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Occurrence and fate of dissolved and particulate antimicrobials in municipal wastewater treatment

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ABSTRACT

Comprehensive study on the occurrence and fate of several classes of antimicrobials, including sulfonamides, trimethoprim, fluoroquinolones and macrolides, in Croatian municipal wastewaters was performed using an integrated approach, which comprised analysis of both dissolved and particulate fractions. A nation-wide screening showed ubiquitous occurrence of human-use antimicrobials in raw wastewater samples with the total concentrations ranging from 2 to 20 μg/L, while veterinary antimicrobials were typically present in much lower concentrations (<100 ng/L). The percentage of the particulate fraction in raw wastewater varied significantly depending on the type of the antimicrobial and the load of suspended solids. A detailed study of the mass flows of dissolved and particulate antimicrobials, performed in the wastewater treatment plant of the city of Zagreb, allowed an improved assessment of the biological and physico-chemical removal mechanisms of investigated compounds during the conventional activated sludge treatment. The overall removal efficiencies of antimicrobials from the water phase were rather variable, ranging from 0% for trimethoprim to 85% for norfloxacin. A significant percentage of fluoroquinolones (norfloxacin and ciprofloxacin) and macrolides (azithromycin and clarithromycin) was associated with the primary and excess secondary sludge, explaining 14–77% of the total removal. The removal, which could be attributed to biological transformation, was relatively poor for all antimicrobials, exceeding 30% only for SMX (32%) and clarithromycin (55%).

1. Introduction

Pharmaceutical compounds represent one of the most important classes of emerging contaminants. In the last decade, they have been detected in different environmental compartments all over the world (Segura et al., 2009). Traces of pharmaceuticals in the environment raised concerns due to their high biological activity and there is an accumulating evidence in the literature that they can cause different adverse effects in non-target species (Fent et al., 2006).

Among different classes of pharmaceuticals, antimicrobial agents are under special scrutiny due to the possible formation of the resistant bacterial strains, which can pose a serious threat to the human health (da Costa et al., 2006). Antimicrobials represent the third biggest group among all pharmaceuticals in human medicine and the most prominent group in veterinary medicine (Thiele-Bruhn, 2003), with the estimated annual world consumption between 100,000 and 200,000 tons (Wise, 2002). Antimicrobials with the highest consumption are β-lactams, followed by tetracyclines, macrolides, fluoroquinolones and sulfonamides. However, due to the instability of the β-lactam ring, which is easily hydrolysed, either chemically or microbiologically, penicillins and cephalosporins are not considered to be a potential threat for the

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environment (Cha et al., 2006). On the contrary, due to their comparatively higher stability, sulfonamides, fluoroquinolones and macrolides are widely recognised as important classes of environmental contaminants (Segura et al., 2009).

The most important global sources of antimicrobials in the environment are municipal wastewaters. After the intake, antimicrobials are only partially metabolised and their residues, excreted via urine or feces, reach municipal wastewater treatment plants (WWTPs). In addition, it was suggested that a significant percentage of pharmaceuticals in the sewage may derive from the illegal disposal of unused medications down the drain (Daughton and Ruhoy, 2009). Numerous reports throughout the world (Hirsch et al., 1999; Miao et al., 2004; Lindberg et al., 2005; Karthikeyan and Meyer, 2006; Xu et al., 2007; Gulkowska et al., 2008; Watkinson et al., 2009; Zuccato et al., 2010; Gros et al., 2010; Gracia-Lor et al., 2012) showed that antimicrobials represent ubiquitous contaminants of municipal wastewaters. Recent study on the occurrence and fate of emerging contaminants in wastewaters of the Western Balkan Region, showed widespread occurrence of different classes of emerging contaminants, including many representatives of antimicrobials (Terzic et al., 2008).

Most of the literature reports suggested that the current design of conventional WWTPs does not assure a complete removal of pharmaceuticals, including several classes of antimicrobials (Castiglioni et al., 2006; Watkinson et al., 2007; Xu et al., 2007; Kasprzyk-Hordern et al., 2009; Gros et al., 2010). Moreover, treatment of municipal and industrial wastewaters in many less developed countries, including Croatia, is often incomplete and consists only of the mechanical treatment (Kastelan-Macan et al., 2007). As a consequence, a large percentage of pharmaceuticals introduced into WWTPs is released into the aquatic environment and pose a significant threat to the receiving ambient waters. Despite a growing number of studies on pharmaceutical in WWTPs, only few studies addressed the issue of particle bound antimicrobials (Göbel et al., 2005; Okuda et al., 2009; Jelic et al., 2011). The reason for that is a general perception that most of the pharmaceuticals belong to comparatively polar compounds, which are primarily expected to occur in the dissolved phase. Moreover, determination of organic micropollutants in the complex solid matrices is often analytically more challenging than their determination in the dissolved fraction. Nevertheless, comprehensive approach, which includes both dissolved and particulate fraction, is the key prerequisite for the accurate assessment of the pharmaceutical behaviour in the wastewater treatment and for understanding their ultimate fate in the aquatic environment.

The aim of this work was to investigate the occurrence of several important classes of antimicrobials, including sulfonamides, trimethoprim, fluoroquinolones and macrolides, in raw municipal wastewaters, and their fate in conventional wastewater treatment. Unlike most of the reports in the literature, our study involved simultaneous determination of these compounds in both dissolved and particulate fraction, thus providing a basis for a comprehensive assessment of the physico-chemical and biological removal processes.

2. Experimental

2.1. Target compounds

This study was focused on antimicrobials with the extensive usage in human medicine in Croatia, but some additional compounds, including several representatives of veterinary antimicrobials and *N*-acetylsulfamethoxazole as the main human metabolite of sulfamethoxazole, were also included. The list of all target compounds, together with their abbreviations and usage is presented in Table 1.

2.2. Sampling

All samples were collected in clean amber glass bottles pre-rinsed with methanol and ultrapure water. The nation-wide screening was performed in April and May 2005. Raw wastewater (RW) samples were collected in the largest Croatian cities (Table S1; Supplementary information). Additionally, where available, effluents from wastewater treatment plants were also collected. During this sampling campaign only a few cities (Bjelovar, Cakovec, Varazdin, Velika Gorica and Vinkovci) had facilities for full mechanical and biological treatment, while wastewaters of the cities of Rijeka and Split were treated only mechanically. For those cities, which did not have any wastewater treatment facilities, grab samples were collected directly from the sewerage system. In WWTPs of the cities Cakovec and Bjelovar 24-h flow-proportional composite samples were collected using automatic devices, while on some other locations (Rijeka, Split, Varazdin) composite samples were prepared by mixing grab samples taken over a diurnal cycle.

A detailed study on the occurrence and fate of antimicrobials during conventional wastewater treatment was performed in the central WWTP of the city of Zagreb, which is fully operational since 2007. This WWTP receives a combined municipal and industrial sewage from the entire city and includes full mechanical and biological treatment based on conventional activated sludge (CAS) treatment. It has a designed capacity of 1,000,000 population equivalents and currently serves about 750,000 inhabitants (Schröder et al., 2001). The average hydraulic load of raw wastewater is about 250,000 m^3/day. The details about the WWTP are summarised in Table S2 (Supplementary information). During the study in this WWTP, several sampling campaigns in the period from March to September 2009 were performed, in which twenty-four-hour composite samples of both RW and biologically treated wastewater (secondary effluent, SE) were collected. In addition, for the investigation of the partitioning behaviour of the selected antimicrobials during CAS treatment, activated sludge samples were taken directly from the aeration tank. In order to keep the original character of the collected sample, the addition of any chemical preservative was strictly avoided.

2.3. Sample pre-treatment and instrumental analysis

All samples were filtered through 2.7 μm glass fibre filters (GF/D, Whatman, USA) immediately after being brought back to the