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Monitoring a large number of pesticides and transformation products in water samples from Spain and Italy

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ABSTRACT

Assessing the presence of pesticides in environmental waters is particularly challenging because of the huge number of substances used which may end up in the environment. Furthermore, the occurrence of pesticide transformation products (TPs) and/or metabolites makes this task even harder. Most studies dealing with the determination of pesticides in water include only a small number of analytes and in many cases no TPs. The present study applied a screening method for the determination of a large number of pesticides and TPs in wastewater (WW) and surface water (SW) from Spain and Italy. Liquid chromatography coupled to high-resolution mass spectrometry (HRMS) was used to screen a database of 450 pesticides and TPs. Detection and identification were based on specific criteria, i.e. mass accuracy, fragmentation, and comparison of retention times when reference standards were available, or a retention time prediction model when standards were not available. Seventeen pesticides and TPs from different classes (fungicides, herbicides and insecticides) were found in WW in Italy and Spain, and twelve in SW. Generally, in both countries more compounds were detected in effluent WW than in influent WW, and in SW than WW. This might be due to the analytical sensitivity in the different matrices, but also to the presence of multiple sources of pollution. HRMS proved a good screening tool to determine a large number of substances in water and identify some priority compounds for further quantitative analysis.

1. Introduction

During the last decade scientific interest in environmental pollution has risen, since a large number of organic contaminants have been found in the environment, some of which induce known or suspected undesirable effects on humans and ecosystems (Meffe and de Bustamante, 2014). Several classes of micropollutants have been investigated, such as pharmaceuticals, personal care products, illicit drugs, artificial sweeteners, nanomaterials, perfluorinated compounds, disinfection byproducts, brominated and emerging flame retardants, microplastics and pesticides (Asimakopoulos and Kannan, 2016; Gago-Ferrero et al., 2015; Hernandez et al., 2014; Kock-Schulmeyer et al., 2013; Luo et al., 2014; Richardson and Kimura, 2016). These substances are usually found in water at low concentrations, from traces in the low ng/L to few μg/L levels, but as they normally occur as complex mixtures they have potential adverse effects on human health in the general population (Lei et al., 2015).

Pesticides are a wide class of chemicals used to limit, inhibit and prevent the growth of harmful animals, insects, invasive plants, weeds and fungi (Meffe and de Bustamante, 2014). The main source of pesticides in the aquatic environment is runoff from agriculture, but their application in other areas is also important. They are used in public health (e.g. for control of disease vectors such as malaria), treatment of large structures (e.g. public and private buildings), maintenance of green areas (e.g. parks, sports grounds and golf courses), maintenance of water reserves (e.g. ponds), livestock and domestic animals (e.g. disinfection of sheep), industry (e.g. paints, resins and for the preservation of fresh foods) and homes (e.g. insect repellents) (Garcia et al., 2012). Pesticides applied in agriculture eventually end up in ground and surface waters (SW) and those applied in urban areas finish up mainly in wastewater treatment plants (WWTPs). Since WWTPs are not designed to remove micropollutants (Eggen et al., 2014; Luo et al., 2014), many of these substances can reach the aquatic environment in discharged treated wastewater.

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More than 1300 active substances are listed in the EU pesticides database, some of which are no longer approved for use (European Commission, 2016). In addition, transformation products (TPs) can be formed in the environment after the degradation of the parent substances and they can even reach higher levels than the parent substances and be even more toxic (Richardson and Ternes, 2014).

Pesticides are one of the most frequently detected classes of micropollutants in water, especially in Mediterranean countries such as Spain (Hernández et al., 2015) and Italy (Meffe and de Bustamante, 2014), on account of their widespread use, particularly in extensive areas of agriculture. In fact, Spain and Italy are the countries with the highest use of pesticides in Europe, according to the Statistical Office of the European Union (Eurostat, 2014).

Comprehensive monitoring of the enormous number of authorized pesticides and TPs would be desirable to gain a full overview of these compounds in the environment, but unfortunately this is far from possible. Triple quadrupole (QqQ), coupled to both gas chromatography (GC) (Hernández et al., 2013) and liquid chromatography (LC) (Marín et al., 2009) is the preferred technique for the quantitative determination of pesticides in water samples when analytical standards are available, since it offers high sensitivity and selectivity, and a wide dynamic range. However, the main disadvantage is the limited number of compounds that can be determined in a single run and the fact that many compounds are ignored in the analysis as they are not part of the target list. Thus, "unknown" compounds (without reference standards), such as TPs, cannot be measured (Masiá et al., 2014; Pitarch et al., 2010).

Full-spectrum acquisition techniques such as high-resolution mass spectrometry (HRMS) with appropriate software tools overcome some of the limitations (Gago-Ferrero et al., 2015; Hernandez et al., 2012; Schymanski et al., 2014). Liquid chromatography coupled with hybrid systems as quadrupole time-of-flight (QTOF) or linear ion trap (LTQ) Orbitrap analyzers have been used for screening huge numbers of micropollutants in the aquatic environment, belonging to different chemical families (Hernández et al., 2015; Wode et al., 2015). HRMS can provide information about water pollution, rapidly and in a single run with reasonable sensitivity. Furthermore, compounds can be screened highly reliably without reference standards, since the method has excellent detection and identification capabilities based on high-resolution accurate mass measurements of (de)protonated molecules and fragment ions (Diaz et al., 2013; Krauss et al., 2010). One of the limitations involves the analysis of complex matrices, where it becomes hard to confirm suspects' identities by comparing experimental MS/MS spectra with those provided in the literature and/or in spectral libraries, because of the heterogeneous information on fragmentation (González-Mariño et al., 2016).

Most research on pesticides in the environment has been based on GC-MS/MS and LC-MS/MS analysis, HRMS having been used less. Moschet et al. (2013) developed a suspect screening approach using LC-HRMS for assessing aquatic contamination with rarely investigated pesticides and their TPs, without the need for reference standards. This approach identified two TPs that had never been found in SW before (Moschet et al., 2013). Other advanced analytical techniques with different mass analyzers can be found in the literature, including the determination of pesticides and TPs in different water samples, but all of them dealt with wider screening of emerging pollutants using HRMS, to check water quality (Cotton et al., 2016; Hernández et al., 2015; Pitarch et al., 2016; Portolés et al., 2014).

The present study focused on a large number of pesticides and TPs, with the main aim to investigate their occurrence in wastewater (WW) (influent and effluent) and surface water (SW) in two areas with high pesticide use (Spain and Italy). An advanced analytical tool (HPLC-QTOF MS) was selected and tested. A comprehensive list of substances was built and used to search compounds according to specific criteria. A complementary tool (retention time prediction) was used when no reference standard was available to help with tentative identification.

2. Materials and methods

2.1. Chemicals and reagents

HPLC-grade methanol (MeOH), ammonia solution (25%) and formic acid (HCOOH, 98–100%) were acquired from Scharlau (Barcelona, Spain) and acetonitrile (ACN) for LC-MS from Riedel de Haen (Seelze, Germany). HPLC-grade water was obtained by purifying demineralised water in a Milli-Q plus system from Millipore (Bedford, MA, USA).

Reference standards of organic contaminants were purchased from Dr. Ehrenstorfer (Augsburg, Germany), Wellington Laboratories (Guelph, Ontario, Canada), Fluka (Buchs, Switzerland), Riedel de Häen (Seelze, Germany) or Sigma (St. Louis, MO, USA). All reference standards had purity higher than 93%.

2.2. Selection of analytes and study areas

Pesticides were selected on the basis of the priority pollutant list of the EU and the United States Environmental Protection Agency (US-EPA) and the United Nations list of persistent organic pollutants (Stockholm Convention). The database was built based on our experience with environmental and food samples LC-MS/MS analysis (Díaz et al., 2012).

The dataset was divided into two lists: the first included pesticides (164 compounds) with known fragmentation (standards were available in the laboratory) from previous studies (Table S1); the second included only information on the parent compound as protonated molecule (286 compounds) (Table S2). The dataset included 399 parent pesticides and 51 TPs.

Spain and Italy were chosen for the study since pesticides were one of the most frequently detected classes of micropollutants in waters (Hernández et al., 2015; Meffe and de Bustamante, 2014). Eurostat data showed that pesticide use in Spain in 2014, when the sampling was done, reached 78.8×10^6 kg, making Spain the country with the highest use of pesticides in Europe. Italy ranked third, after France, applying 64.1×10^6 kg of pesticides in the same year (Eurostat, 2014).

2.3. Sample collection

2.3.1. Wastewater

Fourteen wastewater samples (seven influent wastewater (IWW) and seven effluent wastewater (EWW)) were taken from the WWTP of Castellón (Valencia region), Eastern Spain, and four wastewater samples (two IWW and two EWW) from Cremona, Northern Italy. Composite 24-h samples of wastewater were collected by automatic sampling devices from each plant, in March 2014 (Castellón) and in May 2014 (Cremona). Samples were collected in high-density polystyrene bottles, frozen immediately and stored at −20 °C until extraction.

2.3.2. Surface water

Five SW samples (grab samples) were taken from the Valencia region, Eastern Spain: Almenara, Burriana Clot, Nules and two sites in Albufera Natural Park. All samples were stored in high-density polystyrene bottles at 4 °C for less than 48 h, until extraction.

2.4. Sample treatment

Wastewater samples were vacuum-filtered through a glass microfiber filter 1.6 μm GF/A (Whatman, Kent, U.K.) and a 0.45 μm mixed cellulose ester membrane filter (Whatman, Kent, U.K.) before extraction, according to the procedures of each laboratory. SW was not filtered. The method is described in detail elsewhere (Bade et al., 2015c). Briefly, solid phase extraction (SPE), using OASIS HLB 3 cc/60 mg cartridges (Waters Corp., Milford, MA, USA), was applied to all