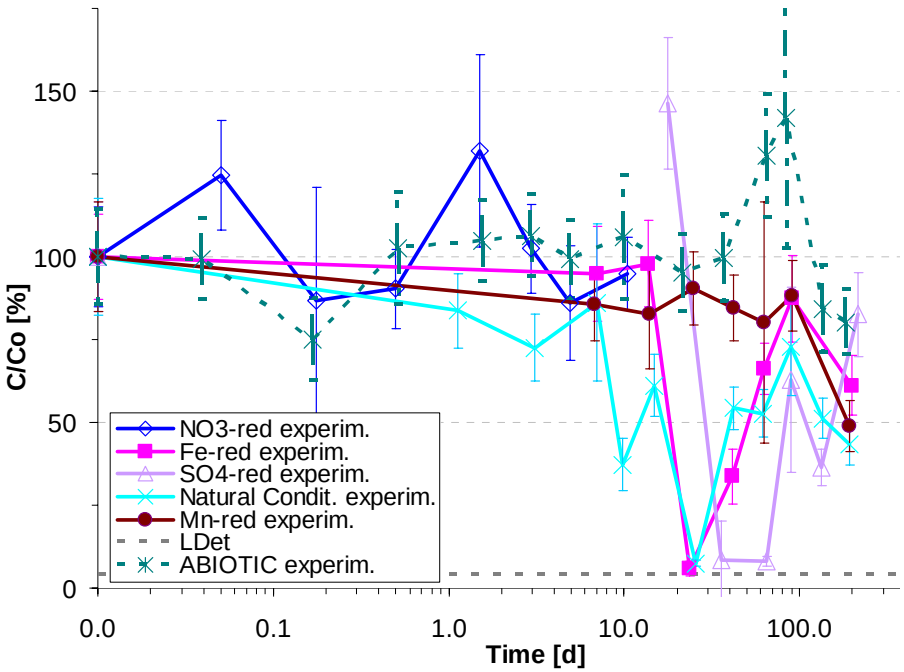


FIGURE C36: Evolution with time of terbuthylazine average normalized concentration (C/Co [%]) in all the experiments (biotics and abiotic) with micropollutants at 1 µg/L individual initial concentration

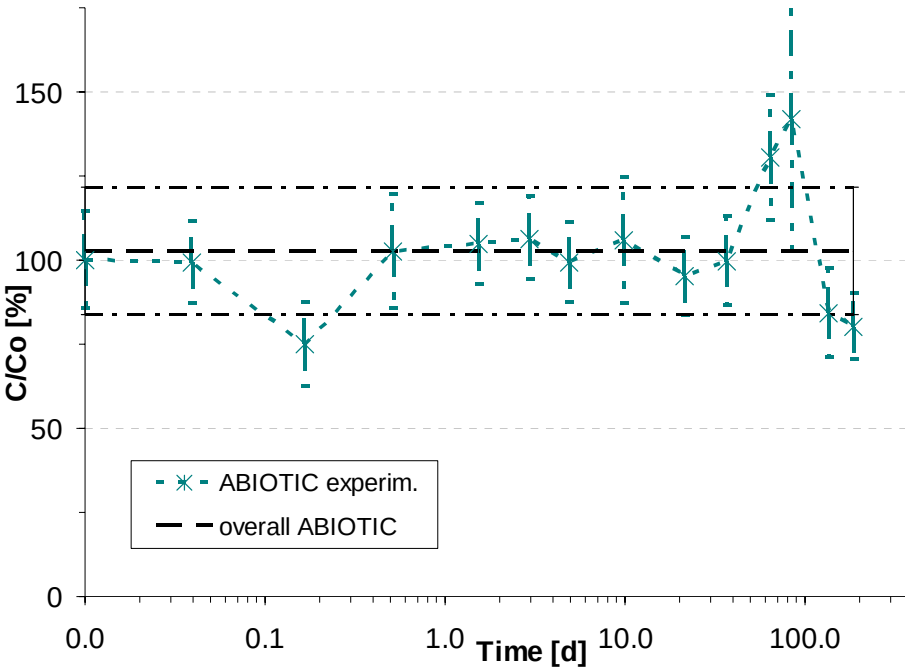


FIGURE C37: Evolution with time of terbuthylazine average normalized concentration in the abiotic experiment and plot of the calculated "overall abiotic" concentration.

COMMENTS to Figure C37:
No abiotic removal could be observed. To simplify the comparison with the biotic experiments, an "overall abiotic" concentration and its corresponent error bar were calculated by using eq. C1 of §C3. They amounted to 103 ± 19%.

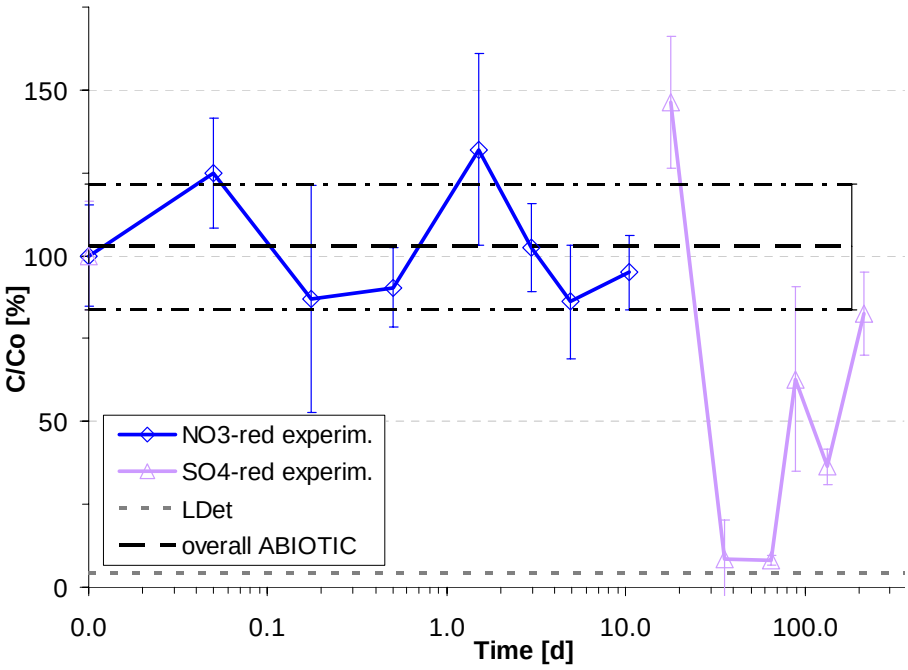


FIGURE C38: Temporal evolution of terbuthylazine average normalized concentration (C/Co [%]) in the NO3- and SO4- reducing experiments versus versus the "overall abiotic" concentration

COMMENTS to Figure C38:
No overall biotic removal was yielded for terbuthylazine by the end of the NO3 and SO4-reducing experiments. Nevertheless, a sudden drop and rebound of its concentrations could be observed bewteen day 17 and 90.

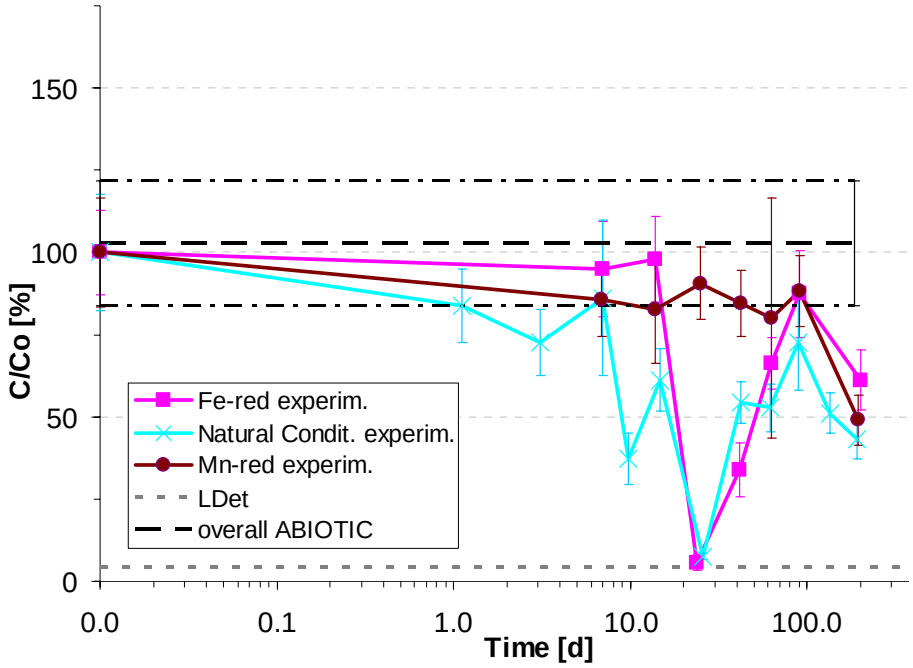


FIGURE C39: Temporal evolution of terbuthylazine average normalized concentration (C/Co [%]) in the Mn-reducing, Fe-reducing and Natural Conditions experiments versus the "overall abiotic" concentration

No overall biotic removal was yielded for terbuthylazine by the end of the NO3 and SO4-reducing experiments. Nevertheless, a sudden drop and rebound of its concentrations could be observed bewteen day 17 and 90 of the SO4 red. experiment. In the same time lapse, a similar behaviour was exhibited in the Fe-red. and the Natural Conditions tests, while in Mn-red. test the concentrations remained almost constant up to day 90. After day 90, a biotic removal of about 40% could be estimated in the three tests, being also characterized by similar removal rates.

C3.3.11 Summary on the fate of chlorotriazines (atrazine, simazine, terbuthylazine)

In the following Figures, the evolutions of the studied chlorotriazines (atrazine, simazine, terbuthylazine) are plotted jointly for each experiment. It could be noted that in almost all tests the fate of these compounds was very similarly. The peculiar drop and recovery of concentrations occured simultaneously for the three of them.

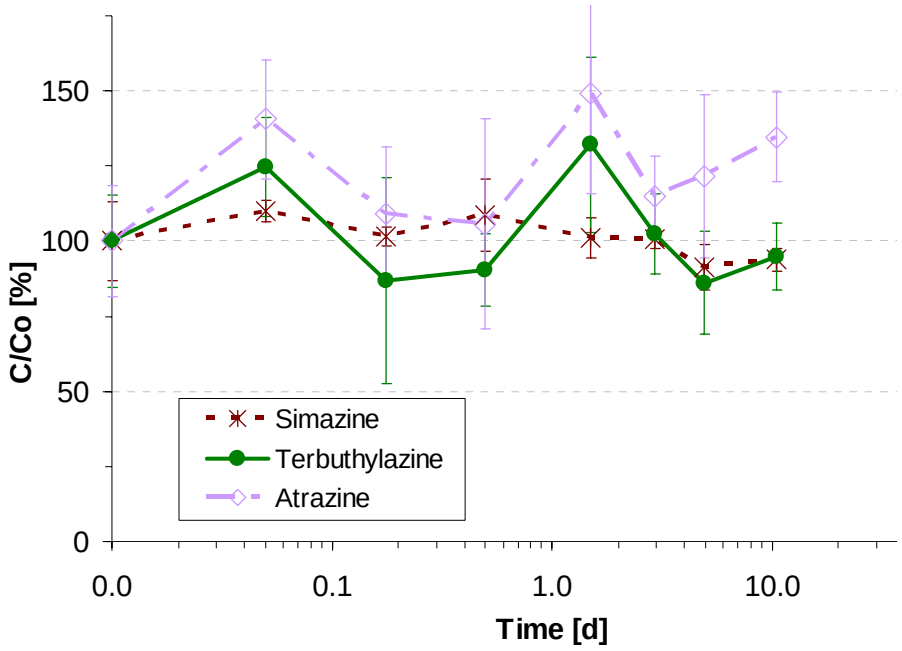


FIGURE C40: Temporal evolution of chlorotriazines average normalized concentrations (C/Co [%]) in the NO3-reducing experiment

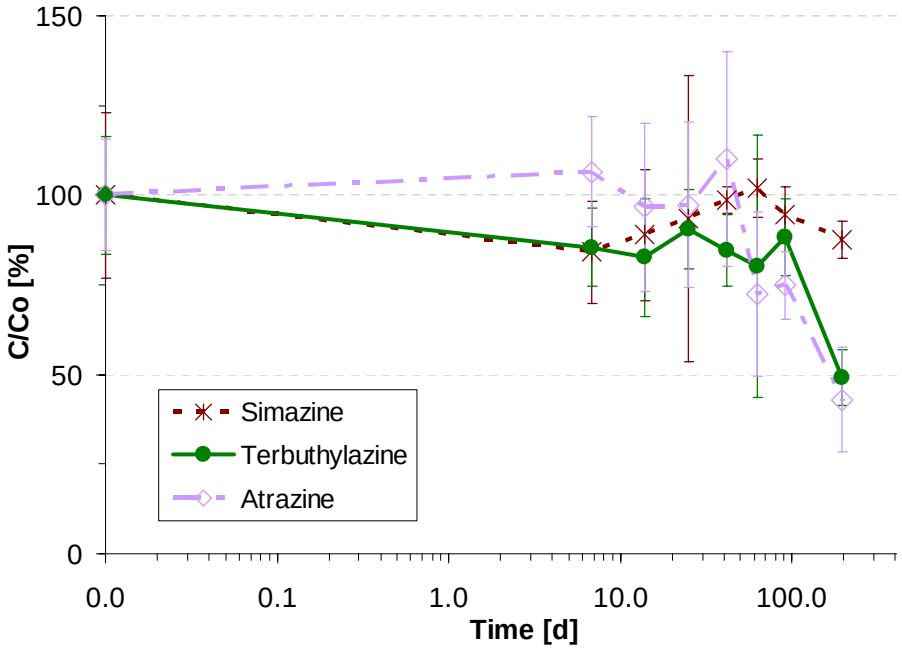


FIGURE C41: Temporal evolution of chlorotriazines average normalized concentrations (C/Co [%]) in the Mn-reducing experiment

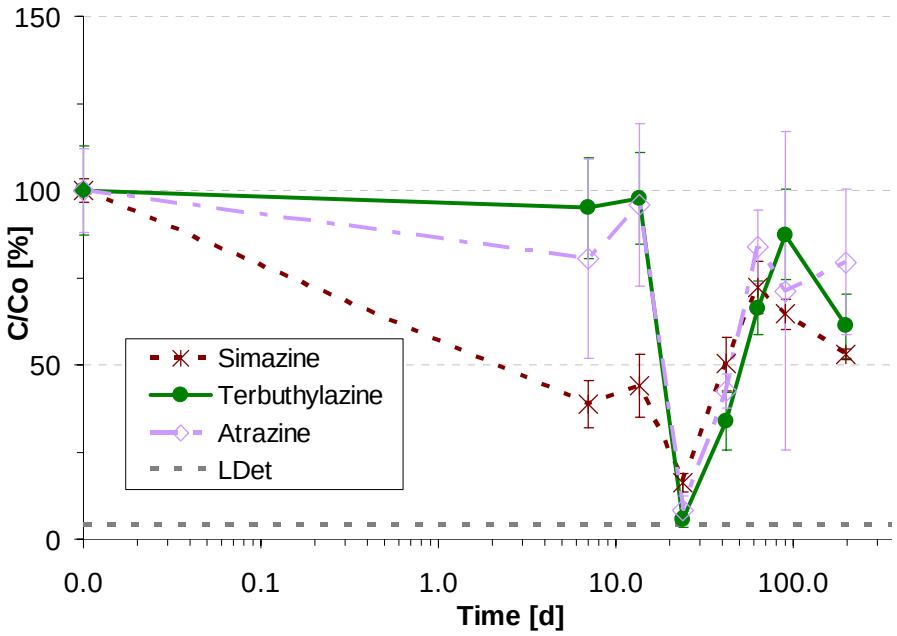


FIGURE C42: Temporal evolution of chlorotriazines average normalized concentrations (C/Co [%]) in the Fe-reducing experiment

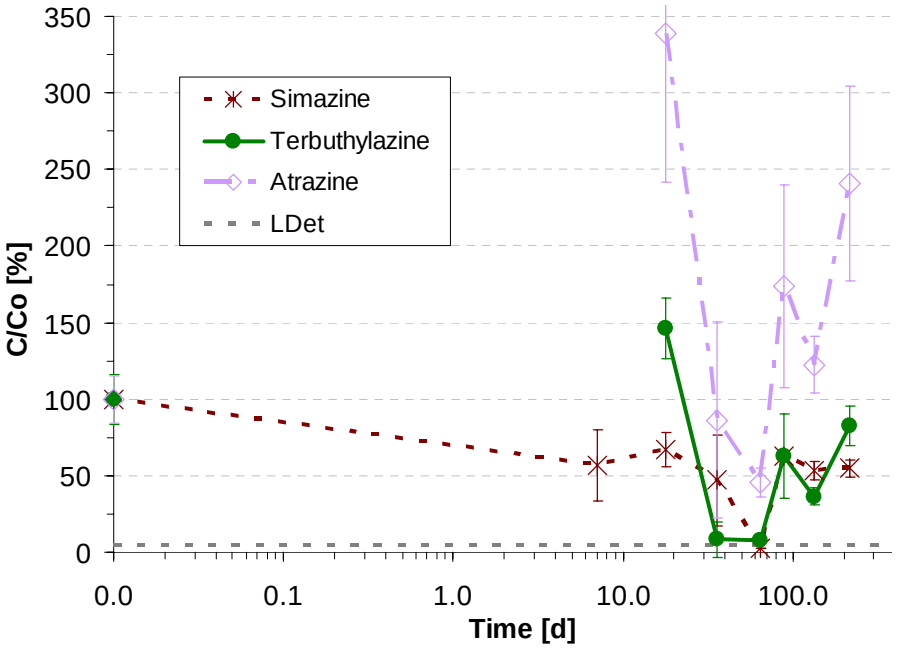


FIGURE C43: Temporal evolution of chlorotriazines average normalized concentrations (C/Co [%]) in the SO4-reducing experiment

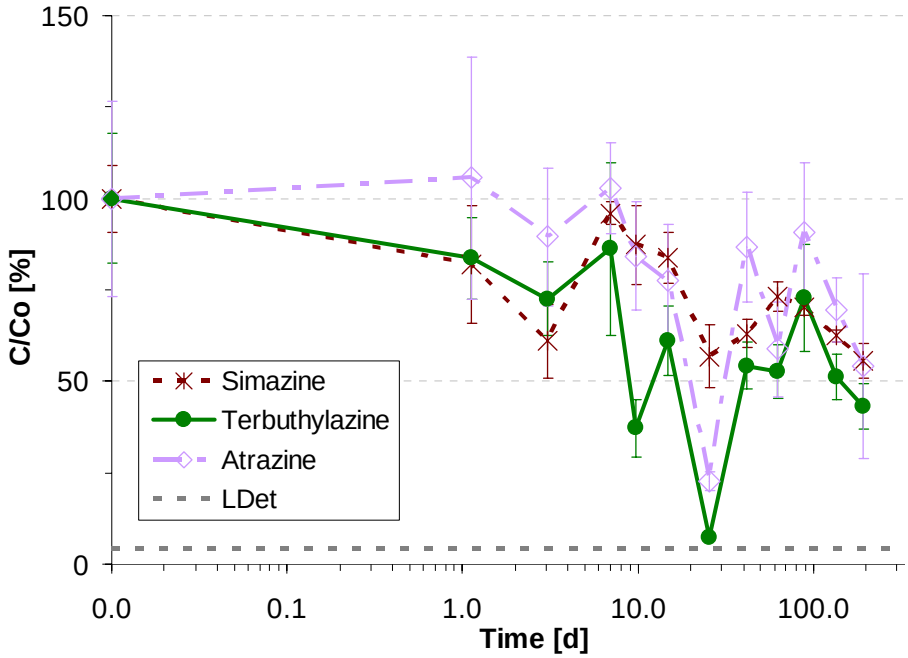


FIGURE C44: Temporal evolution of chlorotriazines average normalized concentrations (C/Co [%]) in the Natural Conditions experiment

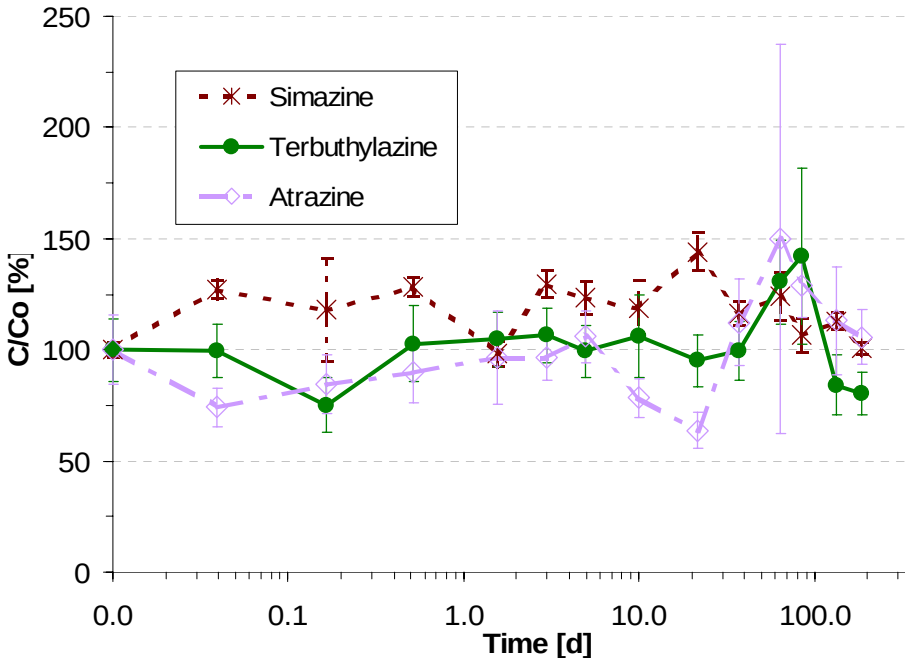


FIGURE C45: Temporal evolution of chlorotriazines average normalized concentrations (C/Co [%]) in the abiotic experiment

C3.3.12 Prometryne
(included in spiking solution “B”)

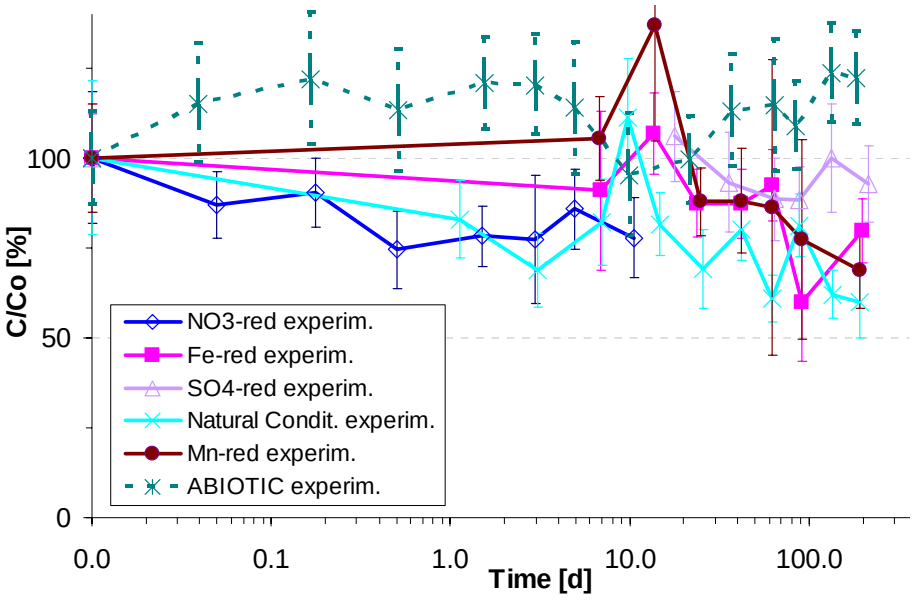


FIGURE C46: Evolution with time of prometryne average normalized concentration (C/Co [%]) in all the experiments (biotics and abiotic) with micropollutants at 1 µg/L individual initial concentration

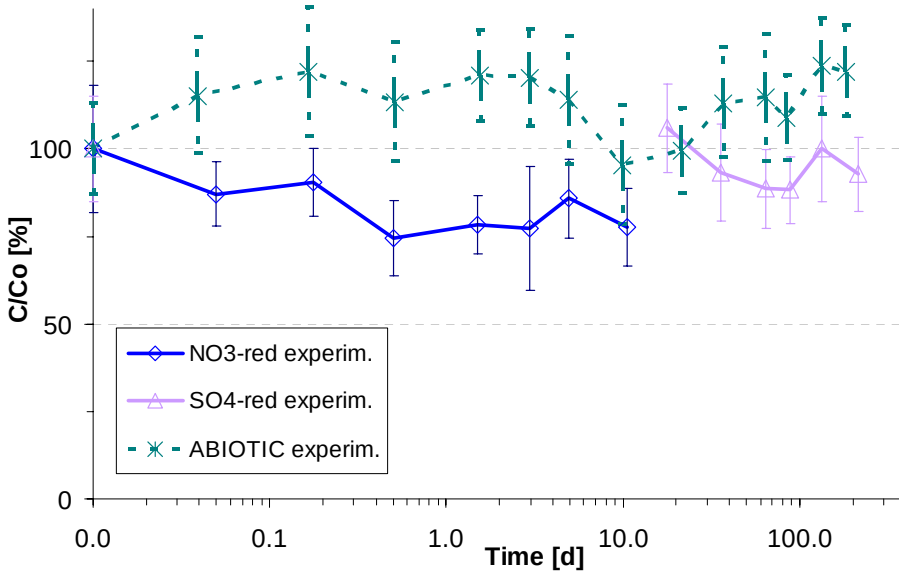


FIGURE C47: Temporal evolution of prometryne average normalized concentration (C/Co [%]) in the NO3- and SO4-reducing experiments versus the evolution in the abiotic experiment

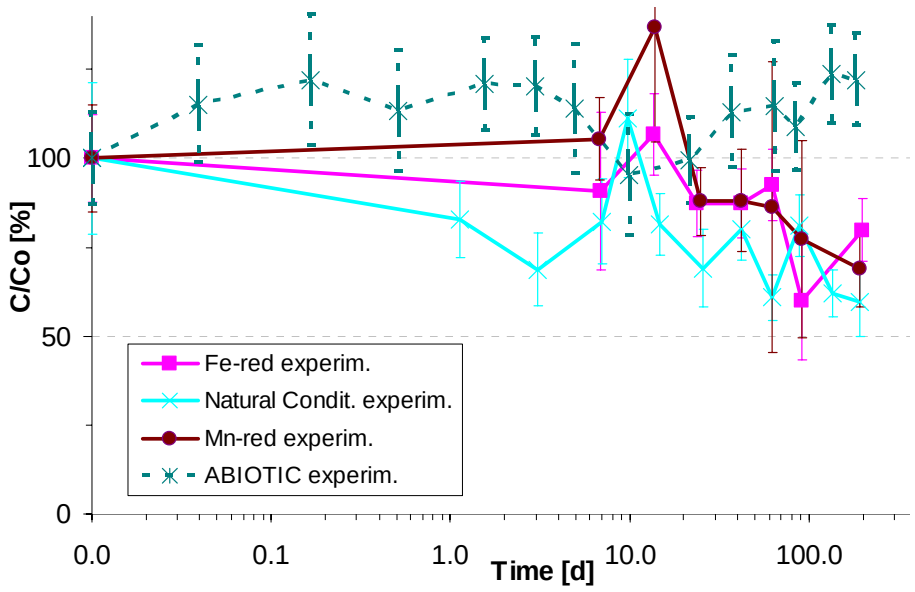


FIGURE C48: Temporal evolution of prometryne average normalized concentration (C/Co [%]) in the Mn-reducing, Fe-reducing and Natural Conditions experiments versus the evolution in the abiotic experiment

COMMENTS to Figure C47 and C48
No abiotic removal for prometryn. Not even biotic removal in the NO3 and SO4-reducing experiments. A maximum of about 40% overall biotic removal could be observed in the Mn- and Fe-reducing as well as in the Natural Conditions experiment

C3.3.13 Diuron
(included in spiking solution "A")

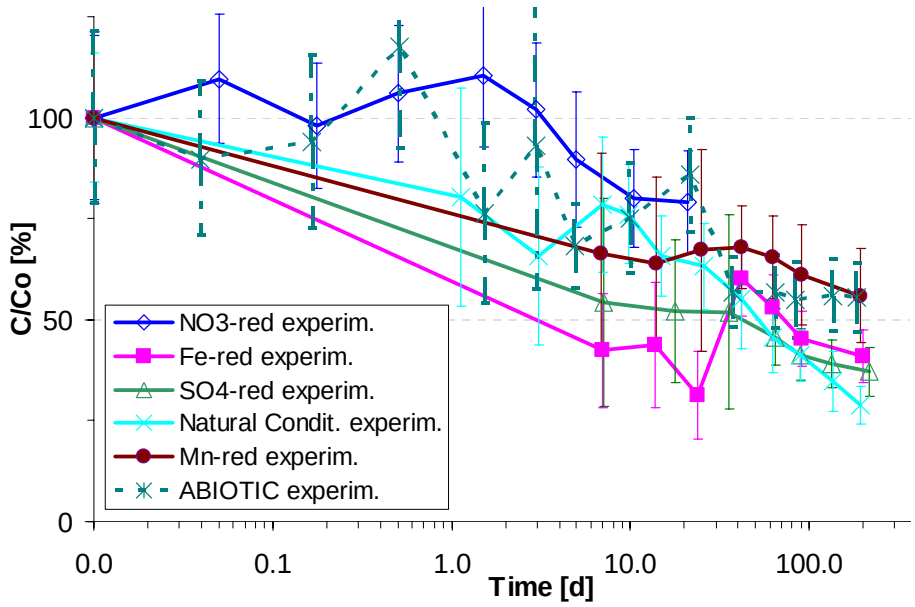


FIGURE C49: Evolution with time of diuron average normalized concentration (C/Co [%]) in all the experiments (biotics and abiotic) with micropollutants at 1 µg/L individual initial concentration

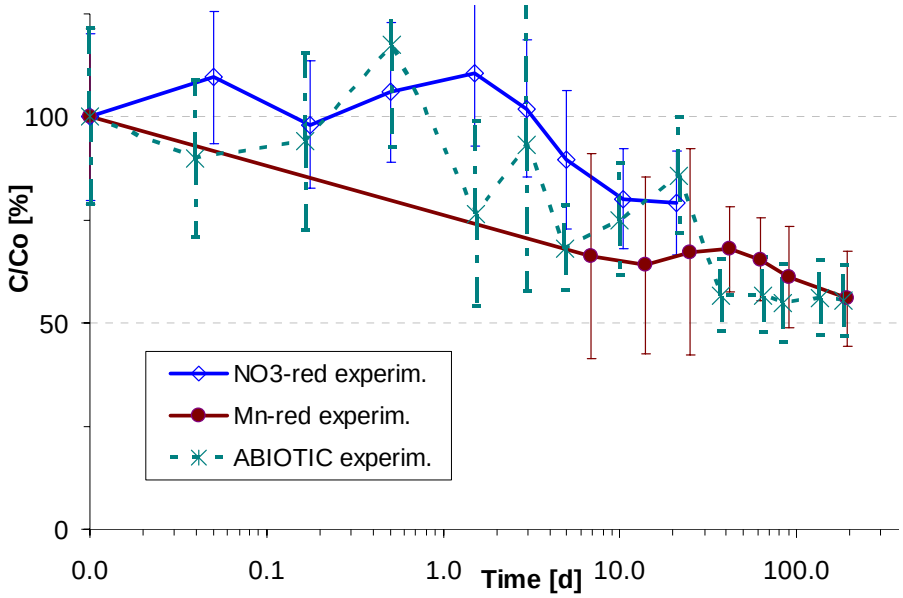


FIGURE C50: Temporal evolution of diuron average normalized concentration (C/Co [%]) in the NO3- and Mn-reducing experiments versus the evolution in the abiotic experiment

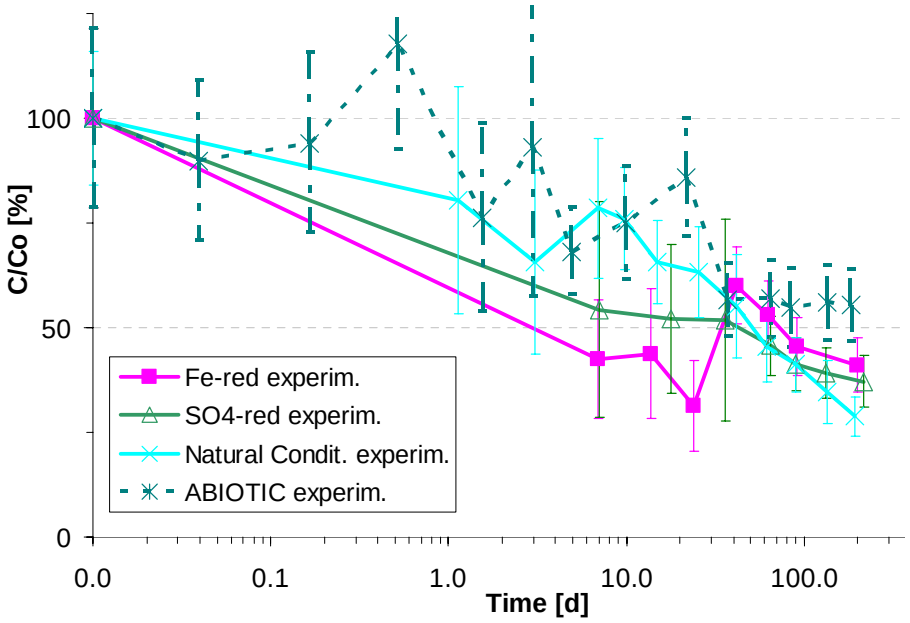


FIGURE C51: Temporal evolution of diuron average normalized concentration (C/Co [%]) in the Fe-reducing, SO4-reducing and Natutal Conditions experiments versus the evolution in the abiotic experiment

COMMENTS to Figure C49, C50 and C51:
The evolution of diuron concentrations is quite similar in all experiments.
Up to day 37, the removal of about 45% suffered by this compound seems to be associated to some abiotic process. By then, no further decreasing could be clearly observed in the Mn- and Fe-reducing experiments. Possibly some additional biotic elimination of diuron occurrs in the SO4-

reducing and the Natural Conditions experiment, amounting to a maximum 20% in the case of Nat. Condit. experiment.

C3.3.14 Chlorphenvinfos
(included in spiking solution “B”)

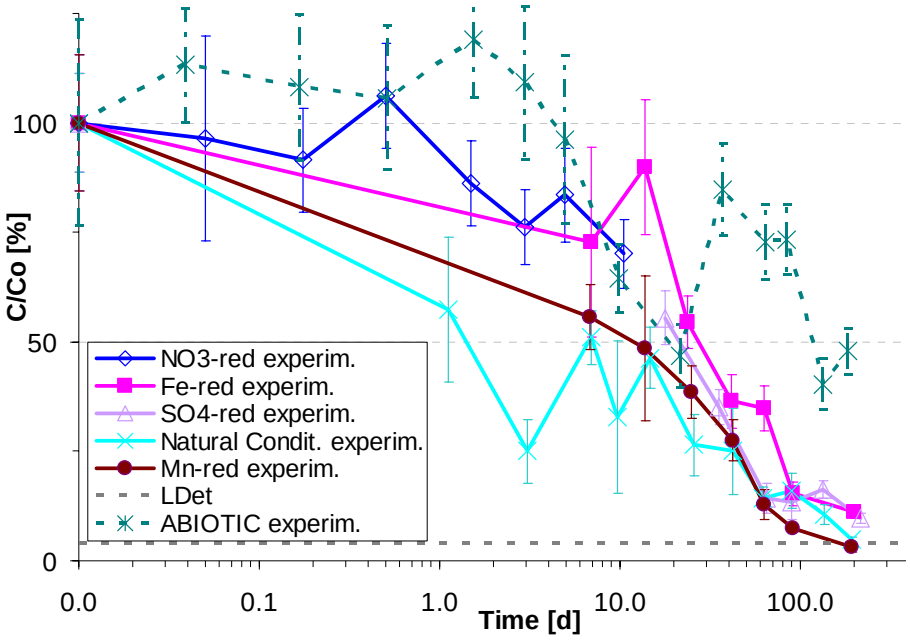


FIGURE C52: Evolution with time of chlorphenvinfos average normalized concentration (C/Co [%]) in all the experiments (biotics and abiotic) with micropollutants at 1 µg/L individual initial concentration

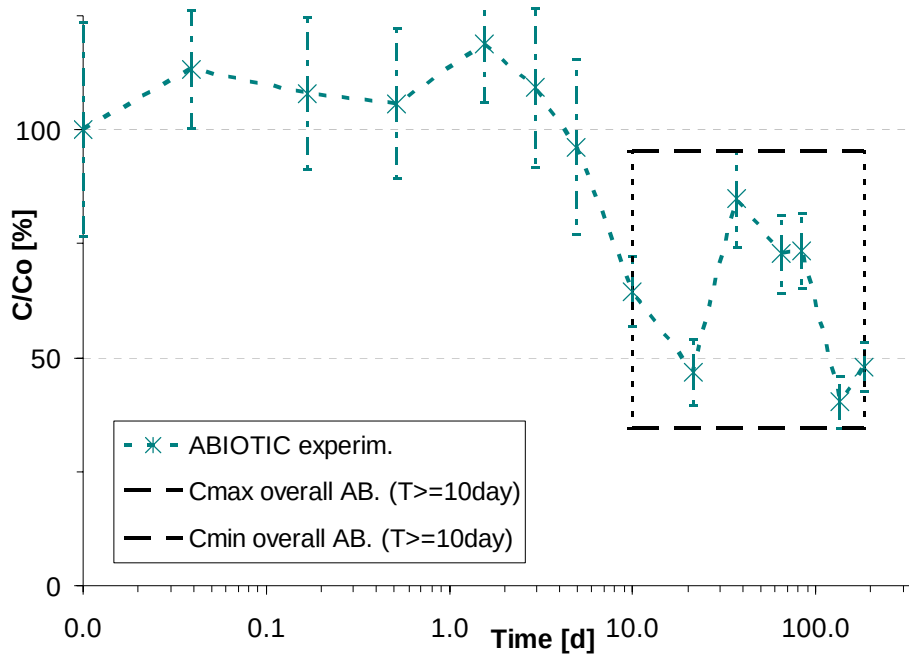


FIGURE C53: Evolution with time of chlorphenvinfos average normalized concentration in the abiotic experiment

COMMENTS to Figure C53:
The fluctuations of concentrations in the abiotic experiment hindered the intrepretation of results for chlorphenvinfos. An abiotic removal could be observed up to day 10 to 21. The final resulting abiotic concentration lied inside the wide range between 34 and 95% (indicated by discontinuous lines in the plot, between “Cmax” and “Cmin”).

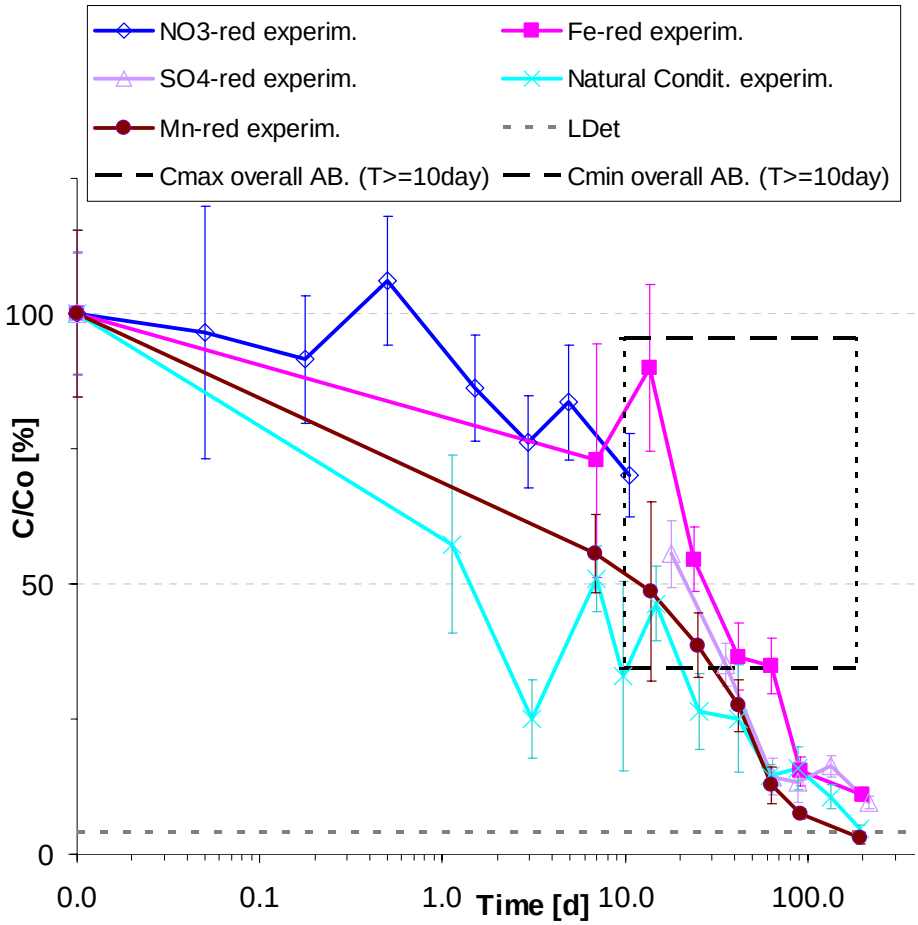


FIGURE C54: Temporal evolution of chlorphenvinfos average normalized concentration (C/Co [%]) in all the biotic experiments versus the range for the final abiotic concentration

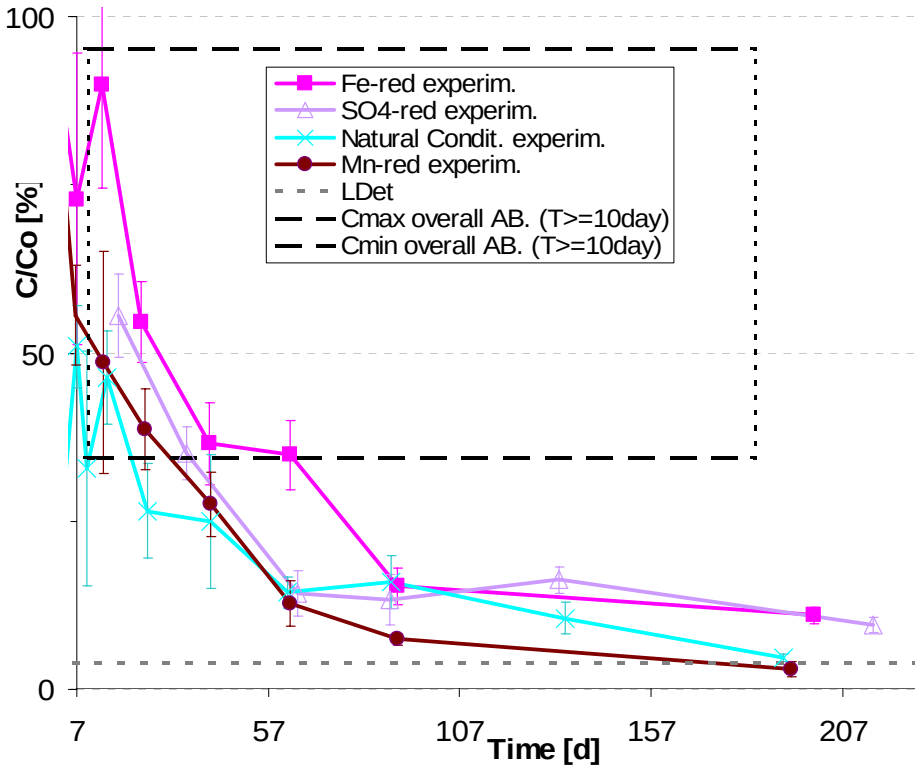


FIGURE C55: Zoom for time > 7days on the temporal evolution of chlorphenvinfos average normalized concentration (C/Co [%]) in the Mn-, Fe-, SO4-reducing and Natural Conditions experiments versus the range for the final abiotic concentration. An arithmetic scale time-concentration is used in the plot

COMMENT to Figure C55:
A part from the NO3-red. experiment which was too short to be compared with the other tests, a further biotic removal could be observed for chlorphyrifos under all reducing conditions, leading to 90% or complete elimination of the compound. The trends were quite similar, being possibly the slower ans smaller elimination that of the Fe-red. experiment. That is, redox conditions seem have only slight effect on the fate of chlorphenvinfos.

C3.3.15 Chlorpyrifos
(included in spiking solution “B”)

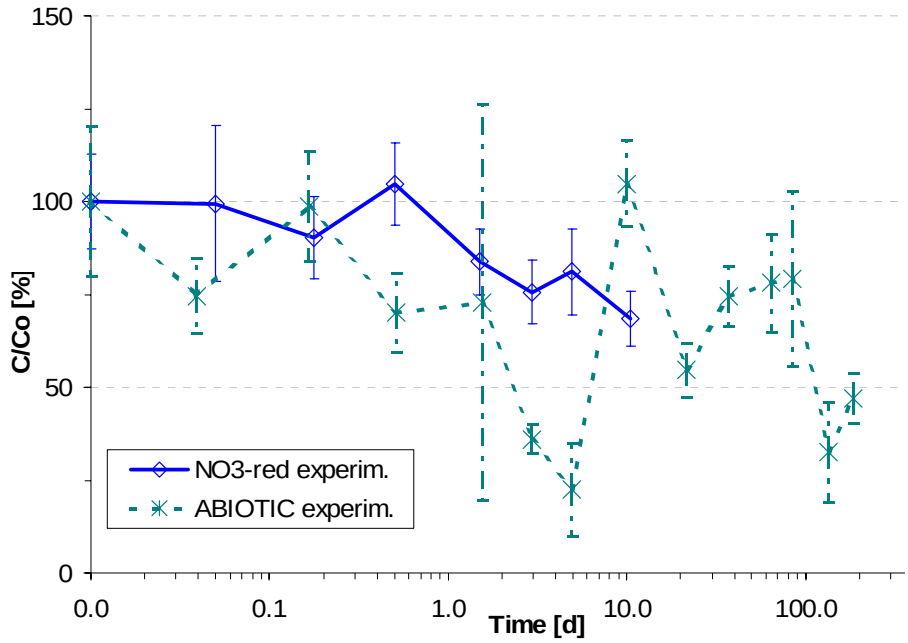


FIGURE C56: Evolution with time of chlorpyrifos average normalized concentration (C/Co [%]) in the NO3-reducing and abiotic experiments with micropollutants at 1 µg/L individual initial concentration

COMMENT to Figure C56:
Among the biotic experiments, only results for the NO3-red. test were reliable. The evolution of the abiotic concentrations is characterized by wide fluctuations and hinder the interpretation of results. Anyway, no removal under NO3-reducing conditions (actually occurring up to day 10 of the NO3-red. experiment) seem to occurr.

C3.3.16 Diazinon
(included in spiking solution “B”)

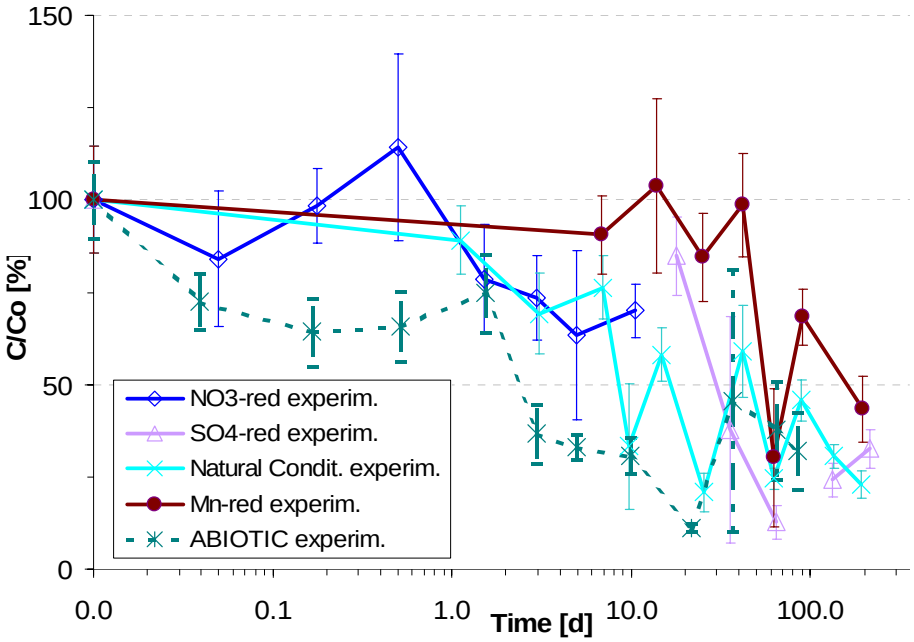


FIGURE C57: Evolution with time of diazinon average normalized concentration (C/Co [%]) in all the experiments (biotics and abiotic) with micropollutants at 1 µg/L individual initial concentration.

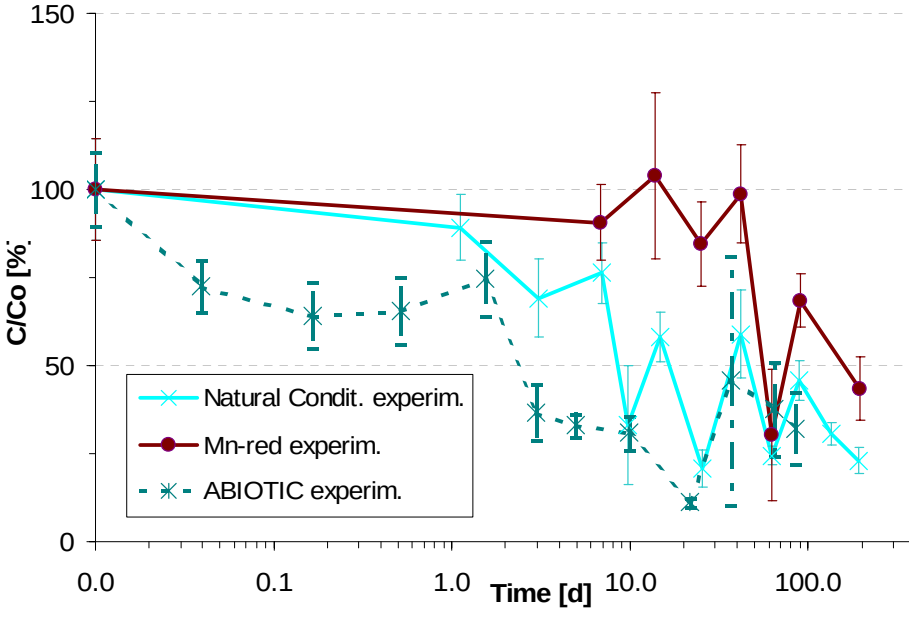


FIGURE C58: Temporal evolution of diazinon average normalized concentration (C/Co [%]) in the Mn-reducing and Natural Conditions experiments versus the evolution in the abiotic experiment

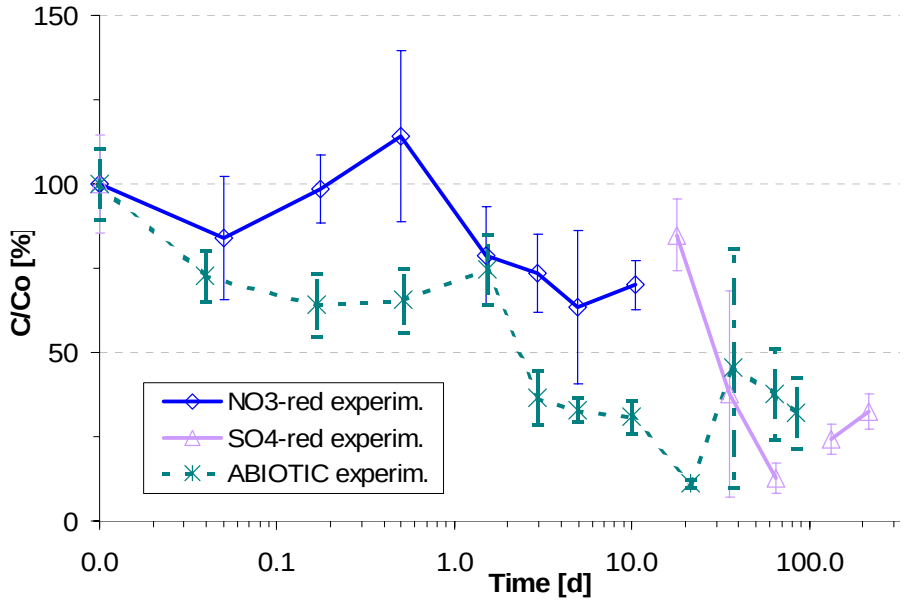


FIGURE C59: Temporal evolution of diazinon average normalized concentration (C/Co [%]) in the NO3- and SO4-Mn-reducing experiments versus the evolution in the abiotic experiment

COMMENTS to Figure C59:
Results from the Fe-red experiment were not realiable (possiible contamination during the analysis). The wide fluctuations of concentrations and the big errors in the remaining experiments hindered the interpretation of diazinon behaviour. Still, an overall (abiotic or biotic?) removal up to 75% could be observed by day 190-210.

C3.3.17 4-tert-Octylphenol (4-t-OP)
(included in spiking solution "B")

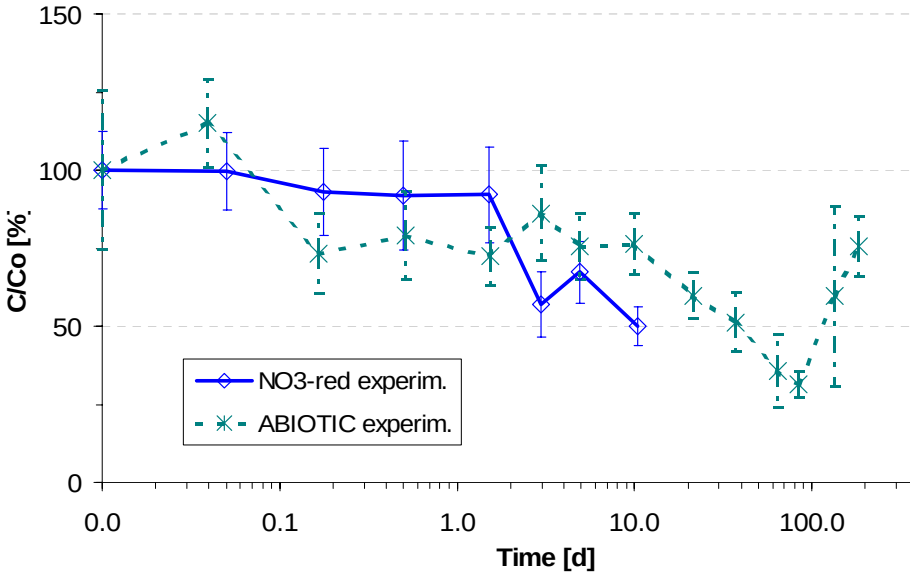


FIGURE C60: Evolution with time of 4-t-OP average normalized concentration (C/Co [%]) in the NO3-reducing and abiotic experiments with micropollutants at 1 µg/L individual initial concentration

COMMENT to Figure C60:
Among the biotic experiments, only results for the NO3-red. test were reliable. The evolution of the abiotic concentrations is characterized by a decreasing and rebound in the last results which do not give total confidence on such data too. Thus, the origin (biotic or abiotic) of the almost 40% of removal observed under NO3-reducing conditions could not be identified

C3.4 Figures and comments on results for the batch experiments with pollutants at 1 mg/L individual initial concentration

C3.4.1 Antihypertensive agents – Beta blockers

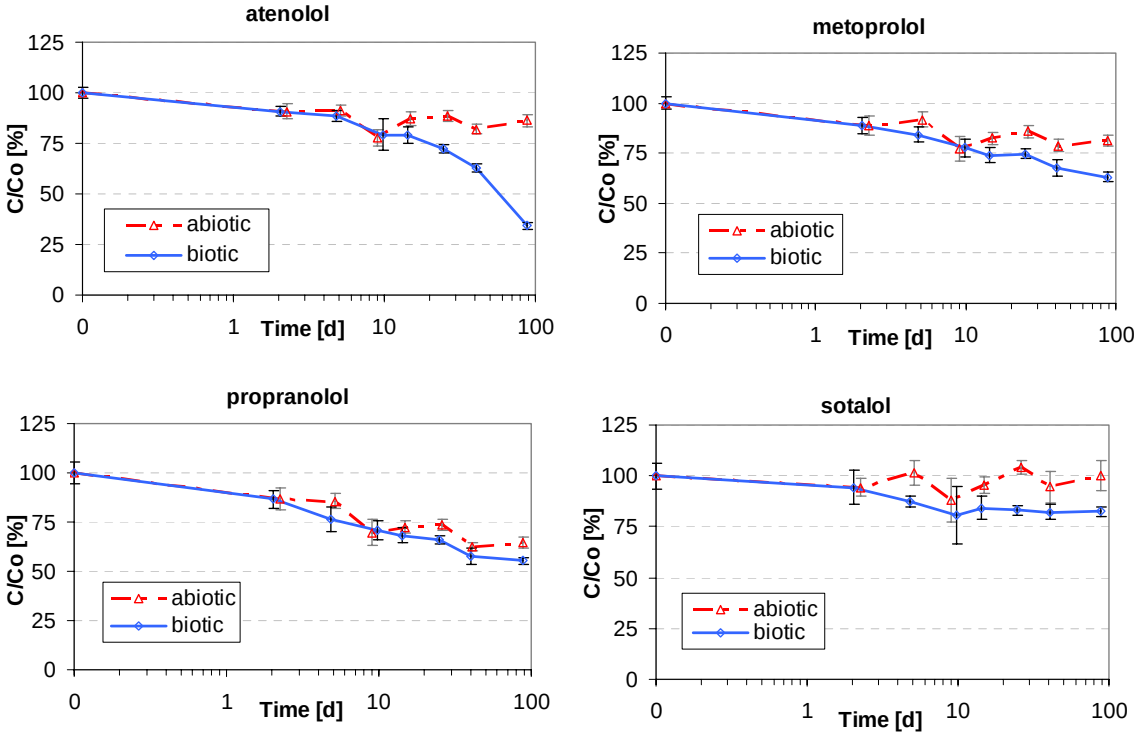


FIGURE C61: Evolution with time of beta-blockers average normalized concentration (C/Co [%]) in the biotic NO3-reducing experiment versus the evolution in the abiotic experiment – Batches with pollutants at 1 mg/L individual initial concentration

Results for beta-blockers are presented in Chapter 4.

C3.4.2 Antibiotics

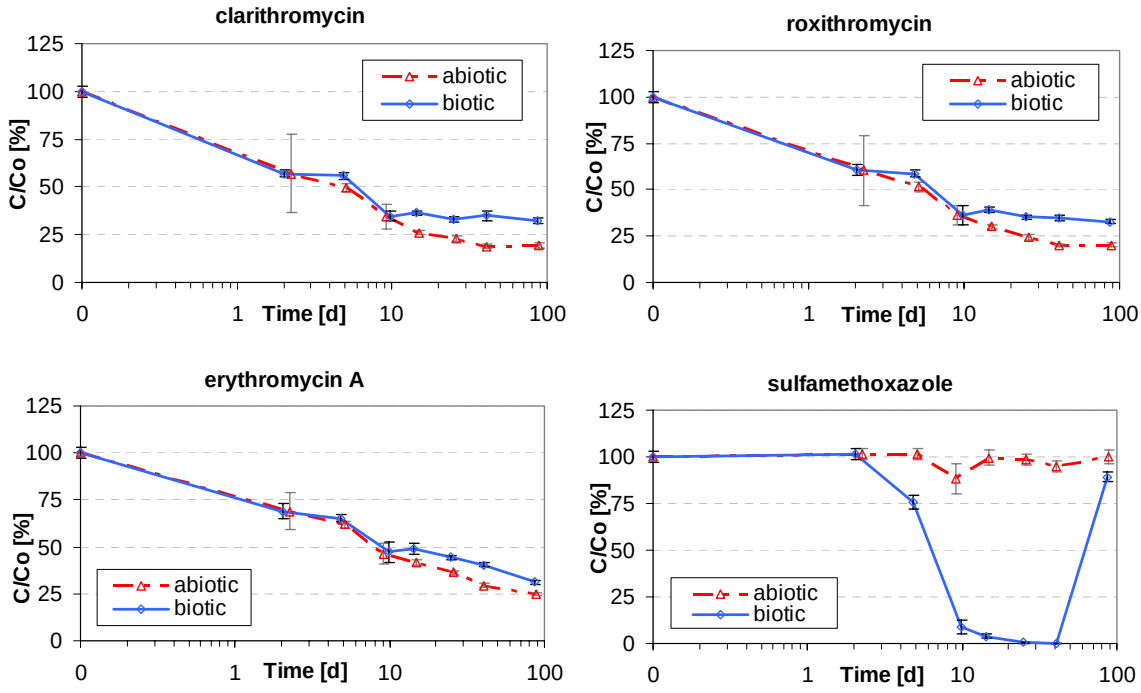


FIGURE C62: Evolution with time of antibiotics average normalized concentration (C/Co [%]) in the biotic NO3-reducing experiment versus the evolution in the abiotic experiment – Batches with pollutants at 1 mg/L individual initial concentration

For the macrolide antibiotics: removal in abiotic experim. (i.e., due to abiotic processes) > removal in the biotic experiment (i.e., due to abiotic + biotic processes). We could not identify the reason. Possibly results could not be considered as reliable.
Details on results for sulfamethoxazole are presented in Chapter 3.

C3.4.3 Neuro-active compounds

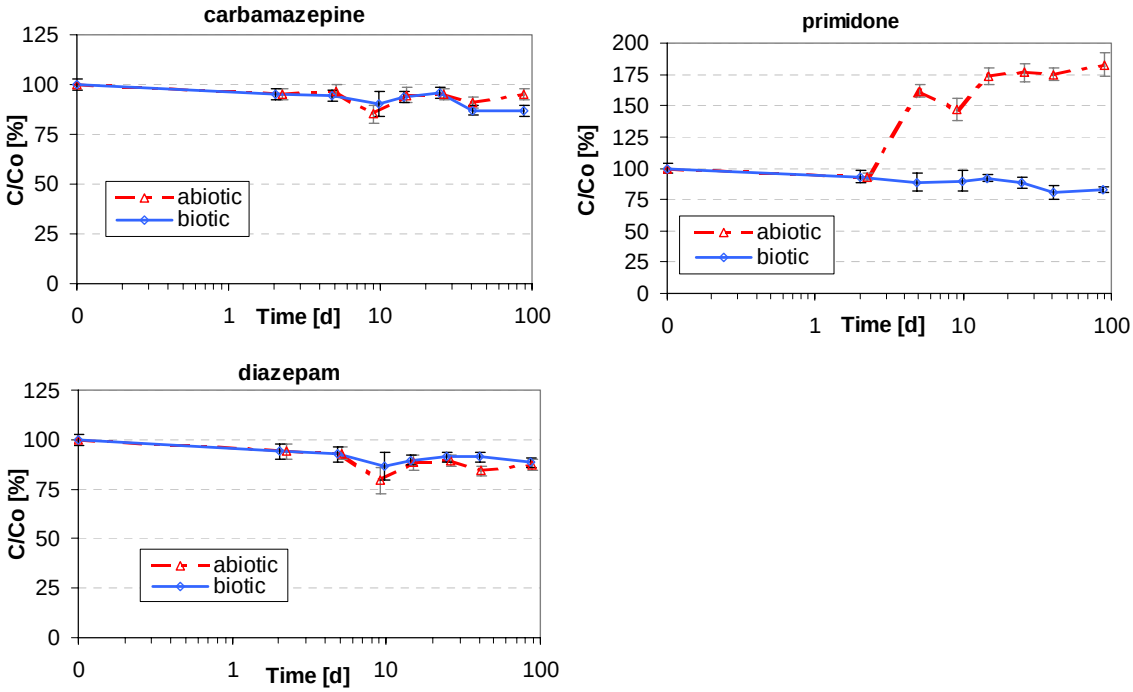


FIGURE C63: Evolution with time of neuroactive compounds average normalized concentration (C/Co [%]) in the biotic NO3-reducing experiment versus the evolution in the abiotic experiment – Batches with pollutants at 1 mg/L individual initial concentration

Carbamazepine and diazepam remained almost constant under the 87 days of nitrate reducing conditions. Actually, a maximum of 10% biotic overall removal could be observed for Carbamazepine.
Abiotic results for Primidone were not reliable. Some 20% of removal could be observed under the NO3-red. conditions dominating in the biotic expeiments. Thus, the nature of the process (biotic or abiotic?) could not be identified.

C3.4.4 Iodionated contrast media

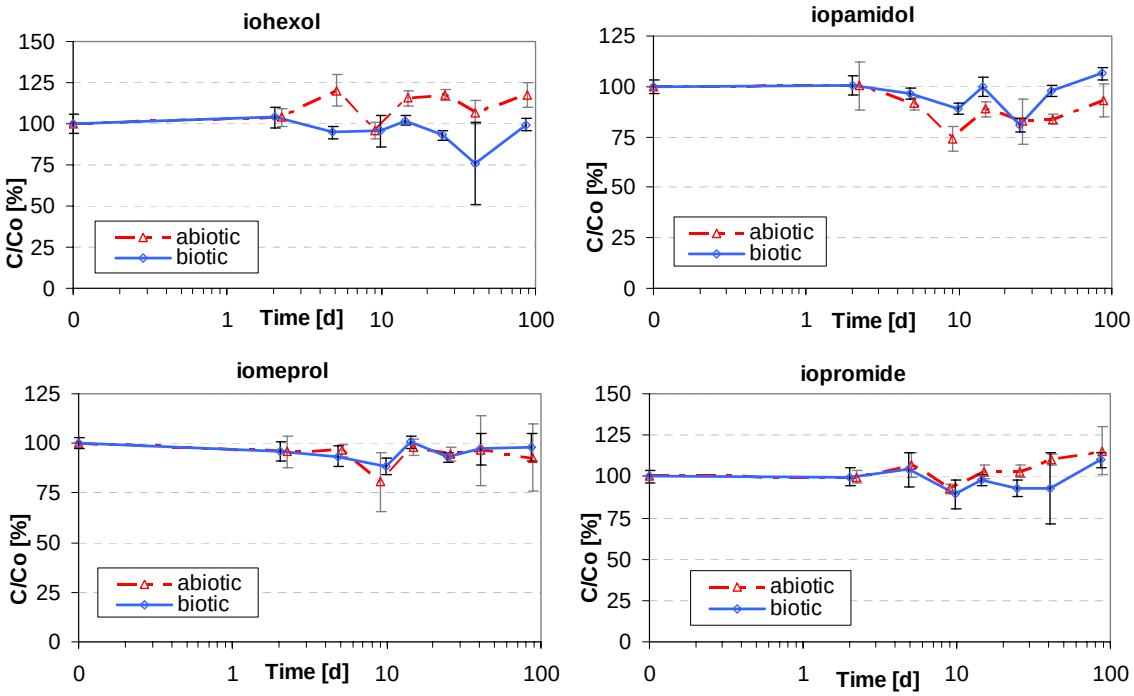


FIGURE C64: Evolution with time of iodinated contrast media average normalized concentration (C/Co [%]) in the biotic NO3-reducing experiment versus the evolution in the abiotic experiment – Batches with pollutants at 1 mg/L individual initial concentration

Overally constant during the whole experiment

C3.4.5 Lipid regulators

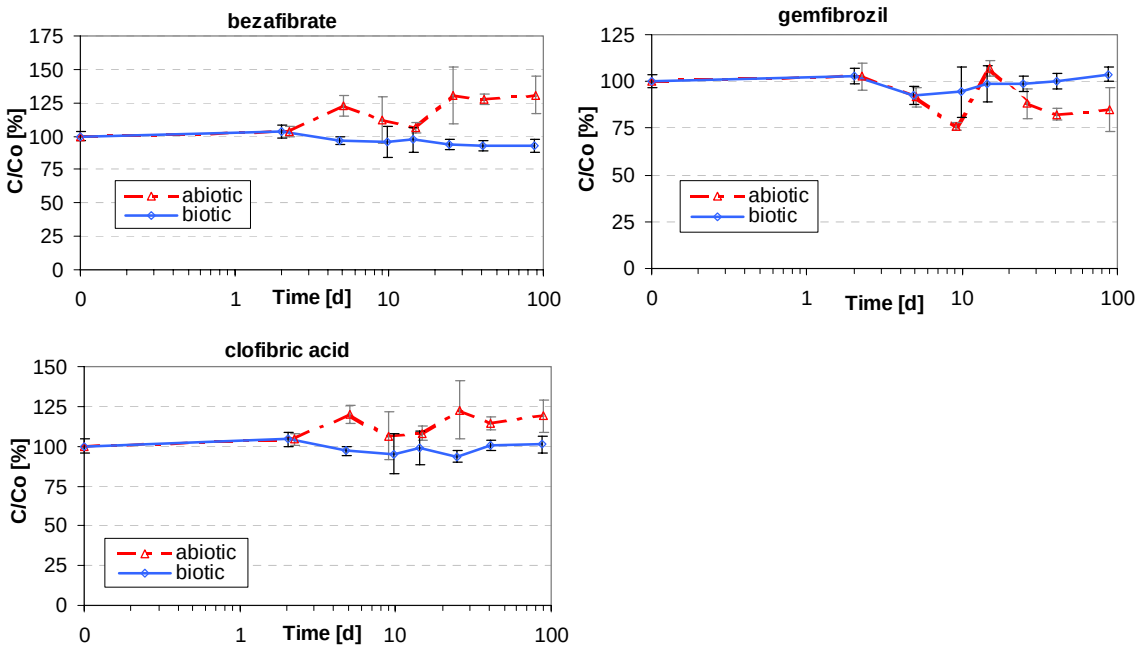


FIGURE C65: Evolution with time of lipid regulators average normalized concentration (C/Co [%]) in the biotic NO3-reducing experiment versus the evolution in the abiotic experiment – Batches with pollutants at 1 mg/L individual initial concentration

The evolution of the abiotic experiment suggests some interaction (probably during analysis) with the Hg present in the samples. No removal could be observed in the biotci experiment.

C3.4.6 Anti-inflammatory

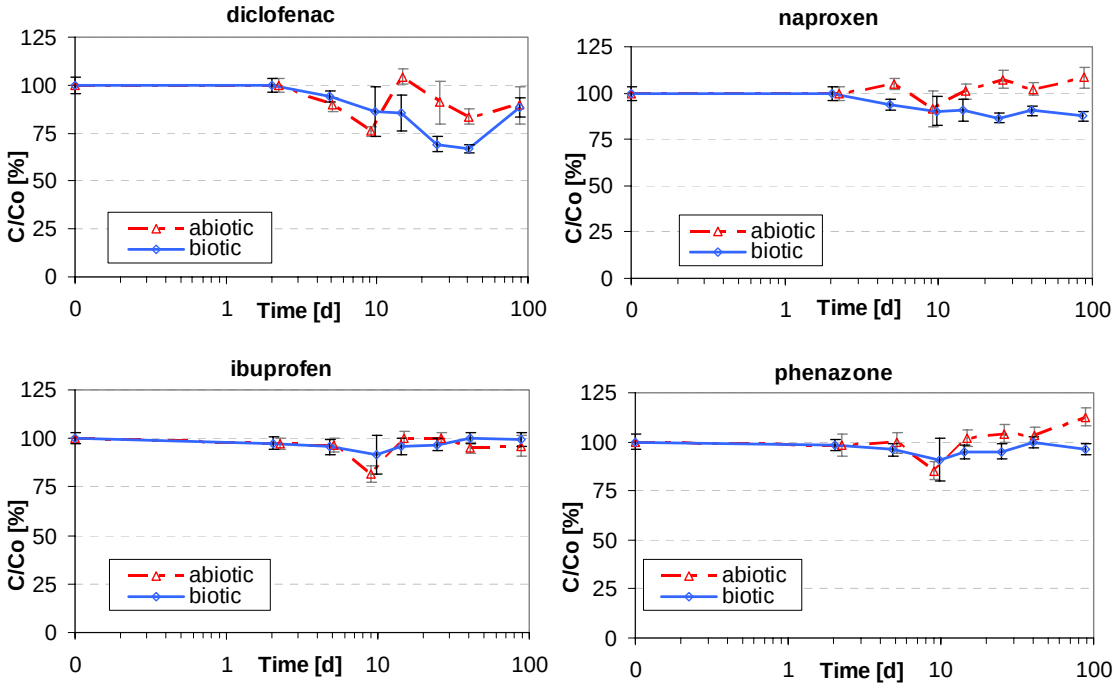


FIGURE C66: Evolution with time of anti-inflammatory average normalized concentration (C/Co [%]) in the biotic NO3-reducing experiment versus the evolution in the abiotic experiment – Batches with pollutants at 1 mg/L individual initial concentration

Details on results for diclofenac are presented in Chapter 3.
Ibuprofen and phenazone were not removed (neither abiotically nor biotically) during 87 days under NO3-reducing conditions. A biotical removal of about 20% could be observed for naproxen.

C3.4.7 Ulcer treatment compounds

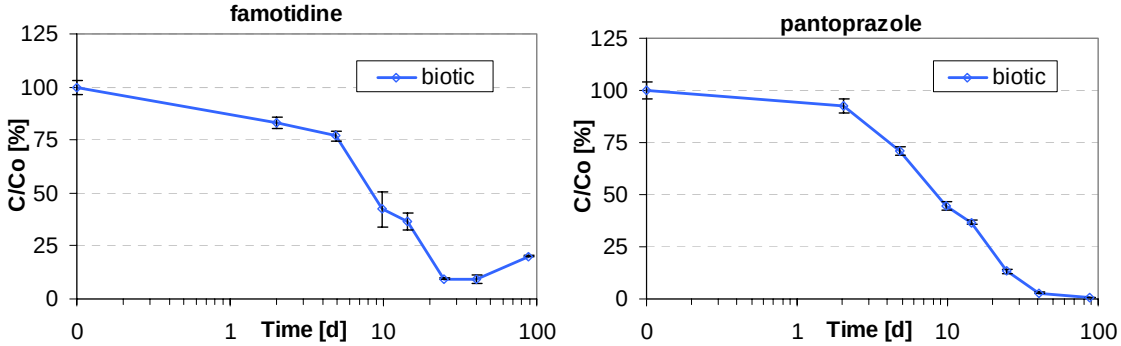


FIGURE C67: Evolution with time of ulcer treatment compounds average normalized concentration (C/Co [%]) in the biotic NO3-reducing experiment versus the evolution in the abiotic experiment – Batches with pollutants at 1 mg/L individual initial concentration

Due to some incompatibility wiht Hg, results from the abiotic experiment were not reliable for famotidine and pantoprazole. Overall removals of 80% and 100% could be observed in the biotic experiment, respectively. Wheter the process responsible of such removals was biotic or abiotic could not be evaluated due to the lack of the abiotic experiment reference.

C3.4.8 Antihistamines

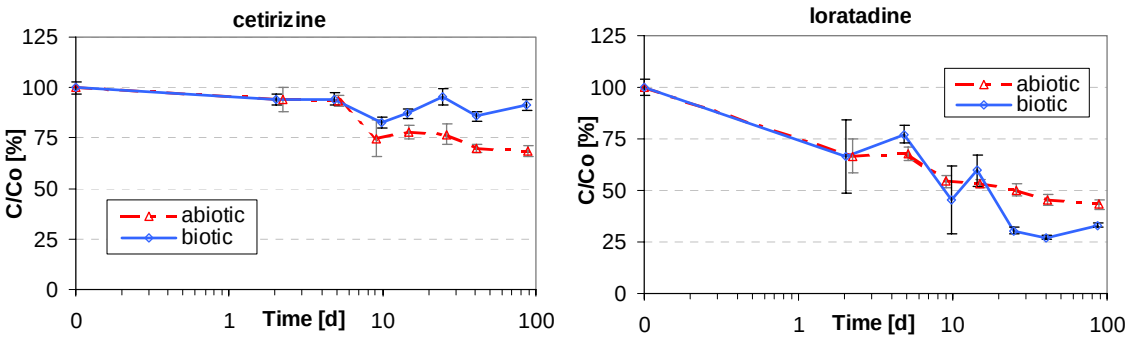


FIGURE C68: Evolution with time of antihistamines average normalized concentration (C/Co [%]) in the biotic NO3-reducing experiment versus the evolution in the abiotic experiment – Batches with pollutants at 1 mg/L individual initial concentration

Comparing the evolution of cetirizine in the biotic and abiotic experiments, data from the latter seem to be not realiable since the removal in the abiotic experiment (i.e., due to abiotic processes) is bigger than the removal in the biotic experiment (i.e., due to abiotic + biotic processes).
By the end of the experiments, loratadine has suffered an overall removal of about 70%, being the main part associated to abiotic processes.

C3.4.9 Analgesic

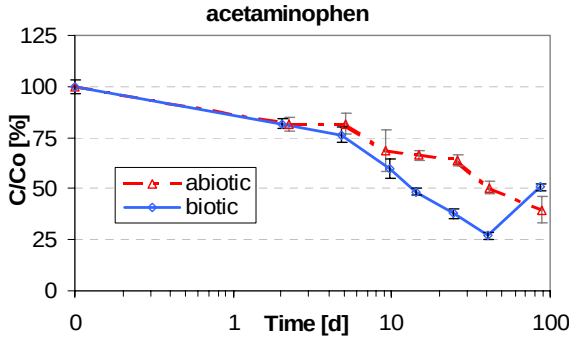


FIGURE C69: Evolution with time of analgesic average normalized concentration (C/Co [%]) in the biotic NO3-reducing experiment versus the evolution in the abiotic experiment – Batches with pollutants at 1 mg/L individual initial concentration

Comments on results for acetaminophen are presented in Chapter 3.
ràfic de Catalunya (unpuplished). Barcelona.

APPENDIX D

NOTE: the present Appendix consists of the paper “Evidence for reversible and non-reversible sulfamethoxazole biotransformation products during denitrification”, by Nödler, K., Licha, T., Barbieri, M., Pérez, S.. Submitted.

Evidence for reversible and non-reversible sulfamethoxazole biotransformation products during denitrification

Karsten Nödler*, [1], Tobias Licha[1], Manuela Barbieri[2, 3], Sandra Pérez[4]

[1] Department Applied Geology, Geoscience Centre of the University of Göttingen, Goldschmidtstr. 3, 37077 Göttingen, Germany
[2] Department of Geosciences, Institute of Environmental Assessment and Water Research, IDAEA-CSIC. Jordi Girona 18-26, 08034 Barcelona, Spain
[3] Department of Geotechnical Engineering and Geo-Sciences - Technical University of Catalonia. Jordi Girona 1-3, Modul D-2, 08034 Barcelona, Spain.
[4] Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research, IDAEA-CSIC. Jordi Girona 18-26, 08034 Barcelona, Spain

Corresponding author phone: +49/551/3919332; fax: +49/551/399373; e-mail: Karsten.noedler@geo.uni-goettingen.de, karsten.noedler@gmx.de.

Abstract: A water/sediment test on the microbial degradation of the widely used antibiotic sulfamethoxazole (SMX) under denitrifying conditions was performed with focus on the transformation products 4-nitro-N-(5-methylisoxazol-3-yl)-benzenesulfonamide (4-nitro-SMX) and N-(5-methylisoxazol-3-yl)-benzenesulfonamide (desamino-SMX). Reference substances of the transformation products were synthesized, identified and used for confirmation and quantification in the degradation batch experiments and in environmental samples. During the denitrifying degradation experiment SMX was no longer detected after 10 days. However, at day 87 the SMX concentration surprisingly recovered to 53 ± 16 % of the initial concentration after most of the nitrate was consumed. A retransformation of the denitrifying transformation product 4-nitro-SMX under anaerobic conditions was postulated and confirmed by an anaerobic water/sediment test. Both denitrifying transformation products were also detected in karst spring samples, highlighting the need and benefit of focusing on biotransformation products in environmental studies. Furthermore, the consideration of the retransformation potential of 4-nitro-SMX can substantially improve the understanding of SMX behavior during processes such as bank filtration and artificial recharge.

Introduction

The antibiotic drug sulfamethoxazole (SMX) is extensively used in both human and veterinary medicine (1,2). Since it cannot be completely eliminated by the typical state-of-the-art wastewater technology, it is frequently detected in various environmental compartments including surface water, groundwater and seawater (3-5).

Various studies at laboratory and field scale were conducted to investigate the fate of SMX in sediment and soil including bank filtration processes (6-11). At the pH range encountered in natural systems, i.e rivers and aquifers, sorption of SMX to organic matter and minerals can be considered to be of minor importance (7,10,12,13). Investigations on biotransformation rates of SMX clearly demonstrated the dependency on its residence time, the dominating redox condition, the adaptation of microorganisms, the availability of an alternative carbon source, the entry routes (accompanying matrix) of SMX, and the initial SMX concentration (6,9,10). However, data on removal rates under anoxic conditions (denitrification) are not consistent, ranging from an insignificant removal in soil column tests to almost full removal in field studies (6,11). Moreover, a study on soil treatment of wastewater reported a sporadically and irregular occurrence of SMX in the pore water, further emphasizing the complexity of its environmental behavior (14). Human metabolites of SMX were identified to be retransforming to the parent compound in a water/sediment test (10). Thus, if the presence of these compounds is not considered in field studies, the potential concentration of SMX may also be underestimated and a sudden appearance of the parent SMX can potentially be related to these human metabolites.

In degradation studies, the elimination or removal of SMX is commonly identified by a decreasing concentration of SMX and transformation products are not described (6-10). However, as the mineralization of SMX by microorganisms is very limited, the identification and quantification of transformation products must become a major issue for a realistic assessment of its environmental fate and associated risk (9). Regarding pharmaceutical residues, a phytotoxic transformation product of diclofenac is an alarming example for an environmental transformation product demonstrating an increased toxicity relative to the parent compound (15).

In this study, a set of denitrifying water/sediment tests was scanned in a broad mass range by high performance liquid chromatography electrospray mass spectrometry (HPLC-ESI-MS) in positive and negative mode at different degradation time steps. The two most significant emerging masses with time dependent abundances were short-listed as possible candidates for SMX-transformation products. These masses were assigned to the likely SMX related desamino- and nitro-species as occurring by metabolic pathways and also known from denitrification studies of

anilines (16-18). The two respective target compounds were later synthesized for the purpose of their confirmation and quantification in laboratory experiments and field samples.

Experimental Methods

Chemicals. LC-MS grade methanol, analytical grade toluene and analytical grade ethyl acetate were purchased from Fisher Scientific (Schwerte, Germany). Ammonium acetate and formic acid were obtained from VWR (Darmstadt, Germany). Sulfamethoxazole, benzenesulfonyl chloride (≥ 99 %), 4-nitrobenzenesulfonyl chloride (97 %), 3-amino-5-methylisoxazole (≥ 98 %) and pyridine (≥ 99 %) were purchased from Sigma Aldrich (Steinheim, Germany). The internal standard sulfamethoxazole-$^{13}C_6$ (SMX-$^{13}C_6$) was obtained from LGC Promochem (Wesel, Germany). Organic and inorganic salts for the preparation of the water/sediment tests (ammonium phosphate, iron(III) chloride, sodium acetate, sodium chloride, potassium dihydrogen phosphate, magnesium chloride hexahydrate, magnesium nitrate hexahydrate, potassium chloride, calcium chloride dihydrate, sodium sulphate, ammonium chloride and mercury(II) chloride) were all purchased from Merck KGaA (Darmstadt, Germany). Nitric acid was also purchased from Merck KGaA. Ultrapure water was obtained from a combined water purification system from Millipore (Schwalbach, Germany). It consisted of Elix 5 (Progard 1 silver cartridge) and Milli-Q Gradient A10 (Quantum Ex Ultrapure Organex + Q-Gard 1 cartridge).

Synthesis of SMX-transformation products as reference substances. The transformation product 4-nitro-N-(5-methylisoxazol-3-yl)-benzenesulfonamide (*4-nitro-SMX*) was synthesized as described by Rieder et al. (19). 6 g of 4-nitrobenzenesulfonyl chloride was mixed with 2.4 g of 3-amino-5-methylisoxazole in 10 ml of pyridine at 0 °C. After a reaction time of approximately 24 h at room temperature the crude product was precipitated in 200 ml of ultrapure water, filtered and washed twice with 25 mL of ultrapure water. The product was then recrystallized using an ethyl acetate:toluene (1:3 v/v) mixture, filtered and washed with ethyl acetate. For the synthesis of the transformation product N-(5-methylisoxazol-3-yl)-benzenesulfonamide (*desamino-SMX*) 4.8 g of benzenesulfonyl chloride was used instead of the 4-nitrobenzenesulfonyl chloride. The synthesis was similar to 4-nitro-SMX. Structures of both compounds are presented in Table C1. The identity and purity of the transformation products were confirmed by 1H-NMR. The purity was > 95 % for 4-nitro-SMX and 88 % for desamino-SMX.

Denitrifying water/sediment batch experiment (SMX). Aim of this water/sediment batch experiment was the qualification and quantification of SMX and its transformation products under

denitrifying conditions over time. The sediment was obtained from a test site for artificial groundwater recharge located in Sant Vicenç dels Horts (Barcelona, Spain) on the banks of the Llobregat river. The aquifer consisted of quaternary alluvial sediments, mainly gravel and sand containing small fractions of lutites. Sediment samples were collected from an oxic unsaturated horizon at about 1 m depth under the bottom of the infiltration pond prior to its operation. The sediment was sieved to < 1 mm and homogenized in steel containers. The air-dried sediment contained < 0.2 % of total nitrogen and total organic carbon in the bulk. Total carbon content was 2.5 %. Concentrations of manganese and ferric iron associated to oxide-hydroxides and oxides in the air-dried sediment were 0.07 and 5.8 mg g^{-1}, respectively.

An aqueous test medium for the batch tests was obtained by dissolving respective amounts of salts given in the “chemicals” section in ultrapure water. In order to remove dissolved oxygen the solution was purged with argon 5.0 for 1 h. Finally, 43 mg sodium acetate and 3 ml of a SMX stock solution in methanol (0.333 mg mL^{-1}) were added per liter solution, resulting in a SMX concentration of 1 mg L^{-1}. Although cell growth initiation is delayed by trace concentrations of SMX, a complete inhibition of microbial growth at the given SMX concentration can be excluded (20,21). The individual concentrations of the respective cations and anions in mg L^{-1} were as follows: 228 (Na^+), 40 (K^+), 120 (Ca^{2+}), 833 (Mg^{2+}), 452 (Cl^-), 4100 (NO_3^-), 200 (SO_4^{2-}), 31 (HCO_3^-) and 2 (NH_4^+ and PO_4^{3-}). The dissolved organic carbon (DOC) concentration was 970 mg L^{-1}.

The assembling of the batches was conducted under argon atmosphere in a glove box. 14 glass bottles (300 mL) were all filled with 120 g of the air-dried and homogenized sediment and 240 mL of the liquid test medium. A headspace of 15 mL argon was left in each bottle. Screw caps with PTFE seals were used to close the bottles. The batches were shaken and wrapped in alumina foil to prevent photochemical reactions over the whole period (22). 10 mL of the liquid test medium were frozen as a reference of the initial concentration of SMX. All batches were incubated at 25 ± 1 °C and sacrificed according to the sampling schedule. On a regular basis, the batches were shaken carefully to avoid destruction of the developed biofilms.

To distinguish biological from non-biological effects, 14 abiotic control batches were prepared in analogy to the biotic batches. The sediment and the liquid test medium for the controls were autoclaved three times for 20 min (T=121 °C and 1 atm overpressure, once a day for three consecutive days). Prior to the assembling, SMX and sodium acetate were added to the sterilized liquid test medium. Furthermore, mercury(II) chloride (60 mg L^{-1}) was applied to prevent microbial activity (23). 10 mL of the liquid test medium were frozen as a reference of the initial concentration of SMX. The sterility of the control batches was verified two times during the first 41 days of the experiment by incubating aliquots of water from the disassembled microcosms on tryptic soy agar

(TSA) plates. The plates were incubated in duplicate at 25 °C for one week under aerobic conditions and two weeks under anaerobic conditions. None of the plates demonstrated a growth of microorganisms.

Biotic batches were sacrificed in duplicate after 2, 5, 10, 14, 25, 41 and 87 days, duplicates of abiotic batches after 2, 5, 9, 15, 26, 41 and 89 days, respectively. For the quantification of SMX and the transformation products, 10 mL aliquots of each batch supernatant were stored at –18 °C in amber glass vials until analysis. Samples for nitrite and nitrate analysis were filtered prior to freezing (0.45 µm PALL Acrodisc® Sterile Syringe Filters with Supor® membrane). Samples for iron analysis were also filtered and acidified to pH 2 with nitric acid (65 %) before storage at 4 °C.

Anaerobic water/sediment batch experiment (4-nitro-SMX). To confirm the suspected retransformation of 4-nitro-SMX to SMX under anaerobic conditions, a further water/sediment experiment was conducted. The aim of the experiment at this stage was to demonstrate the reduction of the compound in general to be independent from the underlying reaction mechanisms (biotic/abiotic, sediment type). For this reason, sediment different to the denitrifying experiment was used and no abiotic control batches were prepared.

14 glass bottles (100 mL) were all filled with 40 g of sediment and 100 mL of an anaerobic soil column outflow, which also served as the microbial inoculum. Prior to assembling, the liquid was spiked with 1 mg L^{-1} of iron(III) chloride and ammonium phosphate, respectively. Furthermore, 40 mg L^{-1} of sodium acetate and 1 mg L^{-1} of the synthesized 4-nitro-SMX were added. The batches were shaken and incubated at 25 ± 1 °C submerged under water to maintain anaerobic conditions. After one hour, two batches were directly sacrificed. Aliquots of the water phase were frozen and used for the determination of the initial SMX and 4-nitro-SMX concentrations. The remaining batches were carefully shaken once a day. After 3, 7, 14, 21, 28 and 35 days, two batches were sacrificed and aliquots of 5 mL supernatant were frozen until analysis. Anaerobic conditions of the batches were verified by the negative redox potential.

Chemical analysis of the batch experiments. Nitrate and nitrite were analyzed by ion chromatography (IC). Iron was analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES).

SMX and its transformation products were analyzed by high performance liquid chromatography electrospray tandem mass spectrometry (HPLC-ESI-MS/MS). Details regarding the instrumentation have been published previously (5) and can be found in the Supporting Information (text S.C1). A flow rate of 200 µL min^{-1} and an injection volume of 100 µL were applied. The separation was

operated at 30 °C. The eluents consisted of 0.015 % formic acid + 5 % methanol (eluent A) and methanol (eluent B). To avoid interferences during the ionization process with the high load of inorganic sample matrix, a slow gradient was chosen over a rapid analysis (24). The elution started with 5 % B followed by a gradient of 27 min to 65 % B. This was followed by a sharp gradient of 1 min to 95 % B, which was held for 5 min. After a gradient of 1 min to 5 % B the system was allowed to equilibrate for 11 min. As an additional means to compensate matrix effects, samples were diluted 1:4 (v/v) with aqueous 5 mM ammonium acetate solution, containing 4 % methanol. Furthermore, SMX-$^{13}C_6$ (125 ng mL^{-1}) was used as the internal standard and the standard solutions for the calibration were prepared in inorganic matrix according to 50 % of the respective aqueous test medium's concentration. Afterwards, the standards were also diluted 1:4 (v/v) with aqueous 5 mM ammonium acetate solution, containing 4 % methanol. Before analysis, all samples and standard solutions were centrifuged at 1500 rpm (Christ RVC 2-18, purchased from Fisher Scientific, Schwerte, Germany) for 30 min at room temperature.

The quantification of analytes was performed in multiple reaction monitoring (MRM) and two transitions per analyte were monitored. The transformation products in the batch experiments are confirmed if the following criteria apply to standard and unknown: a) identical retention time in the chromatogram, b) identical quantifier and qualifier mass transitions and c) intensity ratios of quantifier and qualifier match (25).

In a previous study using an acidic mobile phase, SMX demonstrated the highest sensitivity in the positive ionization mode (5). However, as 4-nitro-SMX and desamino-SMX demonstrated only poor ionization efficiencies in this mode, all compounds were analyzed in the negative ion mode. The compounds were quantified by using their most intensive mass transition. The applied capillary voltages were -40 V for 4-nitro-SMX and -35 V for all other compounds, respectively. Individual parameters are presented in Table C1. The internal standard SMX-$^{13}C_6$ was quantified by using the mass transition 258 → 160 (13.5 V collision energy).

Environmental samples. Between May and October 2010, 62 water samples from a karst spring (Gallusquelle, Swabian Alb, Germany) were taken. Earlier studies in this area demonstrated an irregular and event-based inflow of wastewater related micro-contaminants and the occasional presence of nitrite indicated denitrifying conditions to exist in the aquifer system (26,27).

Sample preparation (solid phase extraction, SPE) was conducted according to Nödler et al. (5). The individual MS-MS-parameters of 4-nitro-SMX and desamino-SMX given in the previous chapter were included with the methodology published previously (5). Further information can be found in the Supporting Information (text S.C2). Eight concentration levels (1 – 250 ng L^{-1}) were used for the

calibration and included the preconcentration step. The correlation coefficients exceeded 0.99 and the method quantitation limits (MQL) according to a signal to noise ratio (S/N) of 10 were 1.0 and 1.5 ng L^{-1} for 4-nitro-SMX and desamino-SMX, respectively.

Results and Discussion

SMX transformation under denitrifying conditions. The presented experiment was designed with the intention to sustain denitrifying conditions over a large period. Furthermore, a high concentration of easily degradable DOC was applied to support high metabolic rates of microorganisms. During the first 41 days of the experiment, dissimilative denitrification was clearly indicated by an increasing nitrite concentration while the nitrate concentration decreased at the same time (28). Nitrite was not detected in the sterile control batches. This and the negative growth controls emphasized the successful growth-inhibition by $HgCl_2$. The results of the monitored parameters are shown in Figure D1. The concentration of SMX in the sterile control decreased slowly but continuously by 7 % within 89 days. Radke et al. reported similar results and they attributed their observations to slow sorption to the sediment (10). All batches were wrapped in alumina foil for the whole experiment. Therefore, a photochemical degradation of SMX can be excluded (22).

Despite the high SMX concentration of 1 mg L^{-1} no SMX was detected after 10 days in this water/sediment test. The fast degradation of SMX disagreed at first sight with recently published results about long-term laboratory column experiments (6). At 4.5 µg L^{-1} SMX Baumgarten et al. observed a half-life of 49 days under anoxic conditions (6). Furthermore, they observed a very long adaptation time of the degrading microorganisms and postulated, that the occurrence of SMX in aquifers mostly reflects insufficient residence time or unfavorable redox conditions. However, the respective applied DOC and nitrate concentrations were approximately 8 and 2 mg L^{-1} and thus 2 and 3 orders of magnitude, respectively, lower than in the presented study. By using an aerated sequencing batch reactor (SRB), Drillia et al. observed that SMX serves as nitrogen- and carbon-source once the medium lacks one or both of the elements, whereas in the presence of alternative nitrogen- and carbon-sources, such as ammonium and acetate, SMX was not degraded (29). On the other hand, higher degradation rates of SMX were observed in the presence of methanol as alternative carbon-source in an aerobic water/sediment test (10). Furthermore, in comparison with test designs on ready and moderate degradation the elimination of SMX in a laboratory scale treatment plant was much more effective and without any significant lag phase (21). These findings underline the significance of cometabolic degradation of SMX in wastewater treatment technology, which was in line with the results presented in this study.

In contrast to the fast and initially complete disappearance of the applied SMX concentration increasing concentrations of the transformation products 4-nitro-SMX and desamino-SMX were observed. A typical MRM chromatogram of a standard solution and a denitrifying batch supernatant can be found as Supporting Information (Figure S.D1). As presented in Figure D1, the concentration of 4-nitro-SMX correlated very well with the nitrite concentration. Pereira et al. observed a fast abiotic transformation of aniline and sulfanilic acid in the presence of nitrite resulting in a yellow coloration of the solution with time and with decreasing pH value (30). In an attempt to identify the transformation products by MS-spectra they suggested the formation of nitro-aryl compounds and a set of different diazonium compounds (30). O'Neill et al. also described the formation of yellow and brown colored degradation products from aniline and suggested the formation of a benzene diazonium salt as the most probable reason (31). In preliminary experiments on the abiotic reaction of SMX with nitrite at different pH values (data not shown) a yellow coloration and the formation of both 4-nitro-SMX and desamino-SMX were observed within minutes to hours. Nitric oxide (NO) plays a key role in the formation of diazonium compounds from aromatic amines and nitrite (32). As NO is also a product of the denitrification process, the transformation of SMX can most likely be attributed to this cometabolic process (28). Accordingly, Lammerding et al. concluded that the actual diazotization step of anilines under denitrifying conditions is independent from biological assistance (33). Woolley and Sigel demonstrated a substantial dependency between the reductive deamination of the sulfonamide antibiotic sulfadiazine (SDZ) in different animals and the presence of nitrite (16). 4-amino-benzenesulfonamide is a common structural element of SDZ and SMX and the deaminated transformation product discussed therein is comparable with the desamino-SMX shown in the presented study. During a denitrifying water/sediment test with desamino-SMX (data not shown) no 4-nitro-SMX was formed. Therefore, the compound can be excluded to be an intermediate transformation product of SMX to 4-nitro-SMX. Based on the formation of a diazonium compound the proposed reaction mechanisms are presented in Table C2.

As demonstrated in Figure D1, a molar imbalance of SMX and the transformation products was noticeable and may be attributed to additionally formed and not yet identified transformation products. Furthermore, although SMX was no longer detected, the concentration of 4-nitro-SMX still increased from experiment days 10 to 25. This gives further indication for the presence of intermediate molecules of SMX transformation to 4-nitro-SMX. However, the diazonium compound of SMX and any other intermediates were not clearly identified. Lammerding et al. proposed that cellular compounds may trap a significant amount of diazonium ions, which could explain the negative results in the presented study (33).

Regarding the molar imbalance of the transformation products, sorption to the sediment may be more relevant for 4-nitro-SMX and desamino-SMX than for SMX and the sediment may have retained a considerable amount of the compounds. As predicted by Scifinder®, both transformation products demonstrate higher log K_{ow} values than SMX (Table C1). Furthermore, nitroaromatic compounds may absorb specifically to natural clay minerals (35). The pK_a of desamino-SMX is predicted to be 7 (Table C1). This would increase the amount of the neutral species susceptible to hydrophobic sorption at neutral water pH.

Anaerobic 4-nitro-SMX retransformation. The reappearance of SMX at day 87 of the denitrifying experiment was highly surprising and the concentration recovered to 53 ± 16 % of the initial concentration (Figure D1). Due to the high effort to minimize adverse matrix effects during HPLC/MS-MS analysis (isotope-labeled internal standard, matrix calibration, slow gradient and sample dilution) analytical issues as a reason for this observation can be virtually excluded. The formation of aromatic amines from nitroaromatic compounds at anaerobic conditions was demonstrated in different studies (36). Heijman et al. demonstrated the anaerobic reduction of 10 different monosubstituted nitrobenzenes in laboratory aquifer columns and all compounds were stoichiometrically reduced to their corresponding amino compounds (37). Accordingly, the reduction of 4-nitro-SMX to its corresponding amino-compound and thus the retransformation of 4-nitro-SMX to SMX was suspected. To verify the hypothesized retransformation, an additional water/sediment test with 4-nitro-SMX under anaerobic conditions was conducted. The results presented in Figure D2 clearly demonstrate the retransformation potential of 4-nitro-SMX.

At day 87 of the denitrification experiment with SMX, nitrate was still present but nitrite was no longer detected (Figure D1) and no iron was yet detected in the respective samples (MQL = 50 µg L^{-1}). However, the aim of the anaerobic experiment at this stage was to demonstrate the reduction of 4-nitro-SMX to SMX in general to be independent from the underlying reaction mechanisms (biotic/abiotic, sediment type). The molar imbalance of 4-nitro-SMX and SMX in the anaerobic experiment may be attributed to degradation of SMX (6). The identification of other transformation products and intermediates was not followed.

Environmental monitoring. In total 62 spring samples were analyzed. Desamino-SMX, 4-nitro-SMX and SMX were detected in 4, 6 and 3 samples, respectively. The respective concentration ranges were 2.9 - 7.7 ng L^{-1}, 3.4 - 5.4 ng L^{-1} and 2.1 - 9.6 ng L^{-1}. Interestingly, no co-occurrence of SMX with either of the transformation products was observed. In two samples both desamino-SMX and 4-nitro-SMX were detected.

Environmental relevance of the presented study. The spring water analysis clearly demonstrated that the transformation products, which were detected in the batch experiment, are also present in the environment. During the period of investigation the concentration ranges of SMX and its transformation products in the spring water were in the same order of magnitude. Furthermore, the higher detection frequencies of the transformation products in comparison to the parent compound highlight the need and benefit of including transformation products in environmental studies. This measure could here substantially improve the understanding of the environmental occurrence, fate and behavior of SMX.

Comparable to the human metabolites N_4-acetyl-SMX and SMX-N_1-glucuronide the environmental transformation product 4-nitro-SMX has the potential to be converted back to SMX (10). During artificial recharge the redox conditions below the injection point are variable with time (38). Accordingly, monitoring of SMX and 4-nitro-SMX potentially simplify and further improve the evaluation of sites for artificial recharge and bank filtration (8,11). Furthermore, discrepancies of laboratory and field studies and even a sporadically and irregular occurrence of SMX could possibly be explained by the demonstrated retransformation (6,11,14).

A serious aspect derives from the supposed reaction mechanism with NO. 4-amino-benzenesulfonamide is a common structural element of many different sulfonamide antibiotics and a reaction with NO similar to the reaction with SMX is very likely (16). A high number of sulfonamide antibiotics is used in veterinary medicine and the use of manure from medicated animals in agriculture bears the risk of groundwater contamination with these compounds together with nitrate (39).

Regarding the toxicological relevance of SMX in potable water, adverse health effects are usually not expected at the typically encountered ng L^{-1} concentrations (40). However, SMX is generally considered to be ecologically harmful (1, 20). To the authors' knowledge there is no current data available on the toxicity of the presented transformation products. In comparison with the corresponding aromatic amines a variety of different nitro-aromatics are clearly more toxic to methanogenic bacteria (41). Among other compounds 4-nitro-SMX was identified as an ozonation product of SMX (42). Regarding their toxicity studies with *Daphnia magna* Abellán et al. concluded a higher toxicity of the transformation products in comparison to the parent compound (42). As 4-nitro-SMX and desamino-SMX were also identified in the environment, a careful toxicological evaluation of both compounds is essential.

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Commission (contract number 518118-1, GABARDINE, “Groundwater Artificial Recharge Based on Alternative Sources of Water: Advanced Integrated Technologies and Management”), and by the German Federal Ministry of Education and Research (promotional references No. 02WM0802 & 02WM1081, SMART, “Sustainable and Integral Management of Available Water Resources Using Innovative Technologies”).

FIGURES

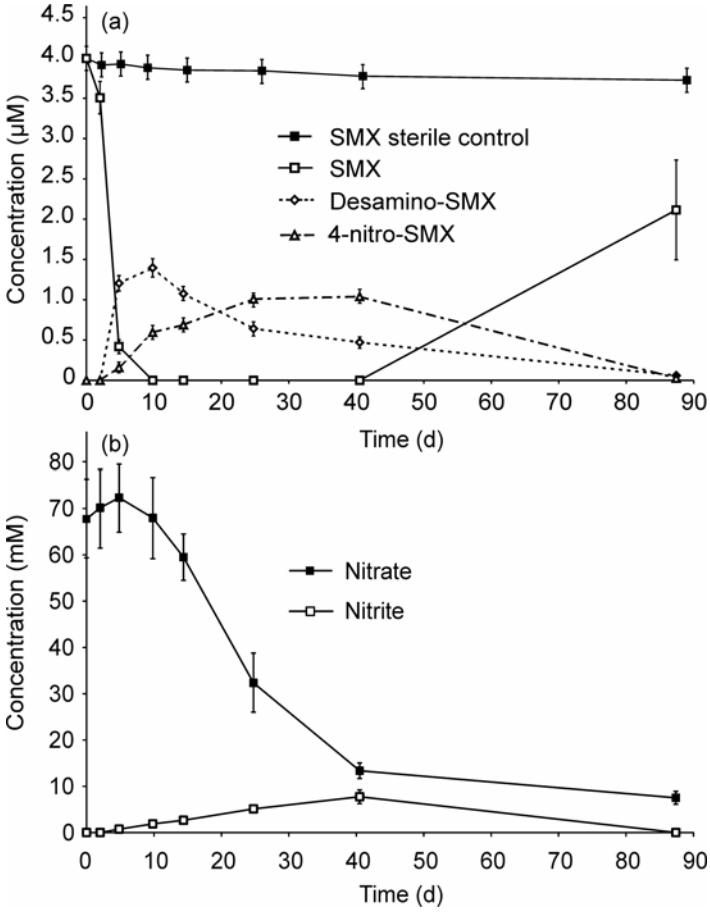


Figure D1. Water/sediment experiment on SMX transformation under denitrifying conditions, chart (a): concentrations of SMX (biotic and sterile control), desamino-SMX and 4-nitro-SMX. Chart (b): concentrations of nitrate and nitrite. Mean values of two batches per time point, error bars represent the respective concentration range of duplicates and the analytical error. The surprising reappearance of SMX at day 87 can be attributed to the reduction of 4-nitro-SMX.

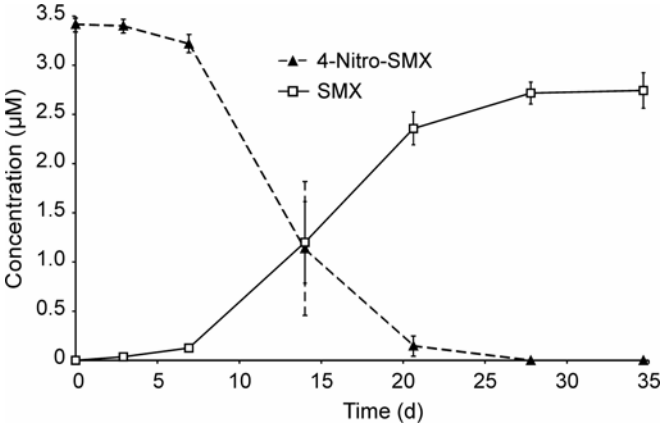
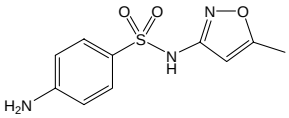
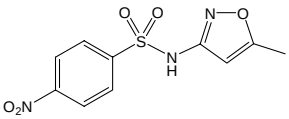
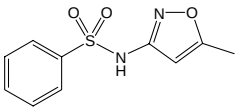


Figure D2. Water/sediment experiment on the anaerobic 4-nitro-SMX retransformation: concentrations of 4-nitro-SMX and SMX. Mean values of two batches per time point, error bars represent the respective concentration range and analytical error.

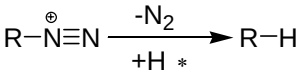
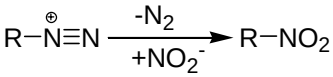
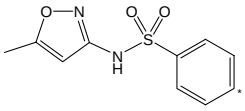
TABLES

Table D1. Structures, pK_a and log K_{OW} values, quantifier and qualifier transitions of the analytes

Compound (CAS)	Structure	pK_a[a,b]	Log K_{ow}[a]	Quantifier (CE)[c]	Qualifier (CE)[c]
Sulfamethoxazole, SMX (723-46-6)		5.81 ± 0.5	0.66 ± 0.41	252 → 154 (13.5 V)	252 → 106 (17.5 V)
4-nitro-SMX (29699-89-6)		5.65 ± 0.4	1.27 ± 0.41	282 → 138 (20.5 V)	282 → 186 (13.0 V)
Desamino-SMX (13053-79-7)		6.92 ± 0.5	1.34 ± 0.40	237 → 141 (13.0 V)	237 → 77 (25.5 V)

[a] Scifinder predicted values. [b] pK_a of the secondary amine. [c] Collision energy

Table D2. Proposed reaction mechanism of the transformation product formation

Transformation product	Proposed reaction mechanism	Reference(s)
Desamino-SMX		Brückner (32) Itoh et al. (34)
4-nitro-SMX		Brückner (32)
R =		

Supporting information.

Further details on the HPLC/MS-MS instrumentation and methodology, a typical MRM chromatogram of a standard solution and a denitrifying batch supernatant are provided in the followig.

Text S.C1: High performance liquid chromatography electrospray tandem mass spectrometry (HPLC-ESI-MS/MS) instrumentation and general instrumental parameters.

All instruments were purchased from Varian, Darmstadt, Germany. The HPLC system consisted of a ProStar 410 autosampler and two ProStar 210 pumps. A Polaris C18-Ether column 150 mm × 2 mm i.d., 3 µm particle was used for chromatographic separation. A L1200 triple quadrupole with electrospray interface (ESI) was used for detection and quantification. The drying and nebulizing gas pressures were 180×10^3 and 386×10^3 Pa, respectively. The drying gas temperature was set to 280 °C. Argon 5.0 with a pressure of 0.27 Pa was used as the collision gas. The spray and shield voltages were -4.5 kV and -0.5 kV, respectively.

Text S.C2: Extraction and analysis of the environmental samples.

Samples were allowed to settle in the refrigerator (4 °C) for no more than 12 h before extraction. 500 mL of the supernatant was spiked with 100 ng of the internal standard (sulfamethoxazole-$^{13}C_6$) and 5 mL of a pH buffer concentrate (13.4 g L^{-1} potassium dihydrogen phosphate and 6.22 g L^{-1} disodium hydrogen phosphate dihydrate in ultrapure water). OASIS HLB 500 mg from Waters,

Eschborn, Germany was used for the extraction. Prior to the extraction the sorbent was conditioned by subsequently flushing with 4 mL methanol and 2 × 4 mL ultrapure water. An extraction flow rate of 15 mL min^{-1} was applied. In order to remove inorganic salt matrix after the extraction, the sorbent was rinsed twice with 1.5 mL ultrapure water. The sorbent was dried by drawing air through the cartridge under vacuum for 30 min. The cartridge was stored at –18 °C until the elution of analytes. The analytes were subsequently eluted with 2 × 2 mL methanol and 2 × 2 mL ethyl acetate. The solvents were evaporated at 40 °C with a gentle stream of nitrogen. The analytes were re-dissolved in 800 µL of aqueous 5 mM ammonium acetate solution, containing 4 % methanol. Before analysis, the extract was centrifuged at 1500 rpm for 30 min at room temperature.

The instrumentation and individual parameters given in Text S.C1 were used for analysis. Eluent A was 0.015 % formic acid + 5 % methanol in ultrapure water and eluent B was methanol. The elution started isocratically for 50 s with 100 % A, which was followed by a gradient of 10 s to 95 % A. This step was followed by a 39-min linear gradient to 95 % B, which was held for 5 min. After a 1 min gradient to 100 % A, the system was allowed to equilibrate for 11 min. A flow rate of 200 µL min^{-1} and an injection volume of 100 µL were applied. The separation was operated at 30 °C.

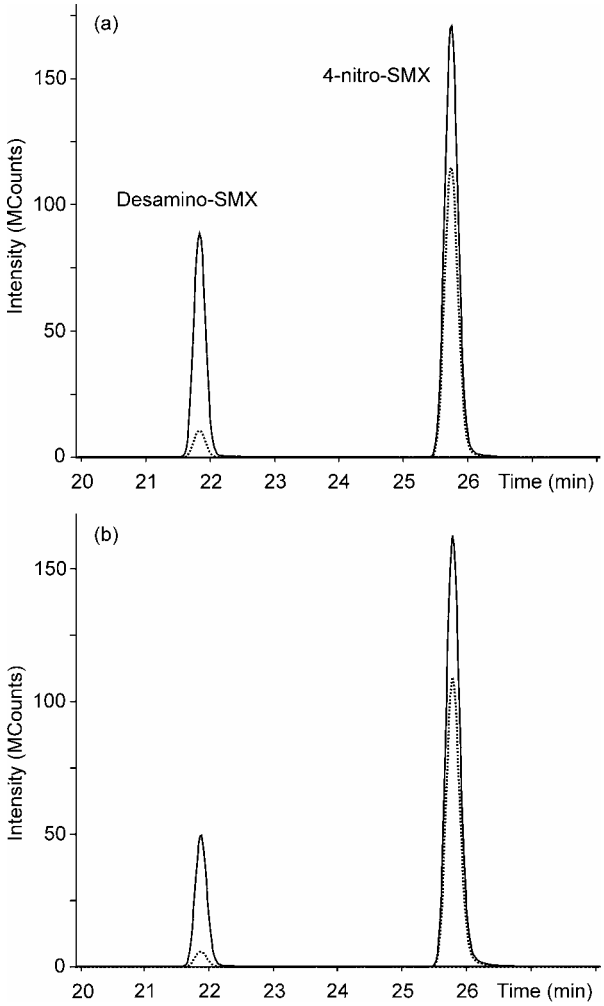


Figure S.D1: Chromatogram of a standard (a) and a sample from the denitrifying degradation experiment of SMX (b). Identical retention times and intensity ratios of quantifier (continuous line) and qualifier transitions (dashed line) confirmed the formation of desamino-SMX and 4-nitro-SMX.

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APPENDIX E

Field work at Sant Vicenç dels Horts test site (Barcelona, Spain)

E1 Introduction

The field test site for artificial recharge is located in Sant Vicenç dels Horts (Barcelona, Spain. Figure E1). The aquifer interested by recharge is the superficial unconfined aquifer of the lower Llobregat river valley. An overview of the geology of the area at local scale is shown in Figure E2. Further information on geology, climate, hydrology, hydrogeology and hydrochemistry at regional scale could be found in the work by UPC-ACA (2005).

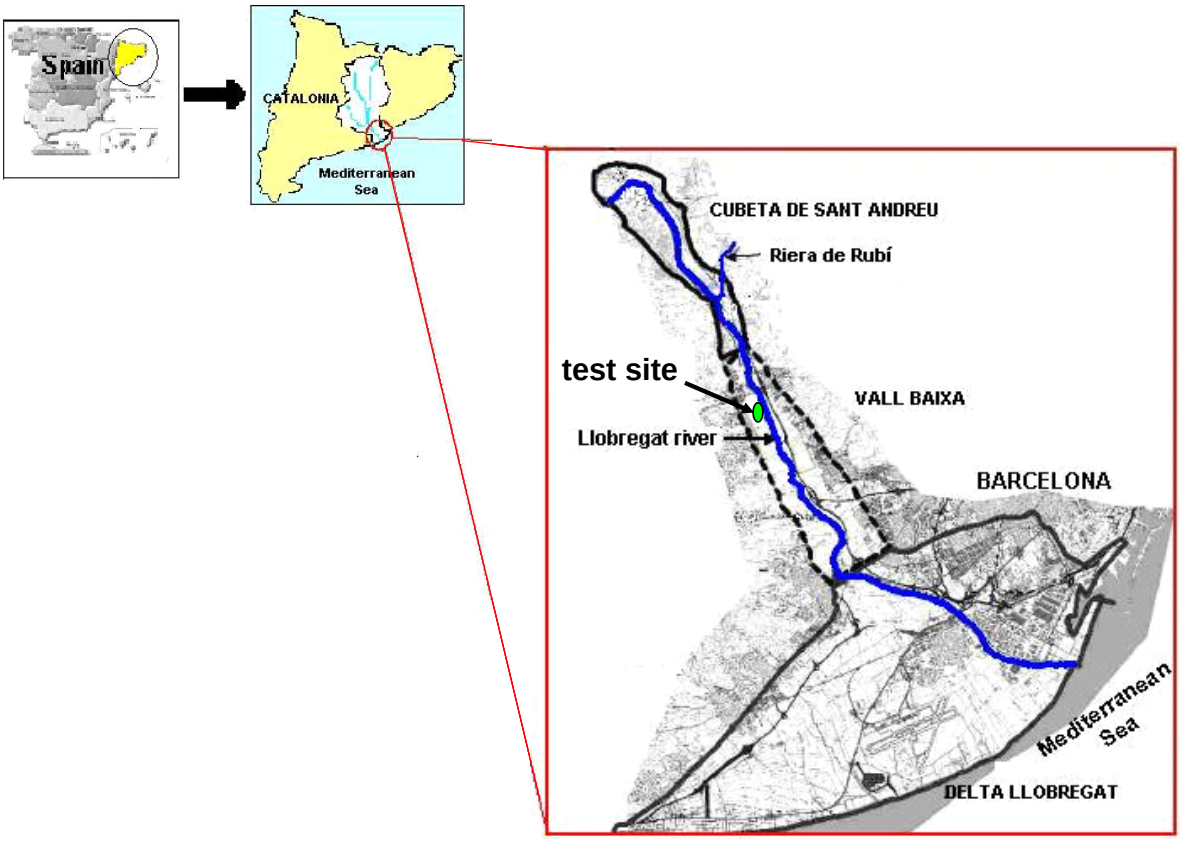
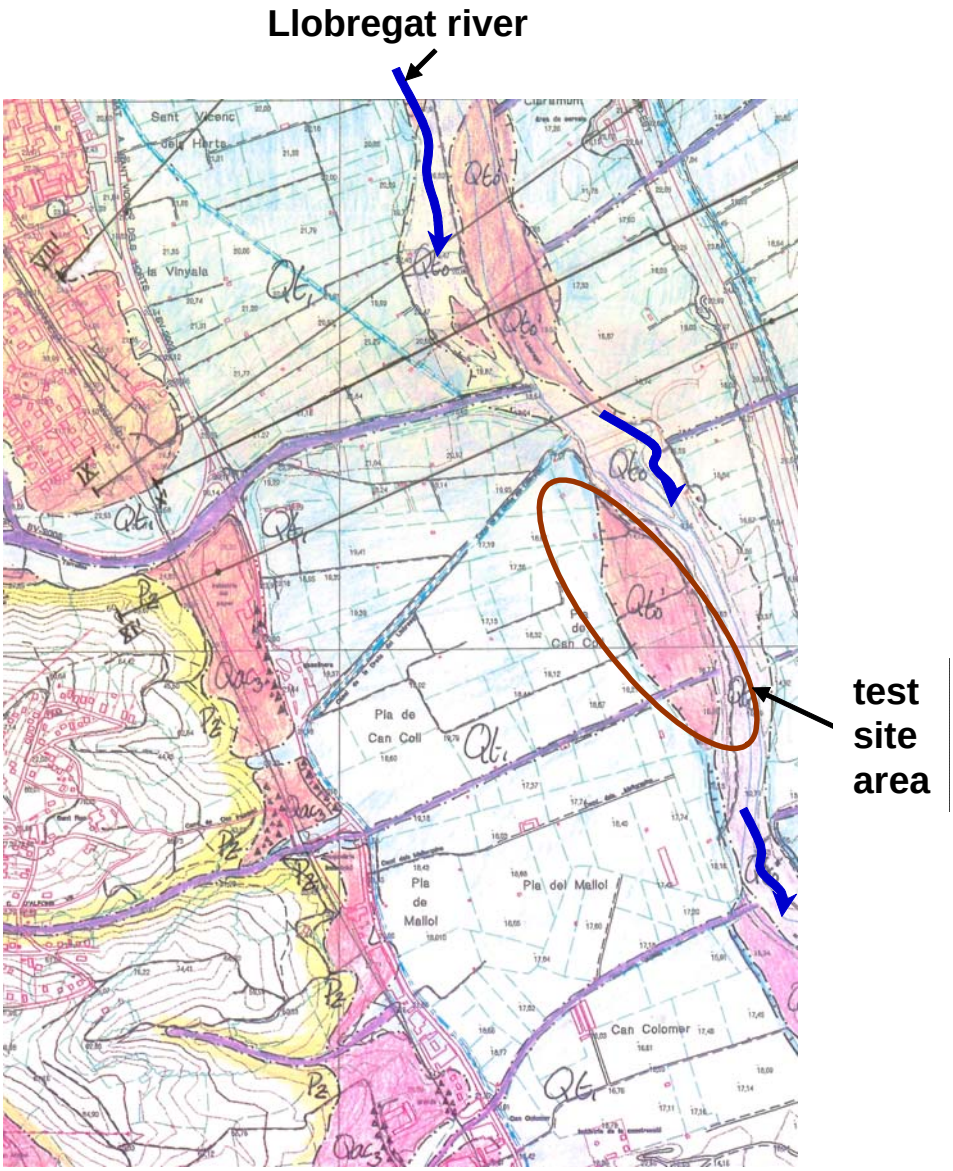


Figure E1: Location of Sant Vicenç dels Horts test site.



Qto Gravels, sands and silts - Recent alluvial
Qto' Gravels and sands - Quaternary
Qt1 Gravels, sands, silts and clay - Upper Holoc.
Qt2 Gravels, sands and silts - Upper Pleist.-Lower Holoc.
Qt3-Qac3 Gravels, sands and silts - Upper Pleist.
Qt4--Qac4 Gravels, sands and silts - Middle Pleist.
Qt5-Qac5 Gravels, sands and silts - Lower-Middle Pleist.
NP Blocks, breccias, conglom., sandstone and mudstone - Neog.-Plioc.
NMccs Mudstone, sandstone and conglom. - Neog.-Upper Mioc.
NMcci Breccias, conglom., sandstone, mudstone and conglom. - Neog.-Lower Mioc.
PZ Slates - Paleozoic

Figure E2: Geology at test site scale (from Galindo, 1998)

The overall objectives at the test site are:

- to improve the local experience on artificial recharge with ponds, looking forward to the operation of bigger infiltration systems in the area.
- to monitor the changes in water quality during the recharge processes, both in the vadose and saturated zone. Special focus is given to the fate of emerging and priority organic micropollutants. The findings at test site will be compared with the results from the laboratory batch experiments presented in this thesis and from other experiments being at ther moment carried out by other PhD students of the Hydrogeology group of UPC (Technical University of Catalonia).

The superficial recharge system is made up of two ponds (Figure E3): the first works as sedimentation pond, while the second one (about 0.5 ha) is the actual infiltration pond. The system has been setup so that two different waters could be used for recharge purposes: Llobregat river water or reclaimed water (tertiary effluent) from the wastewater treatment plant of El Prat de Llobregat (near Barcelona). The ponds were built as compensatory measure for the reduction in natural recharge caused by the construction of the high speed train line, but they have never been operated up to march 2009. Since then and up to now, Llobregat river water has been intermittently recharged in the facilities under the supervision of the Catalonian Water Agency (ACA) and the Lower Llobregat aquifers End-Users Community (CUADLL).

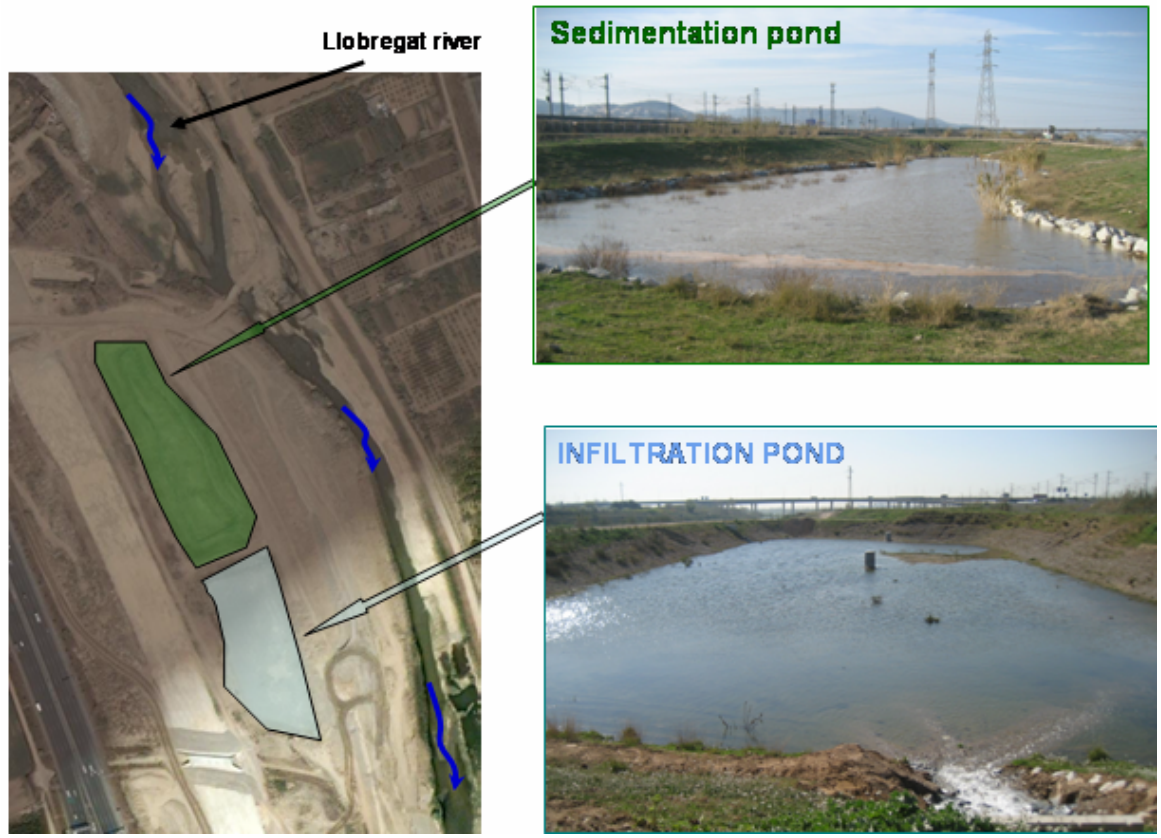


Figure E3: Recharge system at Sant Vicenç dels Horts test site.

E2 Work carried out at test site by the PhD candidate

The activities carried out at the field test site of Sant Vicenç dels Horts during the course of the PhD studies related to the present thesis are detailed in the following:

- Collection of the existing information at regional and local scale (geology, hydrology, hydrogeology, water chemistry, etc.).
- Design of the monitoring network to assess water flow and quality (vadose and saturated zone) in the facilities area.
- Characterization of the lithological profiles of 6 pits excavated inside the infiltration ponds; grain-size distribution of some sediment samples.
- Assessement of the installation of 4 new piezometers (2 of them multilevel piezometers) around the ponds; characterization of the lithological columns from the boreholes. The definitive monitoring network is exhibited in Figure E4.
- Instrumentation of the vadose zone underneath the infiltration pond (in 2 different niches. Figure E4 and E5). Tensiometers, water content probes and suction cups were installed (further details in paragraph E1.3). Concrete rings of 1 m diameter and about 2m high were installed inside the pond in order to create small “islands” for the dataloggers and the tips of sampling tubes from the suction cups.
- Design and installation of a lysimeter at about 1m depth inside the infiltration pond, in the nearby of the central niche.
- Installation of pressure/temperature/electrical conductivity transducers in the piezometer network (TD and CTD divers by Schlumberger Water Services).
- Monitoring of recharge water (volumes entering the pond and water quality), vadose zone (flow and water quality), and groundwater (water levels, temperature, electrical conductivity, and quality) during the first stage of the ponds’ operation (inundation test). Samples collected during the water sampling campaing were analysed for major and minor components, microbiological and general parameters, and organic micropollutants.

Further activities as well as an ampliation of the monitoring network at test site have been undertaken during the last two years and are at present ongoing, carried out by other PhD students of the Hydrogeology group of UPC (Technical University of Catalonia).

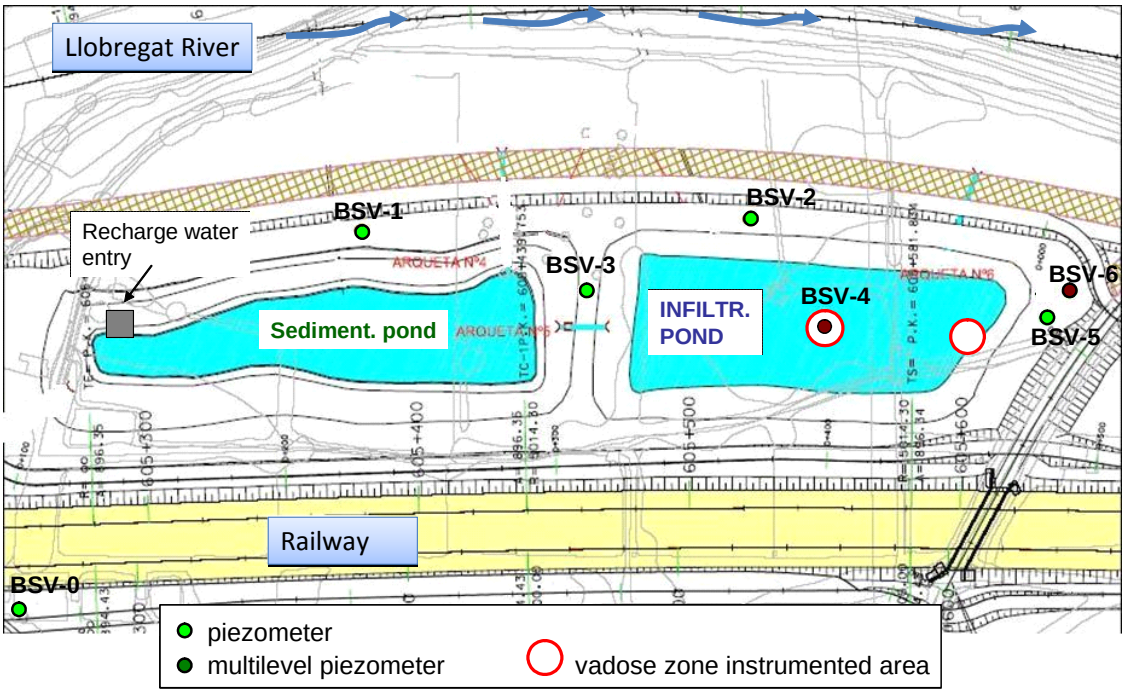


Figure E4: Scheme of the recharge system and the monitoring network at Sant Vicenç dels Horts test site.

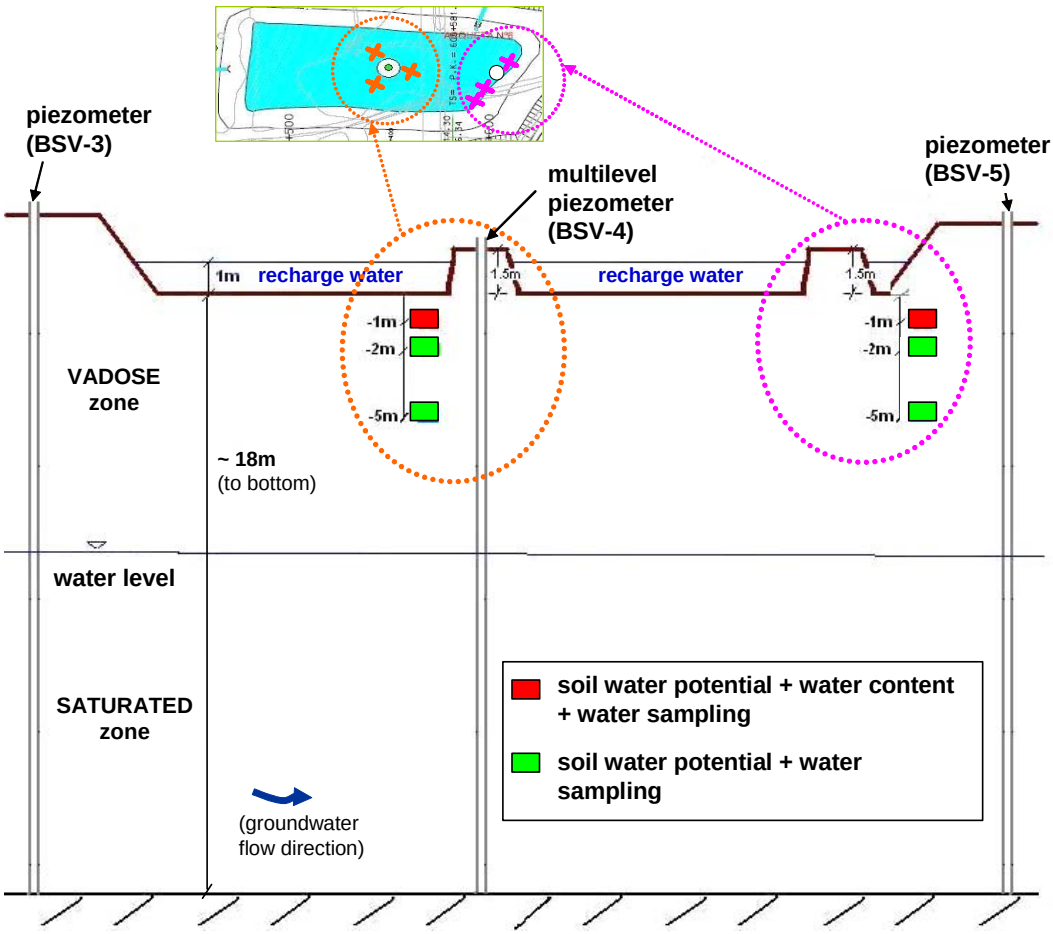


Figure E5: Scheme of the vadose zone monitoring network installed underneath the infiltration pond of the test site in Sant Vicenç dels Horts.

E3 Additional information on the equipment installed in the vadose zone

A) Tensiometer T4e (Umwelt Monitoring Systeme) + Datalogger DL6-te (6 channels)

Technical specifications

Measuring range	-1000 hPa ... +850 hPa
Output signal	-100 mV ... +85 mV
Accuracy	±5 hPa
Supply	typ. 10.6 VDC / 1.3 mA
Shaft	Ø 25 mm; acrylic
Shaft lengths	10 ... 200 cm
Shaft extensions	Possible on request
Porous ceramic	Ø 24 mm; length 60 mm
Bubble point	15 bar

Water refilling:	**External**
Max. installation depth:	**>8m**

B) Dielectric Sensor MPS-1 (UMS) + Datalogger Decagon Em-50 (5 channels)

Measuring range:	**-100 to -5000 hPa**
Accuracy:	**± kPa from -10 to -50 kPa**
	± 20% of reading fom -50 to -500kPa
Resolution:	**1 kPa from 0 to -100kPa**
	4kPa from -100 to -500kPa
Max. installation depth:	**>8m**

Figure E6: Equipments installed in the vadose zone underneath the infiltration pond to monitor soil water potential.

Capacitance probe ECH_2O-EC5 (Decagon Devices Inc.) + Datalogger Decagon Em-50 (5 channels)

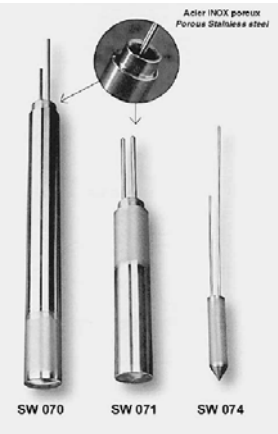
Measuring range: **0-100 water content**
Dimensions: **5cm**

Figure E7: Equipment installed in the vadose zone underneath the infiltration pond to monitor soil water content.

A) SKPE25 (UMS)

Cup: **ceramic**
Shaft diameter: **25mm**
Length: **to be specified**
Vol. retained in reservoir: **390mL (for shaft lenght 80cm)**
Suction tube: **PE**
Operating depth: **down to 8m**
Bubble point: **1bar**

B) Porous Stainless steel SW070 (SDEC)



Cup: **porous stainless steel**
Outside diameter: **50.8mm**
Length: **457.2mm**
Vol. retained in reservoir: **~600mL**
Suitable for organics and most inorganics
Operating depth: **>3m**
Bubble point: **500mbar**

Figure E8: Equipment installed in the vadose zone underneath the infiltration pond for water sampling.

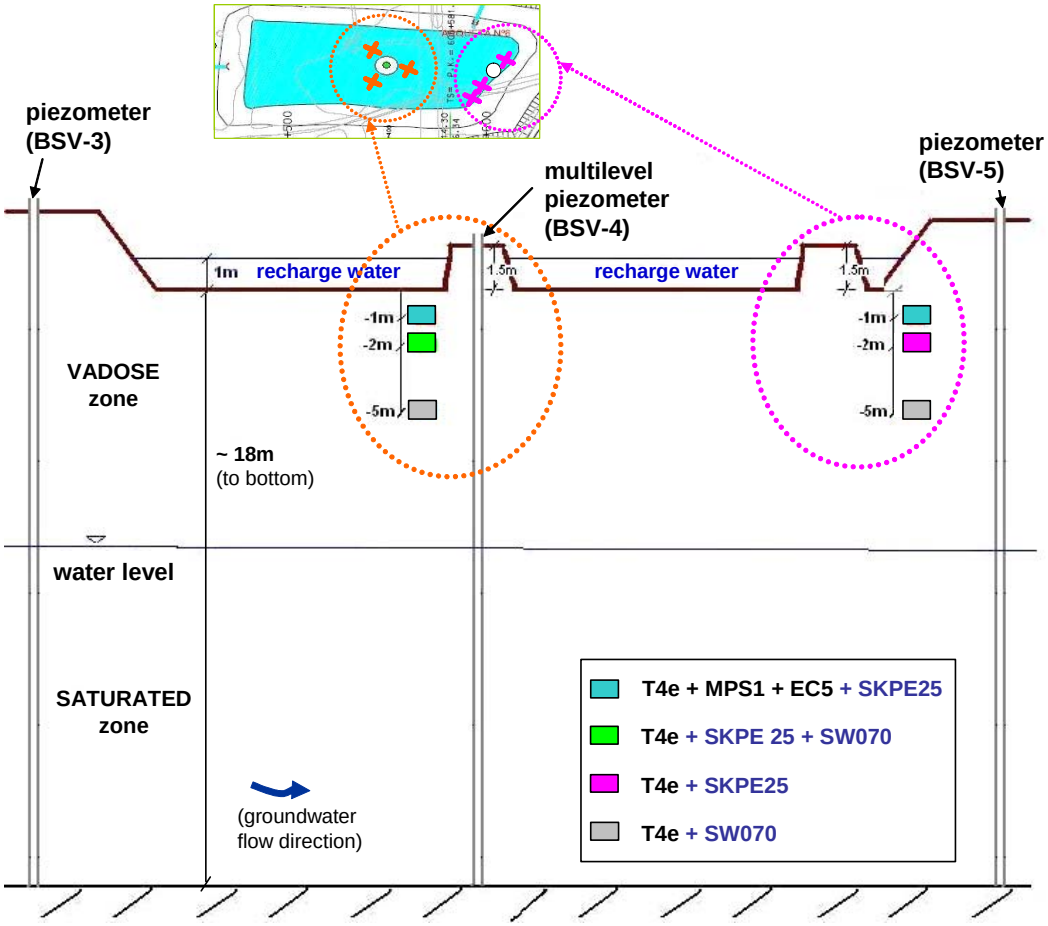


Figure E9: Location of the equipment installed in the vadose zone underneath the infiltration pond.

References – Appendix E

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