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Chemical characterization of organic microcontaminant sources and biological effects in riverine sediments impacted by urban sewage and pulp mill discharges

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HIGHLIGHTS

- Forty-five organic microcontaminants were identified in seven sediment samples from Biobío River.
- A PCA study was conducted.
- Domestic wastewater and Kraft pulp mills were the most important factors.
- Domestic wastewater impact presents the highest estrogenic and dioxin-like activities.

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ABSTRACT

The Biobío River basin is highly impacted by a variety of anthropogenic activities such as pulp mills and urban wastewaters subjected to different treatment processes. This work assesses for the first time, the contamination source and biological effects (estrogenic and dioxin-like activities) in the river basin by the determination of 45 organic microcontaminants in seven sediment samples. Pressurized solvent extraction combined with two-dimensional comprehensive gas chromatography coupled to time of flight mass spectrometry was employed for this purpose. The organic microcontaminants identified comprise monoterpenes, sesquiterpenes, diterpenes, ionones, lineal alkyl benzenes, polycyclic aromatic hydrocarbons, musk fragrances, sterols and phathalate esters. The presence of pine and eucalyptus pulp mill effluents increased the abundance of resin-derived neutral compounds and monoterpenes respectively. A principal component analysis showed that the Biobío River basin was impacted by domestic wastewater treatment plants (WWTPs), pine or eucalyptus Kraft pulp mills and pyrolytic and pyrogenic processes. Finally, the recombinant yeast assays showed that the presence of estrogenic and dioxin-like activity was mostly located in sediments impacted by domestic WWTP effluents.

1. Introduction

Urban and industrial wastewater effluents subjected to different type of treatment processes are commonly discharged into rivers or lakes from inland cities (Laws, 2000). These waters contain a large variety of organic microcontaminants at trace or ultra-trace concentration levels (ng L^{-1} up to μg L^{-1}) (Hollender et al., 2008). Nevertheless, concern about the adverse effects related to the occurrence of some of these microcontaminants on the aquatic ecosystems has not arisen until the last decade (Schwarzenbach et al., 2006). For example, terpenoids, phytosterols and related compounds have been identified as a potential source of endocrine disruption effects caused by pulp and paper mill effluents (Hewitt et al., 2006; Orrego et al., 2009; Chamorro et al., 2010, 2013). On the other hand, estrogenic effects have also been identified in domestic wastewater treatment plant (WWTP) effluents due to the presence of steroid hormones and non-ionic surfactant degradation intermediates (Purdom et al., 1994; Tilton et al., 2002; Campbell et al., 2006; Silva et al., 2012). Little attention has been paid on the fate of these microcontaminants in sediments impacted by these sources of pollution. The accumulation of organic microcontaminants in sediments depends on their content and type of organic matter. Consequently, it is generally accepted that sediments constitute a sink for the most hydrophobic compounds that can, depending on the hydrographic conditions, be resuspended on and reach the food chain (Bayona et al., 1991).

Due to the high chemical complexity of the sediment extracts, the characterization of trace organic contaminants requires the use of high resolution and selectivity analytical techniques (Richardson, 2012). In this regard, two-dimensional comprehensive

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gas chromatography coupled to time of flight mass spectrometry (GCxGC-ToF-MS) has emerged as a powerful separation technique that is especially well-suited for complex sample characterization (Dallüge et al., 2003; Dimandja, 2004; Adahchour et al., 2006). Its potential has already been shown in the determination of several environmental contaminants in water samples (Matamoros et al., 2009; Gómez et al., 2011) and sediments (Skoczynska et al., 2008; da Silva et al., 2011). However, the capability of GCxGC-ToF-MS for the identification of Kraft pulp mill and wastewater compounds present in sediment samples has not been addressed yet. The aim of this study is to identify and assess the occurrence of organic microcontaminants in river sediments collected along the Biobío River basin. Furthermore, recombinant yeast assays (RYAs) have been used for the determination of the estrogenic and dioxin-like activities from the sediments extracts (Noguerol et al., 2006). The Biobío River basin has been chosen due to the growing concern of the effects that anthropogenic stressors, such as the discharge of microcontaminants by Kraft pulp mills and domestic WWTPs, as well as diffusive water pollution from agricultural and forestry activities (Orrego et al., 2005) may cause on the aquatic ecosystem. Among them, it is worth mentioning that in the Biobío basin is located the 83% of Chile's pulp production (Karrasch et al., 2006).

2. Materials and methods

2.1. Sampling site description

The Biobío River flows from the Icalma and Galletue lakes in the Andes to the Pacific Ocean. The River Biobío is the second largest river in Chile with a length of 380 km and a hydrographic basin of 24 260 km^2 (Focardi et al., 1996). On its way to the Pacific Ocean, the river flows through steep, narrow gorges and forests and in its lower reaches passes through agricultural land, towns, cities, and industrial areas. The basin is inhabited by over a million people that use the resources of the Biobío River for different purposes and services (e.g. drinking and irrigation water, discharge of industrial and municipal sewage, generation of electricity, recreation and fishing). Samples were taken at six locations along the river basin (midstream to downstream) (Fig. 1).

2.2. Sampling strategy

All sediment samples were collected during the summer season, either in December 2007 (samples M1, and M4) or December 2008 (M2, M3, M5, M6 and M7) in order to check annual variability. Furthermore, since recent publications have shown that the induction of endocrine activity in fish is located near the pulp mills (Milestone et al., 2012), some sampling points were located downstream (M2 and M5) and upstream (M3 and M6) from the Kraft pulp mills effluents in the river.

Grab samples were collected using a dredge in the places where the river flow rate was greater. All samples were stored in aluminium containers and transported in refrigerated conditions to the laboratory. Samples were lyophilized and homogenized in a 200 mesh stainless steel sieve, after which they were stored at −20 °C in a freezer until extraction. The selection of the sampling sites was based on different criteria related with the geomorphology of the main channel, the hydrological regime, and the localization of the urban and industrial discharges as summarized in Table 1.

2.3. Analytical methodology

2.3.1. Reagents and chemicals

Gas chromatography (GC) grade (Suprasolv) hexane, acetone and ethyl acetate were obtained from Merck (Darmstadt, Germany). Triphenylamine was purchased from Sigma–Aldrich (Steinheim, Germany).

2.3.2. Sample preparation

The extraction was performed on a PSE-One (Applied Instruments, USA) by duplicate. Briefly, 5 g of freeze-dry sediment was sequentially extracted, firstly with *n*-hexane/acetone 1:1 (FI) and then with *n*-hexane/acetone 1:5 (FII). The extraction conditions were 110 °C, 140 bar, and 3 cycles with solvent mixtures of 15 min each. The clean-up of the extracts was carried by using a glass Pasteur pipette filled with 0.7 g of florisil and 0.3 g of sodium sulphate and then eluted with hexane/ethyl acetate (1:1) The elutants were concentrated with a gentle nitrogen stream and resuspended with ethyl acetate for the chemical analysis or methanol for the toxicological analysis (Section 2.5). Then, 250 ng of triphenylamine was added to the extract as internal standard (IS) and 2 μL of the sample was injected into a GCxGC-ToF-MS for analyte identification, but not for quantification.

2.3.3. Chemical analysis

The FI and FII extracts were analyzed by GCxGC-ToF-MS. This determination was performed on a computerized system consisting of a gas chromatograph HP 6890N (Agilent Technologies, CA, USA) fitted with a split/splitless injector. The second dimension column is fitted to a secondary furnace with a thermal modulator ZX1 (Zoex, TX, USA). The pulses freeze liquid nitrogen were automatically pumped from a Dewar using a standard driver supplied with liquid nitrogen stored in a 60 L storage tank. The mass detector coupled to a Pegasus 4D system was a TOF (LECO MI, USA) with an electron energy of 70 eV. The ion source was set at 200 °C. The mass range was set between 50 and 550 amu at 100 Hz with a 1600 V voltage detector. For the first dimension, a column of 30 m × 0.25 mm ID coated with 0.25 μm film thickness TRB5-MS (5% diphenyl-polydimethylsiloxane) was used. For the second dimension, a 2 m × 0.10 mm ID coated with 0.10 μm film thickness TRB-50 HT (high temperature 50% diphenylpolydimethylsiloxane) was installed. Both columns were obtained from Teknokroma (Sant Cugat del Vallès, Spain). The oven temperature was raised from 65 °C (1 min) to 290 °C at 5 °C min^{-1} with a final holding time of 20 min; the secondary oven was kept at 10 °C above the first dimension temperature during the whole experiment. Modulation time was 6 s. Data were acquired and processed using the ChromaTOF 3.32 software. NIST 05 version was used for the identification of unknown compounds. The signal-to-noise ratio of the data processing method was set to 100.

2.3.4. Organic carbon and nitrogen content

Total organic carbon (TOC) analysis of the sediment was determined as described by Boronat et al. (2009). Samples were treated with HCl to remove inorganic carbon, neutralized with Milli-Q water and dried at 60 °C. The determination of TOC was performed by flash combustion at 1025 °C followed by thermal conductivity detection in a CHNS Elemental Analyzer EA1108.

2.4. Toxicological analysis

The resuspended extracts with methanol (FI and FII) were analyzed for the toxicological effects. The Estrogen Receptor Assay (ER-RYA) was performed using the yeast strain BY4741 (MATa ura3Δ0 leu2Δ0 his3Δ1 met15Δ0) from EUROSCARF (Frankfurt, Germany) transformed with the plasmids pH5HE0 (hER) and pVitBX2 (ERE-LacZ) (Noguerol et al., 2006). For the AhR yeast assay (AhR-RYA), we used the YCM4 yeast strain (Miller, 1997), harbouring a chromosomally integrated construct that co-expresses the hAHR and ARNT genes under the Gal1-10 promoter and the pDRE23-Z (XRE5-CYC1-LacZ) plasmid. These two RYA assays were