



Formation of diclofenac and sulfamethoxazole reversible transformation products in aquifer material under denitrifying conditions: Batch experiments

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ABSTRACT

Soil-aquifer processes have proven to work as a natural treatment for the attenuation of numerous contaminants during artificial recharge of groundwater. Nowadays, significant scientific effort is being devoted to understanding the fate of pharmaceuticals in subsurface environments, and to verify if such semipersistent organic micropollutants could also be efficiently removed from water. In this context we carried out a series of batch experiments involving aquifer material, selected drugs (initial concentration of 1 µg/L and 1 mg/L), and denitrifying conditions. Diclofenac and sulfamethoxazole exhibited an unreported and peculiar behavior. Their concentrations consistently dropped in the middle of the tests but recovered toward the end, which suggest a complex effect of denitrifying conditions on aromatic amines. The transformation products Nitro-Diclofenac and 4-Nitro-Sulfamethoxazole were detected in the biotic experiments, while nitrite was present in the water. Their concentrations developed almost opposite to those of their respective parent compounds. We conjecture that this temporal and reversible effect of denitrifying conditions on the studied aromatic amines could have significant environmental implications, and could explain at least partially the wide range of removals in subsurface environments reported in literature for DCF and SMX, as well as some apparent discrepancies on SMX behavior.

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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). An important source of these organic compounds is the discharge of effluents from wastewater treatment plants into surface water bodies (Gros et al., 2010; Onésios et al., 2009; Petrovic et al., 2009 and references therein). They also come 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 in the ng/L to µg/L range. Still, they are a cause 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; Yamamoto et al., 2009 and references therein). In this respect, intensive work is associated to managed aquifer recharge (Díaz-Cruz and Barceló, 2008 and ref. therein; Hoppe-Jones et al., 2010 and ref. therein; Patterson et al., 2009). 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 on the behavior of drugs in the subsurface, their degradation pathways and the potential formation of transformation products is still limited.

This work is motivated by artificial recharge of groundwater practices using Llobregat river water (Barcelona area, Spain). We have carried out microcosm experiments involving natural aquifer material to investigate the fate of selected organic pollutants, mainly pharmaceuticals, under nitrate reducing conditions, which we expect will develop under recharge basins. We present in this paper the unreported and peculiar behavior exhibited during the experiments

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by two of the studied compounds, diclofenac and sulfamethoxazole. Their concentrations consistently dropped in the middle of the tests, but recovered toward the end.

Diclofenac (from now on 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. Both compounds are included as Class 1: high priority pharmaceuticals in the common list of pharmaceuticals relevant to the water cycle prepared by The Global Water Research Coalition (GWRC, 2004). Their physicochemical characteristics are reported in Table 1. Recent monitorings reported the occurrence of these two drugs in the Llobregat river basin at concentrations in the ng/L range (Köck-Schulmeyer et al., 2011; López-Roldán et al., 2010), with punctual maxima of some µg/L (Muñoz et al., 2009). The existing literature on their fate in subsurface environments describes sorption onto aquifer material to be not significant at pH ranging between 7 and 8.3 (Baumgarten et al., 2011; Rauch-Williams et al., 2010). Field and laboratory studies on managed aquifer recharge (Heberer and Adam, 2004; Preuss et al., 2001; Rauch-Williams et al., 2010, 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%). Regarding SMX, however, mixed and sometimes contradictory results have been reported on its fate in different natural systems. For example, 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. However, field data on bank filtration and aquifer recharge through ponds in Germany suggested SMX to be degradable preferably 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) rather than under aerobic ones (Grünheid et al., 2005: 53% removed in 50 days; Heberer et al., 2008: 52% removed in 1 month). Finally, 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).

We conjecture that the wide range of reported removals for DCF and SMX as well as the apparent discrepancies in the literature on

SMX behavior could be explained, at least partially, by a transient and reversible effect of denitrifying conditions on aromatic amines.

2. Materials and methods

The experiments consisted of sets of microcosms containing natural sediments, synthetic water and organic pollutants. The latter were spiked at individual concentrations of 1 µg/L, i.e. environmental concentrations, in “Experim. 1 µg/L”. To check the representativeness of studies at concentrations easier to be tested and analyzed, a markedly higher individual concentration of 1 mg/L was used for pollutants in “Experim. 1 mg/L”. 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. A more exhaustive description of the experimental design and methodology as well as details on the complete set of the organic pollutants included in the experiments could be found in Barbieri et al. (2011) and references therein.

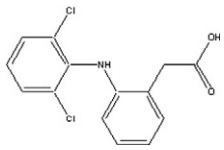
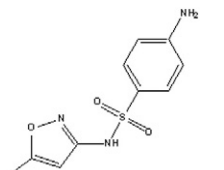
2.1. Organic pollutants

DCF and SMX (purity >96%), diclofenac-*d4*, sulfamethoxazole-*d4* and ibuprofen-*d3* were purchased from Sigma Aldrich. SMX-¹³C₆ was supplied by LGC Promochem. Nitro-Diclofenac (from now on Nitro-DCF) and 4-Nitro-Sulfamethoxazole (4-Nitro-SMX) were synthesized according to Osorio and Pérez (in preparation) and Nödler et al. (2012). 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. Stock and working standard solutions were stored at –18 °C in the dark.

2.2. Sediments and water

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 before any artificial recharge activities, from an oxic unsaturated horizon at about 1 m below the infiltration pond. They were sieved to <1 mm and immediately used for assembling

Table 1
Physicochemical properties of the compounds of interest.

Compound	Structure	CAS number	logK _{ow} ^{a,b}	pK _a ^a	Formula
Diclofenac (DCF)		15307-86-5	4.06 ± 0.41	4.2 ± 0.1	C ₁₄ H ₁₀ Cl ₂ NO ₂
Sulfamethoxazole (SMX)		723-46-6	0.89 ± 0.42	5.8 ± 0.5 ^c 1.4 ± 0.1 ^d	C ₁₀ H ₁₁ N ₃ O ₃ S

^a SciFinder predicted values.

^b log K_{ow} value of the neutral species.

^c pK_a value of the secondary amine.

^d Basic pK_a value of the primary amine (pK_a of the conjugate acid).

the experiments, or stored before use for a maximum of two days at 25 °C inside aluminum foil. Their mineralogical and chemical characteristics are summarized in Table S1 of the Supporting Information.

The experiment water was artificially prepared to mimic recharge water (Llobregat river water) at test site except for the organic carbon, which was initially zero. This water was prepared in a glass amber bottle by dissolving respective amounts of salts (sodium bicarbonate, sodium sulfate, sodium chloride, calcium chloride dihydrate, magnesium nitrate hexahydrate, potassium chloride, magnesium chloride hexahydrate, potassium dihydrogen phosphate and ammonium chloride). All salts were purchased from Sigma Aldrich in ultrapure water. Its composition is shown in Table S2 of the Supporting Information. Nitrate concentration was then increased by further dissolution of magnesium nitrate hexahydrate (additional 7.3 mmol/L and 66 mmol/L of nitrate for the waters of “*Experim. 1 µg/L*” and “*Experim. 1 mg/L*”, respectively). Finally, the water was purged with Ar (purity ≥99.999%) for about 1 h to remove all oxygen from the solution and the bottle headspace.

2.3. Experimental procedure

The sediments were homogenized in steel containers and distributed in fractions of 120 g (dry weight) into 0.3 L glass bottles. The 0.3 L bottles as well as the glass amber bottle containing the previously prepared water (Section 2.2) were then placed inside a glove box under Argon atmosphere (maximum 0.1% of O₂). 2.1 mmol/L of anhydrous sodium acetate was added to the synthetic water. Afterwards, the organic pollutants were also added to the water from their methanol working standard mixtures (Section 2.1) to yield final aqueous individual concentrations of 1 µg/L in “*Experim. 1 µg/L*” and 1 mg/L in “*Experim. 1 mg/L*”. The expected Dissolved Organic Carbon (DOC) concentration resulting from the addition of acetate and of the methanol contained in the pollutants spiking solution resulted in 6.9 mmol/L and 80 mmol/L for “*Experim. 1 µg/L*” and “*Experim. 1 mg/L*”, respectively. After measuring parameters and sampling for chemical analysis, 0.24 L of the initial water obtained was then added to each 0.3 L glass bottle already containing the sediments. The assembling procedure was concluded by properly sealing the bottles against oxygen with screw-caps plus a PTFE protection seal, and gently shaking. A remaining headspace of about 15 mL was left in each bottle.

The microcosms were removed from the glove box and wrapped with aluminum foil to prevent photodegradation. Then, they were incubated under controlled temperature (25 ± 2 °C) and gently shaken a 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 days) as well as the day before dismantlement.

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

Duplicate bottles were sacrificed according to pre-defined schedules (at days 0.05, 0.2, 0.5, 1.5, 3, 5, 10 and 21 for “*Experim. 1 µg/L*”; at days 2, 5, 10, 15, 25, 41 and 87 for “*Experim. 1 mg/L*”). One at a time, the two bottles were opened under Ar atmosphere, chemical parameters were measured, and aqueous samples for bulk chemistry and pollutants analysis were collected and stored as described in Section 2.4 until analysis. Sterility of the abiotic series was verified six times in “*Experim. 1 µg/L*” series and twice in “*Experim. 1 mg/L*” series. To this end, an aliquot of microcosms water was spread on tryptic soy agar (TSA) plates and incubated in duplicate at 25 °C for 1 week under aerobic conditions and for 2 weeks under anaerobic conditions. None of the plates demonstrated microorganisms' growth.

2.4. Analytical methods

2.4.1. Bulk water chemistry analysis

Biotic samples collected during microcosms' assembling/disassembling for the analysis of Cl⁻, NO₃⁻, NO₂⁻, SO₄²⁻, PO₄³⁻, F⁻, NH₄⁺ and DOC (Dissolved Organic Carbon) were filtered through 0.45 µm PALL Acrodisc® Sterile Syringe Filters with Supor® membrane and frozen. Anions were analyzed by ion chromatography using a Dionex ICS-1000 instrument. The analytical error was estimated to be 14% for PO₄³⁻ and 13% for the remaining anions. Detection limits for NO₃⁻ and NO₂⁻ were 0.5 mg/L and 0.05 mg/L, respectively. NH₄⁺ concentration was analyzed with a selective electrode Orion 9512. Its detection limit was 0.1 mg/L. DOC was analyzed by 680 °C combustion catalytic oxidation/NDIR method using a Shimadzu TOC-V CSH instrument. The estimated analytical error was 20%. Biotic and abiotic samples for analyzing COD (Chemical Oxygen Demand) were also filtered 0.45 µm and frozen, to be further on analyzed by colorimetry with the spectrophotometer Spectroquant Nova 60. Abiotic samples for Cl⁻, NO₃⁻, SO₄²⁻ and F⁻ were frozen and then analyzed by ion chromatography using a Dionex DX-320 instrument with conductometric detection, a Dionex AS11-HC (2 × 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). The analytical error was estimated to be 8%.

Samples for Ca, Mg, Na, K and minor elements were filtered at 0.45 µm, acidified and stored at 4 °C. 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.

The parameters measured during the assembling/disassembling of the microcosms were: pH (Thermo Scientific 9157BN Triode pH electrode, refillable), dissolved oxygen (Hanna Instruments, HI 76407/4 DO probe) and alkalinity (drop test kit Taylor K-1726, precision of 0.5 mmol/L).

2.4.2. Micropollutants analysis in “*Experim. 1 µg/L*”

The aqueous calibration standards for DCF and SMX (concentration range 1–1500 ng/L) were prepared by using the methanol working standard mixtures described in Section 2.1. Diclofenac-*d4* and sulfamethoxazole-*d4* were used as surrogate standards for DCF and SMX quantification, respectively. The surrogate standard mixture in methanol was also prepared according to the procedure described in Section 2.1, and then added to the water samples from the experiment and to the aqueous calibration standards just before analysis to yield final concentration of 200 ng/L.

Samples collected during microcosms' assembling/disassembling for analysis of organic pollutants were filtered at 0.45 µm using WATERS Syringe filter with PTFE membrane and frozen. Their analysis was then performed by on-line solid phase extraction-liquid chromatography-tandem mass spectrometry (SPE-LC-MS/MS). Briefly, water samples and all the aqueous calibration standards, spiked with the standard mixture of the isotopically labeled 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. The main advantage of the method is that aqueous calibration and surrogates standards are processed in the same way as samples. Sample and calibration standards preconcentration were 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 (125×2 mm, 5 μm 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 (see experimental conditions in Table S3 of the Supporting Information) 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.

The analytical method developed showed good validation parameters in terms of accuracy (relative recovery – comparison of the absolute recoveries of the analytes with those of their surrogates – between 106.7% and 108.3%) and repeatability (RSD<25%). The validation was carried out with a fivefold study (n=5) and synthetic water with an initial concentration of the analytes of 1 μg/L. The main advantage of the developed method is its improved accuracy due to the use of the isotope dilution method.

2.4.3. Pollutants analysis in “*Experim. 1 mg/L*”

For the quantification of DCF and SMX, the internal standards 100 ng/mL ibuprofen-*d*3 and 125 ng/mL SMX-¹³C₆ were used, respectively. The suitability of ibuprofen-*d*3 for the quantification of DCF was demonstrated previously (Nödler et al., 2010). Calibration levels of DCF and SMX (concentration range 20–1000 ng/mL) were prepared by successively diluting methanolic stock solutions in inorganic matrix according to 50% of “*Experim. 1 mg/L*” initial water concentrations. This measure was undertaken in order to generate an inorganic matrix similar to the samples, as inorganic matrix may interfere with the analytes in electrospray ionization and thus negatively influencing their quantification. Internal and external matrix adjusted calibration ensured data quality. The overall Relative Standard Deviation (RSD) of multiple injections was <5% for all analyses. The linear correlation coefficients of the calibrations exceeded 0.99.

Samples collected during microcosms' assembling/disassembling for analysis of organic pollutants were kept frozen until analysis. Prior to DCF and SMX analysis, samples and calibration levels were diluted (v/v) 1:2 (DCF) and 1:4 (SMX) with aqueous 5 mM ammonium acetate solution, containing 4% methanol. Before analysis, all samples and calibration levels were centrifuged at 1500 rpm for 30 min at room temperature. Analysis of 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. The parameters of the mass spectrometer for DCF and SMX analysis are provided in Table S4 of the Supporting Information.

3. Results and discussion

Results are presented below in terms of averages of data from the duplicate batches. Namely, the temporal evolution of the organic pollutants is reported in terms of their average concentration divided by the corresponding initial concentration C_0 . The objective of this normalization is to remove systematic errors from the analyses.

The error bars reported in the figures have been calculated by considering the analytical errors and the difference between batches results (details are given in Text S2 of the Supporting Information).

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

3.1. Bulk water chemistry

The evolution of water chemistry in the biotic series of “*Experim. 1 μg/L*” (pollutants at 1 μg/L individual initial concentration) is shown in Fig. 1a. During the first 10 days, DOC decreased from 9.7 mmol/L to 1.5 mmol/L while alkalinity increased from 1 mmol/L to 3.75 mmol/L. Afterwards, both remained practically constant. No oxygen was detected during the whole experiment. By day 10, the initial 6.7 mmol/L of nitrate had disappeared. Nitrite concentration began to increase after some 12 h, reaching a maximum at day 5 and becoming depleted by 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. Sulfate 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 were established after 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, reflects the actual denitrification pathway, with nitrite being an intermediate product between nitrate and nitrogen. More reducing conditions were established after nitrate and nitrite were exhausted, as suggested by the slight increase of Mn and Fe.

The complete hydrochemical evolution and mass balance of the biotic test are not reported here (details are given in Text S1 of the Supporting Information). The essence of those mass balance calculations is that about 27% of the consumed organic carbon was converted into biomass during the 10 days of nitrate reducing conditions. The remaining part was mineralized and then precipitated as carbonates, transferred to the gas phase in the headspace of the bottles, or remained in solution to increase alkalinity. Under these assumptions, the overall inorganic carbon mass balance could be closed with an error of about 15%.

Results for the water chemistry of the biotic series of “*Experim. 1 mg/L*” (pollutants at 1 mg/L individual initial concentration) are shown in Fig. 1b. Qualitatively, its evolution is consistent with that of the biotic series of “*Experim. 1 μg/L*” though displaced in the time scale. DOC and nitrate decreased throughout the experiment, starting (appreciably) after day 5 but still remaining by day 87 with final concentrations of 27.2 mmol/L and 7.5 mmol/L, respectively. Alkalinity increased continuously from 0.8 mmol/L to 22 mmol/L starting at day 2. Nitrite concentrations also began to increase at day 2, reaching a maximum at day 41, and becoming depleted at day 87. Dissolved oxygen, manganese and iron were not detected, sulfate remained constant, and pH ranged between 7.3 and 8.3 during the experiment.

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 somehow longer adaptation time of microbial communities, compared to that characterizing “*Experim. 1 μg/L*”, could be likely related to the different proportion of methanol present in “*Experim. 1 mg/L*” or possibly to some growth inhibition by SMX (Underwood et al., 2011). The practical depletion of nitrite between days 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 during such period.

In this case, about 5% to 10% of the organic carbon was calculated 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 10%.

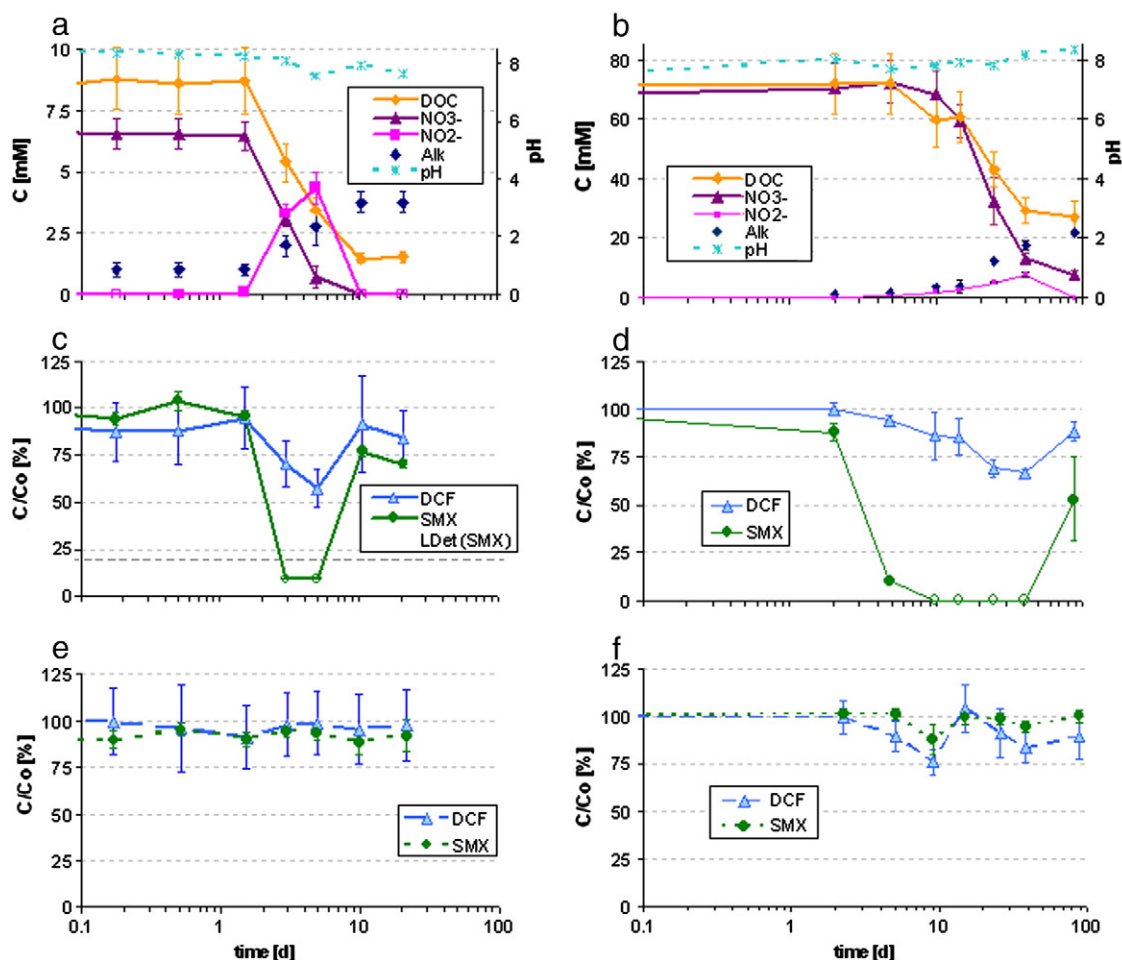


Fig. 1. Chemical evolution with time in the biotic series of “Experim. 1 µg/L” (a) and “Experim. 1 mg/L” (b). Evolution with time of the average normalized concentration (with respect to the initial value C_0) of DCF and SMX in the biotic series of “Experim. 1 µg/L” (c) and “Experim. 1 mg/L” (d); idem in the abiotic series of “Experim. 1 µg/L” (e) and “Experim. 1 mg/L” (f). Concentrations smaller than the correspondent Limit of Determination (LDet) have been plotted as LDet/2 and by using open symbols.

3.2. Organic pollutants

The time evolutions of DCF and SMX in the biotic and abiotic series of “Experim. 1 µg/L” (pollutants at 1 µg/L individual initial concentration) and “Experim. 1 mg/L” (pollutants at 1 mg/L individual initial concentration) are reported in Fig. 1c, e, d and f, respectively. Both compounds were negatively charged within the pH range of the experiments.

Overall, DCF was not removed in “Experim. 1 µg/L” after 10 days of nitrate reducing conditions. Its final normalized concentration in both biotic and abiotic series (Fig. 1c and e) remained around 100% (considering the error bars). Nevertheless, its concentration in the biotic series suffered a sudden drop at day 1.5, followed by complete recovery by day 10.

An analogous pattern could be observed for DCF in “Experim. 1 mg/L” (Fig. 1d and f), but displaced in the time scale. On the whole, DCF was not or only slightly (10%) removed during the 87 days of both biotic and abiotic tests. But its concentration in the biotic set of microcosms also suffered a drop between day 2 and day 41 followed by a recovery between days 41 and 87.

The overall removal of SMX was about 25% during the 10 days of nitrate reducing conditions in the biotic series of “Experim. 1 µg/L” (Fig. 1c), and $47 \pm 20\%$ after 87 of nitrate reducing conditions in the biotic series of “Experim. 1 mg/L” (Fig. 1d). But SMX concentration also dropped, even more sharply than DCF (down to near zero concentration), between days 1.5 and 10 of the biotic test of “Experim. 1 µg/L”, rebounding afterwards. Again, the same non-monotonic

behavior but displaced in the time scale could be observed for SMX in the biotic test of “Experim. 1 mg/L”. As for DCF in “Experim. 1 mg/L”, the SMX drop and recovery occurred between days 2 and 87. In the abiotic series of both tests (Fig. 1e and f), on the other hand, SMX concentrations remained constant all time long.

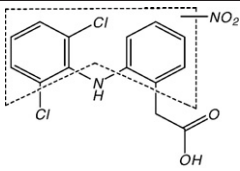
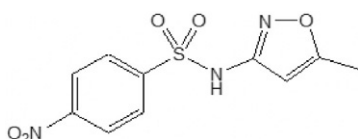
The evolution of the two pharmaceuticals and the bulk water chemistry (Fig. 1) shows that such unexpected reversible process of drop and rebound of both DCF and SMX concentrations occurs only in the biotic series, concurrently and opposite to the evolution of nitrite. Among all the organic pollutants included in the experiments (results not shown), such peculiar trend could be observed only for DCF and SMX, which were the only aromatic amines in the tests.

3.3. Discussion upon the effect of denitrifying conditions on DCF and SMX

A further analysis of the water samples exhibited the presence of additional peaks emerging in the chromatograms after day 0.5 and after day 2 in the biotic series of “Experim. 1 µg/L” and “Experim. 1 mg/L”, respectively, representing possible candidates for DCF and SMX transformation products. Based on ongoing studies and previous literature (Nödler et al., 2012; Osorio and Pérez, in preparation; Pérez and Barceló, 2008), two of the newly emerging peaks suggested nitro analogs of DCF and SMX. Indeed, among them we could detect the presence of Nitro-DCF and 4-Nitro-SMX with authentic standards (Section 2.1). Multiple injections resulted in a RSD of 20%. The

Table 2

Physicochemical properties of the transformation products Nitro-DCF and 4-Nitro-SMX detected in the biotic series.

Compound	Structure	CAS number	logK _{ow} ^a	pK _a
Nitro-Diclofenac (NO ₂ -DCF)		174316-61-1	4.86 ^b	3.82 ^b
4-Nitro-Sulfamethoxazole (4-NO ₂ -SMX)		29699-89-6	1.27 ± 0.41 ^c	5.65 ± 0.4 ^c

^a log K_{ow} value of the neutral species.^b SPARC predicted values for 3-NO₂-DCF (<http://archemcalc.com/sparc>).^c SciFinder predicted values.

characteristics and structures of the two compounds, being anionic within the pH range of our experiments, are reported in Table 2.

As presented in Fig. 2, 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. As such, their concentration increases with nitrite, while that of their parent compounds decreases. Furthermore, the two nitro derivatives become completely depleted at the same time as nitrite, which coincides with the rebound of the parent compounds.

It has to be noted that in both “Experim. 1 µg/L” and “Experim. 1 mg/L” 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 suggests the presence of some intermediate transformation product of SMX to 4-Nitro-SMX. Unfortunately we could neither identify such possible intermediate nor further DCF transformation products, hindering a full assessment of DCF and SMX fate. In spite of these restraints, it is important to stress that

the trends observed for both drugs and their respective nitro derivatives could be confirmed in experiments testing two markedly different initial concentrations of DCF and SMX (i.e., 1 µg/L to 1 mg/L), and characterized by a different evolution of denitrification in the time scale (Section 3.1).

The presented findings suggest thus a reversible nature of Nitro-DCF and 4-Nitro-SMX formation. In summary, even if the specific mechanisms are still unclear, we propose that the drop in concentration of DCF and SMX is at least partially caused by the formation of nitro products in presence of nitrite, a process already observed for aromatic amines by Pereira et al. (2011) and Pérez and Barceló (2008). Further on, such nitro-derivatives return the parent compounds when the concentration of nitrite drops. It is worth mentioning that DCF, containing a secondary amine, presented only a partial decrease of concentration in presence of nitrite while SMX, containing a primary amine, experienced a sharper drop, down to zero concentration.

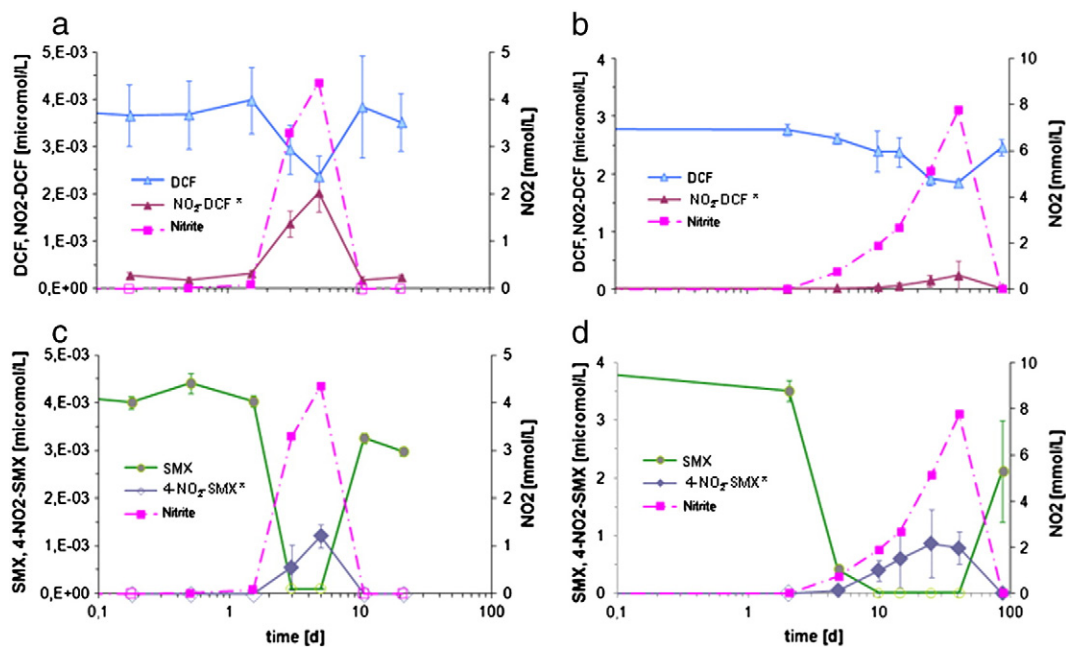


Fig. 2. Evolution of DCF, Nitro-DCF (NO₂-DCF), and nitrite in the biotic series of “Experim. 1 µg/L” (a) and “Experim. 1 mg/L” (b). Evolution of SMX, 4-Nitro-SMX (4-NO₂-SMX), and nitrite in the biotic series of “Experim. 1 µg/L” (c) and “Experim. 1 mg/L” (d). Concentrations smaller than the correspondent Limit of Determination (LDet) have been plotted as LDet/2 and by using open symbols. The superscript “*” indicates estimated concentrations relative to a standard from synthesized compounds (Section 2.1), and to the internal standards used for quantification of the parent compounds.

4. Implications

- We have observed that the fate in aquifer material of the aromatic amines diclofenac and sulfamethoxazole could be temporarily and reversibly affected by denitrifying conditions. Namely, the concentration of these two drugs decreases when nitrite builds up, but rebounds when nitrite is reduced toward nitrogen.
- Nitro-DCF and 4-Nitro-SMX, transformation products of DCF and SMX, were detected. 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.
- DCF, containing a secondary amine, presented only a partial decrease of concentration while SMX, containing a primary amine, experienced a drop down to zero concentration. The specific mechanisms of these reactions are still unclear, but nitrite could be likely involved/responsible of the formation of the two detected nitro compounds from the studied aromatic amines.

There could be a significant environmental implication of the presented findings related to the fate in aquifers under denitrifying conditions of DCF and SMX, and possibly other aromatic amines. Ignoring the observed transient drop of these compounds could lead experimenters to overestimate their actual elimination in field and laboratory studies. This may explain the wide ranges of removals reported for DCF and SMX, as well as the inconsistencies on literature reports about their elimination (e.g. in the case of SMX). This also stress the importance of a thorough monitoring of the inorganic chemistry, including nitrite species, in field and laboratory studies, and the advisability of the identification and/or quantitative analysis of transformation products when investigating the fate of organic micropollutants.

We finally conjecture that the transformation products found in the presented experiments may also be formed in wastewater treatment plants, since nitrification and denitrification processes occur during biological treatment. This can lead to a wrong estimation of the actual efficiency of wastewater treatment plants in removing DCF and SMX, and possibly other aromatic amines, as their nitro derivatives can transform back into the parent compounds when released in the environment.

Supplementary materials related to this article can be found online at doi:10.1016/j.scitotenv.2012.02.058.

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References

Barber LB, Keefe SH, LeBlanc DR, Bradley PM, Chapelle FH, Meyer MT, et al. Fate of sulfamethoxazole, 4-nonylphenol, and 17 β -estradiol in groundwater contaminated by wastewater treatment plant effluent. *Environ Sci Technol* 2009;43(13):4843–50.

Barbieri M, Carrera J, Sanchez-Vila X, Ayora C, Cama J, Köck-Schulmeyer M, et al. Microcosm experiments to control anaerobic redox conditions when studying the fate of organic micropollutants in aquifer material. *J Contam Hydrol* 2011;126:330–45.

Baumgarten B, Jährgig J, Reemtsma T, Jekel M. Long term laboratory column experiments to simulate bank filtration: factors controlling removal of sulfamethoxazole. *Water Res* 2011;45(1):211–20.

Benotti MJ, Brownawell BJ. Microbial degradation of pharmaceuticals in estuarine and coastal seawater. *Environ Pollut* 2009;157(3):994–1002.

Christensen TH, Kjeldsen P, Bjerg P, Jensen D, Christensen JB, Baun A, et al. Biogeochemistry of landfill leachate plumes. *Appl Geochem* 2001;16(7):659–718.

Cordy GE, Duran NL, Bouwer H, Rice RC, Furlong ET, Zaugg SD, et al. Do pharmaceuticals, pathogens, and other organic waste water compounds persist when waste water is used for recharge? *Ground Water Monit Rem* 2004;24(2):58–69.

Díaz-Cruz MS, Barceló D. Trace organic chemicals contamination in ground water recharge. *Chemosphere* 2008;72(3):333–42.

Farré M, Pérez S, Kantiani L, Barceló D. Fate and toxicity of emerging pollutants, their metabolites and transformation products in the aquatic environment. *TrAC Trends Anal Chem* 2008;27(11):991–1007.

Fent K, Weston AA, Caminada D. Ecotoxicology of human pharmaceuticals. *Aquat Toxicol* 2006;76(2):122–59.

Focazio MJ, Kolpin DW, Barnes KK, Furlong ET, Meyer MT, Zaugg SD, et al. A national reconnaissance for pharmaceuticals and other organic wastewater contaminants in the United States – II. Untreated drinking water sources. *Sci Total Environ* 2008;402(2–3):201–16.

Gros M, Petrovic M, Ginebreda A, Barceló D. Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. *Environ Int* 2010;36(1):15–26.

Grünheid S, Amy G, Jekel M. Removal of bulk dissolved organic carbon (DOC) and trace organic compounds by bank filtration and artificial recharge. *Water Res* 2005;39(14):3219–28.

GWRC. Pharmaceuticals and personal care products in the water cycle. An international review, London, UK; 2004.

Heberer T, Adam M. Transport and attenuation of pharmaceutical residues during artificial groundwater replenishment. *Environ Chem* 2004(1):22–5.

Heberer T, Massmann G, Fanck B, Taute T, Dünnbier U. Behaviour and redox sensitivity of antimicrobial residues during bank filtration. *Chemosphere* 2008;73(4):451–60.

Holm JV, Ruegge K, Bjerg PL, Christensen TH. Occurrence and distribution of pharmaceutical organic compounds in the groundwater downgradient of a landfill (Grindsted, Denmark). *Environ Sci Technol* 1995;29(5):1415–20.

Hoppe-Jones C, Oldham G, Drewes JE. Attenuation of total organic carbon and unregulated trace organic chemicals in U.S. riverbank filtration systems. *Water Res* 2010;44(15):4643–59.

Hua J, An P, Winter J, Gallert C. Elimination of COD, microorganisms and pharmaceuticals from sewage by trickling through sandy soil below leaking sewers. *Water Res* 2003;37(18):4395–404.

Jekel M, Grünheid S, Baumgarten B, Hübner U, Wiese B. Removal of bulk and trace organics in underground treatment systems. *Micropol & Ecohazard* 2009 – 6th IWA/GRA Specialized Conference on Assessment and Control of Micropollutants/Hazardous Substances in Water. June 8–10, 2009 – San Francisco, CA; 2009.

Kemper N. Veterinary antibiotics in the aquatic and terrestrial environment. *Ecol Indic* 2008;8(1):1–13.

Köck-Schulmeyer M, Ginebreda A, Postigo C, López-Serna R, Pérez S, Brix R, et al. Wastewater reuse in Mediterranean semi-arid areas: the impact of discharges of tertiary treated sewage on the load of polar micro pollutants in the Llobregat river (NE Spain). *Chemosphere* 2011;82(5):670–8.

Kumar A, Chang B, Xagorarakis I. Human health risk assessment of pharmaceuticals in water: issues and challenges ahead. *Int J Environ Res Public Health* 2010;7(11):3929–53.

Loos R, Gawlik BM, Locoro G, Rimaviciute E, Contini S, Bidoglio G. EU-wide survey of polar organic persistent pollutants in European river waters. *Environ Pollut* 2009;157(2):561–8.

López-Roldán R, López de Alda MJ, Gros M, Petrovic M, Martín-Alonso J, Barceló D. Advanced monitoring of pharmaceuticals and estrogens in the Llobregat River basin (Spain) by liquid chromatography-triple quadrupole-tandem mass spectrometry in combination with ultra performance liquid chromatography-time of flight-mass spectrometry. *Chemosphere* 2010;80:1337–44.

Muñoz I, López-Doval JC, Ricart M, Villagrasa M, Brix R, Geiszinger A, et al. Bridging levels of pharmaceuticals in river water with biological community structure in the Llobregat river basin (northeast Spain). *Environ Toxicol Chem* 2009;28(12):2706–14.

Nödler K, Licha T, Bester K, Sauter M. Development of a multi-residue analytical method, based on liquid chromatography-tandem mass spectrometry, for the simultaneous determination of 46 micro-contaminants in aqueous samples. *J Chromatogr A* 2010;1217:6511–21.

Nödler K, Licha T, Barbieri M, Pérez S. Evidence for the microbially mediated abiotic formation of reversible and non-reversible sulfamethoxazole transformation products during denitrification. *Water Res* 2012;46(7):2131–9.

Onesios K, Yu J, Bouwer E. Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: a review. *Biodegradation* 2009;20(4):441–66.

Osorio V, Pérez S. Development of an analytical method to determine occurrence of Diclofenac, its Metabolites and its Transformation products in wastewater. In preparation.

Patterson B, Shackleton M, Furness A, Pearce J, Descourvieres C, Lingge K, et al. Fate of nine recycled water trace organic contaminants and metal(loid)s during managed aquifer recharge into an anaerobic aquifer: column studies. *Water Res* 2009;44(5):1471–81.

- Pereira R, Pereira L, van der Zee FP, Madalena Alves M. Fate of aniline and sulfanilic acid in UASB bioreactors under denitrifying conditions. *Water Res* 2011;45(1):191–200.
- Pérez S, Barceló D. First evidence for occurrence of hydroxylated human metabolites of diclofenac and aceclofenac in wastewater using QqLIT-MS and QqTOF-MS. *Anal Chem* 2008;80(21):8135–45.
- Petrovic M, Eljarrat E, Lopez de Alda MJ, Barceló D. Endocrine disrupting compounds and other emerging contaminants in the environment: a survey on new monitoring strategies and occurrence data. *Anal Bioanal Chem* 2004;378:549–62.
- Petrovic M, Lopez de Alda M, Diaz-Cruz S, Postigo C, J., R., Gros M, et al. Fate and removal of pharmaceuticals and illicit drugs in conventional and membrane bioreactor wastewater treatment plants and by riverbank filtration. *Philos Trans R Soc A* 2009;367:3979–4003.
- Preuss G, Willme U, Zullei-Seibert N. Verhalten ausgewählter Arzneimittel bei der künstlichen Grundwasseranreicherung – Eliminierung und Effekte auf die mikrobielle Besiedlung. Behaviour of some pharmaceuticals during Artificial Groundwater Recharge – elimination and effects on microbiology. *Acta Hydrochim Hydrobiol* 2001;29(5):269–77.
- Rauch-Williams T, Hoppe-Jones C, Drewes J. The role of organic matter in the removal of emerging trace organic chemicals during managed aquifer recharge. *Water Res* 2010;44(2):449–60.
- Scheytt T, Mersmann P, Rejman-Rasinski E, These A. Tracing pharmaceuticals in the unsaturated zone. *J Soils Sediments* 2007;7(2):75–84.
- Schmidt C, Lange F, Brauch H-J. Assessing the impact of different redox conditions and residence times on the fate of organic micropollutants during riverbank filtration. Fourth International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water, October 13–15, 2004 – Minneapolis, Minnesota; 2004.
- Ternes TA, Bonerz M, Herrmann N, Teiser B, Andersen HR. Irrigation of treated wastewater in Braunschweig, Germany: an option to remove pharmaceuticals and musk fragrances. *Chemosphere* 2007;66(5):894–904.
- Tiehm A, Schmidt N, Stieber M, Sacher F, Wolf L, Hoetzel H. Biodegradation of pharmaceutical compounds and their occurrence in the Jordan valley. *Water Resour Manage* 2010:1–9.
- Underwood JC, Harvey RW, Metge DW, Repert DA, Baumgartner LK, Smith RL, et al. Effects of the antimicrobial sulfamethoxazole on groundwater bacterial enrichment. *Environ Sci Technol* 2011;45(7):3096–101.
- Yamamoto H, Nakamura Y, Moriguchi S, Nakamura Y, Honda Y, Tamura I, et al. Persistence and partitioning of eight selected pharmaceuticals in the aquatic environment: laboratory photolysis, biodegradation, and sorption experiments. *Water Res* 2009;43(2):351–62.