

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.

Keywords: Pesticides; Environment; High-Resolution Mass Spectrometry; Wastewater; Surface Water.

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 $\mu\text{g/L}$ 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.

More than 1,300 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

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.

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 water samples in order to extract the analytes. Cartridges were conditioned with 6 mL of MeOH and equilibrated with 6 mL of Milli-Q water. Then 100 mL of the samples (IWW was diluted four times with Milli-Q water, i.e. 25 mL sample in 100 mL) were passed through the cartridges by gravity and vacuum-dried for approximately 15 min. The analytes were eluted with 6 mL of MeOH and the extracts were evaporated to dryness at 35°C under a gentle nitrogen stream and finally reconstituted in 1 mL of 10 % MeOH aqueous solution.

2.5 Instrumentation and analytical method

A Waters Acquity UPLC system (Waters, Milford, MA, USA) was interfaced to a hybrid quadrupole-orthogonal acceleration-TOF mass spectrometer (XEVO G2 QTOF, Waters Micromass, Manchester, UK), using a Z-Spray ESI interface operating in positive ion mode. The chromatographic separation was done using a Waters Acquity UPLC BEH C₁₈ (100 \times 2.1 mm, 1.7 μm) column (Table S3).

MS data were acquired over a m/z range of 50 – 1,000. Capillary voltage was 0.7 kV and cone voltage 20 V. Collision gas was argon 99.995% (Praxair, Valencia, Spain) and nitrogen was used as drying gas and nebulizing gas. The desolvation temperature was 600°C and the source temperature 135°C. The column temperature was 40°C.

For MS^E experiments, two full-scan acquisition functions with different collision energies were created: the low-energy function (LE), selecting a collision energy of 4 eV, and the high-energy (HE) function, with a collision energy ramp from 15 to 40 eV in order to obtain a greater range of fragment ions. The total scan time was 0.4 s.

QTOF MS data were processed using ChromaLynx XS application manager (within MassLynx v 4.1; Waters Corporation). The following parameters were used: target retention time (t_R) tolerance (1.50 min) from empirical retention time (from injected standards), mass tolerance 0.020 Da (for positive ID 0.010 Da), peak width at 5% height 6 s, peak-to-peak baseline noise 1,000 and threshold absolute area 250 for SW and 500 for IWW and EWW.

For more details about instrumentation and analytical method see (Bade et al., 2015c).

2.6 Criteria for detection/identification

The detection and identification of the compounds was based on the confidence levels for small molecules in HRMS analysis proposed by Schymanski et al. (2014) and the European Commission Decision 2002/657/EC (Commission, 2002). The criteria included the presence of the protonated molecule at accurate mass, accurate mass fragment ion(s) and retention time deviation (European Commission, 2014). When no reference standard was available, a retention time prediction model was used as a complementary tool (Bade et al., 2015a).

2.6.1. Standard available

A compound was classified as “*detected*” when the accurate-mass protonated molecule $[M+H]^+$ (mass error <5 ppm) was found and the retention time was in agreement with the reference standard (± 0.2 min).

A compound was classified as “*identified*” when the accurate-mass protonated molecule $[M+H]^+$ (mass error <5 ppm) was found together with at least one fragment ion at accurate mass (mass error <5 ppm) and the retention time was in agreement with the reference standard (± 0.2 min).

2.6.2. *Standard not available*

Compounds were classified as “*tentatively detected/identified*” when a protonated molecule $[M+H]^+$ at accurate mass (<5 ppm) was found, and at least one fragment ion could be justified (<5 ppm).

2.6.3. *Retention time predictor*

A retention time (t_R) prediction approach, based on a previously developed artificial neural network (ANN) method using 544 compounds (Bade et al., 2015a), was employed to aid in the tentative detection and/or identification of compounds, when no reference standards were available. In brief, canonical simplified molecular input line entry system strings (SMILES) were created using ChemSpider (Royal Society of Chemistry, UK) and/or ChemSketch (ACD Labs) freeware. From these, 16 molecular descriptors (as ANN inputs) were generated: the number of double and triple bonds (nDB or nTB), the number of carbon and oxygen atoms (nC or nO), the number of 4–9 member rings (nR04–nR09), unsaturation index (UI), hydrophilic factor (Hy), Moriguchi and Ghose–Crippen logP (MlogP and AlogP respectively), and software predicted

$\log K_{ow}$ data (Tetko et al., 2005). Prediction of t_R (as the designated single output) via neural networks was performed using Alyuda NeuroIntelligence 2.2 (Cupertino, CA).

3 Results and discussion

The general screening applied in this work made use of a sample pre-concentration with Oasis HLB cartridges. Oasis HLB is a universal sorbent that has been extensively used for retention/pre-concentration of organic contaminants, its hydrophilic/lipophilic character makes it suitable for interaction with analytes of a wide polarity range (Bade et al. 2015c; Rousis et al. 2016a; Hernandez et al. 2015). Recently, it has been reported that “solid phase extraction (SPE) with Oasis HLB cartridges continues to be the most popular means of extraction and concentration for emerging contaminants in water” (Richardson and Kimura 2016). In addition, in our previous study, HLB showed better recoveries for the majority of pesticides and TPs, compared to other more selective cartridges, such as OASIS WAX, MAX and MCX cartridges (Rousis et al. 2016a).

The pesticides in the matrices were initially investigated using the information from standards about fragmentation and retention times according to the criteria described above. Examples of a substance “*detected*” and one “*identified*” are reported in Figure 1. Occasionally, the isotopic pattern of chlorine was used for additional confirmation and proved advantageous, especially when no other data were available. Many compounds containing chlorine(s) were considered false-positive and removed (i.e. phosfon, tepraloxymid), since no Cl isotopic pattern was displayed.

From the initial list of 164 compounds (Table S1), seventeen pesticides and TPs were detected and identified in the samples (Tables 1-2). These were fungicides (imazalil, metalaxyl, propiconazole, thiabendazole, carbendazim), herbicides (metolachlor, molinate, simazine, terbutylazine, terbutryn), insecticides (carbaryl, diazinon, imidacloprid) and TPs (2-hydroxy-simazine, 2-hydroxy-terbutylazine, deethyl terbutylazine, deethyl terbumeton). In most cases, once a compound was detected, it could also be identified using the reference standards (fragment ions). However, in some cases only the exact mass of the protonated molecule and the t_R could be used to assess the presence of the substance. Although these findings cannot be classified as level 1 (Schymanski et al., 2014) and more research is needed for reliable confirmation, the information obtained (i.e low mass errors and low t_R deviation) gave sufficient confidence to report these compounds as detected.

The second list of pesticides (Table S2) was also screened and seven masses were frequently detected (Table 3). These could be assigned to a “*tentatively detected*” compound according to the exact mass, since no fragments were available in our database. Furthermore, the retention times were the same in all the samples for each of these seven compounds and the mass errors were always lower than 5 ppm. Unfortunately, these substances could not be fully identified only on the basis of the exact mass and analytical standards would be needed to confirm their identity. However, t_R prediction was used as a way to increase the confidence for these tentatively detected compounds, using a 2-min window (Bade et al., 2015a, 2015b) with 5-OH-clethodim sulfon found within this threshold. The “classical” pesticides in the priority pollutant lists of EU, US-EPA and United Nations, and many organophosphorus compounds widely used in recent years, were not detected in the samples analyzed.

3.1 Results in Wastewater

In total, eleven pesticides and TPs were detected and identified in WW samples from Spain and Italy (Table 1). A few compounds were found in IWW: terbutryn, terbutylazine and its TP 2-hydroxy-terbutylazine were detected in Italy, while none of the selected pesticides were found in Spain. Respectively nine and four pesticides were detected in EWW samples from Spain and Italy (Table 1). In Italy the compounds found in IWW were also found in EWW, indicating that removal was not complete during wastewater treatment. Metolachlor was only identified in EWW. Generally, in both countries more compounds were detected in EWW than IWW.

Nine substances (bendiocarb, desmethylprimicarb, dibenzylamine, ethofenprox, ethoxyquin, kresoxim-methyl, spiroxamine, thiofanox, thiofanox sulfone) were tentatively detected from the first database (Table S1), but reference standards were no longer available to confirm them. Therefore, a t_R prediction model was applied, utilizing a ± 2 min window, as in previous works (Bade et al., 2015a, 2015b) and concluded that seven of the nine were within the window (Table S4). The strength of t_R prediction as a complementary tool is thus underscored, as two compounds (bendiocarb and thiofanox) could be removed from further investigation, without the need for reference standards.

Considering that pesticide concentrations in wastewater are usually very low, the full MS scan option used in HRMS can fail to identify multiple substances, thus giving a false-negative result due to the lack of sensitivity compared to, for example, LC-MS/MS with QqQ. A number of factors may also explain why more substances were detected in EWW than IWW. First of all, raw wastewater (IWW) is a complex matrix and therefore harder to analyze than EWW. Thus, the frequency of detection was probably higher in EWW owing to the fact that it is a cleaner matrix.

However, it should be taken into account that the different sample volume taken for IWW and EWW (25 mL and 100 mL, respectively), also result in lower concentration factor for IWW. Another hypothesis relates to the behavior of pesticides during the treatment processes; if a compound is retained in the activated sludge or reverts to the parent compound from conjugated forms during treatments, its concentration may be higher in EWW than in IWW. Since our method is based on qualitative detection and identification of compounds, and no concentrations were available, the behavior of pesticides during the WWTP processes could not be fully evaluated (Campo et al., 2013; Kock-Schulmeyer et al., 2013), but the phenomenon is well known for other contaminants such as pharmaceuticals (Castiglioni et al., 2006; Gracia-Lor et al., 2012). Other possible reasons are related to the sampling procedure, the wastewater treatment technology, environmental conditions (e.g. rainfall, high temperature), and hydrolysis and transformation during treatments (Kasprzyk-Hordern et al., 2009; Kock-Schulmeyer et al., 2013; Luo et al., 2014; Moschet et al., 2014; Ort et al., 2010).

3.2 Results in Surface Water

Surface water samples were taken from five areas in Spain, and twelve pesticides and TPs were detected and identified (Table 2). In almost all the cases the compounds detected were also identified, except for simazine (Burriana Clot and Albufera Natural Park 1) and imazalil (Albufera Natural Park 1), probably because of their very low concentrations. Up to eleven pesticides were found in Albufera Natural Park compared to the other areas. Some differences were observed among the two sites of this park; 2-hydroxy-terbutylazine, molinate, simazine and terbutylazine were found only in the first site, while terbutryn was detected only in the second. Seven substances

were found in Burriana Clot and Nules, while in Almenara none of the substances investigated were found.

3.3 Comparison of results in Spain and Italy

In general, more compounds were detected (9) and identified (5) in Spanish EWW (7 samples) than Italian EWW (2 samples) (4 detected and 4 identified) (Table 1). Terbutryn and 2-hydroxy-terbutylazine were found in both countries, but terbutylazine, the parent compound of 2-hydroxy-terbutylazine, was identified only in Italy. The chloroacetanilide herbicide metolachlor was only identified in one EWW sample from Italy, and 2-hydroxy-simazine, carbendazim, imidacloprid and thiabendazole were only identified in Spanish EWW. In addition, carbaryl, deethylterbumeton and diazinon were also detected only in the Spanish samples. These results could also be explained by the fact that more samples were analyzed from Spain and the WWTPs are of different size. The WWTP in Castellon serves 180,000 inhabitants and the WWTP in Cremona 85,000.

More pesticides were found in SW than WW. This is in line with the fact that additional sources of contamination can affect SW, such as direct runoff from cultivations, while WW is mainly affected by the urban use of these substances. Eleven compounds were found in Spanish SW. Five of these were also found in Spanish EWW samples. The compounds deethyl terbutylazine, imazalil, metalaxyl, molinate, propiconazole and simazine were identified only in Spain and in SW, indicating that their main source was agriculture.

The use of simazine, carbaryl, carbendazim, terbumeton, diazinon, metolachlor, molinate and terbutryn is currently prohibited in EU (European Commission, 2016). Nevertheless, the presence of carbendazim and molinate can probably be explained by the fact that during the

sampling year (2014) their use was still permitted. The other compounds in water might imply spills and disposals of unused pesticides, transportation through the wind, illegal use of banned pesticides, high environmental persistence, transportation with foodstuffs and/or application during storage and transport from countries in which their use is allowed (Barco-Bonilla et al., 2013b; Botero-Coy et al., 2015; Coscollà et al., 2013; Wittmer et al., 2010).

3.4 Comparison with other studies

3.4.1. Italy

The presence of pesticides in WW in Italy has been assessed only in a few studies to date. Benvenuto et al. (2010) analyzed IWW and EWW samples for triazine herbicides and their TPs. Terbutryn and 2-hydroxy-terbutylazine were quantified, while terbutylazine concentrations were below the LOQ in IWW. Deethyl terbutylazine and deethyl terbumeton were detected in EWW (Benvenuto et al., 2010). The same compounds were identified in the present study and were the only ones identified in IWW and EWW. The chloroacetanilide herbicide metolachlor was identified in EWW in our study, while it has been detected before in many Italian SW samples (De Liguoro et al., 2014), despite the fact that its use is prohibited in the EU.

Other studies investigated atrazine and some triazine TPs, pyrethroid metabolites, the specific metabolite of diazinon (2-isopropyl-6-methyl-4-pyrimidinol), and the specific metabolite of chlorpyrifos and chlorpyrifos methyl (3,5,6-trichloro-2-pyridinol) in IWW from seven Italian cities (Rousis et al., 2016a). Atrazine was detected at very low concentrations (commonly < 10 ng/L) and therefore it could not be detected by a HRMS instrument. The other metabolites were not included in the present study because they were mainly specific human metabolites, while in this study the focus was mainly on environmental TPs. However, the parent compounds diazinon,

chlorpyrifos and chlorpyrifos methyl were included (Table S1) and were not detected. The metabolite of cyfluthrin, permethrin and cypermethrin (3-(2,2-dichlorovinyl)-2,2-dimethyl-(1-cyclopropane)carboxylic acid (DCCA)) was detected in IWW in all the samples (Rousis et al., 2016a, 2016b), but cyfluthrin was not detected in the present study. This might be due to its log octanol-water partition coefficient ($\text{Log } K_{ow}$) which is 6 (WHO (World Health Organization), 2003), so this substance is lipophilic and its adsorption to sludge particles can be strong. Since the free dissolved water concentration of cyfluthrin is expected to be very low this was probably one reason for the negative detection in our samples.

Studies on pesticides in Italian aquatic environments are still scarce and more research is needed. However, the most frequent compounds in SW and groundwater are glyphosate and its metabolite, aminomethylphosphonic acid (AMPA) and some triazines with their TPs (Meffe and de Bustamante, 2014). The use of atrazine in Italy was prohibited in the early 1990s and it was rapidly replaced by terbutylazine. Since then, terbutylazine and its TP, deethyl terbutylazine, have been detected at high frequency and in some cases the concentrations exceeded the EU limits (Bottoni et al., 2013). Other triazines detected in Italian SW were atrazine, simazine, atrazine desethyl, desisopropyl atrazine and terbutryn, but the concentrations were very low (a few ng/L) (Benvenuto et al., 2010; Bono and Magi, 2013; Bottoni et al., 2013; De Liguoro et al., 2014). Imidacloprid, propiconazole, carbendazim, tetraconazole, thiabendazole, metolachlor, diazinon, penconazol, and dimethoate were also detected at very low concentrations in SW (Bono and Magi, 2013; De Liguoro et al., 2014; Montuori et al., 2015).

3.4.2. *Spain*

Pesticides in WW and SW in Spain have been investigated more than in Italy. Seven IWW samples were screened in this study and the presence of pesticides was not confirmed. Campo et al. (2013) investigated around 50 pesticides in IWW from different WWTPs in 2010 and 2011. Most of the pesticides found in IWW belonged to different classes and in many cases the frequency of detection differed impressively over the two years (i.e. prochloraz) (Campo et al., 2013). Benvenuto et al. (2010) examined triazines and their TPs in IWW, and terbutryn and 2-hydroxy-terbutylazine were quantified in all the samples.

The results of the present study in EWW from Spain were in line with other studies, where triazines, carbendazim, thiabendazole, diazinon and/or imidacloprid were also identified (Barco-Bonilla et al., 2013a, 2013b; Benvenuto et al., 2010; Masia et al., 2013). The carbamate insecticide carbaryl was detected only in one EWW sample, but it was not investigated in other studies. Other pesticides detected with high frequency in EWW were 2-phenylphenol, chlorfenvinphos, diuron, buprofezin, chlorpyrifos, dimethoate and pyriproxyfen (Barco-Bonilla et al., 2013a, 2013b; Masia et al., 2013).

The presence of triazine herbicides and their TPs in SW of Spain has been thoroughly studied. These compounds were detected in almost all studies at different concentrations and frequency of detection (Benvenuto et al., 2010; Herrero-Hernandez et al., 2013; Pitarch et al., 2016; Robles-Molina et al., 2014). Some of these classes of pesticides and TPs were also identified in the present work. The present results were also in line with other studies for several substances such as carbendazim (Pitarch et al., 2016), imazalil (Belenguer et al., 2014) and metalaxyl (Herrero-Hernandez et al., 2013; Pitarch et al., 2016). Although diazinon (Belenguer et al., 2014; Masia et al., 2013; Pitarch et al., 2016; Robles-Molina et al., 2014) and dimethoate (Herrero-

Hernandez et al., 2013; Masia et al., 2013; Robles-Molina et al., 2014) were detected in many previous studies, they were not confirmed in the SW samples investigated and diazinon was only detected in five out of seven EWW samples. The pesticides chlorfenvinphos, diclofenthion, prochloraz and pyriproxyfen were quantified at concentrations ranging from 50 to 100 ng/L in all the samples from the Jucar River (Belenguer et al., 2014), but they were not found in our SW samples. This may be because the occurrence of pesticides in SW is closely correlated with their use in the surrounding area, which may differ according to the type of cultivation and current legislation.

4 Conclusions

We investigated 450 pesticides in WW and SW in Spain and Italy by HPLC-QTOF MS. Seventeen pesticides and TPs belonging to different classes (fungicides, herbicides and insecticides) were found. More pesticides were detected in SW and EWW than in IWW, probably because of the complexity of the matrix which makes it hard to reach high sensitivity. Another reason is that SW receives direct inputs from wide usage in agriculture, while WW receives mainly discharge from urban use of pesticides.

We used a simple and fast t_R prediction model to facilitate identification when reference standards were not available, and was able to exclude some false-positives. The wide-scope screening method based on HRMS was an efficient tool for screening a large number of pesticides and selecting priority substances to be investigated in a subsequent quantitative target, when more sensitive methods are required (i.e. based on LC-QqQ-MS/MS).

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Table 1. Compounds detected/identified* in influent (IWW) and effluent (EWW) wastewater samples from Spain and Italy by UHPLC-(Q) TOF MS

Compound	Spain IWW (n = 7)	Spain EWW (n = 7)	Italy IWW (n = 2)	Italy EWW (n = 2)
2-hydroxy-simazine		3/1		
2-hydroxy-terbutylazine		4/0	1/0	2/1
Carbaryl		1/0		
Carbendazim		7/7		
Deethylterbumeton		1/0		
Diazinon		5/0		
Imidacloprid		1/1		
Metolachlor				1/1
Terbutylazine			2/2	2/2
Terbutryn		7/7	1/1	1/1
Thiabendazole		6/6		
Total	0/0	9/5	3/2	4/4

*Detected: exact mass of protonated molecule with mass error < 5 ppm and retention time within ± 0.2 min.; Identified: exact mass of protonated molecule with mass error < 5 ppm, retention time within ± 0.2 min, at least one fragment ion identified with mass error < 5 ppm

Table 2. Compounds detected/identified* in surface water (SW) samples from Spain by UHPLC-(Q) TOF MS

Compound	Burriana Clot	Nules	Almenara	Albufera Natural Park 1	Albufera Natural Park 2
2-hydroxy-terbutylazine	1/1	1/1		1/1	
Carbendazim	1/1			1/1	1/1
Deethyl terbutylazine	1/1	1/1		1/1	1/1
Deethylterbumeton	1/1	1/1		1/1	1/1
Imazalil				1/0	1/1
Metalaxyl		1/1		1/1	1/1
Molinate				1/1	
Propiconazole				1/1	1/1
Simazine	1/0			1/0	
Terbutylazine	1/1	1/1		1/1	
Terbutryn		1/1			1/1
Thiabendazole	1/1	1/1		1/1	1/1
Total	7/6	7/7	0/0	11/9	8/8

*Detected: exact mass of protonated molecule with mass error < 5 ppm and retention time within 0.2 min; identified: exact mass of protonated molecule with mass error < 5 ppm, retention time within 0.2 min, at least one fragment ion identified with mass error < 5 ppm

Table3. Compounds (exact masses) detected in different samples with high frequency and intensity and the potential corresponding pesticides

Exact mass	Spain			Italy		Potential corresponding pesticides (according to Table S2)	Retention time (min)	
	IWW (n = 7)	EWW (n = 7)	SW (n =)	IWW (n = 2)	EWW (n = 2)		Sample	Predicted
408.1248	4	5			2	5-OH-clethodim sulfon	5.30	5.77
282.2797	7	7	5	2	2	Dodemorph	15.90	12.56
190.1266	4		2			EPTC	8.76	10.87
304.2640			5	2	2	Fenpropimorph	15.90	12.10
165.1028	5	3			1	Fenuron	1.70	5.39
204.1025	7	7		2		Hormodin	1.45	8.83
203.0933	3		1	2		Metamitron	8.18	4.08

Figure Legend

Figure 1. a) Identification of carbendazim (m/z 192.077, t_R 3.73) from a surface water sample (RIGHT), with fragment ions 165.051 and 132.056 and retention time comparable to the standard (LEFT); b) Detection of diazinon (m/z 305.109, t_R 12.51) from a EWW sample (RIGHT). Neither of the fragment ions (169.08 and 153.103) of the standard (LEFT) could be seen in the sample.

