ENVIRONMENTAL FATE OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IN AEROBIC RIVER WATER/SEDIMENT SYSTEM

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ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) are compounds which present significant scientific interest due to their toxicological and chemical characteristics and their persistent detection in the aquatic environment. At present, few data are available for the degradability of NSAIDs in the aquatic environment after treated wastewater disposal to the surface water. Therefore, the present work aimed at studying the fate of four NSAIDs in aquatic sediment system and in specific to investigate the role of sorption and biodegradation on their removal under aerobic conditions. The river water was sampled from Sperchios River (Central Eastern Greece) and the sediment was collected from the banks of the rural stream where the discharge point of the wastewater treatment plant of the town Lamia is located. Biodegradation studies of the selected compounds were conducted under aerobic conditions, while parallel sterile controls were carried out through the whole experimental cycle to assess abiotic degradation or other non-biological removal of the test compounds. Regarding biodegradation and abiotic degradation experiments, it can be stated that all selected compounds found to be biodegradable under aerobic conditions in river sediment.

All compounds were removed at dissipation half-lives between 3.3 and 10.8 days. A comparison between sterile and non-sterile experiment shows that the concentration of the substances remained constant or decreased only marginally in the sterile controls and this excludes abiotic processes such as hydrolysis or sorption as relevant loss mechanisms from water phase and assign the dissipation from water to microbial action in the river sediment.

Keywords: non-steroidal anti-inflammatory drugs, aerobic, biotransformation, river water sediment

1. Introduction

Emerging contaminants (ECs) is a large, diverse and heterogeneous group of chemicals consisting of persistent organic pollutants, pharmaceuticals and personal care products, endocrine disruptors, pesticides, disinfection by-products and a host of other compounds (Thomaidis *et al*., 2012). ECs are used in large quantities and the main sources of these chemicals to the environment originate from industrial, municipal, domestic and animal farming activities (Tijani *et al*., 2013). The widespread presence of non-steroidal anti-inflammatory drugs (NSAIDs) in the aquatic environment and specially in the surface waters, groundwater, wastewater and in some cases in the drinking waters has well documented during the last years (Kim *et al*., 2007; Kasprzyk-Hordern *et al*., 2009; Stasinakis *et al*., 2012). In general, photodegradation, sorption and biodegradation are the main transformation processes of such chemicals from surface waters. Most NSAIDs have been found to be photoactive (Matamoros *et al*., 2009; Jacobs *et al*., 2011; Zhang *et al*., 2011; Koumaki *et al*., 2015) while sorption depends on compound's physico-chemical properties and also the type of the sediment.

Biodegradation in surface waters has been shown to be an efficient attenuation process for some emerging contaminants and it can be attributed to microorganisms presented in water/sediment systems (Gröning *et al*., 2007; Kunkel & Radke, 2008; Murdoch & Hay, 2013). Although the

effectiveness of biodegradation has been demonstrated, few data are available for the degradability of NSAIDs in the aquatic environment after treated wastewater disposal to the surface water and in particular no information exists on the degradability of these compounds in Greeκ environmental media. Therefore, the present work aimed at studying the fate of four pharmaceuticals in aquatic sediment system and in specific to investigate the role of sorption and biodegradation on their removal under aerobic conditions. The compounds selected in this study as representatives are the following: Naproxen (NPX), Ketoprofen (KTP), Diclofenac (DCF) and Ibuprofen (IBU).

2. Materials and methods

2.1. Materials and reagents

Methanol (MeOH) and ethyl acetate were of high performance liquid chromatography (HPLC) grade (Merck, Darmstadt, Germany) and were used as received. Bis(trimethylsilyl) trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) and pyridine, used for silylation, were purchased by Supelco (Bellefonte, PA, USA) and Carlo Erba-SDS (Peypin, France), respectively. Analytical standards of IBU, NPX, KTP, DCF, and meclofenamic acid (MCF) were supplied by Dr. Ehrenstorfer (Germany). All compounds were used without further purification (minimum purity >99%). Stock solutions of individual compounds were prepared in methanol at 1000 mg L^{-1} and kept at -18 ˚C. HPLC grade water was prepared in the laboratory using a MilliQ/Milli-RO Millipore system (Millipore, Billerica, Massachusetts USA). Ultra-pure HCl (32%) was used for acidification of the samples (Merck, Germany).

2.2. Biodegradation experiments

The river water was sampled from Sperchios River (Central Eastern Greece), at a location which does not receive any regular input of municipal wastewater. The sediment was collected from the banks of the rural stream nearby the discharge point of the Lamia municipal wastewater treatment plant. Sediment samples were generally collected from the entire 5-10 cm upper layer of the sediment, sieved to <2 mm and stored at 4 °C until used. River water samples were filtered under suction through 0.45 μm membranes to remove particulate and algal materials. Biodegradation studies of the aforementioned compounds were conducted under aerobic conditions using a modification of an Environmental Protection Agency guideline (2008). Aerobic degradation treatments were carried out in 1 L glass bottles with a sediment:water volume ratio of 1:3 (200 g of wet sediment and 600 mL of river water) and a sediment layer of 2.5 cm. For the purpose of this test, the water phase was continuously aerated with pressurized air. All incubations were carried out without shaking at 15±2 °C in darkness. The measured pH in the water phase remained relatively constant 8.0±0.3 during the course of the tests. The nominal concentration of each compound was around 40μg L^{-1}. All compounds were added as a mixture. Sterile controls experiments were carried out through the whole experimental period to assess abiotic degradation or other non-biological processes such as hydrolysis, volatilization and sorption. The biological activity stopped by autoclaving (121 °C; 30 min) the test water and sediment. Samples for analysis were taken after being spiked (t=0) and in regular intervals over a period of up to 21 days, filtered and stored frozen until solid phase extraction and GC/MS analysis.

2.3. Kinetic analysis

To quantitatively describe the degradation kinetics of the selected compounds, pseudo first-order kinetic (until 90% degradation been achieved) was used and only pseudo first-order degradation rate constants (k_{deg}) at a significance level of $p<0.05$ are reported. The rate constant for the loss (k_{deg}) was determined as the best fit slope of a liner regression of *ln(C)* versus *t*. k_{deg} was then converted into $t_{1/2}$ (half-life value; the time it takes until the concentration in the aqueous phase has decreased by 50%; $t_{1/2} = -\ln(2)/k_{deg}$).

2.4. Analytical methods

For the determination of the target compounds, water samples were analyzed using a chromatographic method developed by Samaras *et al*. (2011). The developed procedure included

solid phase extraction followed by silylation which was performed at 70˚C for 20 min by adding 50 μL of BSTFA + 1%TMCS along with 10 μL of pyridine. MCF was used as surrogate for the determination of NSAIDs. For the qualitative and quantitative analyses, an Agilent Gas Chromatograph 7890A connected to an Agilent 5975C Mass Selective Detector (MSD) was used. Water and sediment characteristics were determined according to Standard Methods (APHA, 2005).

3. Results

Time profiles of the biodegradation and sterile experiments for the selected compounds are presented in Figure 1. According to the results it can be stated that the biotransformation rate of Diclofenac, Naproxen and Ketoprofen was slow and increased substantially towards the end of the experimental period.

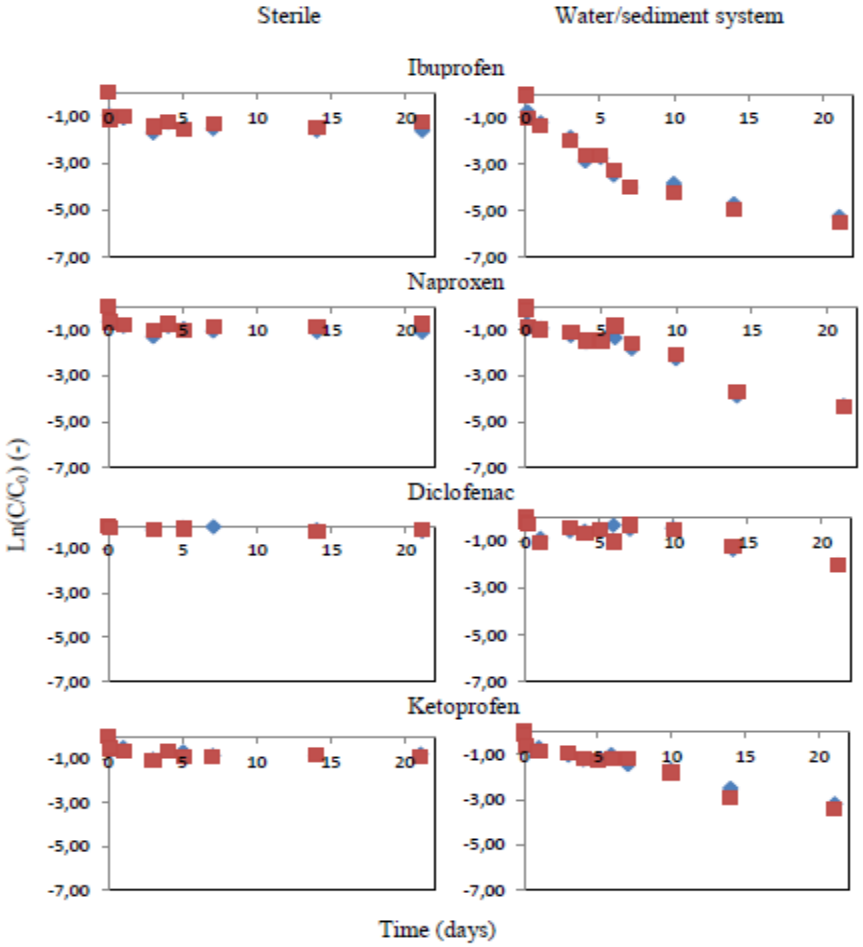

Figure 1: Time profile of the aqueous concentration of the target compounds [unsterile (right) and sterile (left) treatments]

On the contrary, Ibuprofen was found to be very labile to biotransformation. Also, a comparison between sterile and non-sterile experiments shows that an initial loss of the compounds was observed in the sterile experiments during the first day of the experimental period. The initial loss which was observed in sterile experiments could be associated with abiotic degradation processes such as hydrolysis or sorption. However, the concentration of the substances remained constant or decreased only marginally in the sterile tests after the first experimental day and it appears that sorption controlled by slow diffusion of the compounds to deeper sediment layers. The estimated half-lives and first order rate constants are shown in Table 1. Based on the results, all compounds were removed at biodegradation half-lives between 3.3 and 10.8 days. The high

biotransformation rate of the selected compounds have also been reported by other researchers (Löffler et. al., 2005; Kim *et al*., 2007; Radke & Maier, 2014).

Table 1: Kinetic pseudo-constants (k_{deg}) and experimental half-lives ($t_{1/2}$)

Compound	k_{deg} (days^{-1})	k_{deg} (days^{-1})	$t_{1/2}$ (days)
Naproxen	0.01935 ± 0.011	0.1857 ± 0.008	4.2 ± 0.2
Ibuprofen	0.0341 ± 0.011	0.24455 ± 0.018	3.3 ± 0.2
Ketoprofen	0.02295 ± 0.0026	0.1408 ± 0.007	5.9 ± 0.3
Diclofenac	0.01170 ± 0.001	0.0762 ± 0.006	10.8 ± 0.8

A possible explanation for the high degradation rates of the selected compounds which observed in the present study are most likely due to the breadth and quantity microbial communities. The microbial population of the sediment is more diverse and acclimated to the selected compounds due to their exposure to a wide range of complex phenolic compounds from treated municipal effluents entering the rural at the sampling location.

4. Conclusions

All four NSAIDs in river water/sediment system were biodegraded and can be nearly depleted in 4-11 days of incubation. The high biodegradation rates of the selected compounds can be attributed to the kind of sediment which was used in this study. The sediment collected from the banks of a rural stream nearby the discharge point of a municipal wastewater treatment plant. This may indicate that the microorganisms presented in the sediment are acclimated to the selected compounds. The high biodegradability under aerobic conditions in river water/sediment system was predictable for Ibuprofen, which present high removal efficiency in wastewater treatment plants. In contrast, Naproxen, Diclofenac and Ketoprofen remained stable across the early experimental period, presented a lag phase during the first 10 days and eventually tailed by a degradation phase.

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REFERENCES

1. Environmental protection Agency (2008) Aerobic Aquatic Metabolism. Harmonised Test Guidelines OPPTS 835.4300. EPA 712-C-08-018.
2. Gröning J., Held C., Garten C., Claussnitzer U., Kaschabek S.R., Schlömann M. (2007), Transformation of diclofenac by the indigenous microflora of river sediments and identification of a major intermediate. Chemosphere **69**, 509-516.
3. Jacobs L. E., Fimmen R. L., Chin Y.-P., Mash H. E., Weavers L. K. (2011), Fulvic acid mediated photolysis of ibuprofen in water. Water Research **45**, 4449-4458.
4. Kasprzyk-Hordern B., Dinsdale R.M., Guwy A.J. (2008), The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK, Water Research **42,** 3498-3518.
5. Kim S. D., Cho J., Kim I. S., Vanderford B. J., Snyder S. A. (2007), Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters. Water Research **41**, 1013-1021.
6. Koumaki E., Mamais D., Noutsopoulos C., Nika M.C., Bletsou A.A., Thomaidis N.S., Eftaxias A., Stratogianni G. (2015), Degradation of emerging contaminants from water under natural sunlight: the effect of season, pH, humic acids and nitrate and identification of photodegradation by-products. Unpublished results, in preparation.

7. Kunkel U. & Radke M. (2008), Biodegradation of Acidic Pharmaceuticals in Bed Sediments: Insight from a Laboratory Experiment. Environmental Science & Technology **42**, 7273–7279.
8. Löffler D, Rombke J, Meller M, Ternes TA (2005) Environmental fate of pharmaceuticals in water/sediment systems. Environmental science & technology **39**: 5209-5218.
9. Matamoros V., Duhec A., Albaiges J., Bayona J.M. (2009), Photodegradation of Carbamazepine, Ibuprofen, Ketoprofen and 17α-Ethinylestradiol in Fresh and Seawater. Water Air & Soil Pollution **196**, 161-168.
10. Murdoch, R. & Hay A.G. (2013). Genetic and chemical characterization of ibuprofen and degradation by Sphingomonas Ibu-2. Microbiology **159** 621-632.
11. Radke M. & Maier M.P. (2014), Lessons learned from water/sediment-testing of pharmaceuticals. Water Research **55**, 63-73.
12. Samaras, V.G., Thomaidis, N.S., Stasinakis, A.S., Lekkas, T.D. (2011), An analytical method for the simultaneous trace determination of acidic pharmaceuticals and phenolic endocrine disrupting chemicals in wastewater and sewage sludge by gas chromatography-mass spectrometry. Anaytical and Bioanaytical Chemistry **399**, 2549-2561.
13. Standard Methods for Examination of Waters and Wastewaters (2005), , 21st edn, American Public Health Association, Washington DC, USA.
14. Stasinakis A. S., Mermigka S., Samaras V. G., Farmaki E., Thomaidis N. S. (2012), Occurrence of endocrine disrupters and selected pharmaceuticals in Aisonas River (Greece) and environmental risk assessment using hazard indexes. Environmental Science and Pollution Research, **19:**1574–1583.
15. Thomaidis N. S., Asimakopoulos A. G., Bletsou A. A. (2012), Emerging contaminants: a tutorial mini-review. Global Nest Journal,**14**:72–79.
16. Tijani J. O., Fatoba O. O., Petrik L. F. (2013), A Review of Pharmaceuticals and Endocrine-Disrupting Compounds: Sources, Effects, Removal, and Detections. Water Air & Soil Pollution **224**, 1770.
17. Zhang N., Liu G., Liu H., Wang Y., He Z., Wang G. (2011), Diclofenac photodegradation under simulated sunlight: Effect of different forms of nitrogen and Kinetics. Journal of Hazardous Materials **192**, 411-418.