TESIS DOCTORAL

Análisis, Distribución, Transporte y Toxicidad de Contaminantes Emergentes en la Cuenca del Río Turia

Parque Natural L'Albufera - Valencia, España

Alexander David Ccanccapa Cartagena

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Análisis, Distribución, Transporte y Toxicidad de Contaminantes Emergentes en la Cuenca del Turia

Analysis, Distribution, Transport and Toxicity of Emerging Pollutant in the Turia Basin

Memoria presentada para optar el título de doctor para

Alexander David Ccanccapa Cartagena

Dirigida por:

Dra. Yolanda Picó García

Catedrática de Farmacia
Universidad de Valencia

Dra. Ana Masiá Reyes

Investigadora
Grupo de Investigación en Seguridad Alimentaria y Medio Ambiental -
Universidad de Valencia

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Ana Masiá Reyes, Investigadora, i Yolanda Picó García, Catedràtica de l'àrea de Nutrició i Bromatologia en el Departament de Medicina Preventiva de la Universitat de València, ambdues Doctores en Farmàcia i investigadores del Centre de Recerques sobre Desertificació (CIDE):

I N F O R M E N :

Que el *D. Alexander David Ccanccapa Cartagena* ha realitzat sota la nostra adreça la tesi doctoral que porta per títol **"ANALISI, DISTRIBUCIÓ, TRANSPORT I TOXICITAT DE CONTAMINANTS EMERGENTS EN LA CONCA DEL RIO TURIA"** que es presenta com un compendi de quatre articles indexades en el JCR i un capítol de llibre en una editorial de prestigi:

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- CCANCCAPA, A., Masià, A., Navarro-Ortega, A., Picó, Y. and Barceló, D. (2016). **Pesticides in the Ebro River basin: Occurrence and risk assessment**. *Environmental Pollution* **211,** 414-424. [**JCR (WOS)** IF 4.839 (2015) en l'àrea de Ciències Mediambientals 17/225 Q1].
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cide@uv.es

CARRETERA DE MONCADA A NÁQUERA
KM 4,5.
46113 MONCADA (VALENCIA)
TEL: 96 342 41 62
FAX: 96 342 41 60
http://www.uv.es/cide/

a Mediterranean River basin. *Environmental Science & Technology* (Enviada). [**JCR (WOS)** IF 5.393 (2015) en l'àrea de Ciències Mediambientals 14/225 Q1]

La contribució del doctorand en els treballs ha consistit en l'examen dels antecedents, el disseny i planificació de l'estudi, la realització de la fase experimental, l'anàlisi dels resultats, i la presentació de conclusions, per el que ha comptat també amb la supervisió de la resta d'autors, tots ells doctors altament qualificats en els seus camps de especialitat. D'aquests treballs, en els quals el doctorand és sempre el primer autor, 3 han sigut ja publicats i 2 estan encara en alguna fase del procés editorial, i cap d'ells s'ha utilitzat o utilitzarà implícita o explícitament per a la realització d'una altra tesi doctoral, per la qual cosa autoritzem la seua presentació per a optar al grau de doctor.

Montcada, 19 de maig de 2017

Dr. Ana Masiá Reyes

Dra. Yolanda Picó García

cide@uv.es

CARRETERA DE MONCADA A NÁQUERA
KM 4,5.
46113 MONCADA (VALENCIA)
TEL: 96 342 41 62
FAX: 96 342 41 60
http://www.uv.es/cide/

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Hace pocos años pensar en convertirse en Dr. era un anhelo a largo plazo, era saberse con muchos años de experiencia y algunas canas encima. Sin embargo, los tiempos han cambiado y hoy con esfuerzo, perseverancia y continuidad se puede conseguir ser un especialista en lo que has descubierto que entregarías algo más que tres años, sí, eso que llamamos futuro. Empiezas a alimentar el futuro con la idea de no dejar la línea de conocimiento que has abrazado y creas un imaginario de tu porvenir; creo que ese es el síntoma que no sólo han sido tres años, que por bondades de la vida y la constante discusión interna de lo que te gusta y disgusta, al fin puedes ver con claridad el oficio al que quieres entregarle los siguientes años de tu vida. Sin embargo, en esta búsqueda no estás solo, hay muchas personas que a lo largo del camino han contribuido de manera transcendental para que hayas moldeado tu personalidad, tus anhelos y sueños. Es a ellos a quienes quiero agradecer en este breve escrito.

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Mi padre duerme. Su semblante augusto
figura un apacible corazón;
está ahora tan dulce…
si hay algo en él de amargo, seré yo.

Hay soledad en el hogar; se reza;
y no hay noticias de los hijos hoy.
Mi padre se despierta, ausculta
la huida a Egipto, el restañante adiós.

Está ahora tan cerca;
si hay algo en él de lejos, seré yo.
Y mi madre pasea allá en los huertos,
saboreando un sabor ya sin sabor.
Está ahora tan suave,
tan ala, tan salida, tan amor.

Hay soledad en el hogar sin bulla,
sin noticias, sin verde, sin niñez.
Y si hay algo quebrado en esta tarde,
y que baja y que cruje,
son dos viejos caminos blancos, curvos.
Por ellos va mi corazón a pie.

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PRESENTACIÓN MEMORIA

OBJETIVOS

Los cambios económicos, sociales y demográficos en los últimos años en todo el mundo han provocado una presión en la calidad del medio ambiente, el cual ha sido impactado principalmente por actividades humanas como la agricultura, industria y estilo de vida urbano [1, 2]. El desarrollo de estas actividades ha sido un motor para la producción de distintos compuestos orgánicos que han mejorado su rendimiento, pero a su vez se han transformado en contaminantes que impactan los diferentes ecosistemas acuáticos, terrestres y atmosféricos [3, 4]. En este contexto, los contaminantes emergentes se definen como compuestos que no están incluidos actualmente en las regulaciones existentes de calidad del agua o no se han estudiado previamente, y se piensa que son amenazas potenciales para la salud humana y los ecosistemas. Plaguicidas, fármacos, productos de cuidado personal (PCP), esteroides y hormonas, surfactantes, sustancias perfluoroalquilicas (PFASs), retardantes de llama, aditivos y agentes industriales, así como sus productos de transformación (TPs) conforman los principales grupos de contaminantes emergentes que se están investigando en la actualidad [2, 5-9]. Uno de los más importantes grupos de estos contaminantes que está en constante crecimiento son los plaguicidas [4, 10]. Estos compuestos incluyen un amplio rango de sustancias químicas utilizadas para limitar, inhibir y prevenir el crecimiento de animales nocivos, insectos, plantas invasoras, malas hierbas y hongos en la agricultura, ganadería y el hogar [11]. El área mediterránea de Europa se caracteriza por desarrollar una agricultura intensiva y a gran escala, siendo España uno de los principales productores agrícolas y el primer consumidor de plaguicidas (78 toneladas, 2013) según Eurostat (Oficina de Estadística de la Unión Europea) [12]. Los últimos estudios vinculados al análisis de plaguicidas ponen de manifiesto que, aunque principalmente están enfocados a su evaluación en alimentos, en el caso de matrices ambientales hay tres vertientes de

enorme importancia: (i) desarrollo de métodos analíticos, (ii) seguimiento de la presencia de estos y otros contaminantes emergentes en las cuencas hidrográficas y (iii) evaluación del riesgo ecotoxicológico. Sin embargo, existen pocas referencias de estudios que aglutinen un análisis integral de las tres vertientes descritas y que posibiliten una comprensión global de la distribución, degradación y acumulación de los plaguicidas en los diferentes compartimentos ambientales, así como un análisis comparativo de este problema en las cuencas mediterráneas.

El río Turia está situado en el sureste de España y es uno de los más importantes que drena sus aguas al Mar Mediterráneo. Pertenece a la Demarcación Hidrográfica del Júcar, la cual se caracteriza por una alta presión demográfica (5.162.163 habitantes, 2009) y una importante agricultura de regadío-secano, que ocupa alrededor de la mitad del territorio (350.000 ha), concentrada principalmente en la zona baja de la cuenca [13-15]. Estas características hacen de esta cuenca un espacio importante para el análisis y estudio del comportamiento integral de los contaminantes emergentes.

En este contexto, el **objetivo principal** del presente estudio es *evaluar la contaminación por plaguicidas y otros contaminantes emergentes en la Cuenca del Río Turia* y está basado (I) en el análisis de diferentes familias de plaguicidas aplicando cromatografía de líquidos con espectrometría de masas en tándem (LC-MS/MS) y en la determinación del perfil de contaminación global mediante cromatografía de líquidos con espectrometría de masas de alta resolución (LC-HRMS), que nos permitirá comprender la (II) distribución espacial a lo largo de la cuenca como las diferentes vías de (III) transporte, y en relación a las concentraciones encontradas evaluar el (IV) riesgo derivado de la presencia de contaminantes emergentes (plaguicidas) en la fauna y flora acuática a través del coeficiente de riesgo (RQ) y las unidades toxicas (TUs). Complementariamente y con objeto de llegar a conclusiones extrapolables a un ámbito más amplio, se realiza (V) un análisis comparativo con otras cuencas del mediterráneo.

Así pues, los **Objetivos Específicos** planteados para la presente investigación son:

1. Desarrollar métodos analíticos para determinar estos compuestos en diferentes matrices (aguas y sedimentos) hasta niveles traza.

2. Establecer el perfil de los contaminantes emergentes en la cuenca del río Turia.
3. Caracterizar las fuentes que determinan que el contaminante sea liberado al medio y averiguar sus rutas de distribución.
4. Definir y cuantificar los procesos que determinan su transporte y acumulación en el medioambiente.
5. Identificar los efectos ecológicos potenciales debido a la exposición a estos compuestos.
6. Realizar un estudio comparativo con otras cuencas del mediterráneo que nos permita establecer patrones de comportamiento.

El desarrollo de estos objetivos permitirá:

1. Comprobar la presencia de contaminantes emergentes en el medioambiente, su dinámica, distribución y destino en la cuenca, como un factor clave para establecer la huella antrópica en la zona de estudio.
2. Evaluar la capacidad de respuesta de la cuenca frente a los diferentes tipos de presiones de origen antrópico (desarrollo urbano, industrial, agrícola, etc.) o natural (erosión, incendios forestales), de cara a su conservación y/o posible recuperación bajo la perspectiva del cambio climático.
3. Desarrollar una base metodológica para la aplicación de la forensía ambiental de modo global y multidisciplinar, para determinar las fuentes puntuales de degradación, química y/o física, que se aplicará a la cuenca del Turia para establecer sus zonas frágiles y su estado de degradación.

ESTRUCTURA

La presente tesis doctoral está estructurada en dos partes:

La primera parte consiste en una profunda revisión bibliográfica que incluye (I) introducción, consumo de plaguicidas (estadísticas), legislación, (II) plaguicidas tradicionales, (III) plaguicidas de nueva generación y (IV) problemática de las cuencas mediterráneas y los contaminantes emergentes.

La segunda parte estructura el desarrollo experimental la tesis e incluye la optimización de los métodos analíticos (extracción, identificación y cuantificación de contaminantes emergentes) y su seguimiento en las cuencas a través del análisis dirigido (LC-MS/MS – secciones de V - VII) y no dirigido (LC-HRMS – sección VIII) de contaminantes emergentes. (V). Finalmente, (IX) se presenta el resumen de los resultados y las conclusiones del proyecto de investigación. Al final de la memoria se detalla la bibliografía utilizada, anexos, lista de abreviaturas, tablas y figuras.

La estructura de ambas partes en mayor detalle implica:

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I) **Introducción:** Esta sección presenta una visión general de los plaguicidas, su desarrollo a lo largo de la historia, las estadísticas de producción y consumo a nivel mundial, en el área mediterránea y España (Comunidad Valenciana). Se explora la legislación actual de la Unión Europea en relación a los contaminantes emergentes (principalmente plaguicidas) en materia de Límites Máximos Permisibles en alimentos y matrices ambientales. Este apartado nos permite situarnos en el contexto actual de la problemática de los contaminantes emergentes y su impacto en los diferentes compartimentos ambientales.

II) **Plaguicidas Tradicionales:** Se desarrolla una remembranza de la primera, segunda y tercera generación de plaguicidas y sus modos de acción como su toxicidad. Se realiza un especial énfasis en plaguicidas organoclorados, organofosforados, carbamatos y piretroides. Los plaguicidas pertenecientes a estos últimas tres familias han sido incluidas en la lista de búsqueda dirigida para el análisis de los ríos Turia, Júcar y Ebro.

III) **Plaguicidas de Nueva Generación:** Se presenta una perspectiva profunda de los nuevos tipos de plaguicidas introducidos en el mercado: lactonas macrocíclicas, cloronicotinils, tetranortriterpenos, sales de amonio cuaternario, dinitroanilinas, acetamidas y oximas, Se presenta sus propiedades físico-química, toxicidad, regulación, métodos de extracción, métodos analíticos y referencias de estudios que han abordado el análisis de estos compuestos en matrices ambientales, de alimentos y biológicas. Esta revisión bibliográfica se publicará en un capítulo de libro "*Pesticides (New Generation) and Related Compounds, Analysis of Pesticides*" en la Encyclopedia of Analytical Chemistry, editada on-line por wiley.

IV) **Problemática de los contaminantes Emergentes en las Cuencas Mediterráneas:** acerca de los estudios previos sobre la presencia de los contaminantes emergentes y sus productos de transformación en las principales cuencas mediterráneas de la Union Europea como: Ebro, Llobregat y Turia en España; Po y Tiber en Italia; Guadiana en Portugal; Rhône en Francia y Aliakmon, Axios en Grecia. Se aborda la problemática de los contaminantes emergentes teniendo como factores importantes las condiciones climáticas del área mediterránea y el actual contexto del cambio climático.

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V) **Desarrollo de un Método Analítico y de Extracción para Piretrinas y Piretroides (*PUBLICACIÓN # 1:* Simultaneous determination of pyrethroids and pyrethrins by dispersive liquid-liquid microextraction and liquid chromatography triple quadrupole mass spectrometry in environmental samples.** *Anal. Bioanal. Chem.* (2017) Aceptada (DOI: 10.1007/s00216-017-0422-7)**:** Debido a su creciente utilización en los últimos años, se presenta el desarrollo de un método para el análisis simultaneo de piretrinas naturales y sintéticas basado en la microextracción líquido-líquido dispersiva (DLLME) y cromatografía liquida y espectrometría de masas optimizado para su aplicación en aguas y sedimentos. Se demuestra la eficacia de estos métodos basados en la DLLME para la extracción de piretrinas naturales y sintéticas mediante la

aplicación del mismo a muestras de aguas del humedal La Albufera y sedimentos del río Turia.

VI) **Análisis de Patrón Espacio Temporal de Residuos de Plaguicidas en las Cuencas de Turia y Júcar (2010-2013) (*PUBLICACIÓN # 2* Spatio-temporal patterns of pesticide residues in the Turia and Júcar Rivers (Spain)"** *Sci. Total Environ. 540 (2016) 200-210***:** presenta el seguimiento de 50 plaguicidas agrupados en las siguientes familias: Anilidas, azol, benzimedazoles, carbamatos, cloroacetanilides, hormonas juvelines mimics, neonicotinoides, organofosforados, tiocarbamatos, triazinas, triazoles, ureas y otros plaguicidas en los ríos Júcar (2010-2011) y Turia (2012-2013). Además, se evaluaron los parámetros físico-químicos de las muestras ambientales y su relación con las concentraciones de plaguicidas encontrados. Asimismo, se procedio a la evaluación de riesgo a través del Coeficiente de Riesgo (RQ) para dafnias, algas y peces en ambos ríos.

VII) **Presencia y valoración de Riesgos de Plaguicidas en la Cuenca del Ebro (*PUBLICACIÓN # 3* Pesticides in the Ebro River basin: Occurrence and risk assessment" en la revista científica** *Environ. Pollut. 211 (2016) 414-424***:** Se realizó el mismo seguimiento de plaguicidas en la cuenca del Ebro como estudio comparativo y complementario. En este estudio se evalúo de riesgo para la biota por medio de las Unidades Toxicas (TU), un concepto más integral de la toxicidad de los contaminantes debido a que considera el "cocktail" de concentraciones y su impacto en los distintos niveles tróficos como las dafnias, algas y peces. También se analizó el coeficiente de riesgo (RQ) en los mismos bioindicadores ambientales.

VIII) **Análisis Dirigido, de Amplio Espectro y no Dirigido en el Río Turia (*PUBLICACIÓN # 4: S*uspect, non-target and target screening of emerging pollutants using Data Independent Acquisition: Assessment of a Mediterranean River basin.** *Environ. Sci. Technol*. (enviada)**:** Aguas y sedimentos del Turia se analizaron en 2016 utilizando un método analítico (UPLC-QqQ-TOF) de análisis dirigido, de amplio espectro, y no dirigido para identificar emergentes utilizando. El análisis de amplio espectro se basó en la utilización de una librería teórica de 2.200 componentes de Water Corporation (plaguicidas, fármacos, drogas de abuso, productos de cuidado personal y toxinas) y el modo de adquisición de datos "Data Independent

Acquisition" (DIA). El análisis no dirigido se realizó con la ayuda de bases de datos como el Chem. Spider. Finalmente, se desarrolló un método de análisis dirigido a 170 plaguicidas y 33 fármacos, incluyendo los encontrados en el análisis de amplio espectro y no dirigido.

IX) **Resumen de Resultados:** En este apartado se presenta un resumen detallado de los resultados obtenidos a largo de la investigación de la presente tesis doctoral.

X) **Conclusiones:** Finalmente, se presentan las conclusiones generales de esta tesis doctoral**.**

Anexos: Se presenta un listado de las tablas, figuras y acrónimos de la presente tesis doctoral.

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PRIMERA PARTE

I.

INTRODUCCIÓN

Esta sección presenta una visión general de los plaguicidas, su desarrollo a lo largo de la historia, las estadísticas de producción y consumo a nivel mundial, en el área mediterránea y España (Comunidad Valenciana). Se explora la legislación actual de la Unión Europea en relación a los contaminantes emergentes (principalmente plaguicidas) en materia de Límites Máximos Permisibles en alimentos y matrices ambientales. Este apartado nos permite situarnos en el contexto actual de la problemática de los contaminantes emergentes y su impacto en los diferentes compartimentos ambientales.

1. Introduction

1.1. Pesticide consumption

Food and Agriculture Organization (FAO) defines pesticides as "any substance or mixture of substances intended to prevent, destroy or control any pest, including vectors of human or animal diseases. Unwanted species of plants or animals which cause injury or otherwise interfere with the production, processing, storage, transport or marketing of food, agricultural products, timber and wood products or animal feed, or can be given to animals to fight insects, arachnids or other pests. The term includes substances intended for use as plant growth regulators, defoliants, desiccants, agents to reduce fruit density or agents to prevent premature fruit falling, and substances applied to crops before or after harvesting to protect the product from deterioration during storage and transport".

The use of chemical fertilizers, which has tremendously increased worldwide crop production since the 1960 is well known as green revolution. The origin was serious food shortages in many developing countries. To avoid them, efforts were made to improve the productivity of the major grain crops, such as flee, wheat, and corn to help feed the people in these nations. The huge increase in production obtained from the same surface of land with the help of mineral fertilizers (based on nitrogen, phosphorus potassium) was the best result shown by the green revolution [1, 2]. The green revolution brought the concept of using best yielding crops worldwide to produce a large amount of food. In this line, protecting the growing crop and securing the yielded gains by using pesticide can additionally help in the production. The use of pesticides, including insecticides, fungicides, herbicides, rodenticides, in order to protect crops from pests, can not only attain a significant reduction of the losses but also improve the

yield of crops such as corn, maize, vegetables, potatoes, and cotton and protect cattle from diseases and ticks. The world has known a continuous growth of pesticide usage, both in the number of chemicals and their quantities, sprayed over the fields. The sale of pesticides increased from 850 x 10^6 $ in 1960 to 31.000 x 10^6 $ in 2005 [3, 4].

However, non-target organisms, including human and wildlife, are susceptible to deleterious effects of pesticide mixtures in their environment. There are reports that showed serious effects in species such as honeybees, birds, animals, and humans [5-11]. Also, residues of pesticides were detected in surface water, ground water, sediments and biota at low ng L^{-1} to several μg L^{-1} range in areas of intensive agricultural activity. These compounds reach to environment via run-off or sub-soil tile drains [12-16]. In addition, the residues can remain entrapped in the crops and water cycle, as result enters the food chain. Finally, these will be ingested by humans along with food and water (**Figure 1**).

One more problem which is now aggravating is the fact that insects and pests are developing resistance to insecticides. Consequently, the dose level of pesticide for pest control needs to be increased and this will surely result in their own accumulation in the environment. One may argue that other way out is the demand for new pesticide molecules. Based on this proposition, chemical companies are continuously synthesizing and testing new chemicals, even though few of them reach the market. In the past six years, a significant rise in the number of commercial pesticides containing fluorine atoms were noted. About 26% of new commercial products contain ‘mixed’ halogen atoms, for example one or more fluorine, chlorine or bromine atoms in addition to one or more additional halogen atoms. The other hand, the second generation

synthetic pyrethroid as *lambda-cyhalothrin* was found to be toxic to aquatic organisms including fish and amphipods [17, 18]

The worldwide consumption of pesticides is about two million tonnes per year: Out of which 45 % is used by Europe alone, 25 % is consumed in the USA, and 25 % in the rest of the world. In the EU, the Member States where the highest quantities of pesticides were sold are Spain (19.5 %), France (18.7 %), Italy (13.8 %), Germany (12.3 %) and Poland (6.2 %). Altogether, they made up 70.5 % of the EU-28's pesticide sales (**Table 1**). However, since many years ago, the number of new pesticides bringing to the market has decreased exponentially mostly due to the long and expensive procedure required to put them in the market. Nowadays, around 500 active substances are authorized by the European Union for their application on various crops according to the Regulation (EC) 1107/2009.

Spanish agriculture occupies approximately 50% of the national area. The south has the largest area of intensive agriculture in Europe [19, 20]. According EUROSAT, in Europe the most pesticides consumed were fungicides (43%), herbicides (33%), insecticides (5%) and plant growth regulators (3%) (**Figure 2**). Spain followed the same pattern (**Figure 3**), in 2014 consumed 38.000 tn of fungicides, 14.000 tn of herbicides and 7.000 tn of insecticides as main groups of pesticides.

In Spain, the Valencian Community is one of the Spanish regions with many intensive agriculture areas and human pressure where surface water and groundwater pollution are vulnerable to EPs. Valencia is the region that produces two thirds of the Spanish oranges and it is the oldest and the largest European producer of citrus. Rice crop is one of the main products, only in the natural park *L'Albufera*, on the Mediterranean coast of Valencia (covers 210 Km^2), 67% is currently rice fields [21-24]. According to the last

survey on crop surfaces and yields (Government of Spain, 2016) in the Valencian Community, 152.000 ha of citrus fruit trees, 43.000 ha of rice crops, 67.000 ha of vineyards and 94.000 ha of olive groves are the most important cultivar areas.

1.2. Legislation

Water is a valuable resource, crucial to all living organisms and for multiple human activities. However, several EPs end up in vital aquatic compartments, such as surface water, ground water and even drinking water, at concentrations from few ng L^{-1} to several mgL^{-1}[16, 25], with negative impact on water quality.

Although there are no legal discharge limits for micro pollutants, some regulations have been published. The Directive 2000/60/EC was the first mark in the European water policy, which set up a strategy to define high-risk substances to be prioritized (Directive, 2000). A set of 33 priority substances/groups of substances (PSs) and the respective environmental quality standards (EQS) were ratified by the Directive 2008/105/EC (Directive, 2008). Two years ago, the European Union Directive 2013/39/EU recommended attention to the monitorization and treatment options for a group of 45 PSs (Directive, 2013), meeting the protection of the aquatic compartments and the human health. In that Directive, two pharmaceuticals (the non-steroid anti-inflammatory diclofenac and the synthetic hormone 17-alpha-ethinylestradiol - EE2) and a natural hormone (17-beta-estradiol - E2) were recommended for inclusion in a first watch list of 10 substances/groups of substances for European Union monitoring, to be launched within two years. In the first quarter of 2015, the watch list of substances for European Union-wide monitoring (as set out in Article 8b of Directive 2008/105/EC) was amended in the Decision 2015/495/EU of 20 March 2015. Besides the abovementioned substances (diclofenac, EE2 and E2), three macrolide antibiotics

(azithromycin, clarithromycin and erythromycin) were included, together with other natural hormone (estrone - E1), pesticides (methiocarb, imidacloprid, acetamiprid, clothianidin, and thiamethoxam), a UV filter (2-Ethylhexyl 4-methoxycinnamate) and an antioxidant (2,6-Ditert-butyl-4-methylphenol) commonly used as food additive. The frequent occurrence of EPs in the environment and the inefficiency of conventional WWTPs to remove such compounds, promoted the amendment of the framework to cover a larger set of hazardous compounds, as well as further recommendations for wastewater treatment steps or even new treatment scenarios.

Pesticides are regulated by the maximum residues levels (MRLs) or tolerances. Both words are considered as synonyms. A MRL is the maximum amount of residue legally permitted on food (including the active substance, metabolites and coadjutants). In general, the MRLs are in the range of 0.01–10 mg kg^{-1} (food samples), depending on the combination commodity and pesticide.

Previously, another directive, 98/83/EC, had set limits for pesticides in water intended for human consumption (100 ng L^{-1} for individual pesticides and 500 ng L^{-1} for the sum of all pesticides). The analysis of EPs (pesticides) residues represents a basic instrument not only for the protection of human health, but also for trade and official control purposes [26, 27].

Table 1. Pesticide sales by major groups, 2014

	Total pesticides sales	Fungicides and bactericides	Herbicides, haulm destructors and moss killers	Insecticides and acaricides	Molluscicides	Plant growth regulators	Other plant protection products	Share in the total EU-28 pesticide sales
EU-28 (1)	395,944.4	173,250.8	131,263.5	20,706.3	1,684.4	12,843.7	56,195.7	100.0
Belgium	7,001.1	3,095.0	2,519.7	555.8	47.7	261.2	521.6	1.8
Bulgaria	1,002.0	186.1	652.4	163.4	:	:	:	0.3
Czech Republic	5,663.4	1,788.3	2,755.3	337.7	15.5	350.3	416.2	1.4
Denmark	1,974.6	530.2	1,242.5	38.3	15.4	114.2	33.9	0.5
Germany	46,078.5	12,739.9	17,876.7	977.2	255.5	2,171.3	12,058.0	11.6
Estonia	596.0	88.2	425.8	25.3	:	56.6	:	0.2
Ireland	2,736.0	635.5	2,039.2	51.4	9.9	:	0.0	0.7
Greece	3,907.1	1,866.4	1,194.6	588.8	1.2	148.5	107.7	1.0
Spain	78,818.3	38,379.7	14,908.0	7,515.1	66.2	156.4	17,793.0	19.9
France	75,287.5	34,430.6	30,965.5	2,610.9	870.2	2,802.9	3,607.5	19.0
Croatia	2,119.1	1,004.8	889.1	143.1	5.4	72.2	4.5	0.5
Italy	64,071.1	37,907.1	7,864.4	2,251.9	75.0	367.4	15,605.2	16.2
Cyprus	1,046.7	698.1	153.4	180.6	1.0	1.2	12.5	0.3
Latvia	1,417.4	224.7	847.5	64.0	0.0	274.5	6.6	0.4
Lithuania	2,545.6	604.8	1,394.2	43.6	0.0	502.9	:	0.6
Luxembourg (2)	176.1	91.0	82.8	:	2.3	:	:	0.0
Hungary	8,959.5	3,634.1	4,011.1	916.5	3.5	203.3	190.9	2.3
Malta	108.4	97.4	7.6	2.9	0.5	0.0	:	0.0
Netherlands	10,665.6	4,869.1	3,266.4	252.0	45.1	452.0	1,780.8	2.7
Austria	3,373.2	1,641.1	1,375.8	240.2	16.2	53.5	46.4	0.9
Poland	23,550.6	7,442.5	12,073.4	1,479.2	35.3	2,128.0	392.3	5.9
Portugal	12,889.2	8,244.4	2,410.8	732.9	35.7	1.4	1,464.0	3.3
Romania	10,021.2	4,131.9	5,025.4	569.0	1.2	270.6	23.1	2.5
Slovenia	1,009.0	723.7	238.5	33.5	2.2	0.6	10.5	0.3
Slovakia	2,198.0	567.2	1,215.1	106.5	:	179.8	129.4	0.6
Finland	3,579.9	198.5	1,305.4	12.8	:	88.6	1,974.5	0.9
Sweden	2,486.7	302.3	2,103.8	34.2	:	29.3	17.1	0.6
United Kingdom	22,662.7	7,128.1	12,418.9	779.4	179.4	2,156.8	:	5.7
Norway	859.8	121.8	692.0	4.8	1.3	39.1	0.7	:
Switzerland	2,240.9	1,002.2	745.4	83.1	55.9	30.7	323.6	:

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Figure 1. Cycle of pesticides in the environment

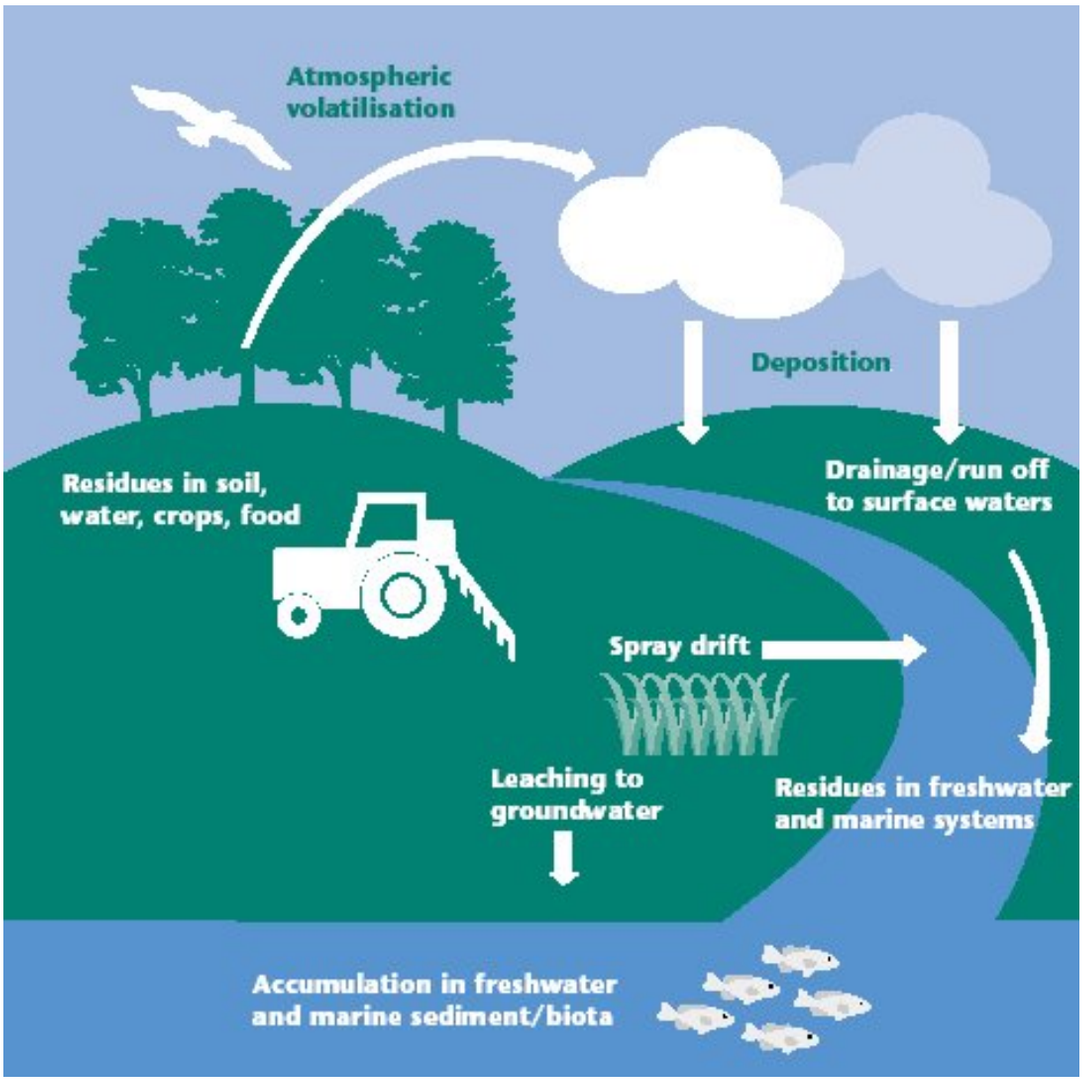

Figure 2. Pesticide sales by major groups, EU-28, 2014

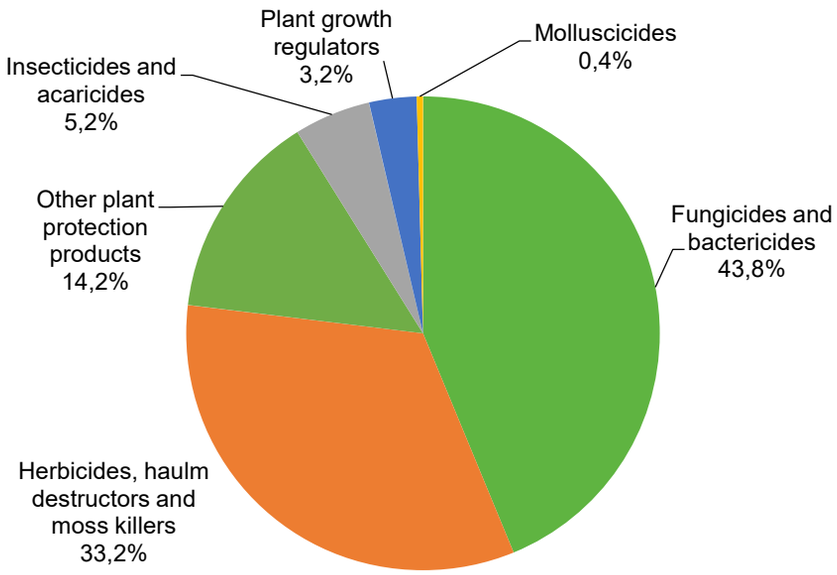

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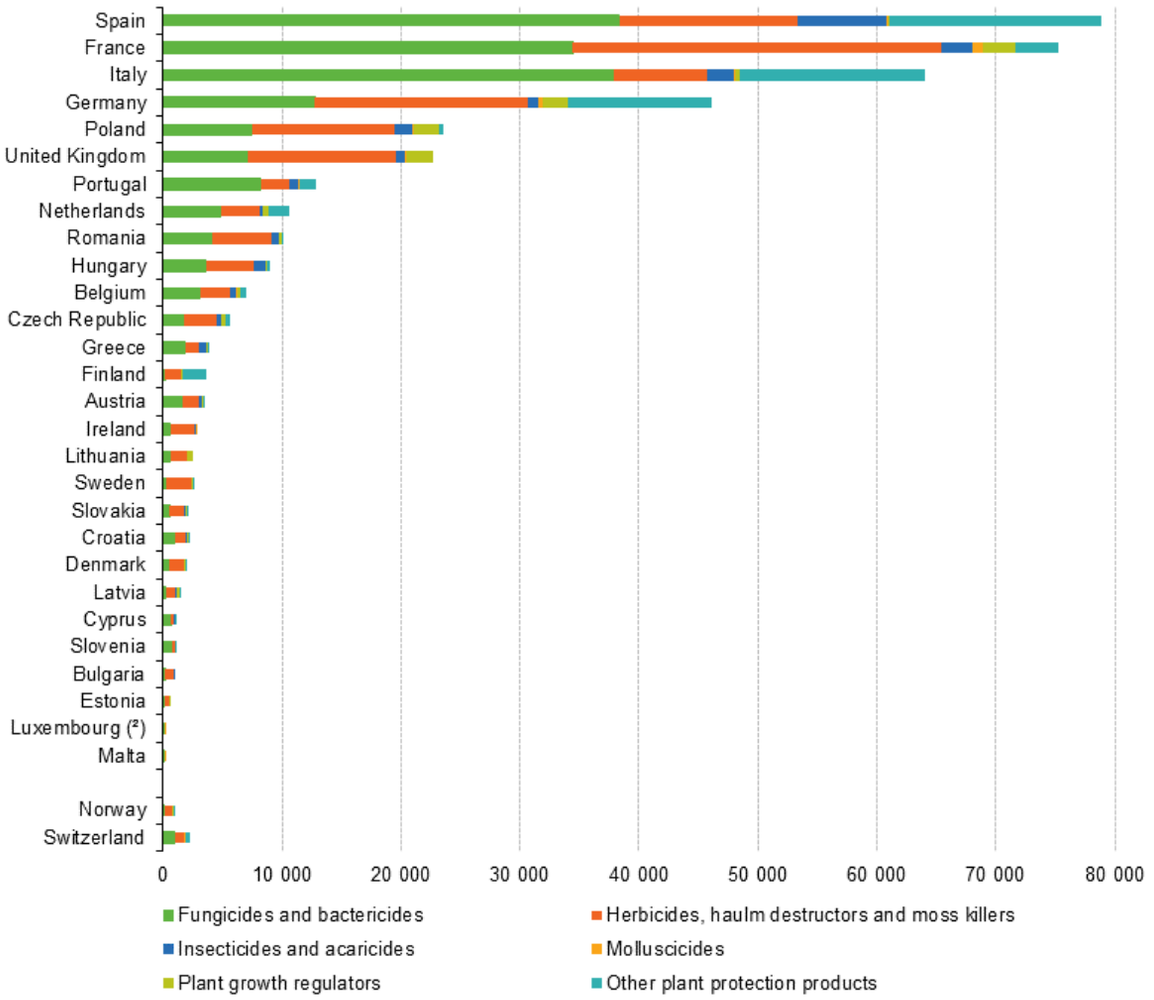
Figure 3. Pesticide sales by major groups, by country, 2014 in Europe (1) (Tonnes)



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II.

PLAGUICIDAS TRADICIONALES

Se desarrolla una remembranza de la primera, segunda y tercera generación de plaguicidas y sus modos de acción como su toxicidad. Se realiza un especial énfasis en plaguicidas organoclorados, organofosforados, carbamatos y piretroides. Los plaguicidas pertenecientes a estos últimas tres familias han sido incluidas en la lista de búsqueda dirigida para el análisis de los ríos Turia, Júcar y Ebro.

1. Types of pesticides

1.1. Traditional Pesticides

First-generation pesticides were primarily used prior to 1940 and consisted of inorganic and organic compounds. Minerals such as arsenic, mercury, and lead are inorganic compounds and were used as early pesticides. These minerals, when used as pesticides, posed a problem, because they did not degrade in the environment. The minerals persist and accumulate in the soil until the levels become so high, that the land is unfertile. The first organic pesticides were from plants and are known as botanicals. Pesticides such as pyrethrin come from chrysanthemum flowers. Rotenone is isolated from jewel vine and nicotine is found in tobacco. Unlike Inorganic compounds, botanicals degrade in the environment and do not persist. However, these pesticides are highly toxic to other organisms, such as, fish and bees. The drawback for many of these products was their high rates of application, lack of selectivity and phytotoxicity [1].

Second generation of pesticides involved the use of synthetic organic compounds. The discovery of the effects of dichlorodiphenyltrichloroethane (DDT), benzene hexachloride (BHC), aldrin, dieldrin, endrin, chlordane, parathion, captan and 2,4-D, had a great impact on the control of pest and soon became widely used worldwide. These products were effective and inexpensive with DDT being the most popular, because of its broad-spectrum activity. DDT was widely used, appeared to have low toxicity to mammals, and reduced insect-born diseases, like malaria, yellow fever and typhus; consequently, in 1949, Dr. Paul Muller won the Nobel Prize in Medicine for discovering its insecticidal properties. However, in 1946 resistance to DDT by house flies was reported and, because of its widespread use, reports of harm to non-target plants and animals and problems with residues started to appear. Organochlorine

pesticides (aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, mirex, toxaphene, and hexachlorobenzene) constitute nine of the 12 chemical substances or group currently defined under the Stockholm Convention on Persistent Organic Pollutants (POPs). Organochlorine pesticides have the ability to biaccumulate and biomagnify and can bioconcentrate up to 70.000 times their original concentration. **Table 1.** shows the chronology of pesticide development in the world.

The term "third generation" pesticides was apparently first coined (Williams, 1967) to describe a juvenile hormone mimic which would be potentially useful as an insecticide. This chemical was expected to differ from "second generation" products (mentioned above) by being target species and immune to the development of resistance. There are three general approaches to the conception, synthesis, and characterization of new pesticide chemicals. Random screening, analog or direct and biochemically rational are the three approach. Random screening is an essentially random biological screening of synthetic or naturally occurring material from available sources. Analog approach consists of synthesizing analogs of natural or synthetic chemicals that have a demonstrated biological activity of interest. Analog of natural products are often called either mimics or antagonists, depending on whether the biological activity exhibited by the natural product is reinforced or inhibited by the synthetic chemical. Finally, biochemically rational approach is based on knowledge or theory of vitally vulnerable life processes of pest, definition of the physical or chemical characteristics or dimensions of the organs and cells involved, and delineation of the pertinent biochemical steps in the life processes.

Many of today's commercial insecticides act by inhibiting the enzyme acetylcholinesterase. Eserine (physotigmine) is a naturally occurring carbamate which

has long been known to cause constriction of the pupil, a test animal response typical of organophosphate and carbamate insecticides. In 1930 eserine was shown to inhibit acetylcholinesterase. Some 20 years later, random screening reveled that l-naphthyl methyl carbamate (carbaryl) had strong insecticidal properties. Consideration of its chemical structure relative to eserine suggested that carbaryl might function as an insecticide by inhibiting acetylcholinesterase. This hypothesis was readily confirmed, and many other commercial carbamate insecticides for agriculture and home use were developed through the analog approach. Since the carbamates are analogs or mimics of naturally occurring product, they could be considered “third generation” products, although they are usually identified as “second generation” or “conventional” pesticide chemicals.

Synthetic pyrethroid products have a similar history. About 50 years ego the chemical structure of pyrethrin I and pyrethrin II were elucidated by two Swiss chemists. Then years later, two additional insecticidal esters in pyrethrum flowers were identified and named cinerin I and cinerin II. Analog approach with cinerin I was used as a model. In 1940s was synthetized a mimic called allenthrin. In 1960s, a new series of pyrethroids were announced by a government scientist in England which eventually led to a product called NRDC143. Before this product, pyrethroids were largely used for household fly control since they were unstable to light, moisture and air, and low toxicity to mammals.

2. Organochlorines

Chlorinated hydrocarbons or organochlorines are a group of compounds that chemically break down very slowly and can remain in the environment for long period of time. Organochlorine pesticides are organic compounds with three, five or more chlorine atoms. All have some common physical properties such as very low water solubility

(0.001-10 ppm) and very high lipid solubility (log P of 4-7). Their acute toxic effects in animals are principally due to hyperexcitation in the nervous system and death is frequently ascribed to respiratory failure after the disruption of nervous system function. The two main groups of organochlorine insecticides are the DDT-type compounds and the chlorinated alicyclics. Their mechanism of action differs slightly: The DDT like compounds work on the peripheral nervous system. At the axon's sodium channel, they prevent gate closure after activation and membrane depolarization. Sodium ions leak through the nerve membrane and create a destabilizing negative "afterpotential" with hyperexcitability of the nerve. This leakage causes repeated discharges in the neuron either spontaneously or after a single stimulus. The other hand, chlorinated cyclodienes include aldrin, dieldrin, endrin, heptachlor, chlordane and endosulfan. Among 2 to 8-hour exposure leads to depressed central nervous system (CNS) activity, followed by hyperexcitability, tremors, and then seizures. The mechanism of action is the insecticide binding at the GABAA site in the gamma-Aminobutyric acid (GABA) chloride ionophore complex, which inhibits chloride flow into the nerve [1, 2].

Organochlorine pesticides are one of the most important persistent organic pollutants (POPs) and have been of great concern around the world owing to their chronic toxicity, persistence and bioaccumulation. Spain, as in most European countries, DDT was banned in the late 1970s (actually, in Spain it was banned in 1977).

3. Organophosphates

Organophosphate (OP) or phosphate ester is the general name for esters of phosphoric acid. Most are only slightly soluble in water and have a high oil-water partition coefficient and a low vapour pressure. Most, with the exception of dichlorvos, are of comparatively low volatile, and all degraded by hydrolysis, yielding water-soluble

products. OP pesticides are used extensively to control agricultural, house- hold and structural pests. These pesticides constitute a diverse group of chemical structures exhibiting a wide range of physicochemical properties, with their primary toxicological action arising from inhibition of the enzyme acetylcholinesterase (*AChE, EC 3.1.1.7*) in the central and peripheral nervous systems. AChE normally rapidly degrades acetylcholine in the synapse; thus, inhibition of AChE allows accumulation of acetylcholine with subsequent excessive stimulation of acetylcholine receptors in associated postsynaptic cells and/or end organs. Some degree of AChE inhibition is tolerated without substantial alteration of cholinergic transmission. With more extensive inhibition (>50–60%) of AChE, signs of toxicity are elicited including autonomic dysfunction (e.g., excessive secretions of the airways, excretory systems, salivary glands, and lacrimal glands), in- voluntary movements (e.g., tremors, convulsions), muscle fasciculations, and ultimately respiratory depression [3, 4].

In comparison with conventional organochlorine pesticides, OP demonstrate relatively low environmental persistence but a high toxicity to mammalian. They can eventually become a threat to human beings. Some of these pesticides are persistent and enter the food chain, environmental compartments and human body [4, 5]. There are several reports about this family of pesticides in food and environmental samples.

4. Carbamates

Carbamates are part of a large group of synthetic pesticides that have developed in the last 40 years. Nowadays there are produced and used on large scale. About 50 individual compounds are used ad pesticides. Carbamates are *N*-substituted esters of the carbamic acid with general formula $R_1NH.CO\text{-}OR_2$. Depending on the chemical nature of R_1, there are three classes of carbamate pesticides. If R_1 is a methyl group, the

compounds has insecticidal activity; if R_1 is an aromatic moiety, it acts as a herbicide, and if it is a benzimidazol moiety acts as a fungicide [6]. Carbamates are crystalline solids with low vapour pressure and low water solubility. They are moderately soluble in benzene, toluene, xylene, chloroform, and dichloromethane and lightly soluble in methanol, ethanol, acetone, and dimethyl formamide (polar organic solvents). This group of pesticides are mainly used in agriculture as insecticide, herbicide, fungicide, and nematocides. They are used for domestic desinsection and vector control in public health, as well.

Carbamates (except benzimidazol compounds) inhibit esterases. They produce carbamylation of acetylcholinesterase similar to the phosphorylation of AChE produced by organophophorous compounds. These chemicals exert general toxicity by acetylcholinesterase (AChE) inactivation leading to acetylcholine accumulation at the synapses in the brain and neuromuscular junctions [7, 8]. Carbamates differ from organophosphorous in the low stability of the carbamylated enzyme and the lack of the aging reaction. The group of carbamates comprises individuals compounds of widely varying acute toxicity. Oral LD_{50} in animals for this group vary less than 1 mg/kg/b.w. to more than 5000 mg/kg/b.w. Aldicarb is the most toxic among them (LD_{50}=0.5 mg/kg/b.w.). Carbamates can also produce a variety of reproductive toxicity, including endocrine disruption and infertility.

Generally carbamates insecticides are used on potato, citrus, coffee and other crops as result residues of these pesticides can enter in surface and ground water. Carbamates are included in the list of the United States Environmental Protection Agency (EPA) for prioritizing control of their application. Some compounds of this group are carbaryl,

carbofuran, aldicarb. Among these, aldicarb is highlighted as the principal target on the list of the controlled pesticides by the EPA due to its elevated toxicity [9-11].

5. Pyrethroids

Pyrethroids are organic synthetic insecticides that have been designed based on the structures of the pyrethrins, which are natural insecticides derived from chrysanthemum flowers. The active ingredient consists of six esters called pyrethrins and identified as pyrethrin I and II, cinerin I and II, and jasmolin I and II, which are obtained from the combination of chrisanthemic or pyrethric acid with three alcohols: cinerolone, pyrethrolone, and jasmolone. They are known for their rapid knock down and lethal action, activity against a broad range of insect pests, non-persistence, and biodegradability through the native enzyme system of mammals [12, 13]. Recently, pyrethroids have increasingly replaced organophosphate, carbamate, and organochlorine pesticides [14, 15]. Owing to their broad spectrum insecticidal activity, they are use indoor to control household pests (flies, mosquitoes, cockroaches, termites, and other harmful insects), to protect livestock, as post-harvest insecticides for grain and as pre-harvest in fruit orchards and vegetables crops [16, 17]**.**

Pyrethrins cause hyperexcitability with very little cytotoxicity. The molecular targets of the pyrethrins and pyrethroids are similar in mammals and insects and include voltage-gated sodium, chloride and calcium channels, GABA-gated chloride channels, nicotinic receptors, membrane depolarization and intercellular gap junctions. Mammals are less susceptible to pyrethrin and pyrethroid toxicoses than insects primarily because they have a faster metabolic clearance, higher body temperatures and a lower affinity for the pyrethrins/pyrethroids. Once absorbed, the pyrethroids are rapidly distributed due to their lipophilicity. Systemic distribution produces effects that can be difficult to control

and may be confused with poisoning by other pesticides, such as organophosphates which also cause increased salivation and hyperexcitability. Cats are very sensitive to pyrethroid exposure [18-21].

Table 1. Chronology of pesticide development

Period	Example	Source	Characteristic
1800 – 1920	Early organics, nitro-phenols, chlorophenols, creosote, naphthalene, petroleum oils	Organic chesmistry, by-products of coal gas production. Etc.	Often lack specificity and were toxic to user or nontarget organisms
1945 - 1955	Chlorinated organics, DDT, HCCH, chlorinated cyclodienes	Organic synthesis	Persistent, good selectivity, goo agricultural properties, goo public health performance, resistance, harmful ecological effects
1945 - 1970	Cholinesterase inhibitor, organophosphorus compounds, carbamates	Organic synthesis, good use of structure-activity relationships	Lower persistence, some user toxicity, some environmental problems
1970 - 1985	Synthetic pyrethroids, avermectins, juveline hormone mimics, biological pesticides	Refinement of structure-activity relationship, new target systems	Some lack of selectivity, resistance, costs, and variable persistance
1985	Genetically engineered organisms	Transfer of genes for biological pesticides to other organisms and into beneficial plants and animals, genetic alteration of plants to resist nontarget effects of pesticides	Possible problems with mutations and escapes, disruption of microbiological ecology, monopoly on products

Source: Stephenson , G.A and Solomon, K.R., Pesticides and environment. Department of Environmental Biology, University of Guelph, Guelph, 1993.

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III.

PLAGUICIDAS DE NUEVA GENERACIÓN

Se presenta una perspectiva profunda de los nuevos tipos de plaguicidas introducidos en el mercado: lactonas macrocíclicas, cloronicotinils, tetranortriterpenos, sales de amonio cuaternario, dinitroanilinas, acetamidas y oximas, Se presenta sus propiedades físico-química, toxicidad, regulación, métodos de extracción, métodos analíticos y referencias de estudios que han abordado el análisis de estos compuestos en matrices ambientales, de alimentos y biológicas. Esta revisión bibliográfica se publicará en un capítulo de libro:

"*Pesticides (New Generation) and Related Compounds, Analysis of* " en la Encyclopedia of Analytical Chemistry, editada on-line por wiley.

Pesticides (New Generation) and Related Compounds, Analysis of

1 Introduction

Pesticides including many new compounds are widely used, as an example, in 2013, the total quantity of pesticide sales amounted to close to 360 000 tonnes. In the EU, the Member States where the highest quantities of pesticides were sold are Spain (19.5 %), France (18.7 %), Italy (13.8 %), Germany (12.3 %) and Poland (6.2 %). All together, they made up 70.5 % of the EU-28's pesticide sales. However, since many years ago, the number of new pesticides bringing to the market has decreased exponentially mostly due to the long and expensive procedure required to put them in the market.

The introduction of a new pesticide in the market involves the synthesis or isolation of the active principle and its testing using a series of complex screens to assess their "biological activity" or potential as a pesticide (including toxicological and environmental assessment).[1, 2] On average only 1 in 70,000 go forward. The cost of this research phase averages 90 million € for each new product. After this, the development of the new product also involved their large-scale production, testing in variety of technical formulations as well as under a wide range of crops, pest and conditions, determining their fate and metabolism in food and the environment and also testing their toxicity and environmental impact. The total time taken from first synthesis to first sale averages about 9 years [2-4]. This high cost justifies the lack of registration of new pesticides and explain why in this third update of this chapter the groups included as

miscellaneous compounds of no so new generation are still macrocyclic lactones, chloronicotinyls, tetranordtriterpenoids, ammonium quaternary salts, dinitroanilines, acetamides, oximes, triazoles, and pyridine-based molecules. **Table 1** lists the chemical structures and some examples of these miscelaneous pesticide.

However, to justify the need of updating this chapter is enough to mention that the advances in analytical techniques apply to their determination compensate the stagnation in the development of new types of pesticides.[3, 5, 6]. In the last 10 years, the evolution of the analytical techniques has been very impressive and dominated by the development of high-throughput methods. Pesticide residue analysis still requires a progression of phases to be successfully accomplished —first, the extraction of pesticides with organic solvents or sorbents from the matrix, followed by the clean-up commonly by any type of solid sorbent and the final high-performance liquid or gas chromatographic (LC or GC) determination, commonly by mass spectrometry (MS). These methods offer quantitative analysis with sensitivity and selectivity, suspected screening and even identification of unknown metabolites.

Other determination techniques are immunoassays (mostly ELISA) that offer certain advantages over conventional instrumental methods because are ideally suited for screening a large number of samples.[7, 8] More and more on-site immunological techniques such as dipstick, immunochromatography, and immunofiltration are gaining interest in the area of pesticide detection in food and environmental matrices. Most of them are basically designed as visual tests.[7]

Biosensors account for an easy method to determine pesticides in environmental and food matrices.[9] The use of biosensors as screening devices is cost effective and decreases the number of samples to be analyzed by traditional analytical techniques

mentioned above.[10] With the explosive growth of smart phones, wireless technologies and sensor technologies have become a fundamental tool for everyday life around the world. The coming wave of interconnected devices, appliances, sensors, meters and countless other “things” represents the next generation of a hyper-connected world, the and Internet of Things (IoT)

The aim of this chapter is to review the analytical methodology used nowadays to determine the pesticides traditional considered as a new generation for comparison with those group chemically well defined, in such a way that the evolution in analytical techniques can also be observed.

2 Characteristics

There are many registered chemicals that can be included under the heading of miscellaneous pesticides and related compounds, which comprise a large group of substances with very different characteristics and applications (**Table 1**).[11-21]. Their common attribute is that they do not belong to the classical chemical categories of pesticides, such as organochlorines, organophosphates, carbamates, or triazines.[13]

2.1 Physical and Chemical Properties

To develop the best residue analytical methods with the most appropriate instruments, knowledge of the physical and chemical properties of these pesticides is essential. For example, it is impossible to develop a GC residue method for imidacloprid without derivatization because of the polar and nonvolatile properties displayed by this molecule. Similarly, a direct GC method for diquat (DQ), paraquat (PQ), or azadirachtin is also impossible because of the polarity of these compounds [3, 22]. The physical and chemical properties of some typical herbicides are listed in **Table 2**.

The avermectins are macrocyclic lactones produced by the soil microorganisms *Streptomyces avermitilis*. They are probably the antiparasitic agents most widely used in the treatment of food-producing animals, poultry, aquaculture, and crops. Ivermectin was the first macrocyclic lactone product to be licensed for use about 20 years ago. A number of alternative products, such as abamectin, doramectin, emamectin, eprinomectin, moxidectin, milbemycin, and selamectin, have been marketed since then.[23, 24] Avermectin B_1, known as *abamectin*, is widely used as an insecticide and a miticide for agricultural crops.[12] Abamectin is a mixture of two components. The major one is avermectin B_{1a}, which makes up 80% or more of the mixture, and the minor component is avermectin B_{1b}, which supplies 20% or less; the two components differ in a single methylene group.[25] Ultraviolet (UV) light below 280 nm rapidly isomerizes the *E* (*trans*) 8,9 and 10,11 double bonds of avermectin to 8,9- and 10,11-*Z* isomers. However, solutions of avermectin in Pyrex flasks are generally stable because Pyrex excludes most of the UV light below 280 nm. Avermectin residues degrade rapidly by both oxidative and photochemical pathways, forming a variety of products when applied to a number of different crops. Therefore, the only residues of toxicological significance are avermectin and 8,9-*Z* avermectin.[26, 27] Avermectins are not readily hydrolysed because they are highly lipophilic substances that dissolve in most organic solvents. Their solubility in water is relatively low at 0.006–0.009 ppm (=mg/l). The chloronicotinyl insecticides act on cholinergic receptors and have good contact properties and powerful systemic action after uptake through the root system. Their common names are acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam. Owing to their insecticidal effectiveness at low concentrations, they are one of the most used family of pesticides.[28] Soil biodegradation studies on the chloronicotinyl imidacloprid have demonstrated that its major metabolite

is 6-chloronicotinic acid and that it leaves no soil residue after the first 3 months.[29] Acetamiprid is stable in solution at pH between 4.5 and 7. It is slowly degraded at pH 9 and 45°C. Azadirachtin is a tetranortriterpenoid (limonoid) present in neem seeds (*Azadirachta indica* A. Juss). Azadirachtin has gained worldwide attention for its insect antifeedant and ecdysis-inhibiting properties. This compound is highly potent at low concentrations against more than 200 agricultural pests and is ecofriendly. It is, therefore, a potentially safe alternative to the toxic synthetic pesticides and a number of commercial formulations have been introduced worldwide.[30, 31] Azadirachtin is nonvolatile and highly polar, soluble in polar organic solvents, and slightly soluble in water. It has UV absorption due to its α, β-unsaturated carbonyl chromophore in the ligate ester and the vinyl ether, but the absorption maximum is at a very short wavelength (UV absorption $\lambda_{max} = 217$ nm, $\varepsilon = 9{,}000$).[32] Azadirachtin hydrolyzes readily at 35°C, and its disappearance follows simple pseudo-first-order kinetics. Its hydrolysis is faster at a basic than at an acidic pH.[31] Azadirachtin is composed of, at least, nine closely related isomers. The types A and B are dominant, with isomer A accounting for 83% and B 16%. [33]

The bipyridylium herbicides DQ, PQ, and difenzoquat (DF) are important cationic pesticides used in agriculture. They are nonselective contact herbicides that are absorbed by foliage with some translocation though the xylem and are used for the broad-spectrum control of broad-leaved weeds and grasses in fruit orchards, plantation crops, ornamentals, and shrubs[34]. DQ is a highly water soluble, dipyridylium crystalline salt and has a mode of action similar to PQ.[35] They have been applied as defoliants on crops, such as cotton and as desiccants for pineapples, potatoes, sugarcane, and sunflower. Chlormequat (CQ) and mepiquat (MQ) are cationic plant growth regulators,

structurally related to the bipyridylium herbicides, which are mainly used to prevent loging in barley and rye and also to increase the yield of cotton.

The PQ salts are hygroscopic, not volatile and very soluble in water. PQ is extremely stable in the presence of acids, but it is destroyed quickly in a basic medium. It is soluble in water and insoluble in organic solvents. DQ is also very thermostable and not volatile. The herbicide activity of PQ and DQ is related to the planar structure of their molecules and the 18 possible resonant structures that stabilize the radical formed in reduction reactions in which they are involved. DF occurs as monovalent cation and is also used as a selective herbicide for post-emergence control of wild oats in barley and fall-seeded wheat. DF salts are very soluble in water and are stable to hydrolysis. DF is stable at weakly acid pH but is degraded in strong acids and in the presence of oxidizers. The three herbicides present an absorption band in the UV region at 260, 310, and 255 nm, respectively, due to the presence of aromatic rings in their structures[36].

Dinitroanilines are selective preemergence herbicides used to control some broad-leaved weeds and the major annual grasses in a wide variety of agronomic crops. The mechanism of action of dinitroanilines is determined by their specific binding to parasite tubulins (the main structural component of microtubules), which causes an antimitotic activity. The chloracetanilide herbicides include benfluralin, butralin, dinitramine, ethalfluralin, fluchloralin, isopropalin, methalpropalin, nitralin, oryzalin, pendimethalin, prodiamine, profluralin, and trifluralin. Although these herbicides are chemically related, they differ in volatility, persistence in soil, and absorption by crops and for this reason may differ in their effects on soil, plants, and air. These compounds are among the least mobile herbicides and, therefore, runoff is the principal route of the contamination of surface waters. Dinitroaniline herbicides are water insoluble, relatively

volatile, and strongly adsorbed to soil colloids. In soil, both chemical reactions and biological processes degrade pendimethalin. In general, dinitroaniline herbicides degradation is more rapid under flooded anaerobic conditions than under aerobic ones.

The group of acetamide pesticides (some also known as *chloroacetamides*) encompasses a considerable number of herbicides and fungicides used to control weeds and fungi in crops, including acetochlor, alachlor, butachlor, metolachlor, and propachlor. The compounds are widely used to control annual grasses and certain broad-leaved weeds in corn, soybeans, and peanuts and to control phytopathogenic fungi (*Peronosporales*) in potatoes, sugar beets, and other crops. They act by inhibiting protein synthesis by the reaction of the activated Cl atom of the chloroacetyl group with reactive sites in proteins.[37, 38] Acetamide herbicides have moderate water solubility and are rapidly absorbed into plants. In susceptible plants, the herbicides act by inhibiting protein synthesis, whereas insensitive plants rapidly inactivate these herbicides via gluthathion conjugation. In sensitive fungi, the structurally related fungicides have effects on RNA synthesis. As these reactions involve various chiral structures, some stereoselectivity is expected in the activity of these compounds.

Oximes are bioactive compounds, originally discovered in insects, that have recently been synthetized, and some are effective herbicides, applied as a good alternative to control glyphosate-resistant pests. Some representatives of this group are alloxydim, butroxydim, clethodim, cloproxydim, cycloxydim, profoxydim, sethoxydim, tepraloxydim, and tralkoxydim. Field trials with these compounds showed that they were not consistently effective, perhaps because of the instability of this active ingredient. Sethoxydim undergoes degradation, including photodegradation. Clethodim is a fatty acid synthesis inhibitor that works via inhibition of acetyl CoA carboxylase

and it is degraded in aqueous solution by acid medium and light. Clethodim degradation increases as pH of the solution decreases and photolysis is more rapid and more complete than hydrolysis. Previous study indicated that clethodim is mainly oxidized to clethodim sulfoxide or clethodim sulphone in the field [39, 40]

Triazole derivatives are aromatic heterocycles widely used as weed killers, fungicides, insecticides, plant growth regulators, and antimicrobial agents.[41] They are nonselective systematic herbicides used against a wide variety of plants, including applications for the treatment and protection of cereals, soybeans, and a variety of fruits. . It is known that most of triazole fungicides are chiral and their optical isomers exhibit different bioactivity and toxicity. For example, the *R*-enantiomer of diniconazole and uniconazole shows stronger fungicidal activities, whereas the *S*-enantiomer has higher plant growth regulating activity. Amitrole belongs to the triazole group [42, 43] and is soluble in water, methanol, ethanol, and chloroform, slightly soluble in ethyl acetate, and insoluble in ether and acetone. Aqueous solutions are neutral. Other examples of these pesticides are cafenstrole, epronaz, and flupoxam.

Pyridine-based molecules are a group of substances that include pyridazines, pyridazones, and pyridones. All of them are herbicides but their applications vary. For example, pyridate is a pyridazine that acts by contact, while pyridazone derivatives, such as norflurazon and cloridazon, are soil-applied herbicides, and fluridone, a pyridone, are an experimental herbicide developed for aquatic plant management systems.[44] Pyridate is a colorless crystalline solid that melts at 27°C and boils at 220°C under 10^{-6} mbar vacuum. Its vapor pressure is 1.3×10^{-9} mbar at 20°C. It is stable in neutral medium but is hydrolyzed in strong acid and strong alkali media.[45]

2.2 Toxicology

Pesticide residues are regulated at the international and national levels according to the toxicity of the pesticide and the human exposure to a particular substance. **Table 3** lists the acute oral and dermal toxicity for different animal species, chronic toxicity for dogs, and the Admissible Daily Intake (ADI).

The macrocyclic lactones are neurotoxins that manifest their action by disrupting the normal function of ϒ-aminobutyric acid (GABA), an important neurotransmitter in the central nervous system of vertebrates and in the peripheral nervous system of invertebrates. Because mammals have only GABA ergic synapses in the central nervous system, the mammalian blood–brain barrier ensures a degree of specificity. A notable feature of this group of compounds is their low LD_{50} (lethal dose 50) values, but they are not usually highly toxic by the dermal route because of their large molecules and poor transdermal absorption. In vitro studies with preparations of rat brain have shown that avermectin B_{1a} stimulates presynaptic binding of GABA and also enhances its postsynaptic binding; the action of avermectin B_{1a} is antagonized by bicuculline and picrotoxin.[25-27]

Avermectin and ivermectin are metabolized in a qualitatively similar way among different species. The major metabolites of both in cattle, sheep, swine, and rats are either 24-hydroxymethyl or 3″-*O*-desmethyl derivatives. However, the enzymes responsible for the metabolism have not been identified in any species.[46] These compounds undergo little metabolism and most of the dose given to the animal is excreted relatively unaltered, primarily in the feces.[47, 48] Abamectin induces teratogenic effects such as cleft palate. Although belonging to the bio-pesticide group, abamectin may be toxic to mammals including human beings. The oral LD50 of abamectin for rats

is about 11 mg/kg, while dogs showed pupillary dilation, weight loss, lethargy, tremors, and recumbency after exposure to 0.5–1 mg/kg/day levels. [49]

The chloronicotinyl insecticides interfere with neuronal functions as do organophosphate, carbamate, and pyrethroid insecticides. Unlike the latter pesticides, they act on nicotinergic acetylcholine receptors in the postsynaptic membrane of an identified insect motor neuron.[28, 50] They generally have low toxicity to mammals (acute and chronic), birds, and fish.[51-53] Imidacloprid can persist in soil depending on soil type, pH, use of organic fertilizers and presence or absence of ground cover. The metabolites of imidacloprid, namely olefin and nitrosimine, have greater insecticidal activity than the parent compound while the guanidine metabolite does not possess insecticidal properties, but has a higher mammalian toxicity than the parent compound. After soil application or seed treatment, a quick degradation of the active substance was observed after root uptake of the active substance. It is a systemic broad-spectrum insecticide and acts as a contact and stomach poison against sucking and some biting insects (rice hoppers, aphids, thrips, whitefly, termites, etc.).[54]

Azadirachtin is nonmutagenic and does not appear to exhibit mammalian toxicity. Insects ingesting azadirachtin and related minor compounds in the seed kernels do not die immediately, but soon stop feeding [55, 56]. This drug interrupts the life cycle of flies by inhibiting the development of the eggs, larvae, or pupae and by blocking themolting of larvae or nymphs, and inhibiting mating and sexual communication.[33]

PQ toxicity in both experimental animals and humans targets primarily the lung, whereas DQ does not. Differences between both the compounds are because the lung selectively accumulates PQ and not DQ. PQ-induced pulmonary injury takes place in two phases: destructive and proliferative. It has been suggested that the biochemical

reactions that lead to the destructive effect of PQ are analogous to its toxic action on plant cells. NADPH (nicotinamide adenine dinucleotide phosphate, reduced form) is the donor for the single-electron reduction of PQ. Reduced PQ is reoxidized rapidly by molecular oxygen and superoxide radicals are formed. The superoxide radicals initiate a chain of reactions that produce toxic reactive intermediates, which include hydrogen peroxide, and hydroxy radicals and also produce lipid peroxidation. They are responsible for the disruption of cellular membranes. In addition, PQ competes for and deprives other systems of essential NADPH and compromises their cellular integrity.[34, 57] DQ poisoning differs from that of PQ, in that the renal effects are more prominent and lung changes do not generally occur. An effect of DQ, which has been extensively investigated, is its ability to produce cataracts in experimental animals.[58] PQ produces chronic effects such as costal cartilage malformation in rats when injected during the gestation. Teratogenicity of PQ results from its effect on collagen biosynthesis.[57]CQ and MQ are widely used as plant growth inhibitors. They are usually used together for controlling unwanted longitudinal shoot growth, improving fruit setting and increasing yield of fruit and vegetables. However, toxicological studies showed that CQ and MQ have adverse effects on animal reproduction. Occupational Safety and Health (NIOSH) indicated that CQ has been classified as a suspected endocrine disruptor.[59] CQ is known to be a competitive inhibitor of cholinesterase in animals. This anticholinesterase chemical causes acetylcholine accumulation at cholinergic receptor sites and, thus, is capable of producing effects equivalent to excessive stimulation of cholinergic receptors through the central and peripheral nervous system.[36] Mepiquat chloride is considered by the World Health Organization (WHO) to be slightly hazardous with a LD_{50} of 1490 mg kg^{-1} in rats. Due to its toxicity, maximum residue limits (MRLs) of mepiquat chloride have been established in different matrices.[60]

Substituted anilines have the general property of causing methemoglobinemia, as do many other aniline derivatives. The probable mechanism of methemoglobinemia is the N-hydroxylation to the corresponding hydroxylamine, which then takes part in an intraerythrocytic cycle with the corresponding nitroso derivative generating methemoglobin at the same time. Alachlor is classified as a "likely" human carcinogen at high doses by the US Environmental Protection Agency (EPA) because of its carcinogenic effect on rodents, where it produces posterior nasal and stomach tumors, possibly by a nongenotoxic mechanism.[6] Acetochlor is absorbed through the roots and shoots just above the seed of the target weeds; they act as a growth inhibitor by suppressing synthesis of protein.[62]

Triazole fungicides are among the flourishing new generations of pesticides applied to fruits, vegetables, and grain crops. Besides their antifungal activity, they are also of concern as a group of compounds that disturb endocrine activity in human beings. Due to their lipophilic nature, these compounds can be bio-accumulated in various tissues of living organisms and they can be transported between various compartments of ecosystems and contaminate food chains.[63] The triazole antifungals myclobutanil, propiconazole, and triadimefon cause various degrees of hepatic toxicity and disrupt steroid hormone homeostasis in rodent in vivo models. Modulation of hepatic sterol and steroid metabolism is a plausible mode of action for changes in serum testosterone and adverse reproductive outcomes observed in rat studies and may be relevant to human risk assessment.[64] There is a solid evidence that amitrole produces thyroid tumors in mice and rats by a nongenotoxic mechanism, which involves interference with the functioning of thyroid peroxidase. However, the International Agency of Research on Cancer (IARC), in its last evaluation,[43, 65] has changed the amitrole classification from "possibly carcinogenic to humans" to "not classifiable as to its carcinogenicity to

humans" because it would not be expected to produce thyroid cancer in humans exposed to concentrations that do not alter thyroid hormone homeostasis.

The literature is scarce on toxicity of the oximes. Clethodim is a cyclohexenone herbicide introduced by Chevron Chemical Co., and has been used as a selective post-emergence herbicide to control annual and perennial grasses in a wide variety of broad leaf crops, including soybeans, cotton, flax, peanuts, sunflowers, sugar beet, potatoes, alfalfa and most. It is also applied on rape to prevent annual gramineae and broadleaf weeds. Clethodim is a fatty acid synthesis inhibitor that works via inhibition of acetyl CoA carboxylase.[40] There are only a few studies demonstrating that sethoxydim produces lesions in bone marrow and the liver of dogs.[66, 67]

There are concerns about the possible effects of pyridine-based molecules. Fluoridone produces cytotoxicity, decreasing the number of cells.[68] A perturbing effect of chloridazon is its interference with the phospholipid moiety of the nerve fibre membrane, leading to interference with total ion transport across the nerve skin junction. [69] However, in the last years these compounds have been reduced for use.

2.3 Regulations

The legal tolerance, or maximum residue limits (MRLs), is defined as the maximum level or the concentration of a pesticide, or its metabolites or derivatives that is permitted in or on a particular food crop, food product, or in a particular environmental compartment. The amount is generally no higher than the concentration that would be expected because of good agricultural practices. Each country establishes its own regulations based on the Codex Standards but with somehow different MRL values for hundreds of pesticides and food products potentially contaminated. Furthermore,

analytical quality and sensitivity of the methods to determine pesticides and MRLs or action limits established for non-authorized compounds is a reciprocal process. The latter determines the former and vice versa. Then, some knowledge of these values is of interest to be aware of the required method's sensitivity.

Comparison of different legislations pointed out that the European Union (EU) establishes lower MRLs than the US of America, Canada, China or Japan. Since December of 2015, a new legislative framework on pesticide residues in food was established in the EU. This regulation completes the harmonization and simplification of pesticide MRLs. With the new rules, MRLs are revised periodically to make sure that all classes of consumers, including the vulnerable ones, like babies and children, are sufficiently protected.[70, 71] The tendency is to diminish the permitted levels. This fact poses substantial analytical problems and demands a methodology of greater sensitivity. **Table 4** lists MRLs of the miscellaneous pesticides in selected food. The whole set of commodities are available in a database that reflects the most updated published legislation under Reg. (EC) N° 396/2005. A general default MRL of 0.01 mg kg^{-1} applies where a pesticide is not specifically mentioned.[72]

The EU has also proposed a rigid limit for pesticides in drinking water —0.1 µg L^{-1} (0.1 ppb) for a single pesticide and 0.5 µg L^{-1} (0.5 ppb) for total pesticides including their degradation products.[73] In the United States, USEPA has set values for maximum contaminant levels for pesticides individually. The USEPA values range approximately from 1 to 1 mg L^{-1}.[74] Such levels are more specific than the EU limits, which are equal for all pesticides without making any distinction among their different toxicities. However, of all the pesticides covered in this article, USEPA has only established a

health advisory level of 20 µg L^{-1} for DQ and 2 µg L^{-1} for Alachlor.[74] There are not established tolerances in other environmental compartments.

Finally, it is important to remark that the EU has established guidelines on the analytical performance of methods used to determine pesticide residues. There are two guidelines applicable: Residue Analytical Methods for Post-Registration Control and Monitoring (Doc. SANCO/825/00; 16 November 2010) and Method Validation and Quality Control Procedures for Pesticide Residues (Doc. SANCO/12571/2013; 19 November 2013) and one Commission Decision of concern in this field[75] whereas other countries as the EU of America, China and Japan have developed official analytical methods that should be followed and incorporate these new compounds.

3 Isolation of the Sample

Sample preparation is an essential step in whole process of sample analysis. It includes removal of interfering compounds from complex matrixes and preconcentration of the targeted analytes. The extraction procedure also depends on the sample type, being different for water and other solid or liquid matrices. **Table 5** summarized the most important procedures reported.

For water sample the most used technique is solid-phase extraction (SPE) using commercially available cartridges that contains the sorbent. Along the history, several formats as cartridges, barrels, syringes or even disks have been traded but currently, most used are syringes containing a hydrophilic lypophylic balanced (HLB) sorbent or a mixed mode one (consisting of HLB and an ionic interchanger). Furthermore, increase specificity of SPE using molecularly imprinted polymers (MIPs) is also an approach used for some difficult matrices.

A variant SPE procedure called magnetic SPE (MSPE), has been developed. This method is based on the use of magnetic or magnetically modified adsorbents. Compared with traditional adsorbents, a distinct advantage of MSPE is that the magnetic materials can be readily separated from sample solutions by the application of an external magnetic field without the need of additional centrifugation or filtration procedures. Therefore, magnetic adsorbents can make phase separation easier and faster.

Recently, attention is being paid to the development of miniaturized, more efficient and environmentally friendly extraction techniques that could greatly reduce the toxic organic solvent consumption. The main improvements of the miniaturized sample preparation techniques are exccedingly low solvent consumption (2-50 µl), flexible volume of sample (from a few mL to one liter), simple to operate and high enrichment factor, which leads to the low detection limit. The main disadvantage is the limited number appropriate extractants. Solid-Phase Microextraction (SPME) or Liquid-Phase Microextraction (LPME) are a good alternative to eliminate or significantly reduce the use of organic solvents. These methods can combine sampling, extraction, preconcentration and sample introduction in a single uninterrupted process that results in a high-sample throughput.

SPME extraction, followed by analysis by Gas Chromatography (GC) or Liquid Chromatography (LC) coupled to a variety of detectors, is very useful for determining pesticide residues in different matrices. This technique is cheap (one fiber can be generally used for hundreds of extractions), rapid and simple; further more, no harmful solvents are needed. SPME is generally based on the partition of the analytes between the sample and the coating material, which is critical in SPME performance

Regarding the LPME, nowadays there are two main alternatives –hollow fiber (HF)-LPME and dispersive liquid-liquid microextraction (DLLME). In HF-LPME, the extracting phase is placed inside the lumen of porous hollow fibers made of polypropylene. HF-LPME is a more robust and reliable alternative to LPME. In addition, the equipment needed is very simple and inexpensive, and the method offers good possibilities for automation compared with other LPME methods. There are two modes of HF-LPME named two-phase and three-phase HF-LPME. In two-phase HF-LPME, the analytes are extracted by passive diffusion from the sample into the organic solvent supported by a fiber, and in three-phase HF-LPME the analytes are extracted from the sample (donor phase) through an organic solvent immobilized in the pores of the fiber and further into a aqueous phase (acceptor phase) in the lumen of the fiber. HF-based LPME uses a different configuration in which only the organic solvent is immobilized in the pores of the hollow fiber as the acceptor phase, and the organic solvent is desorbed from the fiber after the extraction step prior to GC or LC analyses. This technique is compatible with a broad range of samples, including plasma, whole blood, urine, saliva, breast milk, tap water, surface water, pond water, seawater, and soil slurries.

DLLME is based on a ternary component solvent system, which involves the rapid injection of an appropriate mixture of disperser (water soluble solvent) and few microliters of extracting solvent into an aqueous solution. It results in the dispersion of water-immiscible extracting solvent throughout the aqueous phase as fine droplets and then the analytes are enriched into it. DLLME present a number of varitions, such as Homogeneous Liquid-Liquid Microextraction Via Flotation Assistance (HLLME-FA), Ionic Liquid Based Vortex Assisted Liquid-Liquid Microextraction (IL-VALLME), Vortex-Assisted Surfactant-Enhanced-Emulsification (VASEE), Liquid-Liquid

Microextraction with Solidification of Floating Organic Droplet (VSLLME-SFO), Air Agitated Liquid-Liquid Microextraction (AALLME), Ionic Liquid Dispersive Liquid-Liquid Microextraction (IL-DLLME), Up and Down Shaker Assisted Dispersive Liquid-Liquid Microextraction (UDSA-DLLME) and other formats are caracterized by preparation procedures based on the liquid-phase extraction consume organic solvents as extractants which are usually volatile or semivolatile at room temperature and flammables. DLLME is increasing used due to the large contact surface area of the two immiscible phases (organic phase and aqueous phase) and high extraction efficiency is achieved in a relatively short time.

For solid matrices, such as food, soils or sediments, Solid Liquid Extraction (SLE) offers a higher potential for chemically tuning the separation by incorporating different specific reagents. However, SLE has some well-known drawbacks, including high consumption of solvents, multiple time-consuming steps and difficulty in automation and online connection to analytical instruments. SLE can be followed by SPE or other clean-up procedure or not. Within this field, many choices have been proposed for the pretreatment and/or the extraction of pesticide residues in environment and food. These procedures involve sample homogenization with an organic solvent, alone or with water or pH-adjusted water. The solvent extraction method is a time consuming, labor-intensive operation, and consumes a large volume of hazardous and non-environmental friendly solvents, requiring clean-up procedures prior to instrumental analysis. Additionally, the use of organic solvents is against the principles of green analytical chemistry. The SLE consumes energy, this energy is commonly mechanical energy (shaken) but can also be ultrasound (US) or other methods, such as pressurized liquid extraction (PLE) or microwave assisted extraction (MAE).

However, currently there is a trend to miniaturization of the extraction methods and use QuEChERS like routine method to analyses new pesticides. The Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method was developed by Anastassiades and Lehotay and was successfully applied to analyse pesticide residues on fruits and vegetables by using primary secondary amine (PSA) as a cleanup sorbent. The method was developed based on the cleanup process known as dispersive solid phase extraction (DSPE), where the sorbent phase in the solid phase extractions is directly mixed with a portion of the extraction to remove the matrix compounds and leave the compounds in the solvent. Recently, the DSPE procedure became popular in detecting miscellaneous pesticides residues with some modification to the original protocol.

SFE is an interesting alternative to other separation processes, because of carbon dioxide that is used as a supercritical fluid. SC-CO_2 is the most commonly used extraction solvent because it is nontoxic, inflammable, and inexpensive, and it operates at low temperature and has high selectivity. CO_2 is a non-polar solvent, so polar compounds as most new generation pesticides needs the addition of a polar solvent to the supercritical fluid, called cosolvent, may improve the extraction. However, SFE was developed many years ago but its use is quite sporadic and not too common.

3.1 Macrocyclic Lactones

Several extraction methods have been reported for avermectins in water samples. SPE was used to detect avermectins in water, various cartridges at different pH have been tested. However, recoveries were not satisfactory (38–67%) because the analytes remained in the cartridges.[76] Contrarily, SPE disks were used to extract emamectin benzoate and abamectin from sea water achieving analyte recoveries was better than 60%.

LLME techniques are gaining popularity. HF-LPME technique was described to detect abamectin, ivermectin, moxidectin, and doramectin in stream water [76]. An Accurel polypropylene membrane was used as the hollow fiber, and dihexyl ether was used as the extraction solvent. The optimal extraction conditions for HF-LPME were 4 cm fiber length, 45 min extraction time, 200 rpm, and 1 min desorption time with methanol as the desorption solvent. Recovery rates were from 80.1 to 93.7%. Recently, HLLME-FA was also introduced to extract abamectin in aquatic samples [77]. Toluene at microliter volume level and acetone were the extraction and homogeneous solvents, respectively. In this research, a special extraction cell was designed to facilitate collection of the low-density extraction solvent. Recoveries ranged from 88 to 94 %.

Actually, the methods described for the extraction of macrocyclic lactones in environmental matrices, vegetables and food, have increased. Commonly are used SLE with solvents such as acetonitrile, methanol, hexane and acetona. QuEChERS or SFE followed by clean-up step consisted of SPE, LLE, DLLME or DSPE.

In this sense, abamectin, ivermectin, moxidectin, and doramectin were extracted in soil samples using SFE. The optimal extraction conditions for SFE were 80°C for temperature, 300 kg/cm^2 for pressure, 40 min as an extraction time, and 30% of a modifier ratio. The recoveries were in the range of 82.5–96.2%.[78] Furthermore, the result in the study shows shorter extraction time for the determination of abamectin in aquatic samples. [79]

Several methods have been described in the literature for the analysis of the macrocyclic lactones in fruits and vegetables. In this field, the most used QuEChERS, and solvents extraction as acetonitrile and hexane.[80-82] A sensitive and efficient SPME was proposed to clean-up extracts of mangoes. The average recovery rates obtained for each pesticide

ranged from 71.6 to 104.3% at three fortification levels.[80] QuEChERS method was used for extraction spinosad, and abamectin B1a in fruit matrixes. The recoveries obtained were in the range of 70-110%.[83] Regarding solvents, acetonitrile is commonly used for vegetables matrixes. The abamectin residues were extracted efficiently with acetonitrile assisting by ultrasound and then directly derivatized without requiring the clean-up step. The recoveries ranged from 83.2-123.7% with satisfactory precision (RSD<16.7%) and bias (−16.8~23.7%). [49] Most fruits and vegetables are non-fatty and have high water content, such as lettuce, tomato, and strawberry. The non-fatty vegetables and fruits contain hydrophilic materials, such as protein, glucide, and pigment, which could be separated easily from lipophilic pesiticides by using organic solvents.

QuEChERS was also applied to milk and yogurt samples to detect the presence of ivermectin, abamectin, doramectin, eprinomectin and moxidectin. Average recovery at three different levels varied from 83% to 112%. Other study that applied after QuEChERS, DLLME as clean-up in the same matrix also reported the recoveries were between 89.5 and 105%. [84, 85]

In biological samples, an effectiveness and comparative study of cleanup sorbents used in DSPE for the determination of avermectins, including emamectin EMA, abamectin ABA, doramectin DOR, moxidectin MOX, and ivermectin IVE, was performed. Three different types of cleanup sorbents, alumina (Al), primary and secondary amine (PSA) and octadecyl (C18), were used to remove the matrix interference in fish samples. Homogenised fish samples were extracted with acetonitrile, magnesium sulphate anhydrous and sodium chloride. The cleanup sorbents were separately applied to the supernatant during the DSPE procedure; the mixtures were shaken and centrifuged, and the supernatant was dried. The extracts were reconstituted with acetonitrile/water. The

recoveries of avermectins were 91.9–102.5%, with a relative standard deviation lower than 19%.[86] The other hand, a simple and inexpensive sample preparation method based on solvent extraction (Acetonitrile), followed by low temperature cleanup, was applied for determining avermectin and milbemycin residues in bovine muscle. Mean recovery was between 89 and 101 % at three different concentration levels. [87]

3.2 Chloronicotinyls

The environmental profile of neonicotinoids indicate that they are persistent and have high leaching and runoff potential. These pesticides were extracted by off-line SPE with Strata-X cartridges with recoveries higher than 92%.[88] Graphene magnetic nanoparticle ($G–Fe_3O_4$) were also used as the adsorbent for the preconcentration of thiamethoxam, imidacloprid, acetamiprid and thiacloprid from environmental water samples by MSPE. The recoveries of the method for the compounds at spiking levels of 0.5 and 5 ng mL^{-1} were in the range of 86%–110%.[89] Ionic liquid (IL)-VALLME consists of a binary component solvent system (IL as the extractants and aqueous samples). This method was developed and validated for determination of four pesticides in a manufacturing wastewater sample: acetamiprid, imidacloprid, linuron and tebufenozide. The optimal conditions for extraction of the pesticides were determined: the aqueous sample volume of 10 mL with the addition of 0.58 g NaCl, 40 µL of the 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide as extractant, 2 min extraction under vigorous mixing applying the vortex agitator, and separation of phases by centrifugation for 2 min at 1,000 rpm. The relative recoveries of the targeted pesticides from the wastewater are in the range from 89 to 123%.[90]

QuEChERS and MSPD were used to extract chloronicotinyls in soil matrices. A study developed QuEChERS method to extract six neonicotinoids (imidacloprid, acetamiprid,

thiamethoxam, thiacloprid, clothianidin and nitenpyram) and their metabolites. The samples were extracted with acetonitrile, clean-up by treatment with primary secondary amine sorbent and graphitized carbon with recoveries from 55.3 to 95.6%.[91] Extraction of neonicotinoids in soil using MSPD provided recoveries between 63-99 %.[88] Both methods can be comparable in terms of validation parameters and simplicity.

QuEChERS was also widely used to determine neonicotinoids in fruits and vegetables. For these more complicated matrices, DSPE cleanup combined with DLLME was frequently reported. In the DSPE-DLLME method, neonicotinoid insecticides were first extracted by QuEChERS, second cleaned-up by a DSPE with primary secondary amine and multi-walled carbon nanotubes (MWCNTs) as sorbents and finally, the resulting extracts was cleaned-up by DLLME using chloroform. Under the optimum conditions, the enrichment factors for the compounds were between 110 and 243. The recoveries of the method for the target neonicotinoid insecticides spiked at 10.0 and 50.0 ng g^{-1} in fruits and vegetables were between 84.6% and 97.5% (n=5).[92].

In order to simultaneously determine all imidacloprid residues containing the 6-chloropicolyl moiety) in lettuces, samples were extracted using QuEChERS, evaporated and oxidized with potassium permanganate to yield 6-chloronicotinic acid. The acid residues were further dissolved in n-hexane-acetone (8:2, v/v) and then silylated with MSTFA (N-methyl-N-(trimethylsilyl)trifluoroacetamide) to 6-chloronicotinic acid trimethylsilyl ester to determined then by GC. Recoveries at two fortification levels ranged between 72.8% and 108.3%.[93]

However, there are not validated extract and analytical methods available for the determination of chloronicotinyls in matrices such as biological, postmortem fluids and tissues of human in literature. One study proposed solvent extraction for acetamiprid

and its metabolite IM-1-2 of the postmortem human blood, liver, stomach contents with dichloromethane. The method showed acceptable precisions and recoveries. [94] Subcritical water extraction (SWE) was also investigated as a novel and alternative technology for the extraction of neonicotinoids from eel matrices. Average recoveries of the seven analytes from fortified samples ranged between 85 and 102 %.[95]

3.3 Tetranortriterpenoids

The tretranortriterpenoids such as azadirachtin, nimbin, salazanin, azadirachtol etc.. have been widely determined in neem seed, extract and its formulations because these compounds determine the value. An important problem in the analysis of azadirachtin and related compounds is their instability or degradation when in contact with the matrix. A method that involves automated extraction and simultaneous cleanup using an accelerated solvent technique with the matrix dispersed in solid phase over a layer of primary-secondary amine silica was applied to extract azadirachtin in foliage and phloem of hardwood tree species. Validation at three levels (0.02, 0.1, and 1 mg/kg), demonstrated satisfactory recoveries (71-103%) with relative standard deviation <20%.[96]

Bovine muscle sample was extracted with 10 mL dichloromethane-isopropanol (95:5). The mixture was stirred for 1 min at 24,000 rpm, allowed to stand for 5 min in ultrasonic bath, and finally centrifuged at 3,000 rpm for 5 min. The analytical recovery for all concentrations studied for azadirachtin A is close to 100%. Coefficients of variation for azadirachtin A were found to range between 1.3-2.8%, and relative error between –0.5 to 2.8%. Moreover, the analytical recovery for azadirachtin B (85.2–101.9%) showed a variation greater than azadirachtin A with a coefficient of variation between 0.4–2.9%.[33]

3.4 Ammonium Quaternary Salts

The extraction of ammonium quaternary salts is complicated because of its cationic character. Most of the existing methods for ammonium quaternary salts determination are time consuming, require complex isolation procedures to separate the analytes from the original. matrix and employ toxic solvents.

PQ could be preconcentrate from water samples using SPE recovery of the whole procedure was higher than 92%. [97] Determination of DQ in water using a polydimethylsiloxane (PDMS) coated fiber were also successfully achieved. The method involved sodium borohydride-nickel chloride ($NaBH_4$–$NiCl_2$) reduction and headspace solid phase microextraction (HS–SPME) of the perhydrogenated products. For the extraction, the SPME fiber was exposed for 20 min at 60°C. Using these conditions, the recovery rates of DQ ranged from 93 to 101 %.[98]

Pesticides are generally thought to accumulate in sediments when they have solubility S<1 mg L^{-1}, coefficient octanol/water log P>3, and a half-life greater than 30 days. Quaternary ammonoium herbicides have special affinity for clay where there are retained by an additional electrostatic mechanism. Hexanoic acid 2-(diethylamino)ethyl ester and mepiquat residues in soil and cotton samples were extracted with water, formic acid and NH_4Cl. The overall average recoveries ranged from 76 to 100 %.[99]

PQ and DQ are widely used herbicides for the control of weeds in crops. Then, their residues are frequently determined in fruits and vetables. PQ was extracted from vegetables simply by sonication in water, followed by SPE clean-up. Recovery ranged from 46 to 74 %.[100] PQ and DQ residues in potatoes, cereals and pulses were extracted with a mixture of methanol/water/ hydrochloric acid at 80 °C. The recoveries obtained were in the range of 92–120 % w for all compound/commodity/spiking concentration

combinations.[101] In recent years, DSPE has also been successfully applied as a clean-up step for ammonium quaternary herbicides extraction. CQ was extracted of the meat samples with acetonitrile, followed by a rapid cleanup through DSPE using octadecyl silice as sorbent. The average recovery of CQ in spiked meat samples was 86−95%.[102]

Various extract methods have been applied to the analysis of PQ in biological samples. SPME was used to extract PQ in plasma and urine samples. Recoveries in plasma and urine samples were 94-100% and 95-100%, respectively. Excellent sample clean-up was observed and good linearities (r = 0.9982 for plasma sample and 0.9987 for urine sample) were obtained in the range of 0.1-50 μg/mL.[103]

3.5 Dinitroanilines

All dinitroaniline herbicides in use are 4-alkyl (or -sulfonyl)-2,6-dinitro-N,N-(mono- or disubstituted) anilines, characterized by very low water solubility and basicity. However, a number of SPE procedures were optimized for the determination of dinitroanilines alone or simultaneously with other herbicides, such as carbamates, triazines, and chloroacetamides. Pendimethalin and trifluralin and other pesticides were extracted by SPE using Oasis HLB cartridges with mean recoveries ranged from 70 to115%.[104, 105]

Physico-chemical behavior of dinitroanilines in soils or sediments is influenced by volatilization and by strong soil adsorption. Nitralin and oryzalin may leach slightly, but dinitroanilines are essentially immobile. MAE were tested for the isolation and preconcentration of target compounds in sediments due to its higher extraction efficiency, since it reduces the extraction time in less than 30 min and the consumption of the solvents below 50 mL. The extraction solvent was acetone-hexane, applied

pressure 100 psi, microwave power 1,600 W, temperature 60 °C, and extraction time 10 min. Recoveries in all samples varied from 67 to 123 %.[106] An other MAE procedure used to extract pendimethalin from soil and vegetables samples used acetone, ethanol, and water as extraction solvents. The maximum temperature that can be used during the heating is 60°C, where the recovery percentages reached 97%.[107] Furthermore, PLE and QuEChERS were also assessed a to extract in soil, sediment and sludge samples. The obtained recoveries were 33–89 % versus 25–120 %.[108] QuECHERS presented other advantages such as less time and reagent consumption and lower energy expenditure.

Acetamide herbicides are used in a variety of crops and are commonly included in many multiresidue methods. There is a large variety of procedures for their extraction. Solid–liquid–solid dispersive extraction (SLSDE) was applied to the extraction of herbicides from tobacco samples using MWCNTs as clean-up adsorbents. The effect of the quantity of MWCNTs on SLSDE, and of type and volume of extraction and disperser solvents and of salt effect on SLSDE were optimized. The recoveries in case of herbicide-spiked tobacco ranged from 79 to 105 %. All the tobacco samples contained butralin and pendimethalin at levels ranging from 15.8 to 500.0 μg kg^{-1}.[109] Modified QuEChERS extraction without clean-up procedure demonstrated its ability for simultaneous determination of the residues of selected herbicides viz. pendimethalin, oxyfluorfen, imazethapyr and quizalofop-*p*-ethyl in peanut. Accuracy of the method in terms of average recoveries of all the four herbicides ranged between 69 –94 %.[110]

As the requirement on the determination of pesticides and veterinary drugs residues in milk is more demanding now than before, the development of sensitive methods to improve separation capability of multiresidue analysis has been very important. In addition, sample pretreatment for the multiresidue analysis in milk is necessary, in order

to eliminate the interferences from the complicated matrices, concentrate the analytes and improve sensitivity. A sensitive method for determination of chloramphenicol, enrofloxacin and denitroaniline pesticides residues in bovine milk was developed. Residues of the targets were extracted from milk with acetonitrile, cleaned up by C18-SPE cartridge. Recoveries for the studied compounds were in the range of 71–107%, except that recoveries of trifluralin ranged between 62% and 70% .[111]

3.6 Acetamides

Contrarily to the previous group, acetamides are highly soluble in water. Acetamide herbicides have been isolated from water samples by SPE with C_{18}, MWCNTs, and nonporous graphitized carbon sorbent (US-EPA Method 535). Actually, a number of novel coatings, such as carbon nanomaterials, metal–organic frameworks, mesoporous materials and polymeric ionic liquids, have been developed for the extraction of different kinds of compounds.[112, 113]. MSPE extraction using magnetic graphene nanocomposite (G-Fe3O4) as the adsorbent was also applied to extract alachlor, acetochlor, pretilachlor, butachlor, and metolachlor in environmental water samples. This novel adsorbent showed a great adsorptive ability toward the analytes. Recoveries of the method for the analytes were in the range of 80.7–105.3%.[114].

Other technique that has been applied to this group of herbicides is SPME. Several SPME fibers are commercially available, however, they are mainly apolar or moderately polar, then, little appropriate for this group of compounds. Therefore, the development of novel coating materials to achieve efficient SPME has attracted much research attention. A graphene composite-coated stainless steel SPME fiber was developed. The applicability of the fiber was evaluated through the solid-phase microextraction of five acetanilide herbicides (alachlor, acetochlor, pretilachlor,

butachlor and metolachlor) in water samples. The recoveries of acetanilides from water samples by the method were from 82 to 112 %.[115]

Sorption of acetamides in soil has been positively correlated to organic matter and clay content Acetochlor and propisochlor were extracted in soil samples with water and acetone, followed by SPE to remove co extractives. The analytes from soil matrices were eluted with petroleum ether-acetic ether with ranged from 74 % to 116 %. [62] In recent years, molecular imprinting technology has been used for sample cleanup with higher selectivity to enrich the target molecules from complex matrices. MISPE was developed for four chloroacetamide herbicides, alachlor, acetochlor, pretilachlor and metolachlor. After solvent extraction SPE was used to clean-up the extract showing that the MISPE cartridges were better than the nonimprinted and C18 cartridges in terms of recovery.[116]

Three herbicides (thifensulfuron-methyl, atrazine and acetochlor) residues were extracted in soybeans. The methodology consists of an acetonitrile extraction and DSPE clean-up. Under the optimized conditions, the average recoveries of three herbicides in spiked soybeans ranged from 70.2 to 105.3%.[117] QuEChERS procedure was applied for simultaneous determination of metolachlor, pendimethalin and oxyfluorfen residues in bulb vegetables (garlic, Chinese onion, onion, garlic stem and leek). Samples were extracted with acetonitrile and cleaned-up with primary secondary amines (PSA). Average recoveries in five bulb vegetables ranged from 78 to 114 %.[118]. Homogeneous liquid–liquid extraction (MHLLE) was evaluated as one of the miniaturized clean-up step for determination of alachlor, butachlor, diazinon and in cow milk samples. Methanol was used as extraction solvent and then, it was extracted and cleaned up by the addition of butyl acetate and after addition of water, butyl acetate was separated

from methanol phase. Reduction of the required volume of organic solvent and high throughput sample preparation are important advantages.[119]

3.7 Oximes

Cyclohexanedione oxime herbicides are polar, nonvolatile, and thermally labile, and applied at low rates, which makes their analysis difficult at the trace levels necessary to monitor their environmental fate. Clethodim has been used as a selective post-emergence herbicide to control annual and perennial grasses in a wide variety of broad leaf crops and most vegetables. Its behaviour in soil and vegetables has been widely studied. QuEChERS was selected for simultaneous determination of clethodim and its oxidation metabolites (clethodim sulfoxide and clethodim sulphone) in soil, rape plant and rape seed.[40] The developed method showed satisfactory validation parameters in terms of specificity,linearity, recovery, sensitivity and repeatability.

Furthermore, MAE, was also applied to extract multigroup pesticide residues, including phoxim, sethoxydim and cycloxydim in sediments. The extraction solvent was acetone-hexane, the applied pressure was 100 psi, microwave power 1,600 W, temperature 60 °C, and extraction time 10 min. Recoveries in all samples varied 67–123 %.[106]

3.8 Triazoles

Triazoles are nonselective polar herbicides that pollute ground and surface waters because of their high solubility in water. Conventional SPE was recently applied to determine 253 pesticides separated in 6 different categories including triazoles in surface waters. The recoveries in all samples were 63–112 %.[106].

New approaches based on a new graphene-based silica coated magnetic nanoparticles (Fe_3O_4@SiO_2–G) for the simultaneous preconcentration of several pesticides including

triazoles were also proposed. Compared to commercial C18 sorbent and Fe_3O_4@SiO_2, the newly synthesized adsorbent showed higher adsorption capacity (13 –19 mg g^{-1}).[120] Furthermore, HF-LPME method was also applied to extract penconazole, hexaconazole, diclobutrazole and diniconazole in farm, river, tap water and grape juice samples. The four triazole fungicides were extracted with toluene without salt addition or pH adjustment for 20 min providing recoveries between 83-114%.[121]

Triazole fungicides are persistent in soil and sediments. Microwave-assisted extraction has been applied for the isolation and preconcentration of target compounds in sediments due to its advantages over other extraction techniques, since it reduces the extraction time in less than 30 min and the consumption of the solvents below 50 mL. MAE was applied to extract the pesticide residues from lake sediment samples. Recoveries in all samples varied 67– 123 %. [106] Ultrasound-assisted extraction (UAE) followed by DSPE cleanup was used for the determination of 16 azoles in sewage sludge. The recoveries of these compounds through the method were between 71.9 and 115.8%, with relative standard deviations lower than 20%.[122] QuEChERS method also was used to extract fenbuconazole, tetraconazole, nuarimol, triticonazole, and simeconazole in soil samples. The recovery rates were 77.4–103.6%.[123]

Almost all triazole fungicides are chiral due to the presence of the asymmetrically substituted carbon atoms in the triazol alkyl moiety. Then, enantiomeric analysis of 8 triazole fungicides (tetraconazole, fenbuconazole, epoxiconazole, diniconazole, hexaconazole, triadimefon, paclobutrazol, and myclobutanil) also by QuEChERS has been reported in soil. In this method C_{18} SPE was used instead of DSPE for soil samples clean-up because of cheapness and the results were satisfactory. Under optimal conditions, the mean recoveries for all sixteen enantiomers from the soil samples were 76.4–108.1%.[124]

Regarding vegetables and food samples, solvent extraction followed by SPE and QuEChERS are most commonly reported methods. However, miniaturization methods also are applied. Ultrasound-assisted extraction (UAE) and pressurized liquid extraction (PLE) were used for the rapid determination of 11 fungicides (metalaxyl, cyprodinil, procymidone, iprovalicarb, myclobutanyl, kresoxim-methyl, benalaxyl, fenhexamide, tebuconazole, iprodione and dimethomorph) in white grape bagasse. The PLE procedure showed much higher efficiency than UAE for the target fungicides. Under the selected extraction conditions, PLE showed satisfactory linearity, repeatability and reproducibility. Recoveries for the majority of studied fungicides were higher than 80%.[125]

Elevated temperature dispersive liquid–liquid microextraction (ET-DLLME) was applied for the extraction, preconcentration, and determination of penconazole, hexaconazole, diniconazole, tebuconazole, and difenoconazole in honey samples. Using 1,2-dibromoethane as extractant, enrichment factors near to 2,000 were achievable with recoveries higher than 90 %.[63] Other method based on air-assisted liquid–liquid microextraction (AA-LLME) was also developed to determine these five fungicides in edible oils. Under the optimum extraction conditions, the recoveries were in the ranges of 71-96%, respectively.[126] A graphene-based magnetic nanocomposites (GFe3O4 MNPs) was synthesized and used as the adsorbent for the extraction of some triazole fungicides (triadimefon, paclobutrazol, hexaconazole, myclobutanil, diniconazole, propiconazole, and tebuconazole) in cucumber, cabbage, and tomato samples. The recoveries of the method for the seven triazoles were in the range from 84.4 to 108.2 % with RSDs between 3.4 and 10.6 %.[127]

4 Identification and Determination

Table 6 summarizes the techniques reported for the determination of new generation pesticides.The wide range of physicochemical properties covered by these compounds explains why a large range of analytical techniques have been used to determine them. The most remarka

Liquid chromatography (LC) is still the most employed to separate and detect miscellaneous pesticides. This may be attributed to the general guidelines to autorize new pesticides that look for substances less hazardous for human beings, less volatile, more water soluble, more polar, and more (bio) degradable. This group of characteristics makes LC an ideal technique for determining them. Reverse phase LC using apolar columns (C_{18}, C_8, etc) and polar mobile phases (mixtures of methanol, acetonitrile and water) are commonly used the separation. The mobile phases can also be added of salts or acids to improve peak shape. There are some innovation within LC applied to the determination of these new compounds, such as, the UHPLC that uses columns of small particle size (<1.7 μm) to achieve better resolution in less analysis time. This technique is relatively recent but widespread used. Most polar columns, such as hydrophilic interaction liquid chromatography (HILIC) column have an important application within quaternary ammonium herbicides due to the ionic characteristics.

The second selected technique is gas chromatography (GC). The new generation pesticides are determined by very traditional approaches within the field that is using conventional 30 m x 0.21 mm columns and applying splitless or PVT injector. The most frequent detectors are electron capture (ECD), nitrogen phosphorus detector (NPD), flame photometric detector (FPD) and mass spectrometry (MS). Among them, MS is the technique of choice for detecting small quantities of these pesticides. Recent

advances in GC are related to an increase in the multidimensionality of the technique. Recent reviews on applications, advantages, and comparison of different GC and MS techniques based on applied separation dimensionalities related to column and/or mass analyzers pointed out that column dimensionality has not been applied to these pesticides yet.[128, 129]

During the last 10 years, MS has gained an outstanding position in many areas of pesticide residue analysis combined with either, LC or GC.[130] Nowadays, pesticide residue analysis using MS techniques has evolved and depending either on the way the analysis is approached or on its final purpose, the determination of pesticide residues may be a target or nontarget analysis. Target analysis is conventional analysis based on establishing a method with standards before analysis and monitoring real samples. Nontarget analysis covers unusual compounds, such as, old or unauthorized pesticides, which are not supposed to be used, or very recent compounds that are not included in target lists and unknown degradation products and impurities. Further progress in this field has been evidenced in the development of large-scale multiresidue methods (i.e. covering more than 80 compounds) for pesticides and their degradation products by LC-MS.[131]

Although some works have reported the use of atmospheric pressure chemical ionization (APCI), electrospray (ESI) is more sensitive and more used. Usually, pesticides included in multiresidue methods are easily ionized in the atmospheric pressure sources commonly used in LC-MS/MS, and the protonated $[M + H]+$ or deprotonated molecule $[M - H]^-$ used to be the most abundant ion, eventually selected as a precursor ion in MS/MS methods. However, the absence of acidic or basic centers

in some pesticides hampers their ionization, requiring the formation of appropriate adducts in order to be measured by LC-MS.

Liquid chromatography tandem mass spectrometry (LC-MS/MS) is a highly selective method when used either in ion monitoring mode or in multiple reaction monitoring mode. Despite its popularity, the technique is limited by the suppression or enhancement of analyte ionization in the electrospray ionization (ESI) source due to co-eluting compounds, known as the matrix effect.[132, 133] Although invisible in the LC/MS signal, this effect very often adversely affects the accuracy and sensitivity of the method. Moreover, it has been observed that the ionization efficiency of polar compounds is more influenced by co-eluting compounds than the ionization of less polar compounds.

Although QqQ instruments are currently the workhorse of residue analysis laboratories, a new generation of high resolution MS (HRMS) instruments based on technologies such as time-of-flight (TOF) or Orbitrap are being introduced. These hybrid instruments, which combine a quadrupole and a high resolution mass spectrometer, i.e. Q-TOF and Q-Orbitrap, are very attractive and can be considered as the high resolution equivalent of the QqQ instruments. In particular, Q-Orbitrap appears especially suitable for highly demanding residue analysis, as it can offer outstanding resolution and accuracy in addition to high sensitivity.[134] A number of reviews have dealt with the subject of non-target analysis using LC-MS,[135, 136]

Other alternative techniques reported to determine these pesticides are immunoassays, sensors and biosensors and in a less extend capillary electrophoresis and voltammetry.[137, 138, 139 3352] Biosensors are still a promising evolution within the field. These device integrate an immobilized biological element (e.g. enzyme, DNA probe,

antibody) that recognizes the analyte (e.g. enzyme substrate, complementary DNA, antigen) and a transduction element used to convert the (bio)chemical signal resulting from the interaction of the analyte with the bioreceptor into an electronic one. According to the signal transduction technique, biosensors are classified into electrochemical, optical, piezoelectric and mechanical biosensors. Nanotechnology is playing an important role in the development of efficient biosensors for the new genration pesticide detection.[137]

4.1 Gas Chromatography

The miscellaneous pesticides commonly determined by GC are dinitroanilines and acetamides and triazoles because they are nonpolar, thermostable and volatile. GC offers advantages relative to LC methods (e.g., better resolution, elimination of the need of organic solvents for mobile phases, etc.). The macrocyclic lactones, chloronycotinil, tetranortriterpenoids and ammonium quaternary salt pesticides are, in general, not amenable for direct GC analysis and then, determination of these pesticides by GC required derivatization. Direct analysis of macrocyclic lactones by GC or GC-MS has not been successful due to their hydrophobic nature (see octanol–water partition coefficient in Table 2) and low volatility (see also melting point in Table 2). However, macrocyclic lactones such as epinomectin and ivermectin have been determined in meat by GC-MS.[140] . The approach is based upon the pre-column derivatization of macrocyclic lactones by reaction with N,O-bis(trimethylsilyl)tri-fluoroacetamide (BSTFA) in the presence of 1-methylimidazole as catalyst and carbon tetrachloride as solvent to form the trimethylsilyl (TMS) derivative. Chloronycotinyls have also low volatility but were determined by GC - MS/MS and GC-ECD in vegetables, water and fruits.[93, 141, 142] This procedure involves oxidation with potassium permanganate to yield 6-chloronicotinic acid. The acid residues were further dissolved in an organic solvent

and then silylated with MSTFA (N-methyl-N-(trimethylsilyl)trifluoroacetamide) to 6-chloronicotinic acid trimethylsilyl ester. Ammonium quaternary salts such as DQ and PQ were are polar and ionized then, their determined by GC-MS requires also derivatization.[98, 103] The method involved the procedure of sodium borohydride–nickel chloride ($NaBH_4$–$NiCl_2$) reduction necessary to convert the quaternary ammonium substances into more volatile compounds.

Although considering as detectors falling into disuse, GC-electron capture detector (ECD) and flame thermoionic detector (FTD) are still reported to determine dinitroaniline herbicides. The determination of dinitroaniline herbicide concentrations in water, soil, tobaco, air, vegetables, leek, chili, strawberry and other types of food has usually been carried out by GC-MS.[104, 143-150] The single quadrupole with electron impact (EI) ionization or negative chemical ionization (NCI) working in selected ion monitoring (SIM) is the most commpn platform because the high sensitivity. [104, 143-150] GC-MS and MS/MS with an ion trap working in selected reaction monitoring (MRM) mode have also been proposed for the determination of pendimethalin, together with other pesticides, in water and soil.[151, 152] GC-MS/MS was also applied for simultaneous determination of metolachlor, pendimethalin and oxyfluorfen residues in bulb vegetables (garlic, onion, garlic stem and leek). The limits of quantitation was validated in five samples matrices was 5 mg kg^{-1} and the limit of detection ranged from 0.5 mg kg^{-1} to 2.3 mg kg^{-1}.[118]. (GC–NCI-MS in SIM has been compared to electron ionization (EI) mode for the determination of 7 dinitroaniline herbicide residues in garlic, olive oil, scallion, leek and chili. The limits of the detection of 7 dinitroanilines were in the range of 0.014 - 0.096 µg kg^{-1}.[145] **Figure 1** illustrates the selectivity of different ionization modes, in standard solutions and blank and spiked garlic extracts. The determination by

GC–NCI/MS had high selectivity and no interfering peaks were found from all five selected matrices.

The analysis of chloroacetamides has also usually been carried out by GC-MS. However, GC-ECD also was applied to determinate environmental samples such as water and soil.[114-116] Alachlor, acetochlor, pretilachlor, butachlor, and metolachlor were frequently determined by GC-ECD. The limits of detection was between 0.06 and 0.10 mg L^{-1}.[115] GC-ECD methods are not suitable for complex matrices due to the matrix interference. However, the combination of highly specific molecularly imprinted solid phase extraction (MISPE) with GC-ECD was able to determine alachlor, acetochlor, pretilachlor and metolachlor, in soil samples at concentrations ranging from 1.0 x 10^{-12} to 5 x 10^{-11} g and 0.0005–0.025 mg kg^{-1}, respectively.[116] GC-MS determination is usually carried out using single quadrupole mass spectrometer operated in the EI mode. The limits of detection of Prometryne/Acetochlor were up to 0.06 and 0.17 μg mL^{-1}, respectively.[153] Double focusing magnetic sector mass spectrometers are most often used for applications where high resolution and sensitivity are the primary requirements. However, this instrument has been applied to the trace level determination of 14 selected (EU-directive) priority organic pollutants including acetochlor in wastewater [154]. An example of the identification of the target species in wastewater samples by GC–HRMS is shown in **Figure 2**. This instrument was used in high-resolution selective ion recording HRSIR at a resolution value of 10,000 permitting the accurate identification of the targeted compounds. For identification and confirmation purposes, the accurate mass of two characteristic ions and their relative abundances were combined with retention time matching.

Triazoles pesticides such as, cyproconazole, hexaconazole, myclobutanil, penconazole, propiconazole and tebuconazole have been determined by GC and different types of detectors as MS, flame thermionic detector (FTD), ECD, flame ionization detector (FID) and nitrogen-phosphorous detection (NPD) in environmental and food samples.[63, 104, 120-122, 126, 127, 155-161] Multiresidual method was delveloped for 28 pesticides include myclobutanil and tebuconazole in tomato samples using a GC coupled to a triple quadrupole MS/MS. The mass spectrometer was operated in MRM.[156] This method provides higher selectivity and sensitivity and attain the incorporation of these new generation pesticides to the multiresidue schemes.

4.2 High-Performance Liquid Chromatography

Macrocylic lactones have been determined by LC-UV, LC-FLD and LC-QqQ-MS/MS in different matrices. LC-UV was used to determinate abamectin in fruit samples with LODs were 0.001 and 0.008 mg/kg. [162] However, other studies establish that UV is not sensitive enough to determine abamactin residues in fruit and vegetables. [49, 163] LC–FLD was used to determinate abamectin residues in vegetables and fruits after direct derivatization with trifluoroacetic anhydride and N-methylimidazole. The LOD and LOQ of the proposed method were 0.0005 and 0.002 μg/g, respectively, much less than the lowest MRLs (0.01 μg/g).[49] The determination of abamectin, emamectin benzoate and ivermectin in soils by LC-FLD was also reported. [163]

LC-MS/MS using MRM is the most common technique, even through most avermectins show low ionization efficiency, and tend to form sodium adducts when using positive ESI, which provides poor linearity and fragmentation.[78] LC-QqQ-MS/MS in positive ionization mode has been applied to the determination of these compounds in cheese,[164] edible oils,[165] dried hops,[166] vegetable, [167] environmental matrices,[168] and dairy products.[169] .

Figure 3 illustrates how the analysis of abamectin and ivermectin in edible oils with LC–MS/MS with satisfactory results in terms of sensitivity, selectivity, precision and accuracy. Negative ESI is an alternative but less sensitive than positive ESI.[169]

Neonicotinoid pesticides in food and environmetal samples have been determined alone or already incorporated in multiclass multiresidue pesticide using almost exclusively LC-MS/MS. Particular mention deserves a rapid LC-ESI (+)-MS/MS method developed and validated for simultaneous determination of dinotefuran, nitenpyram, thiamethoxam, clothianidin, imidacloprid, acetamiprid, thiacloprid and imidaclothiz in tea samples. The LOQs of tea were below 0.01 mg kg^{-1}.[170] The same analytical method was applied for the determination of acetamiprid residues in zucchini and zucchini leaves grown under greenhouse conditions. The limits of detection and limits of quantification were 0.01 and 0.03 mg/g and 0.02 and 0.06 mg/g, for the zucchini and zucchini leaves, respectively.[171] The sensitive and selective analysis applying ULC-MS/MS have also been reported for these compounds together with pyrethrins in Beebread.[172] The optimisation of the chromatographic conditions for the multi-residue analysis of pyrethroids and neonicotinoids is challenging due to the different physico-chemical properties of the two families. **Figure 4** shows that appropriate chromatographic conditions attains not only the simultaneous analysis of the two families of substances but also the separation of individual insecticides within the same family.

Regarding the analysis of tetranortriterpenoid pesticides, LC-MS has also been shown to be the most suitable technique. Most LC-MS methods for azadirachtin determination based on an electrospray source monitor its sodium adduct, although some authors have selected the ammonium adduct. However, the sodium adduct is the preferred option in terms of sensitivity.[96, 173, 174] .LC-QTOF MS has also been used for confirmation of the identity of the

compounds detected and to investigate the presence of other azadirachtin-related compounds in the samples..[175] **Figure 5** shows the BPI (Base-Peak Ion) chromatogram as well as the low energy (LE) extracted ion chromatograms (20 mDa mass window) for the azadirachtin A exact mass ([M + Na]+ m/z 743.2449) and azadirachtin B ($[M + Na]^+$ m/z 685.2418) in a Norway Maple foliage sample. Additional related peaks were observed in the chromatogram. Regarding the high energy (HE) spectra, both azadirachtins presented fragment ions corresponding to losses of H_2O (18.0106 Da), CH_3COOH (60.0211 Da) and CH_3–CH—$C_2H_4O_2$ (100.0524 Da).

LC-MS or LC-MS/MS is the analytical instrument of choice for ammonium quaternary salts because they are readily soluble in water and in other polar solvent and are not volatile. On the stationary phases, there are two clear options widely used to determine these compounds. The high polarity of PQ rendered poor retention on conventional C_{18} columns, ion-pairing agent can mitigate the situation, but lead to signal suppression and low sensitivity when coupled with mass spectrometric detectors. Hydrophilic interaction liquid chromatography (HILIC) columns showed promising retention of polar compounds like quaternary ammonium, but a buffer with high salt concentration is required to maintain optimal performance of the column. When coupled with mass spectrometer, the ion-pairing agents and high salt concentration decrease sensitivity and cause clogging of the spray tip and the MS orifice. So, an LC protocol without ion-pairing agent and low buffer concentration is needed to achieve sensitive detection of PQ with LC–MS. There are many works where vegetable samples have been analysed by LC-MS/MS. Kolberg et al.[101] determined PQ residues in potato and cereals with LC–MS/MS-based methods.. PQ was also determined in edible leafy vegetables (cabbage, lettuce, spinach and Chinese cabbage) by LC–MS/MS. Chromatographic separation of PQ was achieved on a HILIC column. The low salt concentration used in the eluting buffer ensured extended LC–MS analysis of PQ in different

matrices without the necessity of frequent source cleaning. The limit of detection was 0.94 ng g^{-1}.[100] Furthermore, the potential of LC-MS/MS for the analysis of six plant growth regulators including chlormequat and mepiquat was studied. Based on the ion suppression produced, no ion-pairing reagent was introduced into the mobile phase. **Figure 6** presents the LC-MS/MS chromatogram of the apple matrix extract sample fortified at the limit of quantification with the six plant growth regulators.

Although dinitroaniline residues have mainly been determined by GC-MS, several methods report the determination of pendimethalin in sediment by LC-UV.[107] The ultraviolet detector was adjusted at 240 nm for absorption measurement. Limits of detection and limits of quantification of 0.059 and 0.17 μg mL^{-1}, respectively. Multiresidue method developed for the determination of several pesticides including trifuralin in sediment and milk by LC-MS/MS have been reported.[106, 111]

Although many GC-MS methods have been reported for chloroacetamides, [104, 114, 115, 176, 177] LC-MS methods are also a cornerstone within these pesticide determination [178-183]. Nowadays, LC methods are generally preferred over GC ones because LC has become the preferred technique in most applications, using a variety of detection methods. LC-UV detection as a fast and inexpensive technique was applied to the simultaneous extraction and determination of traces of two common herbicides, alachlor and atrazine, in aqueous samples.[180] On-line SPE–LC–ESI-MS/MS was applied in groundwater samples for the determination of alachlor, metolachlor and 20 medium to highly polar pesticides. The lowest LOD corresponded to propanil (0.02 ng L^{-1}) and the highest to metolachlor (3.91 ng L^{-1}). [183] The analytical method involving HF-LPME coupled online to high-performance liquid chromatography (LC) for the one-step sample pretreatment and direct determination of

alachlor (2-chloro-2′,6′-diethyl-N -(methoxymethyl)acetanilide) and its metabolite 2,6-diethylaniline (2,6-DEA) in microbial culture medium has been developed.

Few papers have reported simultaneous determination of clethodim and its oxidation metabolites to date. A method was developed for determination of the herbicide clethodim and its oxidation metabolites clethodim sulfoxide and clethodim sulfone in agricultural products by LC-MS/MS with ESI. The detection limit of clethodim sulfone in crops was 0.01 ppm. Recently, other study applied a simultaneous determination of clethodim and its oxidation metabolites (clethodim sulfoxide and clethodim sulphone) in soil rape plant and rape seed using LC-MS/MS. The target analytes were separated using acetonitrile–water (containing 0.1% formic acid). The addition of formic acid was helpful to improve the protonation of target compounds in LC–ESI-MS/MS analysis. The MS/MS condition was established in ESI positive mode because of subsequent experiments results demonstrated higher responses in positive mode than in negative mode. The $[M+H]^+$ ion was chosen as the precursor ion for all analytes because of its high relative intensity and then the fragmentor was optimized. As shown in **Figure 7**, there was no interference to the analysis of the target compound when the mobile phase was set at 20/80 (v/v) mixture of water (containing 0.1% formic acid) and acetonitrile. The use of a liquid chromatography column containing particles with a diameter of <2 mm enabled each run time was to be less than 3 min.The limits of detection of the proposed method ranged from 0.002 to 0.01 mg kg^{-1}.[40]

The triazole fungicides have been widely determined by LC-MS/MS in the same way as that described for the neonicotinoids. Multiclass, multiresidue methods able to determine more than 253 pesticide have been developed using LC- QqQ-MS/MS.[106] Different categories (carbamates, triazines/conazoles/triazoles, organophosphorus, anilides/pyrimides, ureas, unclassified/various) of pesticides were determinated in surface water by UHPLC-MS/MS.

The time of analysis was 10 min. The LOD varied 0.30 - 2.47 ng L^{-1}. [106] Tebuconazole and bisphenol A were determined by LC-MS/MS in vegetables and juice samples. The LOQ for tebuconazole and bisphenol A was 0.5 and 0.5 nmol L^{-1} respectively, with LOD of 0.2 and 0.2 nmol L^{-1} respectively.[184] Recently, other method using gel permeation chromatography (GPC) clean-up followed by LC-MS/MS was developed for the determination of myclobutanil, hexaconazole, diniconazole, epoxiconazole and tetraconazole enantiomers in soil and earthworms. The LODs of the enantiomers were between 0.001 - 0.003 mg/kg.[185] An enantioselective method for the determination of tebuconazole and tetraconazole enantiomers in strawberry has been developed. The enantiomers were resolved by high-performance liquid chromatography on a cellulose tris (3-chloro-4-methylphenylcarbamate) (Lux Cellulose-2) column using methanol–0.1% formic acid solution as mobile phase. The limits of quantification for tebuconazole and tetraconazole enantiomers in strawberry were both 2.5 μg kg^{-1}.[186]An enantioselective method was developed for the simultaneous detection of five chiral fungicides in soil, including fenbuconazole, tetraconazole, nuarimol, triticonazole, and simeconazole by LC–MS/MS on a chiral stationary phase of cellulose tris-(3-chloro-4-methylphenylcarbamate) with a gradient elution.[123] Typical chromatograms of the spiked soil sample are shown in **Figure 8**.

4.3 Immunoassay

Immunoassays, an alternative to traditional analytical methods, are known to be rapid, sensitive and specific for the analysis of a large number of samples for low levels of specific analytes. These methods involve the use of antibodies, which are the key components of all immunoassays because their quality greatly contributes to the sensitivity and selectivity. An enzyme immunoassay uses either a labeled antibody or a labeled analog of the target compound as the detection marker and can be performed in different formats. The most

common format reported is the competitive enzyme-linked immunosorbent assay (ELISA), in which the antibodies specific to the analyte are immobilized onto a solid phase. The enzyme–hapten conjugate and the analyte are added to the antibody-coated tube or the plate for incubation. After incubation, the unreacted material is removed, substrate and chromogen are added and allowed to react for a short period of time during which the enzyme converts the substrate–chromogen to a colored product. The absorbance can be either measured with a spectrophotometer or estimated visually.[187, 188]

An indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) was developed and used for the analysis of parathion and imidacloprid simultaneously. A multi-determinant immunogen was prepared by haptens of both pesticides conjugated to bovine serum albumin. Under the optimized conditions, the 50% inhibition concentration (IC_{50}) value for parathion and imidacloprid was 0.052 and 1.70 mg L^{-1}, with a limit of detection (LOD, IC_{10}) of 0.0005 and 0.0045 mg L^{-1}, respectively.[188]

ELISAs based on monoclonal antibodies for the detection of macrocyclic lactones, chloronicotinoid insecticides, and PQ and acetamides fungicides have been developed.[187-191] Several monoclonal antibodies (MAbs) with the ability to sensitively bind several compounds with different specificities were obtained. Both analyte- and class-specific ELISAs were developed. For example, a broad-selective ELISA for three avermectins using a polyclonal antibody (Pab) was developed in milk samples. Under the optimized conditions, the IC_{50} values in assay buffer were estimated to be 3.05 ng mL^{-1} for abamectin, 13.10 ng mL^{-1} for ivermectin, 38.96 ng mL^{-1} for eprinomectin, 61.00 ng mL^{-1} for doramectin, 14.38 ng mL^{-1} for emamectin benzoate. ELISA sensitivity attains detection of less than 5 ng mL^{-1} and 2 ng mL^{-1} in milk samples prepared by simple dilution and solvent extraction.[187]

Regarding the environmental samples, an indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) has been developed for detection of pretilachlor in water and soil. An immunogen was prepared from haptens of pretilachlor conjugated to bovine serum albumin (BSA). The specific polyclonal antibodies were obtained by immunizing New Zealand white rabbits. Under optimal conditions, the ELISA demonstrated an 50% inhibitory concentration (IC_{50}) value of 0.0359 mg L^{-1} with a limit of detection (LOD, IC_{10}) of 6.9 ng L^{-1}. The cross-reactivities to some analogs of pretilachlor (acetochlor, butachlor, metazachlor and metalaxyl) were below 1.5%.[191]

The analytical methods based on the ELISA kit achieved rapidity and simplicity to determine a high number of samples and will contribute to provide rapid and smooth distribution of agricultural products in market in the future. Although ELISA analysis has been applied to several matrices such as environmental samples, bovine liver, fruit juices, and agricultural products, immunoassay methods still need to expand their adaptability to not only various agricultural samples but also processed foods.

4.4 Sensors and Biosensors

A chemosensor is composed of two main parts: the first, where the selective chemistry occurs and the second is the transducer. The chemical reaction produces a signal such as color change, fluorescence, or change in the oscillation frequency of the crystal, and the transducer translates the physicochemical event into a recognizable physical signal. A biosensor can be defined as a self-contained intregrated device that is capable of providing specific quantitative or semiquantitative analytical information using a biological regonition element (biochemical receptor), which is retained in direct spatial contact with a transduction element. In the special case of immunosensors, the biochemical receptor is an antibody. Nowadays, these techniques are emerging in the field and have shown very promising results so far.[192-194]

Chloronicotinylis pesticides such as acetamiprid and thiamethoxam were determined by sensor techniques in vegetables samples. An electrochemical method for the indirect determination of acetamiprid was studied, using titanium dioxide photocatalysts coupled with a carbon paste electrode. The cyclic voltammetric results indicated that the photocatalytic degradation compound of acetamiprid had electroactivity in neutral solutions. The experimental parameters were optimized with regard to the photocatalytic degradation time, pH of buffer solution, accumulation potential and accumulation time. Under optimal conditions, the proposed electrochemical method detected acetamiprid concentrations ranging from 0.01 to 2.0 μM, with a detection limit (3 S/N) of 0.2 nM.[195] The determination of thiamethoxam, a widely known neonicotinoid pesticide, by a multicommutated optosensing device implemented with photochemically induced fluorescence (PIF) was developed. The combination of both methodologies allowed, on one hand a quick on-line photo- degradation of TMX and, on the other hand, the preconcentration, quantification and desorption of the fluorescent photoproduct generated once retained on C18 silica gel filling the flow-cell which was monitored at 353 and 407 nm for excitation and emission wavelengths, respectively. The proposed analytical method presented a detection limit of 3.6 ng mL^{-1}.[196] Clothianidin, imidacloprid, thiamethoxam, nitenpyram and dinotefuran were dertemined in water samples by cathodic differential pulse (DP) voltammetry at screen-printed disposable sensors featuring a puttered bismuth thick-film working electrode. The LOQ in 4 water matrices (distilled water, tap water, mineral water and surface water) were in the range 0.76 to 2.10 mg L-1.[197]

DNA usually adsorbs to gold nanaparticles by virtue of mercapto or amino group at one end of a DNA molecule. A study reported a highly ordered biosensor constructed using unmodified DNA molecules with consecutive adenines and three-dimensional gold nanoparticles (3D GNPs). The unmodified DNA-3DGNP composite was fabricated on gold

electrodes and characterized through the use of scanning electron microscopy (SEM), atomic force microscopy (AFM), and electrochemical methods. The modified electrode exhibited an ultrasensitive response to PA. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were used to study the linear relationships between the concentrations and the reduction peak currents. The linear relationship for DPV is 7.0 x 10^{-9} M to 1.5 x 10^{-6} M with a detection limit of 2.0 x 10^{-10} M. The proposed assay could be applied to human serum, human urine, and natural samples.[10] Other sensor method to determined PQ was fluorescence sensor based on glutathione-capped CdS quantum dots (QDs). The methodology enabled the use of a simple synthesis procedure for water solubilization of CdS QDs via a fast route using glutathione as a capping agent within 15 min. The resulting water-soluble QDs exhibited a strong fluorescence emission at 536 nm with high and reproducible photostability. PA is an important class of electron acceptors for QDs. Thus, the fluorescence intensity of the glutathione-capped CdS QDs probe could be dramatically quenched by PA due to the electron transfer mechanism. The fluorescence intensity of the CdS QDs system was proportional to PA concentration in the range of 0.025 to 1.5 μg mL^{-1}, with a detection limit of 0.01 μg mL^{-1}.[198]

A method base on a composite of carbon paste and copper nanowire as a sensitive sensor was proposed for the determination of trifluralin in soil samples. The presence of copper nanowire in the composite film enhance the conductivity and as a result increased the electron transfer rate constant and so the current will increase. The composite exhibits a promising higher electrocatalytic activity towards the oxidation of trifluralin in pH 4.0 aqueous solution. As a result, the sensor showed a valuable response in linear concentration range of 100 - 0.02 nmol L^{-1} with a LOD of 0.008 nmol L^{-1} and LOQ of 0.15 nmol L^{-1} for trifluralin.[199]

4.5 Other Techniques

4.5.1 *Capillary Electrophoresis*

Capillary electrophoresis (CE) can separate compounds that have been traditionally difficult to handle by chromatographic techniques, such as highly polar and water-soluble substances. However, the major drawbacks in CE — lower sensitivity and lower repeatability of migration time — make it a less common method to be used for the determination of miscellaneous pesticides compared with the other chromatographic techniques. CE has many advantages such as high separation efficiency, short run time, minimum operation cost, small sample volume required and minimization of environmental pollution. Ultraviolet (UV) absorbance is the most common detection technique for the analysis of herbicide in CE. Another tool for CE is electrochemiluminescence (ECL), which offers a broad linear range and high detection sensitivity, but also simple and inexpensive instrumentation. Since pretilachlor has a tertiary amine group, it may be sensitively detected by this system. Pretilachlor was determined by CE with ECL detection in soil samples. The limits of detection of the proposed method were 0.01 mg kg^{-1} in rice matrix, and 0.008 mg kg^{-1} in soil matrix, respectively.[200]PQ and other two pesticides were determinated in marijuana samples by capillary electrophoresis. For PQ analysis, sample was extracted with aqueous acetic acid solution and analyzed by capillary zone electrophoresis with direct UV detection. The LOD and LOQ were 0.8 and 5 μg g^{-1} [f] for PQ, respectively.[201] PQ have also been determinate by CE in matrices such us oral fluid, plasma, urine and water. [202, 203]

Pressure assisted capillary electrophoresis was applied to determination of acid dissociation constants (pK_a) of six widely used triazole fungicides (cyproconazole, epoxiconazole, flusilazole, tebuconazole, penconazole and propiconazole) in aqueous medium. The pK_a values were determined from the dependence of effective electrophoretic mobility of the

triazole fungicides on pH of the background electrolyte (BGE) using non-linear regression analysis. The electrophoretic measurements showed that the triazole fungicides are very weak bases – their pK_a values were in the range 1.05 - 1.97. [204]

4.5.2 *Voltammetry*

To date, the range of electroanalytical methods that have been employed for the determination of pesticide residues includes amperometry, polarography, potentiomentry, and electrochemical (potentiometric and voltammetric) stripping analysis.

Square-wave voltammetry (SWV) is a fast pulse technique which is especially sensitive for reversible redox systems such as PQ because the analytical response is given by the sum of the anodic and cathodic currents. In water samples PQ was determined by sequential injection–square wave voltammetry method employing the hanging mercury drop electrode. The LOD and LOQ were 2.0 and 7.0 μg L^{-1}, respectively.[205] Determination of PQ by SWV using a variety of electrode materials, such us, carbon paste, chitin modified carbon paste and bare boron-doped diamond electrodes have been reported. [206-208] An interesting method reports SWV using carbon paste electrode modified with silver particles impregnated onto natural phosphate Ag/NP–carbon paste electrode for PQ determination at trace amounts in tomatoes. The limits of detection and quantification were 1.34×10^{-10} and 4.49×10^{-10} mol L^{-1}, respectively.[206]

Neonicotinoids have also been determined by voltammetry methods using different electrodes such us copper (II) phthalocyanine modified carbon ceramic and bismuth in environmental and vegetables samples.[197, 209, 210] Bismuth electrodes were introduced as an alternative to toxic mercury electrodes for the determination of trace metals by stripping voltammetry. More recently, bismuth-based electrodes have been applied to the analysis of

organic compounds. Regarding neonicotinoid pesticides, carbon based electrodes modified with bismuth powder and glassy carbon electrodes electroplated with a bismuth film have been reported.[210]

5 Food and Environmental Applications

5.1 Macrocyclic Lactones

These substances are used control phytophagous mites and insect pests in veterinary and on a variety of agricultural and horticultural crops worldwide. Residues from their used for animal husbandry enter the environment either directly by spreading of manure or after collection and storage in the form of sludge. The active ingredients are applied to farmlands and reach the upper soil layer where they either accumulate, dissolve in surface water, or leach into groundwater, where they can affect both human health and the environment. Abamectin, ivermectin, moxidectin, and doramectin were evaluated in stream water collected near of livestock farms.[76] However, none of the collected field samples contained any of the targeted analytes. This may have been due to the short half-life of the compounds in water. A previous study determined a 3–5 day half-life for ivermectin in water but of 187 days in sediments.

Products of animal origin are specially monitored to ensure they are free of these products. A study validated an analytical method for determination of ivermectin, abamectin, doramectin, eprinomectin and moxidectin in dairy products and verify the occurrence of these compounds in milk and yogurt available in the Brazilian market. A total of 342 samples were analyzed in duplicate. Only moxidectin at a level of 2.2 mg L^{-1} was detected in one sample of pasteurized milk. No residue of the analyzed compounds was found in UHT milk or yogurt samples.[84] Furthermore, the analysis of real pork muscle, pork liver, fish and milk samples collected from different local food manufacturers. Two samples were positive: abamectin was detected

at a concentration of 5.7 mg kg^{-1} in one fish sample, and ivermectin was detected at a concentration of 3.4 mg kg^{-1} in one pork liver sample.[211]

5.2 Chloronicotinyls

The chloronicotyonyl pesticide residues in the environment are of special interest due to their high toxicity for bees. Acetamiprid, imidacloprid, thiacloprid and thiamethoxam has been determined in river water and peanut milk samples prior to LC-UV. Acetamiprid was found at 0.61 ng mL^{-1} of in the river water wheres thiometoxam (8.67 ng mL^{-1}) and acetamipprid (3.69 ng mL^{-1}) were found in the peanut milk sample.[212]. As well as, chloronicotyonyl pesticides were determined in reservoir, river and sea water samples. The results showed that no residues of the neonicotinoids were detected in either sea or reservoir water samples and only low concentration of acetamiprid (0.09 ng mL^{-1}) was found in river water sample.[89]

Modified QuEChERS followed by LC–MS/MS has been applied to a number of pesticides including chloronicotinyls and fruit and vegetable matrices. The method was applied to the analysis 109 pesticides of 345 tomato samples obtained from local markets and tomato traders. Residues of acetamiprid, azoxystrobin and triadimefon were identified and measured in 9.6% of tomato samples, ranging from 0.015 to 0.37 mg kg^{-1}.[213] Ten systemic pesticides, including thiamethoxam and acetamiprid, were analyzed in 13 baby foods (cereals, boiled potatoes, fruit and milk) collected from localmarkets in Seoul, Republic of Korea. None of the tested pesticides were detected in the samples.[214]

The honeybee exposure to neonicotinoids is one hot environmental issue. Furthermore, these substances can contaminate apiarian products, especially honey, which is the most commonly consumed bee product. Because of the potential threat to human health, the European Union established maximum residue limits (MRLs) for acetamiprid, clothianidin, imidacloprid,

thiacloprid and thiamethoxam in the range of 10 - 200 µg kg^{-1}.[215] Honey samples were analyzed by LC-DAD, DLLME and QuEChERS for determining dinotefuran, nitenpyram, thiametoxam, clothianidin, imidacloprid, acetamiprid and thiacloprid. The developed method was applied to the analysis of 104 honey samples of different plant origin (51 sunflower, 26 wildflowers, 22 acacia and 5 linden) collected from multiple locations in the Autonomous Province of Vojvodina, Republic of Serbia. From the investigated neonicotinoids, thiacloprid, imidacloprid and thiamethoxam were detected at low levels in several samples.[216] Simultaneous determination and confirmation of imidacloprid, clothianidin, acetamiprid, thiametoxam, thiacloprid, nitenpyram and dinotefuran and some of their metabolites (imidacloprid guanidine, imidacloprid olefin, imidacloprid urea, desnitro-imidacloprid hydrochloride, thiacloprid-amid and acetamiprid-N-desmethyl) were determined in honey bee and honey by LC-MS/MS. For sample preparation step and validation experiments honey bees and honey samples were collected from hives localised on experimental apiary on the laboratory (Swarzedz, Poland). The results of this study indicated that in both honey bee and honey samples from experiment, the presence of clothianidin were confirmed (4.0 — 13.1 µg kg^{-1} in honeybees, 13.7 — 192.8 µg kg^{-1} in honey). However, the monitoring of massive honeybee death episode shows higher concentrations 27.0 µg kg^{-1} of imidacloprid and 4.5 µg kg^{-1} of imidacloprid urea.[215]

Pesticide residues and bee mortality were also monitored in four apiaries for six months located in areas of intensive agriculture in Valencian Community (Spain). A total of 34 bee samples, obtained along the monitoring period, were analyzed for 58 pesticides by QuEChERS and LC–MS/MS. The organophosphates chlorpyrifos and dimethoate, as well as the neonicotinoid imidacloprid, were the most frequently detected agrochemicals. Almost 80% of the samples had chlorpyrifos, 68% dimethoate, and 32% imidacloprid. Maximum concentrations for these three compounds were 751, 403, 223 ng g^{-1} respectively. Influence of

these pesticides on acute honey bee mortality was demonstrated by comparing coincidence between death rate and concentrations of chlorpyrifos, dimethoate and imidacloprid.[217]

5.3 Tetranortriterpenoids

This compound is found in the seeds (0.2 to 0.8 percent by weight) of the neem tree, *Azadirachta indica*. Many more compounds, related to azadirachtin, are present in the seeds as well as in the leaves and the bark of the neem tree, which also show strong biological activities among various pest insects. One interesting study quantified the uptake, translocation and expression of azadirachtin residues in twigs and foliage of several tree species susceptible to attack by exotic invasive species – the Asian Longhorned Beetle – followed by stem injection with the azadirachtin-based TreeAzin™ formulation specically developed for use against wood boring insect pests. [175] The highest mean concentration of azadirachtin A (6.23 mg kg^{-1}) in foliage was observed in Norway Maple. Futhermore, the formulated product applied in the field experiments was also analyzed by LC-QTOF MS. With these analyses, we confirmed that the five unknown peaks detected in samples (peaks at m/z 699 at 8.69 and 9.43; m/z 743 at 9.56 min; and m/z 685 at 7.66 and 9.61 min) were also present in the technical product.

Azadirachtins and related compounds are very sensitive to sunlight, degrading rapidly, with half-lives of the order of 11.3 h for azadirachtin A and 5.5 h for azadirachtin B and few minutes for the other limonoids. Azadirachtin and other pesticide residues were evaluated in strawberries after model treatment in field trials conducted over 3 years. The concentration levels of insecticide residues were generally below the maximum residue limits and azadirachtin were not detectable in most of the experimental samples.[174]

Azadirachtin is a neutral triterpene and chemotherapeutic agent effective in controlling some pest flies in horses, stables, horns and fruit. Several extraction and analytical method

developed that achieve the quantitation of azadirachtin with precision and accuracy, establishing a lower limit of quantitation of azadirachtin, extracted from the biological matrix have been reported. However, these studies did not report finding of azadirachtin in treated samples.[33, 173]

5.4 Ammonium Quaternary Salts

PQ and DQ are two of most widely used herbicides in the world and toxic for humans and animals. Their presence in water has been widely monitored because both exists as cation at water pH and are very water soluble. DQ was determined in the Hun River water at the average concentration was 0.506 μg mL^{-1}.[98] Other survey on PQ and DQ reported concentration of DQ and PQ of 4.28 and 1.49 ng mL^{-1}, respectively. The other hand, different methods have been proved to determinate PQ and DQ in water samples such us simple flow injection colorimetric system, injection square wave voltammetry, gas chromatography and spectrophotometric determination.[205, 218, 219]

Vegetables are an important food category of human diet, and can also be the PQ carrier, because PQ can enter into plant cell quickly, simple washing may not remove it. PQ has been determined in four edible vegetables: cabbage, lettuce, spinach and Chinese cabbage. The results showed only in a few vegetable samples, small amounts of PQ (20 > ng g^{-1}) were detected.[100]

Regarding biological samples, chlormequat, DQ and PQ were analyzed by LC-MS/MS. The plant growth regulator clormequat was analyzed in 100 samples of 24h-human urine from non-occupationally exposed individuals in the general population in southern Sweden. All samples had detectable levels above 0.1ng mL^{-1}. The median levels were 4ng mL^{-1} of

clormequat in unadjusted urine.[220] PQ and DQ were determined in human serum. The concentration of PQ and DQ in serum ranged from 0.005 to 72 μg ml^{-1}.[221]

5.5 Dinitroanilines

Pendimethalin was analyzed in wheat, straw, soil and water to study its dissipation behaviour and the residues that remain at harvest time. Pendimethalin was not found at harvest time following single application at different rates. In soil, initial deposits of 4.069 and 10.473 mg kg^{-1} of pendimethalin persisted up to 90 days and dissipation followed first order kinetics with half-life period between 12.03 and 13.00 days. Residues of pendimethalin studied in water under laboratory conditions persisted up to 90 days.[151] Pendimethalin was also determined in river water in the towns of Turvo and Meleiro in the southern region of Santa Catarina State, Brazil showing contamination by pendimethalin at levels that ranged from 0.06 to 0.38 mg L^{-1}.[105]

Dinitroaniline herbicides could be absorbed by the soil and hydrosphere, transferred into vegetables, fruits and other agricultural products, aquatic organisms and finally concentrated in human bodies as the result of food chain enrichment.[222] Seven dinitroanilines have been determined in garlic, olive oil, scallion, leek, chili and eel. No dinitroaniline herbicides were detected except for trifluralin in eel.[222] Trifluralin was determined in carrots together with other multiclass pesticides. Trifluralin was not found in any sample.[150]

To date, many researches reported for the analysis of organophosphorus and organochlorine pesticides in milk, which had been banned for a long time, and many new pesticides usually used in feed and grass, such as trifluralin, and sulfonylurea herbicides, have not been considered. A method for determination of 29 pesticides including trifluralin in bovine milk also pointed out the absence of this pesticide.[111]

5.6 Acetamides

Acetamide herbicides have also been widely monitored in water samples. Several studies report the presence of these herbicides in water using extraction procedures based on the use of graphene carbon based solid-phases and GC-ECD. Alachlor, acetochlor, pretilachlor, butachlor, and metolachlor were determined in different types of environmental water. As a result in real samples, no herbicide residues were detected in tap or rain water samples, only acetochlor was found to be 0.12 ng mL^{-1} in reservoir water.[114] Similarly, alachlor, acetochlor, pretilachlor, butachlor and metolachlor were monitored in rain, reservoir and lake water. As a result, the rain and reservoir water samples were all free of contamination by the acetanilide herbicides. In lake water, butachlor was found to be 0.42 mg L^{-1}.[115]

However, these herbicides are fully incorporated in multiresidue schemes that involve a large number of pesticides belonging to many different families. A multi-residue method, based on GC-MS/MS, was developed for the determination of 70 organic micropollutants from various chemical classes including acetamides in surface waters. The method was employed to investigate the water quality in the basin of a transboundary river, Strymonas, in NE Greece during three sampling campaigns conducted in the year 2013. The results showed thirty-nine compounds detected in the river water. Metolachlor, diuron, isoproturon, salicylic acid, chlorfenvinphos, 1,2-benzanthracene, pyrene, diflubenzuron, and carbaryl exhibited the highest detection frequencies.[176] These pesticides have also been incorporated in the "suspected screening" or "non-target screening" methods based on high resolution mass spectrometry. In this sense, the quantification of 35 polar pesticides and 9 metabolites by UHPLC–QqTOF using passive sampler exposed in freshwater (POCIS: Polar Organic Chemical Integrative Sampler) was studied. Methazachlor and metholachlor were frequently detected in water samples at concentrations < 5 ng/L.[223]

Acetamide herbicides have been also widely monitored in other environmental matrices, such as soil or weeds. However, the environmental reported levels are low. For example, pretilachlor was determined in rice and soil samples by MSPD and CE-ECL. The two real sample stuffs were assayed under the optimized experimental conditions, and the results indicate that pretilachlor was not detected because no corresponding peak was found in two stuffs matrices.[200]

5.7 Oximes

Several analytical methods have been described for the determination of cyclohexanedione oxime herbicides and its major active metabolites.[40, 106] These herbicides are photodegrated very rapidly in both, water and soil and show a week tendency to accumulate in soil and sediment. Then, their presence in water has been very scarcely reported and most of the studies focus on sediments, soil or in their dissipation in plants.

Several sediments from lakes (Kerkini, Doirani, and Volvi)., located in northern Greece, were monitored for 253 pesticides including a high number of oximes. The samples were collected in two-time periods (fall/winter 2010 and spring/summer 2011). Main pesticides that were detected included sethoxydim and cycloxydim. [106] These findings confirm the environmental impact of these compounds.

The dissipation of clethodim and its oxidation metabolites (clethodim sulfoxide and clethodim sulphone) in soil, rape plant and rape seed were reported. The trial results showed that clethodim dissipated so rapidly that few clethodim residues were detectable. Clethodim sulfoxide dissipated quickly in rape plant and soil with half-lives of 4.3 and 4.0 days, respectively. Clethodim sulphone showed a tendency of rapid increase initially followed by a decrease in rape plant but could not be detected in soil.[40]

5.8 Triazoles

Triazoles are fungicides that have been widely monitored in the multiclass multiresidue schemes used to determine pesticides in the environment. In one of these studies, fifty-eight compounds, including herbicides, fungicides, insecticides and some of their degradation products, were surveyed to evaluate the quality of natural waters throughout the wine growing region of La Rioja (Rioja DOCa). The results revealed the presence of pesticides in most of the samples investigated. In 64% of groundwaters and 62% of surface waters, the sum of compounds detected was higher than 0.5 μg L^{-1} (the limit established by EU legislation for the sum of all pesticides detected in waters for human use). The fungicide tebuconazole was the compound most frequently detected in water samples (present in more than 60% of the samples).[224] Other study carried out in drinking water in Sao Paulo were analyzed to determine among other pesticides, difenoconazole, epoxiconazole, tebuconazole, which are approved for use in Brazilian crops. For drinking water, three of the twelve pesticides (tebuconazole, atrazine and carbendazim) were determined in concentrations from 4 to 87 ng L^{-1}. [225]

Other group of studies focus on the enantiomeric differences of triazole fungicides. The enantioselective degradation and accumulation has been widely studied in environmental matrices. Tebuconazole enantiomers were studied in water and zebrafish using supercritical fluid chromatography (SFC)-MS/MS. (+)-S-Tebuconazole and (−)-R-tebuconazole in the water and zebrafish samples were detected after 24 h at concentrations of 0.33 ± 0.05 and 0.35 ± 0.04 mg kg^{-1} and 3.67 ± 0.46 and 3.06 ± 0.42 mg kg^{-1}, respectively.[226] These results pointed out the selective bioacculation of these compounds that tend to favor the S isomer.

Enantiomeric triazole fungicides (tetraconazole, fenbuconazole, epoxiconazole, diniconazole, hexaconazole, triadimefon, paclobutrazol, and myclobutanil) were analyzed in soil and water

using chiral LC-MS/MS. Water samples were from the Jingmi Irrigation Canal in Beijing (China). Results showed 3, 2, and 3 soil samples containing enantiomers of fenbuconazole, myclobutanil and triadimefon in the range 10.49 - 23.54, 12.53 - 18.56, and 8.79 - 17.16 μg kg^{-1}, respectively. The enantiomers were not detected in real water samples using the proposed method. In addition, it was observed that the enantiomer fractions (EF) of fenbuconazole, myclobutanil and triadimefon were ranged from 0.517 to 0.531, 0.501 to 0.511, and 0.445 to 0.480, respectively, indicating that dissipation and of fenbuconazole, myclobutanil and triadimefon may be enantioselective in the soil.[124]

Contrarily, myclobutanil, hexaconazole, diniconazole, epoxiconazole and tetraconazole enantiomers were determined in soil and earthworms. Hexaconzole and diniconazole might have the bioaccumulation potential, whereas tetraconazole, epoxiconazole and myclobutanil were most likely to undergo enantioselective biotransformation. On the contrary, the degradation of the triazoles in soil was achiral, and less than 10% of them were degraded after 21 days.[185]

6 Conclusions

The trace determination of miscellaneous pesticides and related compounds continues to be a topic for analytical chemists. Many of these pesticides are now integrated into the common multiclass, multiresidue schemes that make possible the analysis of large number of pesticides. However, there is no universally accepted analytical method for pesticide residues analysis today. New generation pesticides belong to many chemical classes and the analytical methodologies need to be applicable to many food and environmental matrices. Therefore, there is still a need for single-residue methods for the analysis of a these pesticides and their by-products. Methods based on conventional solvent extraction are still the routine procedure in many laboratories to extract pesticides from solid samples. The extraction of these

pesticides from complex matrices is demanding. Extraction techniques such as MAE, SFE, and PLE that are aided mostly of pressure and/or temperature to improve extraction efficiency are widely used.

The extraction of liquid samples and clean-up of the extract obtained by solvent extraction are mostly carried out by conventional SPE. The trend towards the use of methods that save time and reduce sample size and the quantity of organic solvents or other reagents in order to miniaturize the extraction process is still one of the pending issues. Few methods are based on SPME or SBSE because of they are not optimized for polar compounds (as most of these new generation pesticides are). However, within the miniaturized techniques LLME has had an important development in a wide variety of designs and formats. Very hot topics within analytical techniques as ionic liquids and its strong versatility will increase irreversible it use.

The most widely used detection technique for the determination of pesticides including these miscellaneous ones is MS combined with GC or LC. The selection of one or other chromatographic technique depends on the characteristics of the pesticides. Volatile and thermostable pesticides are ideal for GC whereas polar and ionic compounds perform better by LC. However, derivatization of the analytes to get a reaction product more appropriate for the determination technique is still in use and evolving. The triple quadrupole (QqQ) is mass analyzers most commonly used with GC and LC field because its sensitivity achieves the compliance with the strict maximum residue limits established for these miscellaneous pesticides and its specificity attains identification of the compounds. HRMS are gaining acceptance for suspected screening and unknown identification, but their use in this field of new generation pesticides is not generalized yet. However, the recently defined untarget, suspected screening, retrospective analysis are undoubtedly a major future trend in pesticide residues where HRMS plays a significant role. All evidence points towards future growth in

the number of applications of HRMS in pesticide residue analysis, as the power of this technique gains wider recognition. Other alternative techniques with good prospects for food and environmental monitoring of these new generation pesticides are biosensors. The use of biosensors the food and environmental field is still limited probably because concentrations of pesticide residues are low.

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Table 1. Class of Compounds, Typical Examples, and Structures Included as Miscellaneous Compounds

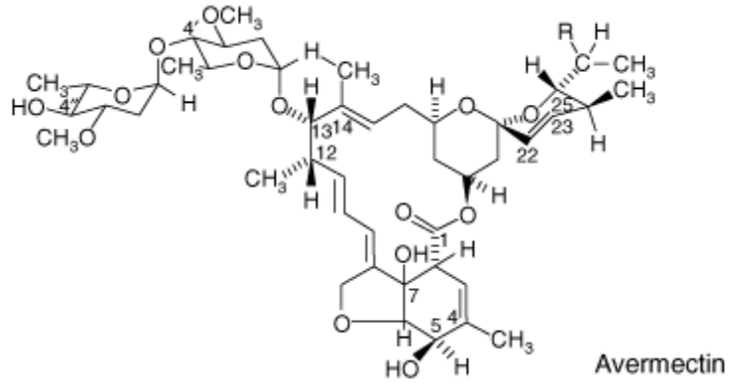
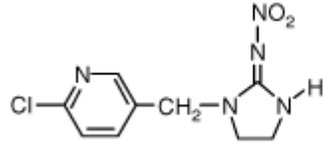
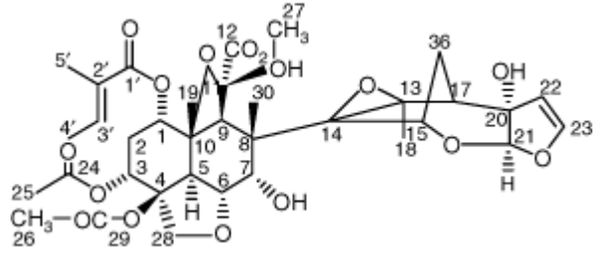
Class	Typical examples	Chemical structure
Macrocyclic lactone	Avermectin Ivermectin Emamectin	 Avermectin
Chloronicotinyl	Imidacloprid Acetamiprid	 Imidacloprid
Tetranortriterpenoid	Azadirachtin	

Table 1. Class of Compounds, Typical Examples, and Structures Included as Miscellaneous Compounds

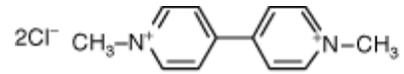
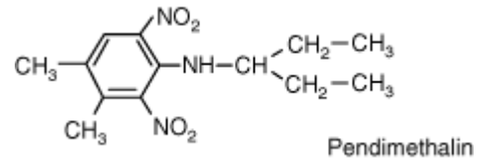
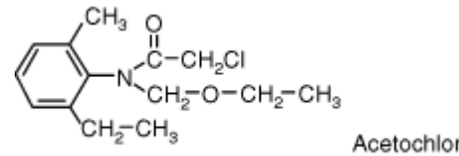
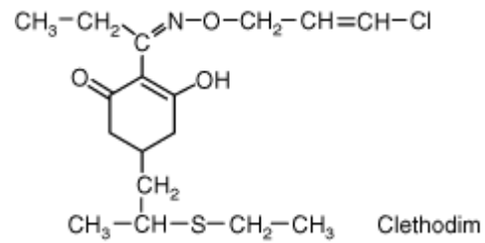
Class	Typical examples	Chemical structure
Ammoniumquaternary salt	DQ, PQ, DF, CQ and MQ	 Paraquat
Dinitroaniline	Butralin Dinitramine Ethalfluralin Oryzalin Pendimethalin Trifluralin	 Pendimethalin
Acetamide	Alachlor Acetochlor Metalaxyl Metolachlor	 Acetochlor
Oxime	Alloxydim Clethodim Sethoxydim	 Clethodim

Table 1. Class of Compounds, Typical Examples, and Structures Included as Miscellaneous Compounds

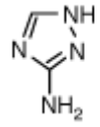
Class	Typical examples	Chemical structure
Triazole	Amitrole Carfentrazone ethyl Fenchlorazol ethyl Flupoxam	 Amitrole

Table 2. Physical and Chemical Properties of some Miscellaneous Compounds

CAS number	Pesticide	MW	Appearance	Mp (°C)	Vapor pressure (20–25°C)	Solubility				LogP_{ow}
						Water (mg L^{-1})	Toluene (g L^{-1})	Acetone (g L^{-1})	Methanol (g L^{-1})	
71751-41-2	Avermectin	873.11	White solid	150–155	1.5 mPa	<0.005	350	100	19.5	3.99
137512-74-4	Emamectin	1008.26	White solid	141–146	4 µPa	300	26	Soluble	Soluble	5
138261-41-3	Imidacloprid	255.66	Pale solid	143.8	0.2 µPa	510	0.5	50	10	0.57
	Acetamiprid	222.68	Pale solid	98.9	<1 µPa	4250	—	Soluble	Soluble	0.8
11141-17-6	Azadirachtin	720.7	Yellow solid	180	—	50	—	—	—	—
2764-72-9 85-00-7 6385-62-2	DQ	344.06	Pale yellow solid	>300	<13 µPa	700 000	Insoluble	—	Slightly soluble	−4.6
4685-14-7 1910-42-5	PQ	257.2	White solid	300	<133 nPa	700 000	Insoluble	—	Slightly soluble	
43222-58-6	DF	360.44	Pale liquid	155–157	13.33 µPa	765 000	Insoluble	9.8	620	0.2838
999-81-5	CQ	158.07	Pale yellow liquid	245	<10 µPa	>1 kg kg^{-1}	—	0.3	—	−1.58
24307-26-5	MQ	149.7	White solid	285	<10 µPa	>1 kg kg^{-1}	—	20	—	−2.82
33629-47-9	Butralin	295.3	Orange solid	60–61/134–136	1.7 mPa	1	—	—	—	—
29091-05-2	Dinitramine	332.2	Yellow solid	98–99/>200	479 µPa	1	—	1040	—	
55283-68-6	Ethalfluralin	333.26	Orange solid	57–59	110 µPa	0.3	>500	>500	82–100	—
19044-88-3	Oryzalin	346.36	Orange solid	141–142	13.3 µPa	2.5	Insoluble	>500	50	
40487-42-1	Pendimethalin	281.31	Yellow solid	54–58/330	4 mPa	0.275	—	700–800	—	5.18
1582-09-8	Trifluralin	335.28	Orange solid	48–49/ 96–97	140 µPa	<1	Soluble	400	—	5.07
15972-60-8	Alachlor	269.77	White solid	41/100	2 mPa	170	Soluble	—	—	—
34256-82-1	Acetochlor	269.77	Liquid	<0	45.3 nPa	223	Soluble	Soluble	—	2.47
57827-19-1	Metalaxyl	279.34	Pale solid	71–72	293 µPa	7100	9.1	—	650	1.75
51218-45-2	Metolachlor	283.8	Oily liquid	282	1.7 mPa	530	Soluble	Soluble	Soluble	3.45
66003-55-2	Alloxydim-sodium	345.5	White solid	185.5	133 µPa	2 000 000	—	14	619	0.63

99129-21-2	Clethodim	359.92	Clear liquid	—	13 µPa	—	Soluble	Soluble	Soluble	4.18
74051-80-2	Sethoxydim	327.5	Oily liquid	90	133.8 µPa	4700	Soluble	Soluble	Soluble	4.51
61-82-5	Amitrole	84.08	Pale solid	157–159	<1 mPa	280 000	Insoluble	Insoluble	Moderately soluble	0.85
103112-35-2	Fenchlorazole-ethyl	403.48	Pale yellow solid	114–116	890 nPa	0.9	270	360	27	3.998
122836-35-5	Sulfentrazone	387.19	Dark brown solid	75–78	133.28 nPa	0.11	—	Slightly soluble	Slightly soluble	—
1698-60-8	Chloridazon	221.65	Pale solid	205–206	<10 µPa	400	—	28	34	1.18
117718-60-2	Thiazopyr	396.4	Brown solid	77.3–79	266 µPa	2.5	—	—	287	3.89
27314-13-2	Norflurazon	303.67	White solid	174–180	2.7 µPa	28	—	50	—	2.4–2.5
59756-60-4	Fluoridone	329.3	White solid	151–155	<13.33 µPa	12	—	—	10–20	1.87
55512-33-9	Pyridate	378.9	Brown solid	20–25	133.28 nPa	Insoluble	Soluble	Soluble	Soluble	3.01

Table 3. Toxicologic Characteristics of Miscellaneous Pesticides

Pesticide	Oral LD_{50} rat (mg kg^{-1})	Oral LD_{50} mice (mg kg^{-1})	Dermal LD_{50} rabbit (mg kg^{-1})	Chronic toxicity NOEL in dog	ADI (mg kg^{-1})
Abamectin	10.6–11.3	13.6	>2000	0.25 mg kg^{-1} day^{-1}	0.0025
Emamectin	70	—	>2000	—	—
Imidacloprid	410–440	98–100	Non irritant	500 mg kg^{-1} diet	0.057
Acetamiprid	146–217	184–198	Non irritant	—	—
Azadirachtin	>5000	—	>5000	—	—
DQ	215–235	125	>400	50 mg kg^{-1} diet	0.008
PQ	100–150	104	Irritant	15 mg kg^{-1} diet	0.004
DF	270–470	31–44	470	2500 mg kg^{-1}	0.1
CQ	807–966	—	>2000	—	0.05
MQ	1490	—	Non irritant	—	0.05
Butralin	12 600	—	10 200	—	0.015
Dinitramine	3000	—	>6800	2000 mg kg^{-1} diet	0.05

Table 3. Toxicologic Characteristics of Miscellaneous Pesticides

Pesticide	Oral LD_{50} rat (mg kg^{-1})	Oral LD_{50} mice (mg kg^{-1})	Dermal LD_{50} rabbit (mg kg^{-1})	Chronic toxicity NOEL in dog	ADI (mg kg^{-1})
Ethalfluralin	10 000	10 000	>2000	5000 mg kg^{-1} day^{-1}	0.05
Oryzalin	>10 000	>10 000	>2000	—	0.15
Pendimethalin	1050–1250	1340–1620	>5000	12.5 mg kg^{-1} day^{-1}	0.0125
Trifluralin	>10 000	500	>2000	1000 mg kg^{-1} day^{-1}	0.25
Alachlor	930–1350	—	>2000	200 mg kg^{-1} diet	0.005
Acetochlor	2148	—	4166	12 mg kg^{-1} day^{-1}	0.01
Metalaxyl	669	788	>6000	62.5 mg kg^{-1} day^{-1}	0.03
Metolachlor	2780	894	>10 000	2.5 mg kg^{-1} day^{-1}	0.015
Alloxydim	2260–2322	3000–3200	>5000	40 mg kg^{-1} day^{-1}	0.125
Clethodim	1360–1630	—	>5000	—	0.01
Sethoxydim	3200–3500	5600–6300	—	—	0.15
Amitrole	1100–24 600	11 000	10 000	—	0.000 03
Carfentrazone	5143	—	—	—	—

Table 3. Toxicologic Characteristics of Miscellaneous Pesticides

Pesticide	Oral LD_{50} rat (mg kg^{-1})	Oral LD_{50} mice (mg kg^{-1})	Dermal LD_{50} rabbit (mg kg^{-1})	Chronic toxicity NOEL in dog	ADI (mg kg^{-1})
ethyl					
Fenchlorazol ethyl	>5000	>2000	>2000	4.5–5.4 mg kg^{-1} day^{-1}	—
Flupoxam	>5000	—	>5000	—	—
Sulfentrazone	2855	—	>2000	—	—
Chloridazon	1100–3830	2500	>2500	—	0.075
Thiazopyr	>5000	—	>5000	—	0.0025
Norfluorazon	8400	—	>20 000	150 mg kg^{-1} day^{-1}	0.0038
Fluridone	>10 000	>10 000	>500	150 mg kg^{-1} day^{-1}	0.08
Pyridate	1800–2100	>10 000	>10 000	7.9 mg kg^{-1} day^{-1}	0.04

NOEL, no observable effect level; ADI, admissible daily intake.

Table 4. Miscellaneous Pesticides Tolerance in Fruit and Vegetables		
Pesticide	**Residue**	**MRL (mg kg^{-1})**
Abamectin	Total toxic residue includes parent abamectin and its δ 8,9-isomers	Fruits and vegetables 0.01–1
		Animal products 0.005–0.02
Spinosad	Sum of spinosad A and spinosad B expressed as Spinosad	Fruits and vegetables 0.03–1
		Animal products 0.02–1
Acetamiprid	Acetamiprid	Fruits and vegetables 0.01–5
		Animal products 0.05–0.2
Imidachloprid	Sum of imidacloprid and its major metabolite 6-chloronicotinic acid expressed as 6-chloronicotinic acid.	Fruits and vegetables 0.05–10Animal products 0.05
Thiachloprid	Thiachloprid	Fruits and vegetables 0.02–3
		Animal products 0.01–0.3
Thiamethoxan	Sum of thiamethoxan and chlorthianidin expressed as thiametoxam	Fruits and vegetables 0.05–0.5
		Animal products 0.01–0.02

Azadirachtin	Azadirachtin	Fruits and vegetables 0.01–1
		Animal products 0.01
DQ	DQ	Fruits and vegetables 0.05
		Animal products 0.05
PQ	PQ	Fruits and vegetables 0.02–0.05
CQ	CQ	Fruits and vegetables 0.01–10
		Animal products 0.05
MQ	MQ	Fruits and vegetables 0.05–0.3
		Animal products 0.05–0.2
Butralin	Butralin	Fruits and vegetables 0.02
		Animal products 0.02
Ethalfluralin	Ethalfluralin	Fruits and vegetables 0.02–0.1
		Animal products 0.01
Oryzalin	Oryzalin	Fruits and vegetables 0.01–0.05
Pendimethalin	Pendimethalin	Fruits and vegetables 0.05
		Animal products 0.05
Trifluralin	Trifluralin	Fruits and vegetables 0.1
		Animal products 0.01

Acetochlor	Acetochlor	Fruits and vegetables 0.01–0.02
		Animal products 0.01
Alachlor	Alachlor	Fruits and vegetables 0.05–0.1
		Animal products 0.01
Metolachlor	Metholachlor including other mixtures of constituents isomers including S-metholachlor (sum of isomers)	Fruits and vegetables 0.05–0.1Animal products 0.05–0.1
Clethodim	Sum of Sethoxydim and Chletodim including degradation products calculated as Sethoxydim	Fruits and vegetables 0.1–1Animal products 0.05–0.2
Tepraloxydim	Tepraloxydim	Fruits and vegetables 0.1
		Animal products 0.1
Amitrole	Amitrole	Fruits and vegetables 0.01
		Animal products 0.01
Cyrproconazole	Cyproconazole	Fruits and vegetables 0.05–10
		Animal products 0.05
Tebuconazole	Tebuconazole	Fruits and vegetables 0.05–5

		Animal products 0.05–0.1
Tetraconazole	Tetraconazole	Fruits and vegetables 0.02–10
		Animal products 0.05–1
Pyridate	Pyridate (sum of pyridate, its hydrolysis product CL 9673 (6-chloro-4-hydroxy-3-phenylpyridazin) and hydrolysable conjugates of CL 9673 expressed as pyridate)	Fruits and vegetables 0.05Animal products 0.05

Table 5. Extraction techniques employed for miscellaneous pesticides and related compounds

Macrocyclic lactone	Extraction Method	Samples	Ref.
Abamectin	HLLME-FA	Water	79
Abamectin, doramectin, Ivermectin and moxidectin	HF-LPME	Water	76
Abamectin, emamectin benzoate, doramectin, epinomectin, ivermectin, selamectin and moxidectin	SPE	Sea water and milk	168, 227
Abamectin, Emamectin Benzoate, Ivermectin, Eprinomectin and Doramectin	SLE-SPE	Food, alfalfa plants, vegetables, soil, pork muscle, pork liver and fish, dried hops and cattle faeces	166, 167, 227-229
Emamectin benzoate	ASE	Marine sediment and tissue	168
Abamectin, doramectin, ivermectin and moxidectin	DSPE	Fish, soil and water	86
Abamecting, emamectin and benzoato	PLE followed by SPE clean-up	Marine sediment and fish tissue	168
Abamectin, doramectin, emamectin benzoato, epinomectin, ivermectin and moxidectin	QuEChERS	Meat, milk, yogurt, cheese, fruits, cottage cheese, cream and curd	83, 84, 164, 169, 230

Macrocyclic lactone	Extraction Method	Samples	Ref.
Abamectin, doramectin, emamectin benzoate, epinomectin, ivermectin and moxidectin	SLE	Butter, edible oils, bovine muscle, liver, eggs, foodstuffs, fruit, vegetables, muscle of largemouth bass, feeds and horse meat	24, 87, 165, 231, 232 140
Abamectin	USA-SPE	Citrus fruits	162
Abamectin and doramectin, Ivermectin and moxidectin	SFE	Soil	78
Abamectin, epinomectin and ivermectin	DLLME	Milk	85
Abamectin, doramectin, emamectin benzoato, epinomectin and ivermectin	LLE	Milk	187
Abamectin	SLE-SPME	Mangoes	80

Chloronicotinyl	Extraction Method	Samples	Ref.
6-Chloronicotinic, acetamiprid, imidachloprid, thiametoxam, clothianidin and thiacloprid	QuEChERS	Soil, lettuce, beebread, cucumber, eggplant, spinach, nutraceutical products, apples, honey, tea, tomatoes, baby foods, honey liqueur, bees, beebread, pollen, high-fructose corn syrup, peppers, spinach, dead honey bees, beehive, coconut tree trunks, dried tea, mulberry leaves, bamboo shoot, mango, pomegranate, soybeans, pulses, olives, olive oil, melon, bumblebees, vegetables, swiss chard grown, baby foods, cereals and fruits	170, 214, 234-245

Chloronicotinyl	Extraction Method	Samples	Ref.
6-Chloronicotinic, acetamiprid, thiamethoxam, imidacloprid and thiacloprid	SPE	Water	88, 212
Thiamethoxam, imidacloprid, clothianidin, acetamiprid, and thiacloprid	SLE-SPE	Tea	246
Thiamethoxam, clothianidin imidacloprid, acetamiprid and thiacloprid	SPE-DLLME	Honey	247
Acetamiprid, clothianidin, imidacloprid, thiacloprid and thiamethoxam	Diatomaceous earth-assisted extraction	Human serum and urine	248
6-Chloronicotinic and imidacloprid	MSPD	Soil, fruit of guava tree and rice	88, 249, 250
Imidacloprid, acetamiprid and thiacloprid and thiamethoxam	SLE-DSPE	Tomato and cucumber samples and soil	112
Thiametoxam, clothianidin, imidacloprid, acetamiprid and thiacloprid	DLLME	Honey liqueur, honey and cucumber	216
Imidacloprid	SDLLME-SFO	Apple and pear	251
Acetamiprid, thiametoxam, clothianidin, imidacloprid, acetamiprid and thiacloprid	QuEChERS-DLLME	Tomatoes, honey and honey liqueur	240

Chloronicotinyl	Extraction Method	Samples	Ref.
Acetamiprid, imidacloprid, thiacloprid, and thiamethoxam	DSPE–DLLME	Tomato and cucumber	92
Thiametoxam, clothianidin, imidacloprid, acetamiprid and thiacloprid	SLE-DLLME	Soil	112
Acetamiprid, imidacloprid, thiacloprid and thiamethoxam	MSPE	Lemon juice, water, pear and tomato	89, 252 253
Acetamiprid, imidacloprid, clothianidin, thiametoxam and thiacloprid	SLE	Grapes, apples, honey bee, honey, chieh-qua, sugarcane, soil, lettuce, tomatoes, beebread, wheat, tea, cucumbers, tomatoes, cabbage, water, bee pollen, beeswax, vegetable, water, postmortem human blood, liver, stomach, dried dendrobium officinale, tap water and river water	215, 254-256 93, 172, 257-262 246, 263 142, 264, 265 188
Acetamiprid and imidacloprid	IL-VALLME	Wastewater	90
Acetamiprid, clothianidin, imidacloprid, and thiamethoxam,	VSLLME-SFO	Fruit juice and water,	266
Acetamiprid, clothianidin, imidacloprid, thiacloprid and thiamethoxam	LLE	Beeswax	267
Thiamethoxam, imidacloprid, acetamiprid and thiacloprid	WBE	Cucumber and eggplant	268

Chloronicotinyl	Extraction Method	Samples	Ref.
Acetamiprid, clothianidin, thiamethoxam, imidacloprid and thiacloprid	DLLME	Honey	269
Thiamethoxam, imidacloprid, clothianidin, acetamiprid and thiacloprid	SWE	Eels	95
Imidacloprid, acetamiprid and thiacloprid	PSE	Particle phase atmospheric	270
Imidacloprid	I-IL Au NPs	Water	271
Imidacloprid	Soxhlet extraction	Soil	272
Imidacloprid and thiacloprid	Membrane assisted solvent extraction	Wine	273
Imidacloprid, acetamiprid, thiacloprid and thiamethoxam	In-coupled syringe assisted octanol–water partition microextraction	Honey	274
Thiamethoxam	SLE - LTP	Pineapple	141
Thiamethoxam	MAE	Vegetable and soil	275

Tetranortriterpenoid	Extraction Method	Samples	Ref.
Azadirachtin	SLE	Long horned beetle and fish	96

Tetranortriterpenoid	Extraction Method	Samples	Ref.
Azadirachtin	QuEChERS	Strawberries, foliage and phloem	174
Azadirachtin	LLE – SPE	Bovine muscle	33

Ammonium quternary salt	Extraction Method	Samples	Ref.
DQ, CQ and PQ	SLE	Cabbage, lettuce, spinach and chinnese cabbage, water, rice and cabbage, human serum and blood, vegetables, cereal, tomatoes, apples, fruits, grapes, cotton crops and soil, wheat, barley, potato and apples	221 276-279 277
DQ and PQ	SPME	Water, plasma and urine	98, 103
PQ	Acetic acid	Marijuana	201
PQ, DF and CQ	SLE-SPE	Water, urine, suface water, drinking water, citruses, baby juices, cotton crops and soil	97, 99, 220, 280-282
PQ	Methanol, water and hydrochloric acid	Potato, cereals and pulses	101
CQ and MQ	QuEChERS	Pears and potatoes and strawberries	59, 283
CQ and MQ	DSPE	Meat	102

Dinitroaniline	Extraction Method	Samples	Ref.
Ethalfluralin, oryzalin and trifluoralin	Soxhlet Extraction	Air	147

Dinitroaniline	Extraction Method	Samples	Ref.
Ethalfluralin and trifluralin	Ultrasonic-assisted extraction-isooctane	Gaseous and particulate phases in the atmosphere	149
Oryzalin, trifluralin and pendimethalin	Thermal-desorption - SPME	Atmospheric samples	284
Pendimethalin	SPME	water, soil and plant products	285
Ethalfluralin and pendimethalin	SPE	Water	104
Pendimethalin and trifluoralin	QuEChERS	Peanut, garlic, olive oil, scallion, leek and chili, bulb vegetables and traditional chinese medicine	110, 118, 144, 145
Trifluralin	SLE/LTP	soil	143
Trifluralin	PLE - MSPD	Carrots	150
Butralin, pendimethalin and Benflurain	SLSDE-DLLME	Tobacco	109
Pendimethalin	MAE	Sediment, soil and garlic	106, 107
Trifluoralin	MSPD	Milk	146
Trifluoralin	Acetonitrile-C18-SPE	Bovine milk	111

Acetamides	Extraction Method	Samples	Ref.
Acetochlor and metolachlor	SLE-MISPE	Soil	116
Acetochlor and alachlor	PSL	Soil, sediment and sludges	178

Acetamides	Extraction Method	Samples	Ref.
Acetochlor, alachlor and metolachlor	QuEChERS	Soil, sediment, sludges, eel and shrimp, white mustard, bulb and vegetables	178 118, 286, 287
Acetochlor, alachlor, metolachlor and pretilachlor	SPME	Water	115
Acetochlor, alachlor, metolachlor and pretilachlor	SLE-SPME	Soil and plant products	285
Acetochlor, alachlor, metolachlor and pretilachlor	SLE-SPE	Soil, corn and tobacco	62, 153, 288 152, 289 289
Acetochlor, alachlor, metolachlor and pretilachlor	MSPE	Water	161
Acetochlor, alachlor, metolachlor and pretilachlor	SPE	water	176 152
Acetochlor, alachlor and butachlor	SLE	Soybeans, pepper, peppers leaf, sediment and wastewater, water, soil and rice	290
Alachlor	DLLE-SFO	Aqueous samples	179
Alachlor and pretilachlor	MSPD	Water, eggs, soil and rice	291 200
Alachlor	DLLME	Aqueous samples	180
Alachlor	LLE	Wastewater	154
Alachlor and metolachlor	On-line SPE	Groundwater	183
Butachlor	μ-SPE	Aqueous samples	292

Acetamides	Extraction Method	Samples	Ref.
Butachlor	MHLLE	Milk	119
Metolachlor	Soxhlet Extraction	Air	147
Metolachlor	HF-LPME	Cucumber	293
Alachlor, acetochlor, pretilachlor, butachlor and metolachlor	MSPE	Water and grapes	114
Metolachlor	ASE-SPE	Cereal crops	294
Pretilachlor	SDME	Water	295

Cilohexanedione oxime	Extraction Method	Samples	Ref.
Clethodim, clethodim sulfoxide and clethodim sulfone	QuEChERS	Soil, rape plant and rape seed	40
Sethoxydim	MAE	Sediment	106

Triazole	Extraction Method	Samples	Ref.
Amitrole, cyproconazole, fenbuconazole, hexconazole and propiconazole	SPE	Water, wine and drinking water and well waters	106 296

Fluconazole, thiabendazole, difenoconazole, hexaconazole, tebuconazole, cyproconazole, prochloraz, tetraconazole, propiconazole and triticonazole	USA-dSPE	Sewage sludge	297

Triazole	**Extraction Method**	**Samples**	**Ref.**
Tebuconazole	MISPE	Vegetable and juice samples	184
Cyproconazole, hexaconazole, penconazole, myclobutanil, propiconazole, tebuconazole and myclobutanil	SLE	Wood extracts, tea, soil, earthworns, baby foods, cucumber, grapes, gherkin, apples, watermelon, wine, garlic and water	159
Cyproconazole, fenbuconazole, hexaconazole, myclobutanil, penconazole, propiconazole and tebuconazole,	QuEChERS	Tomato, soil, foods, water, aquatic products, jujube, strawberries, soil, tomato, milk, olive oils, grapes, chicken, meat, egg, pork, pig liver, leaf lard, pig kidney, vegetation and biota samples, beehive, mango, zebrafish, pollen, single bumbles, tomato, canned and peach	156 123, 124, 157, 186, 287, 298 124, 156, 159, 226, 298-304
Tebuconazole, propiconazole and penconazole	MAE	Water, grape, sediment and vegetables	106, 127, 159, 305
Hexaconazole, myclobutanil and tebuconazole	SLE-MSPE	Water, cucumber, cabbage and tomato	161
Penconazole and tebuconazole	DSPE-DLLME	Tea	306
Penconazole and tebuconazole	DLLME	Honey, water and wine	307

Myclobutanil, penconazole and tebuconazole	SLE-SPME	Liquid fruit extract and wine	308, 309

Triazole	**Extraction Method**	**Samples**	**Ref.**
Tebuconazole, myclobutanil and buprofezin	Thermal desorption-SPME	Atmospheric samples	158
Hexaconazole, myclobutanil and penconazole	UETC - IL-DLLME	Rat blood and water	310, 311
Penconazole, hexaconazole, diniconazole and tebuconazole	AALLME	Aqueous samples and edible oils	126
Tebuconazole	SLE-HF-LPME	Cucumber	293
Hexaconazole, penconazole and tebuconazole	HF-LPME	Water, tap water and grape juice	121
Myclobutanil, penconazole and tebuconazole	USAEME	Fruit juice and water	312, 313
Penconazole and tebuconazole	DSPE	Tea and Jujube	157, 306
Penconazole	MSPD	Grape	159
Tebuconazole	PLE	Grape	125
Tebuconazole	UDSA-DLLME	Wine	314
Penconazole	SFODME	Water	315

Triazole	Extraction Method	Samples	Ref.
Tebuconazole	USAEME-SFO	Liquid samples	313
Myclobutanil	ATD	Atmospheric samples	158
Myclobutanil and penconazole	BaμE-LD/LVI	Aqueous samples and wine	316

Table 6. Determination techniques employed for miscellaneous pesticides and related compounds

Macrocyclic lactone	Analytical technique	Samples	Refs.
Abamectin, doramectin, emamectin benzoate, eprinomectin, ivermectin, moxidectin and spinosad	LC-UV/LC-DAD	Water, citrus fruits, milk, mangoes, sediment, fish, cabbage, muscle of largemouth bass, sheep milk, pure powder, tablets, olives, olive oil	76, 78-80, 85-87, 164, 165, 167-169, 233, 317-321
Abamectin, doramectin, emamectin benzoate, eprinomectin, ivermectin and moxidectin,	LC-FL	Butter, milk and yogurt, edible oils, soil, vegetables and fruit, muscle, eggs, Bovine muscle, bovine milk, liver, muscle, foodstuffs, liver and cattle faeces	24, 79, 84, 87, 163, 227-232, 322-324
Abamectin, doramectin, emamectin benzoate, eprinomectin, ivermectin, moxidectin, selamectin and spinosad	LC-MS/MS	Goat and sheep milk, edible oils, vegetable, fish, soil, water, sediment, meat. cheese, liver, muscle, fruits, dried hops, meat, milk, yoghurt, cottage cheese, cream cheese, and curd, fish, soil, water, food, bovine muscle, sea water, marine sediment, tissue, cabagge, alfalfa plants, pork muscle, pork liver, fish, milk, honey, peppers	78, 81-83, 86, 87, 164-169, 211, 227, 230, 231, 244, 325-328
Spinosad	LC-MS	Bee pollen and beeswax	329
Eprinomectin, ivermectin and moxidectin	GC-MS	Horse meat	140
Ivermectin, doramectin, emamectin benzoate, eprinomectin and ivermectin	ELISA	Milk	187

Chloronicotinyl	Analytical technique	Samples	Refs.
6-Chloronicotinic acid (metabolite of imidachloprid), acetamiprid, imidacloprid, thiacloprid and thiamethoxam	LC-UV/LC-DAD	Soil, cucumber, eggplant, spinach, vegetables, honey, postmortem human blood, liver, stomach, wastewater, bees, lattuce, honey, fruit juice, water, tamatoes, straw mushroom, oyster mushroom, tea	54, 89, 90, 92, 94, 112, 142, 196, 212, 216, 234, 242, 243, 246, 249, 251-253, 258-262, 266, 268, 274, 275, 321, 330-333 88, 112, 334-337
		mulberry leaves, tea leaves, pear, tomato, sugarcane, apple, peanut milk, sugarcane leaves, lemon juice, fruit of guava tree, wheat, mango, pomegranate, edible fungi, olives, olive oil, sourcsesop exotic fruit,	
Imidacloprid and thiamethoxam	LC-FL	Water, vegetables	196, 338
6-Chloronicotinic acid (metabolite of imidachloprid), acetamiprid, imidachloprid, thiacloprid and thiamethoxam	LC-MS/MS	Beebread, dendrobium officinale, apples, beeswax, tea, honey bee, honey, baby foods, human serum, urine, honey liqueur, eels, wine, grapes, beebread¸ pollen high-fructose corn syrup, chesmut, shallot, ginger and tea, zucchini, peppers, nutraceutical products, tomatoes, pear, lemon, orange, cucumber, eggplant, potatoes, dead honey bees, , particle phase atmospheric, beehive, beeswax, chieh-qua, coconut tree trunks, pollen, cabbage, dried tea, bamboo shoot, soybeans, pulses, wine, rice, zebrafish embryos, melon, bumblebees, shallot, swiss chard grown, baby foods	172, 255, 339, 340 92, 95, 170-172, 213-215, 217, 235-238, 240, 241, 243-246, 248, 250, 254, 256, 257, 263, 265, 267, 269, 270, 273, 302, 328, 339-351
6-Chloronicotinic acid (metabolite of imidachloprid) and imidachloprid	GC-MS/MS	Lattuce, Pu-erh tea, tea leaves, water,	93, 142, 332, 352

Chloronicotinyl	Analytical technique	Samples	Refs.
Acetamiprid and thiamethoxam	GC-MS	Human blood, liver and stomach, water	94, 142
Acetamiprid	GC-ECD	Apples, pineaple,	141, 255
Imidachloprid and thiamethoxam	ELISA	Tap water, pond water, grape, cucumber, soil, river water, cabbage, Juveline chinook, tomatoes,	188, 189, 264, 353
Acetamiprid	Raman spectroscopy combined with chemometric	Apples	354
Acetamiprid	SERS	Fruits and vegetables	355
Tetranortriterpenoid			
Azadirachtin	LC-UV	Bovine muscle,	33
Azadirachtin	LC-MS/MS	Longhorned beetle, strawberries, fish, foliage and phloem,	96, 173-175
Azadirachtin	GC-MS	Strawberries	174
Ammonium quaternary salt	**Analytical technique**	**Samples**	**Refs.**
PQ	LC-UV	Whole blood	356
DQ, PQ, CQ and MQ	LC-MS/MS	Cabbage, lettuce, spinach, chinnese, drinking water, potato, cereals, pulses, urine, surface water, tomatoes, fruits, grapes, cotton crops, soil, pear cotton crops, apples,	59, 60, 99-102, 276-281, 283, 357, 358

Ammonium quaternary salt	Analytical technique	Samples	Refs.
DQ and PQ	LC-TOF-MS	Human serum	221
DQ and PQ	GC/MS	Water, plasma and urine,	98, 103
DQ and PQ	SWV	Tomatoes, olive oil, olives, potato, sugar cane, lemon, orange, tangerine, pineapple juice, water, tomato, Human saliva, citric fruit, water, olive oil and olives,	205-208, 359-361
DQ and PQ	Pectrofluorophotometer-UV	Water, rice and cabbage, agricultural products, urine, citruses, baby juices,	198, 282, 362, 363
PQ	Spectroscopy	Water	97
PQ	Flow injection colorimetric system	Water	218
DQ	Spectrophotometric	Drinking water	219
DQ and PQ	CE-DAD	Oral fluid, plasma, urine, marijuana	201, 202
PQ	ELISA	Wheat, barley and potato,	190
PQ	EMIS	Potato	193
DQ, DF, MQ and CQ	HPTLC	Water	364
Butralin	**Analytical technique**	**Samples**	**Refs.**
Pendimethalin	LC-UV	Soil	107
Ethalfluoralin, pendimethalin, fluchloralin and trifluralin	LC-MS/MS	Water, air, peanut, sediment, soil, garlic and bovine milk,	106, 110, 111, 147, 365

Butralin	Analytical technique	Samples	Refs.
Butralin, pendimethalin, ethalfluoralin, oryzalin, pendimethalin and trifluralin	GC-MS	Water, gaseous, particulate phases in the atmosphere, atmospheric samples, wheat, straw, soil and water, garlic, olive oil, scallion, leek and chili, tobacco, bulb vegetables, soil, traditional chinese medicine, milk, water, strawberry, plant products and carrots	104, 118, 144-150, 152, 284, 285, 365, 366
Butralin, pendimethalin	GC-MS/MS	Tobacco	109
Pendimethalin	GC-ECD	Wheat, strawberry, soil and water	105, 148, 151
Butralin, trifluralin, Fluchloralin and Pendimethalin,	SWV	Water, ground water, grains,soil, baby food, sediment, urine and soil, ground water	367-370
Pendimethalin	DPV	Pulse	371
Trifluralin	Spectophotometry	Food	372
Acetamides	**Analytical technique**	**Samples**	**Refs.**
Alachlor	LC-UV	Aqueous samples, microbial culture médium, sediment and wastewater	179-182, 291
Acetochlor, alachlor and metolachlor	LC-MS/MS	Soil, sediment and sludges, soybeans, sludge, groundwater, water, air, aquatic products	117, 147, 178, 183, 287, 288, 373, 374
Acetochlor	UPLC-Q-TOF	Freshwater	223
Acetochlor, alachlor, metolachlor and pretilachlor	GC-ECD	Water, soil, corn, white mustard, cereal crops, peppers and peppers	62, 114-116, 177, 286, 291, 292, 294
Acetochlor, alachlor, pretilachlor, butachlor, metolachlor and pretilachlor	GC-MS	Soil, water, plant product, eel and shrimp, leaf, wastewater, rice, tobacco, air, bulb vegetables,	104, 116, 118, 147, 152, 161, 176, 289, 290, 295, 375, 376

Acetamides	Analytical technique	Samples	Refs.
Alachlor	GC–HRMS	wastewater	154
Alachlor and metolachlor	GC-MS/MS	Surfacewaters	176
Butachlor and alachlor	GC-TSD	Milk	119
Pretilachlor, acetochlor and alachlor	ELISA	Water, soil	191, 377
Alachlor	UPC	Tobacco	289
Metolachlor	IMS	Cucumber	293
Pretilachlor	CE	Soil and rice	200
Cilohexanedione oxime			
Clethodim, Clethodim sulfoxide, Clethodim sulfone and sethoxydim	LC-MS/MS	Soil, rape plant and rape seed	40

Triazole	Analytical technique	Samples	Refs.
Hexaconazole, myclobutanil and tebuconazole	LC-UV/LC-DAD	Water, fruit, soil and grape	308, 311, 313, 378-380
Amitrole, cyproconazole, fembuconazole, herxaconazole, myclobutanil, tebuconazole, and propiconazole	LC-MS/MS	Water, sewage sludge, soil, foods, aquatic products, vegetables, juice, earthworms, rat blood, strawberries, atmospheric samples, baby food, cucumber, grapes, tea, beehive, pollen, single bumblees, drinking water, sediment, chicken, meat, egg, milk, pork, pig liver, leaf lard and pig kidney, wastewater,	106, 122-124, 184-186, 225, 237, 287, 298, 301, 302, 305, 306, 310, 313, 381-390
Propiconazole	LC-Q-LIT-MS	Food, vegetables and biota samples	298

Triazole	Analytical technique	Samples	Refs.
Hexaconazole, myclobutanil, penconazole and tebuconazole	GC-ECD	Water, milk, wine, apples	120, 255, 296, 299, 309
Hexaconazole, myclobutanil, penconazole and tebuconazole	GC-FID	Oils, aqueous samples, fruit juice, Edible oils,	126, 312, 391
Myclobutanil and penconazole	GC-ITMS	Wine,	392
Cyproconazole, hexaconazole, cyproconazole, myclobutanyl, penconazole, propiconazole and tebuconazole	GC-MS	Wood extracts, Jujube, wood extract, honey, water, tap water, grape juice, atmospheric samples, vegetables, tomato, grapes, olive oils, wine	63, 104, 121, 127, 155-159, 315, 316, 385 63, 125, 127, 157, 158, 160, 161, 297, 300, 303, 304, 307, 314, 393
		gherkin, mango, soil, teas, jujube, cucumber, cabbage, honey, watermelon, canned and peach	
Cyproconazole	GC-MS/MS	Tomato	156
Penconazole and tebuconazole	GC-NPD	Honey, garlic, soil and water	63, 394
Cyproconazole	CE	Aqueous medium	204
Tebuconazole	SFC-MS/MS	Water and Zebrafish	226
Tebuconazole	LTP-MS/MS	Wine	395

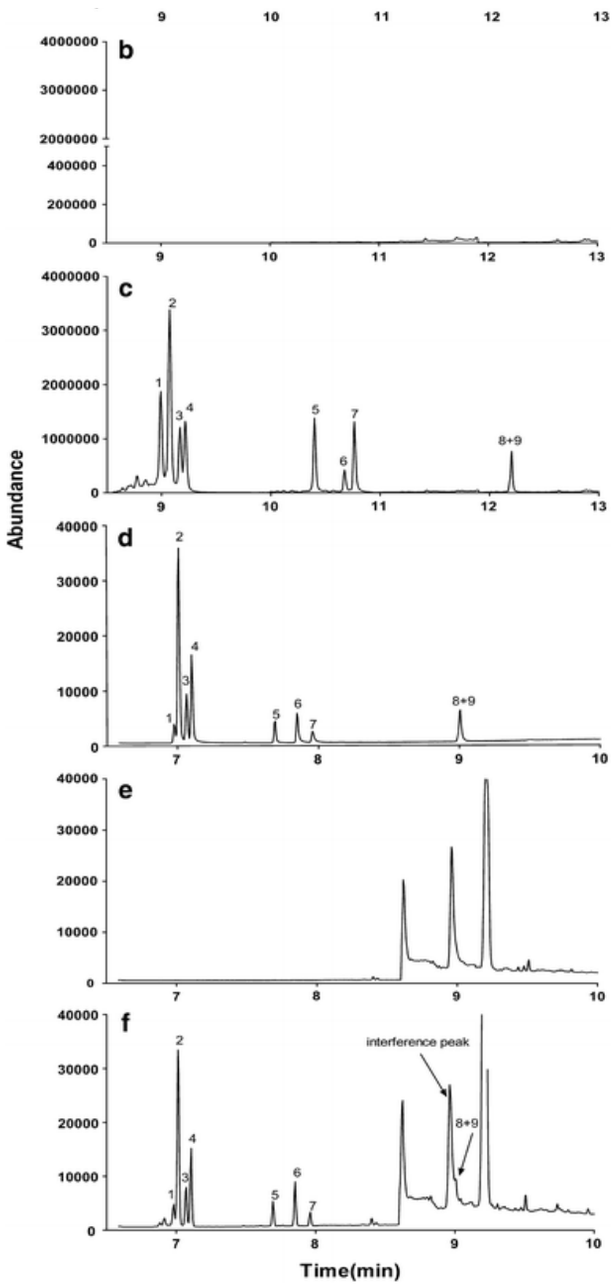


Fig. 1. GC-NCI/MS total ion chromatograms (TICs) of mixed standard solution (0.2 mg L^{-1}) in NCI (a), blank garlic sample in NCI (b), spiked garlic sample at 40 μg kg^{-1} in NCI (c), mixed standard solution (0.2 mg L^{-1}) in EI (d), bank garlic sample in EI (e), spiked garlic sample at 40 μg kg^{-1} in EI (f). Peaks: 1 Ethalfluralin; 2 Trifluralin-d_{14}; 3 Trifluralin; 4 Benfluralin; 5 Profluralin; 6 Fluchloralin; 7 Dinitramine; 8 Pendimethalin-$_{d5}$; 9 Pendimethalin.[1]

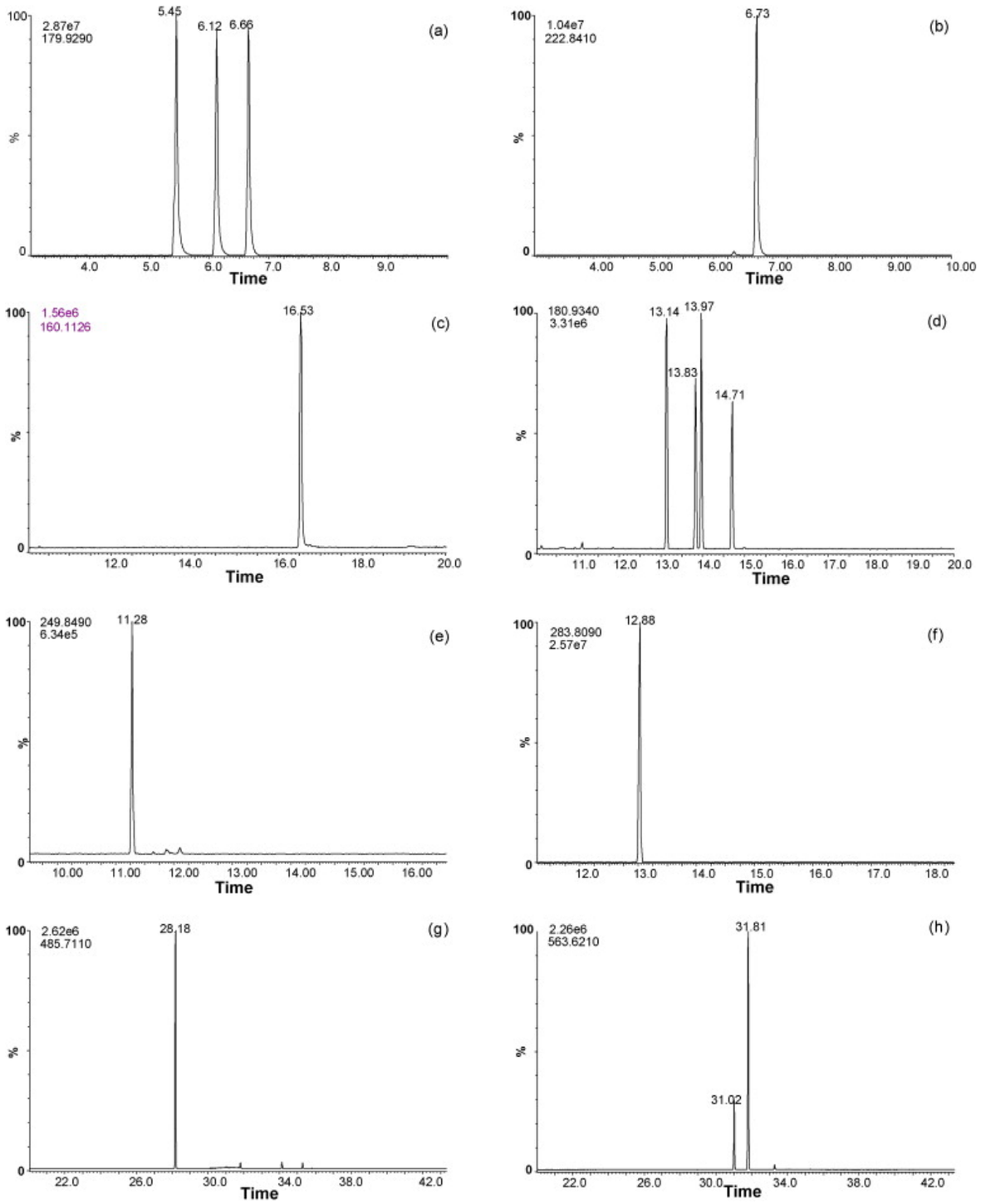


Fig. 2. GC–HRMS extracted ion chromatograms of a sewage effluent matrix-matched standard mixture at 0.5 μg L^{-1} containing all target compounds: (a) trichlorobenzene; (b) hexachlorobutadiene; (c) alachlor; (d) HCHs; (e) pentachlorobenzene; (f) hexachlorobenzene; (g) tetra-BDE; (h) penta- and hepta-BDE.[2]

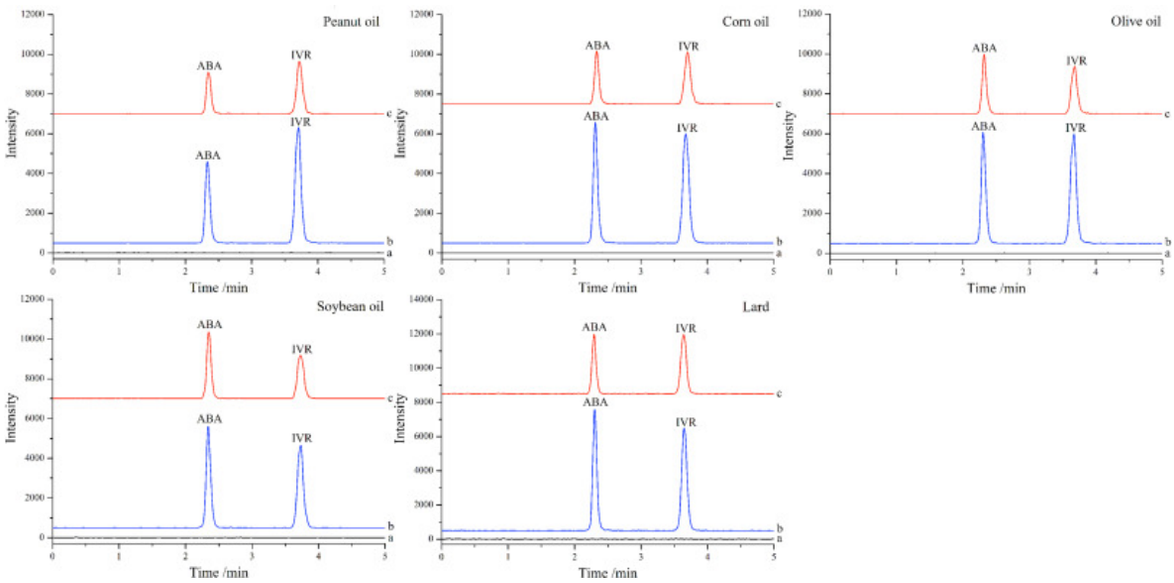


Fig. 3. LC-MS/MS chromatograms of abamectin and ivermectin in spiked edible oil samples. (a) Blank sample, (b) standard solution of 10 µg L^{-1} and (c) sample spiked with 10 µg kg^{-1}.[3]

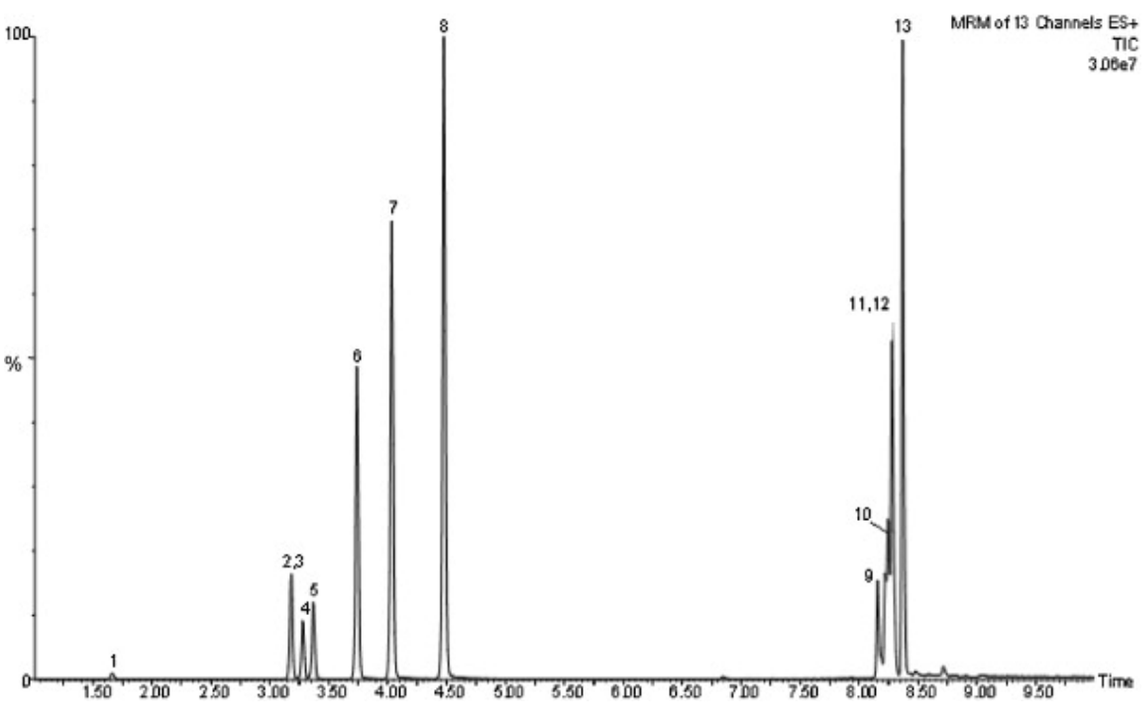


Fig. 4. LC-MS/MS chromatogram corresponding to a mixture of the of the 13 target compounds in H_2O/MeOH (80/20) at 100 µg L^{-1} and with a 2 µL injection volume (1: 6-chloronicotinic acid; 2: thiamethoxam; 3: olefin; 4: 5-hydroxy-imidacloprid; 5: chlothianidine; 6: imidacloprid; 7: acetamiprid; 8: thiacloprid; 9: lambda-cyhalothrine; 10: cypermethrine; 11: deltamethrin; 12: esfenvalerate; 13: bifenthrine).[4]

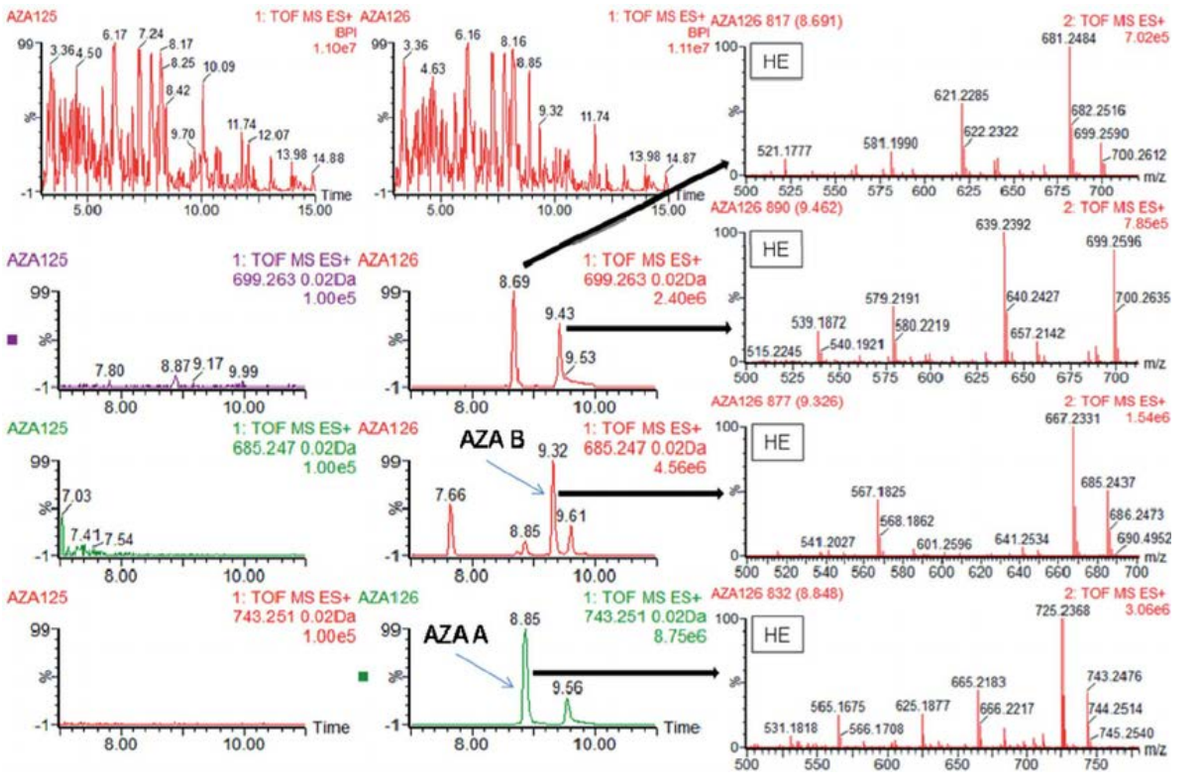


Fig. 5. UHPLC-QTOF MS experiments. Base-peak ion chromatograms (BPI) and extracted ion chromatograms (XIC) at 20mDa mass window for m/z 743.251, 685.247 and 699.263, for control (left) and analyte (middle) Norway Maple foliage samples. High energy (HE) spectra for selected analytes (right).[5]

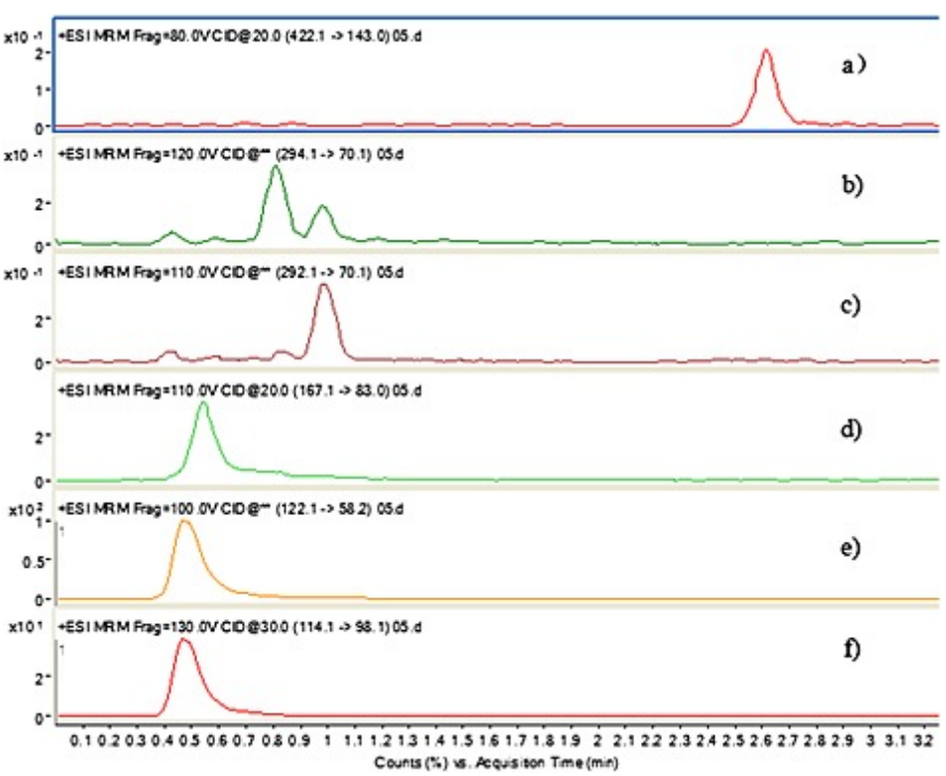


Figure 6: The LC/MS/MS chromatogram of apple matrix extract spiked at the LOQ level: (a) flumetralin 0.1 mg kg^{-1}; (b) paclobutrazol 0.005 mg kg^{-1}; (c) uniconazole 0.005 mg kg^{-1}; (d) ethephon 0.5 mg kg^{-1}; (e) chlormequat 0.01 mg kg^{-1}; and (f) mepiquat 0.005 mg kg^{-1}.[6]

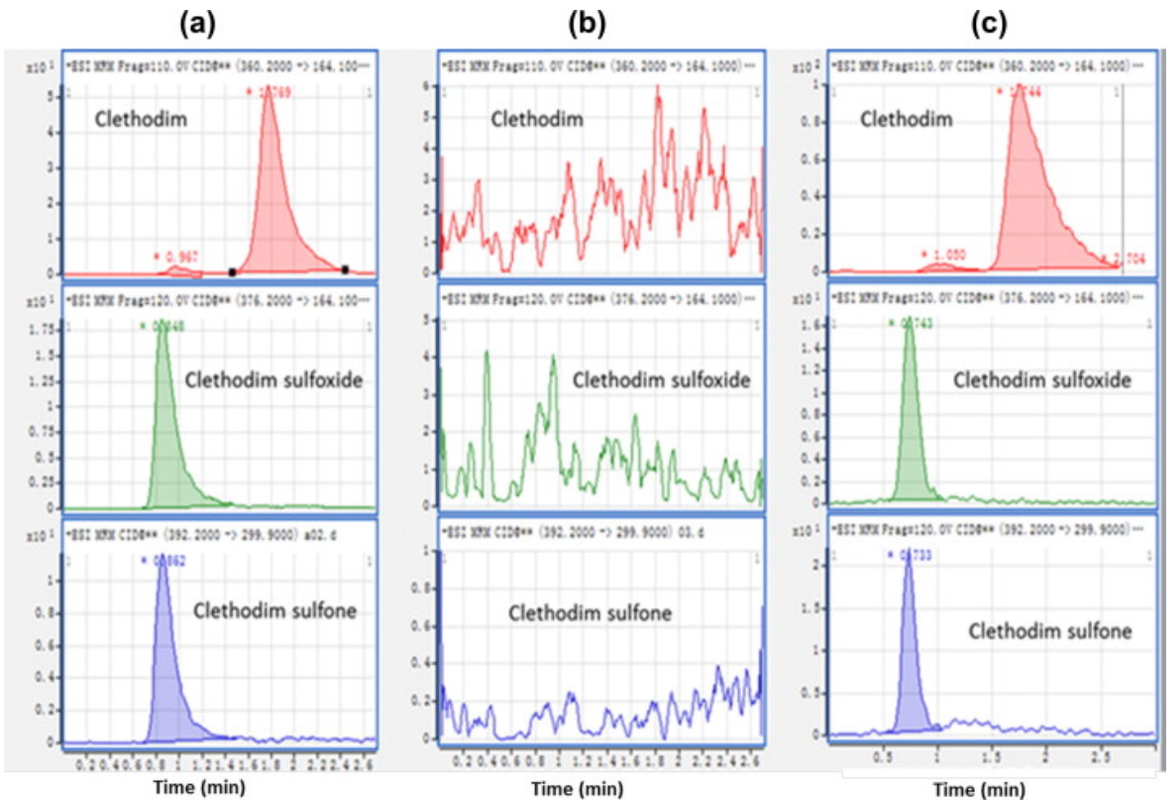


Figure 7. LC–MS/MS chromatograms of clethodim and its two oxidation metabolites (clethodim sulfoxide and clethodim sulphone) in matrix-matched standard (0.1 mg kg^{-1}) (a), blank rape plant (b) and fortified rape plant sample at 0.10 mg kg^{-1} for clethodim and 0.05 mg kg^{-1} for sulfoxide and clethodim sulphone (c).[7]

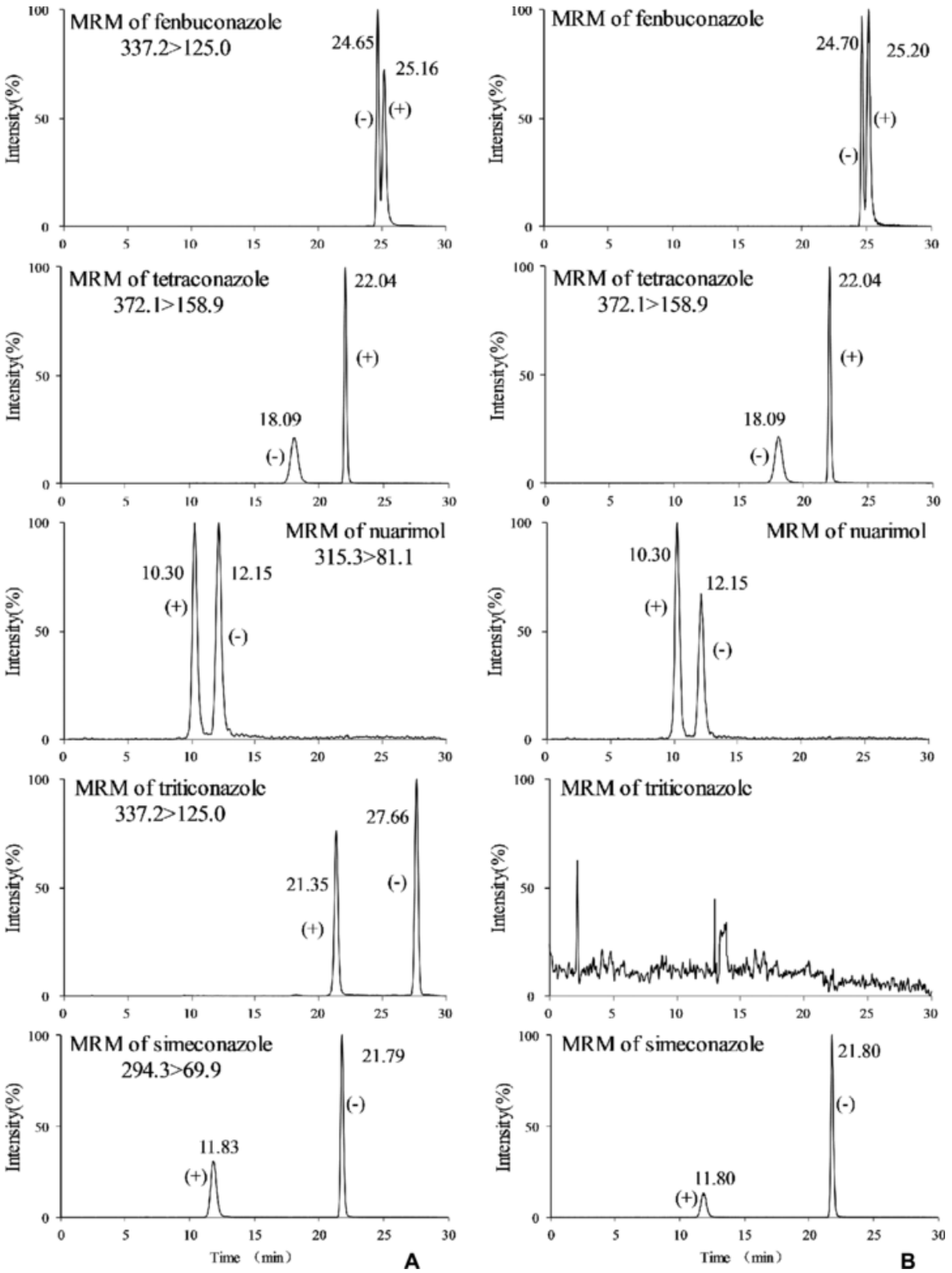


Figure 8. LC–MS/MS chromatograms of spiked soil with chiral fungicides at 0.1 μg g^{-1} (A) and incubated soil after 7 days (B).[8]

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- Biological Matrices: Pesticides Content Sampling, Sample Preparation and Preservation
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- Multiclass, Multiresidue Analysis of Pesticides, Strategies for
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- Quality Assurance in Environmental Analysis
- Sample Preparation for Environmental Analysis in Solids (Soils, Sediments, and Sludges)
- Soil and Sediments: Pesticides Content Sampling, Sample Preparation, and Preservation

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IV.

PROBLEMÁTICA DE LOS CONTAMINANTES EMERGENTES EN LAS CUENCAS MEDITERRÁNEAS

Acerca de los estudios previos sobre la presencia de los contaminantes emergentes y sus productos de transformación en las principales cuencas mediterráneas de la Union Europea como: Ebro, Llobregat y Turia en España; Po y Tiber en Italia; Guadiana en Portugal; Rhône en Francia y Aliakmon, Axios en Grecia. Se aborda la problemática de los contaminantes emergentes teniendo como factores importantes las condiciones climáticas del área mediterránea y el actual contexto del cambio climático.

Emerging pollutants in Mediterranean Basins

Alexander Ccanccapa[*,1], Yolanda Picó[1],

[1]Food and Environmental Safety Research Group (SAMA-UV), Facultat de Farmàcia, Universitat de València, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain.

[*] Corresponding autor: Alexander Ccanccapa
Tel: +34 963543092; Fax: +34 963544954
E-mail: Alexander.Ccanccapa@uv.es

1. Introduction

In the last decades the global society is characterized by a growing use of chemicals in their urban, industry and agriculture activities. The consumption of these chemicals through different ways may reach the aquatic and different environmental components from both point and non-point sources resulting on a potential threat to the water cycle and the aquatic ecosystems. Among the production of a wide variety of chemicals the so called "emerging pollutants" (EPs) and its transformation products (TPs) are of special concern [1, 2]. EPs are chemicals, whose environmental relevance has been only recently highlighted due to either new scientific findings and they are not yet covered by guidelines or legislative intervention that are currently available to regulate their presence in the environment [3-6].

The Mediterranean basin stretches for about 3.800 km from east to west, from the tip of Portugal to the beaches of Lebanon, and a thousand miles from north to south, from Italy to Morocco and Libya. Within the European Union, the Mediterranean region comprises seven member states, Spain, France, Italy, Portugal, Cyprus, Greece and Malta. The Mediterranean rivers are vulnerable to the impact of the EPs due to two main characteristics such us climatic conditions and demography (densely populated cities). Mediterranean rivers are characterized by low summer flow and large floods in autumn and winter seasons as a consequence of Mediterranean climate [7-10]. In comparison to other regions of the world, the Mediterranean basin is one of the most vulnerable to climate changes. In particular, Mediterranean rivers and streams suffer severe alterations in the flow regime because of a decreasing number of precipitation days and an increase of heavy rain events. Resulting imbalance of available water during low flow periods and increasing anthropogenic pressures and demands for water

lead to severe ecological and socioeconomical problems. As a consequence, water scarcity and its quality preservation are becoming important issues of Mediterranean countries.

Many reports pointed out the occurrence of pesticides (PS) [11-18], pharmaceuticals (PHs) [19-27], drugs (DGs) [12, 28] and personal care products (PCPs) [29-35] in Mediterranean basins as Ebro, Llobregat and Turia in Spain; Po and Tiber in Italy; Guadiana in Portugal; Rhône in France and Aliakmon and Axios in Greece.

The analytical techniques used to determinate EPs are usually liquid or gas chromatography coupled mass spectrometry (LC-MS or GC-MS) [9, 10, 36-38]. Presently, GC-MS continues being used widely because it is selective, relatively inexpensive and easy to operate, as LC-MS, provides confirmation of multiple classes of EPs in a single analytical run. With these techniques, polar, apolar, thermally unstable or little volatile compounds could be determined. In the last years, the number of LC-MS applications to determine EPs has increased, becoming a routine technique in most laboratories. Recently, new techniques are incorporating as routine techniques due to their capacities to perform target and non-targeted analysis. The introduction of high-resolution mass spectrometry (HRMS) analyzers, such as time-of-flight (TOF) and Orbitrap, has allowed the development of non-target screening and unknown identification schemes. Data dependent/ independent acquisition (DDA or DIA) are the main modes to work with these instrumentals. DDA mode selects those precursor ions detected in a survey scan meeting some previously defined characteristics for subsequent isolation and fragmentation in a serial manner. DIA mode avoids specific selection during LC-MS analysis by co-selection and co-fragmentation. These two strategies are powerful to acquire chromatograms with very rich in information, which contain thousands of ions

from any compound present in the sample. These last techniques were used by Masiá et al. [39, 40] and Andres-Costa et al. [39, 40] to analysis different kinds of EPs in the Turia river through libraries using DDA mode. In the same way, water samples and sediment samples from Danube (central and eastern Europe) and Dore Rivers (France) were analyzed by HRMS [27, 41].

This review offers an overview of the occurrence of the EPs in the main Mediterranean basin of the European Union (Spain, France, Italy, Portugal, Cyprus and Greece), linked with the climate change and adverse climatic conditions of this area. Also, the risk assessment in different trophic levels (algaes, daphnias and fish) as environmental tool to know the concentrations impact on the aquatic flora and fauna population on the Mediterranean rivers. Generally, the bibliography selected comprised the studies developed in Mediterranean basins the last 3 years focused on pesticides, pharmaceutical, drugs and personal care products. Recent examples illustrate and help to describe the fact situation of the EPs under climate change conditions.

2. Mediterranean Area

The surface area of the Mediterranean area is around 2.51 million Km^2 and has a coastline of 46,000 km. There are 69 rivers that shed an approximate flow of 283 Km^3 per year. Within the European Union, the Mediterranean region comprises seven member states, Spain, France, Italy, Portugal, Cyprus, Greece and Malta. The main basins as Ebro, Llobregat and Turia in Spain; Po and Tiber in Italy; Guadiana in Portugal; Rhône in France and Aliakmon and Axios in Greece (**Table 1**) are sharing the same climate, demographic pattern, topography and land use. Water resources management (quality and toxicity) in the Mediterranean rim of Europe faces several challenges due to variable rainfall patterns and high irrigation demand. The most

distinctive feature of Mediterranean climate is its seasonality, characterized by summer drought. However, the temporal and spatial variability of rainfall in Mediterranean regions is influenced by surface relief, as high relief areas are commonly associated with higher than mean rainfall values [42]. Flooding is the greatest natural risk in Mediterranean environments. In Spain over the last 20 years, floods have accounted for over 81% of the resources required to mitigate damages as a result of extraordinary risks [43]. The case of Mediterranean ephemeral streams has certain peculiarities compared to other river systems, both from a physical (land use) and human (anthropic pressure) viewpoint, which must be taken into account so as to correctly analyze of the environmental hazard, vulnerability and exposure components. The Intergovernmental Panel on Climate Change (IPCC) predicts that the Mediterranean will be particularly sensitive to climate change. IPCC foresees a decrease of annual precipitation and an increase of average temperature, with a higher frequency of extreme events, meaning that water resources will be not only less abundant but also less available. In fact, the need for effective adaptation will be greatest in Southern Europe as a result of increased production vulnerability, reduced water supply and increased demands for irrigation. Increasing flood and drought risks will further contribute to the need for robust management practices [44, 45]. However, increasing flood hazards may present challenges for agriculture, and summer irrigation shortages may result from earlier spring runoff peaks in some regions. Conversely, the need for effective adaptation will be greatest in Southern Europe as a result of increased production vulnerability, reduced water supply and increased demands for irrigation. Increasing flood and drought risks will further contribute to the need for robust management practices. All these features of the Mediterranean rivers are associated with the loads of the emerging pollutants that carry

out along its path and arriving to the Mediterranean Sea modifying the different aquatic and marine ecosystem and finally accentuating the effects of climate change.

3. Occurrence of emerging contaminants in the Mediterranean Basins

This study selected the main rivers of the Mediterranean area within the European Union. The other criteria to select these rivers were the reports available in the last 3 years. In that sense, all the data of the monitoring of the rivers could help to understand the actual profile of the contamination and the interaction with climate change scenario. The risk assessment in different bio indicator as daphnias, algaes and fish could help to understand the impact of the discharges of emerging pollutants in aquatic ecosystems.

3.1. Occurrence of Pesticides

The occurrence of pesticides in the main Mediterranean basins is well documented. The Ebro River runs throughout 950 km from its source to its mouth (NW–SE) and is fed by 347 major tributaries. The agricultural sector (including grazing livestock) makes up for the 58% of the land use and for the 8% of the employed population. Irrigation is a relevant economic tool in the Spanish agriculture, as the productivity of irrigated land is 7.3 fold higher on average than that of non-irrigated areas. In the Ebro River basin up to a 9.16% of agricultural land is irrigated, particularly in the central areas of the basin (especially in Aragon and Catalonia). Population in the basin is close to 3 million inhabitants. Many studies showed a wide range of pesticides concentration in the Ebro River [8, 10, 46-52]. Kuster et al. [51] investigated the occurrence of 52 pesticides in the Ebro delta, in the rice cultivation area, during the main growing season of rice from May to August. The study showed high levels, in the μg L^{-1} range, of bentazone, MCPA (bentazone, (4-chloro-2-methylphenoxy) acetic acid)), propanil, molinate and atrazine,

in basically all the samples investigated. The sampling campaign performed in July showed comparatively higher levels than the other three campaigns. The pesticides used for rice crop were, as expected, the most relevant in this study (concentrations up to 127µg L^{-1} were found for bentazone). Feo et al. [53] analyzed twelve pyrethroids pesticides in surface water and sediment samples in the Ebro delta, this family of pesticides is actually a great concern because of other pesticides as organophosphorus are being replaced by pyrethroids due to their low toxicity and persistence. Cypermethrin was detected in 22 water samples, while deltamethrin was present only in three water samples at concentrations ranging from 0.73 ng L^{-1} to 57.2 ng L^{-1} and 2 ng L^{-1} to 58.8 ng L^{-1} for cypermethrin and deltamethrin. These concentration levels were higher than median lethal concentration (LC50) values found for deltamethrin when short time toxic effects are considered.

In the Turia Basin, Aznar et al. [54, 55], as well as, analyzed pyrethroids pesticides in surface and ground water samples of the La Albufera wetland (mouth of the Turia River) impacted by rice crops. The samples were collected during two periods (flooding and dry soil conditions). Pyrethroids were detected at concentrations ranging from 14 to 1450 ng L^{-1} in surface water and from 6 to 833 ng L^{-1} in ground water. During dry soil conditions, cyfluthrin, cypermethrin and esfenvalerate were the compounds detected more often, in around 80 % of the samples, and with concentration levels higher than during flooding soil conditions. This could be related on apolar nature of the pyrethroids family.

The occurrence of fifty pesticides in water, sediment and biota samples belong to the Llobregat river were analyzed by Masiá et al. [56]. Pesticides were detected primarily in water samples (up to 56% of the analytes), whereas their presence in sediments was more intermittent, and in biota was scarce. Those at high concentrations in water were

the benzimidazoles (carbendazim in 22% of the samples up to 697 ng L^{-1}), the organophosphorus (malathion in 54% of the samples up to 320 ng L^{-1}), and the ureas (diuron in 54% of the samples up to 159 ng L^{-1}). However, this pattern differed in sediments and biota, which were contaminated primarily with organophosphorus (higher K_{ow}) as chlorpyrifos, which was found in 93% of sediments samples and up to 131 ng g^{-1} of concentration.

The Tiber River (Central Italy) has a length of 409 km and passes through the city of Rome, which has 2,863,322 inhabitants and an ancient agricultural tradition that is still the main resource for the socio-economic development. Indeed, with 37,000 ha of utilized agricultural surface, Rome is the largest agricultural district in Europe. Montuori et al. [57] investigated

organophosphate pesticides pollution in the Tiber River and its environmental impact on the Tyrrhenian Sea (Central Mediterranean Sea). Eight organophosphate pesticides (diazinon, dimethoate, malathion, chlorpyrifos, pirimiphos-methyl, fenitrothion, methidathion, tolclofos-methyl) were determined in the water dissolved phase, suspended particulate matter and sediment samples collected from 21 sites in different seasons. Total organophosphate pesticides concentrations ranged from 0.40 to 224.48 ng L^{-1} in water (as the sum of the water dissolved phase and suspended particulate matter) and from1.42 to 68.46 ng g^{-1} in sediment samples. Contaminant discharges of organophosphate pesticides into the sea were estimated in about 545.36 kg $year^{-1}$ showing that this river should be consider as one of the main contribution sources of organophosphate pesticides to the Tyrrhenian Sea.

Palma et al. [15] studied the potential impact of the pesticides on the aquatic organisms in the Alqueva reservoir, which is located in southern Portugal, along 83 km of the main course of the Guadiana River Basin. The hydrologic regime of the Alqueva reservoir

reflects the regional expression of the Mediterranean climate. Twenty-five pesticides and some degradation products were analyzed. Of the all pesticides evaluated, 23 were present in the samples analyzed. Cyanazine and FOX (a degradation product of fenitrothion) were occasionally detected in the samples, probably as a consequence of their use in the intensive olive grove cultivation taking place in Spain. The most abundant pesticides were bentazone, terbuthylazine, metolachlor, MCPA and 2,4-D. Terbuthylazine was the only compound detected in all water samples, with a maximum individual concentration of 532 ng L^{-1}. Bentazone, metolachlor and MCPA had the highest concentration, 1769 ng L^{-1}, 291 ng L^{-1} and 580 ng L^{-1}, respectively. Regarding the limit of 100 ng L^{-1} set for individual pesticides in water intended for human consumption by the Directive98/83/EC (ECC, 1998) was surpassed by terbuthylazine, bentazone and metolachlor in 62%, 33% and 12% of the samples, respectively.

The Rhône river delta and its lagoon system are located in the southern part of France. The lagoon system (422 km^2) is divided into two sub-systems, which are directly connected to the Mediterranean Sea. The Rhône delta is devoted to intensive flooded rice cultivation. In the northern section of the delta (310 km^2), irrigation water is pumped from the Rhône river and drainage water is returned to the river through a main ditch. Chiron et al. [58] monitored the occurrence of pesticides in Rhône River and reported on the results of CMP study and its nitroderivative (CMNP). It was evidenced the following transformation sequence: MCPA = 4-chloro-2-methylphenol (CMP) = 4-chloro-2-methyl-6-nitrophenol (CMNP). Interestingly CMP disappeared about as quickly as MCPA, while CMNP was environmentally more persistent than the parent molecules. MCPA is extensively applied in the rice fields of the Rhône delta as a post emergent herbicide, and its transformation by-products contribute to the chlorophenols burden of the delta.

Konstantinou et al. [59] monitored the occurrence of pesticides in different rivers in Greece as Aliakmon, Axios, Loudias, Louros, Arachthos and Kalamas. The river deltas are regions protected by international conventions as they constitute important aquatic ecosystems. Pesticide classes mostly detected involve herbicides used extensively in corn, cotton and rice production, organophosphorus insecticides as well as the banned organochlorines insecticides due to their persistence in the aquatic environment. The herbicides most frequently detected were atrazine, simazine, alachlor, metolachlor and trifluralin, the insecticides, diazinon, parathion methyl and the organochlorines, lindane, endosulfan and aldrin. Rivers were more polluted than lakes. The detected concentrations of most pesticides follow a seasonal variation, with maximum values occurring during the late spring and summer period followed by a decrease during winter. The other side, elevated concentrations were found in areas of high pesticide use and intense agricultural practices.

Generally, similar trends and levels of pesticides were found in all the Mediterranean rivers selected. Usually the pesticides more used are organophosphorus, azoles, triazines, carbamates, benzimidazoles, neonicotinoids and pyrethrins. These results of the different studies that confirmed the presence of a wide range of pesticides using target screening and untargeted screening are in concordance with the sales of pesticides in the countries of the European Union EU (survey carried out by Eurosat). Figures 1, 2 and 3 showed to Spain, Italy, France, Portugal and Greece as main consumer of fungicides, herbicides and insecticides within the EU, that means that the Mediterranean area is vulnerable due to high consume of pesticides and its impact in the ecosystem and human health. The other side, all the studies took into accounts the seasonality as a main climatic factor to analysis the behavior of pesticides. All of them concluded that extreme climatic condition are linked with the concentration of pesticides, in latest

spring and summer the pesticides were found at high concentration and in autumn and winter they were present at high frequency and low concentration.

3.2. Occurrence of pharmaceutical

As pesticides, pharmaceutical compounds have been reported along the different Mediterranean rivers. In the Ebro river, García-Galán et al. [60] investigated the presence of sulfonamides (SAs), one of the antibiotic families frequently found in all kind of environmental waters. Sulfamethoxazole and sulfapyridine were the SAs most frequently detected in WWTPs (96–100%), showing also the highest concentrations, ranging from 27.2 ng L^{-1} to 596 ng L^{-1} for sulfamethoxazole and from 3.7 ng L^{-1} to 227 ng L^{-1} for sulfapyridine. Sulfamethoxazole was also the SA most frequently detected in surface waters (85% of the samples) at concentrations between11 ng L^{-1} and 112 ng L^{-1}. López-Serna et al. [61] as well as studied the occurrence of pharmaceuticals, their metabolites and transformation products (TPs) in the same river. In total, 17 metabolites (7 of them with pharmacologic activity) and 2 TPs, along with 58 parent pharmaceuticals were analyzed. Metabolites and TPs were found at concentrations of the same order of magnitude as their corresponding parent pharmaceuticals, with the exception of 10,11-epoxi-carbamazepine which was found at approximately 10 times higher concentration than its corresponding parent pharmaceutical carbamazepine. In general, levels of all target compounds were below 100 ng L^{-1}, with the exception of 14 compounds; among them the aforementioned 10,11-epoxicarbamazepine with a maximum concentration of more than 1600 ng L^{-1}. The analgesic propyphenazone, the psychiatric drug carbamazepine, the antibiotics clarithromycin and sulfadiazine, the

cardiovascular drug propranolol, the antineoplastic tamoxifen and 1 pharmacologically active metabolite salicylic acid were found to be ubiquitous (detected in all samples).

Carmona et al. [30] investigated the occurrence of 21 acidic pharmaceuticals, including illicit drugs, and personal care products (PPCPs) in waste, surface and drinking water and in sediments of the Turia River Basin using LC-MS/MS as analytical technique. PPCPs were detected in WWTPs influents up to 7.26 $\mu g L^{-1}$. Mainly ibuprofen, naproxen and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THCOOH) were detected. Similarly, diclofenac, gemfibrozil, ibuprofen, naproxen and propylparaben were detected quite frequently from the low ng L^{-1} range to 7 $\mu g\ L^{-1}$ in the surface waters of Turia River. Mineral and tap waters also presented significant amounts (approx. 100 ng L^{-1}) of ibuprofen, naproxen, propylparaben and butylparaben. The occurrence at trace levels of several PPCPs in drinking water raises concerns about possible implications for human health.

López-Roldán et al. [62] analyzed the occurrence of 28 pharmaceuticals and 10 estrogens in waters samples from the lower part of the Llobregat River basin, where the main intakes for production of drinking water for Barcelona (Spain) are located. They analyzed the samples using a LC-MS/MS and ultra performance liquid chromatography–mass spectrometry with a time-of-flight analyzer (UPLC–TOF–MS). Within the class of pharmaceuticals, 23 out of the 28 compounds investigated, were detected in at least one sample. The highest concentrations were observed for the b-blockers metoprolol (8042 ng L^{-1}) and sotalol (788 ng L^{-1}), the antibiotic ofloxacin (1904 ng L^{-1}), and the lipid regulator gemfibrozil (1014 ng L^{-1}).

Estrogens as estrone and estrone-3-sulfate were at concentrations ranged from 0.82–5.81 ng L^{-1}, these concentrations are considered sufficient to induce estrogenic effects in aquatic organisms (1 - 10 ng L^{-1}).

Eleven antibiotics and the antiepileptic carbamazepine were analyzed by Verlicchi et al. [25] in wastewater and surface water from the Po River in Italy. Information on the consumption and sales of pharmaceuticals in Italy, along with data related to their excretion and removal during wastewater treatment, were used to predict the concentrations of the selected pharmaceuticals for the studied site. The measured and predicted concentrations were compared for all sampling points, and according to a criterion available in literature, the prediction was considered "acceptable". The results showed that the concentrations were accurately predicted for ciprofloxacin in wastewaters, and for azithromycin, trimethoprim and carbamazepine in surface water. The highest concentrations values were detected for ciprofloxacin (2.2 μg L^{-1}), ofloxacin (0.98 μg L^{-1}) and carbamazepine (0.57 μg L^{-1}) in the influent samples, ciprofloxacin (0.63 μg L^{-1}), ofloxacin (0.4 μg L^{-1}) and carbamazepine (0.37 μg L^{-1}) in the effluent samples, and ciprofloxacin (0.25μg L^{-1}) in the surfacewater samples.

Pérez-Fernández et al. [63] analyzed six antithyroid drugs (ATDs) in surface water samples of the Tiber River. The most widespread compound was tapazole, one of the most common ATDs used in human medicine, but also thiouracil and mercaptobenzimidazole were often detected in the analyzed samples. Tapazole was found in all of the samples and its highest concentration was 5 μg L^{-1}. Moreover, the concentrations of mercaptobenzimidazole were quite high with the greatest value determined in the samples (18.5 μg L^{-1}).

In Aliakmon Basin - Greece, eighteen pharmaceuticals and personal care products were analyzed. During the study, a total number of 64 influent and effluent samples were collected from the eight WWTPs, covering a monitoring program for the four seasons over 1-year monitoring period (2010–2011) in eight WWTPs. The results showed the occurrence of all target compounds in the wastewater samples with concentrations up to

96.65 μg L^{-1}. Paracetamol, caffeine, trimethoprim, sulfamethoxazole, carbamazepine, diclofenac and salicylic acid were the dominant compounds, while tolfenamic acid, fenofibrate and simvastatin were the less frequently detected compounds with concentrations in effluents below the LOQ.

In the Mediterranean Basins, pharmaceuticals and personal care products were analyzed and confirmed their presence. Generally the samples were taken from the WWTPs, because is well known that these compounds are released mostly through urban wastewater and many of them can further spread through the water cycle, even reaching drinking water, due to their hydrophilic character and low removal at WWTPs. The main pharmaceuticals were analgesics and anti-inflammatory in water samples from the different Mediterranean rivers.

4. Risk assessment of EPs in Mediterranean Basin

Due to the great number of chemical compounds potentially occurring in the environment, there is a need to prioritize them for management optimization purposes. Therefore, identifying the chemicals of concern for a given river basin requires performing a suitable combination of monitoring and reliable assessment of risk. Risk assessment procedures consider both the potential hazard effect of a given substance and its exposure level. While exposure can be obtained either from measurement (monitoring) or modelling, hazard is derived from its intrinsic properties. Typically, these encompass persistence, bioaccumulation and toxicity (referred to as PBT approach). However, in practice, due to the aforementioned continuous introduction into the environment of many compounds persistence becomes less relevant (i.e., many pollutants are ubiquitous in the environment due to their continuous input). On the other hand, bioaccumulation and toxicity are often correlated. For that reason, many risk-

assessment procedures are focused on ecotoxicity as a hazard measure, while persistence and bioaccumulation are disregarded. In that sense, different approaches have been developed to identify compounds of environmental concern and to establish priorities for monitoring. Most of these approaches are based on the occurrence of such compounds in natural systems and on their ecological and toxicological effects using the Risk Quotient (RQ) or Toxic Units (TUs) for representative taxons, such as algaes, daphnias and fish.

4.1. Risk assessment of pesticides

In the Llobregat River, Köck-Schulmeyer et al. [64] evaluated the TUs for algaes, daphnias and fish. They determined sixteen pesticides (triazines, phenylureas, organophosphates, chloroacetanilides and thiocarbamates) in water samples. The octanol–water partition coefficient K_{ow} (usually expressed as $\log K_{ow}$) is often used in environmental fate studies as an indicator of the potential bioaccumulation of a substance. In this way, pesticides can be classified according to their log K_{ow} as compounds having low (<2.7), moderate (2.7–3.0) and high (>3.0) bioaccumulation potential. The pesticides analyzed in this study had moderate to high potential for bioaccumulation. The results showed the expected toxicity in fish is negligible as compared to that calculated for algae and Daphnia. The study showed that herbicides terbutylazine and diuron were toxic for algae according to the EC50. Organophosphate insecticides as diazinon ($\log K_{ow} = 3.69$) and malathion ($\log K_{ow} = 2.75$) were toxic for macro-invertebrates (daphnia). In addition, these two pesticides would have, according to the log K_{ow} values high bioaccumulation potential. Finally, the results indicated that algae would be the organism most affected by herbicides (presence of diuron) whereas

micro-invertebrates would be the most sensitive organisms to the presence of organophosphates such as diazinon and dimethoate.

Montuori et al. [57] calculated the risk assessment through Risk Quotient Index (RQ) for organophosphate pesticides in algaes, daphnias and fish. In order to evaluate negative impact of organophosphate on Tiber River ecosystem, an environmental risk assessment was performed employing the NOEC values obtained from chronic toxicity tests for producing the corresponding PNECs. The results obtained for all the detected compounds exhibiting low to high risk at either average or extreme conditions, as calculated from their corresponding mean and maximum concentrations. However, malathion, chlorpyrifos, pirimiphos-methyl and fenitrothion would be present in some samples at levels that are representatively high risk (RQ > 1) using maximum MEC values. The application of the RQ method in this study showed that although all pesticide concentrations complied with the EQS, the potential risk associated with the pesticides should not be neglected.

To assess the real impact of pesticides on the aquatic life of the Alqueva reservoir (Guadiana Basin), both exposure (i.e., concentrations) and harmful effects on aquatic organisms, Palma et al. [15] calculated the sum of the risk quotient RQ of each individual pesticide found in their study. According to this coefficient, pesticides can be classified as compounds having low, moderate, or high bio-accumulation potential. Six out of the 25 pesticides analysed have a high potential for bioaccumulation, namely, terbuthylazine, alachlor, metolachlor, diazinon, fenitrothion and chlorfenvinphos. Among these pesticides, the more noticeable were terbuthylazine because of its relatively high concentrations (often above 100 ng L^{-1}), and the organophosphates diazinon, fenitrothion and chlorfenvinphos because of their high toxicity to aquatic

organisms (with PNEC in the ng L^{-1} range). In that sense, diazinon, chlorfenvinphos and terbuthylazine were present in some samples at levels that are representatively high risk (RQ values > 1). In the case of diazinon and chlorfenvinphos, this non-acceptable risk is primarily attributed to their high toxicity to fish and especially to aquatic invertebrates; whereas, in the case of terbuthylazine, the high risk results from the combination of the relatively high MEC values and low PNEC values (due to general low toxicity to the aquatic organisms). Considering the aquatic risk determined for the water samples, the results showed that none of the samples presented negligible risk: all had RQ > 0.1. The pollution pattern is strongly dependent on local conditions such as climate, hydrology, land-use and economical activities [65].

4.2. Risk assessment of pharmaceuticals

Continuous input of pharmaceuticals into rivers, through wastewater treatment systems, may cause adverse effects on the aquatic ecosystems of the receiving water bodies, due to the intrinsic biological activity of these compounds. Ginebreda et al. [66] carried out an environmental risk assessment in the lower part of the Llobregat River basin using RQ for pharmaceuticals, belonging to different therapeutic classes (analgesics and non-steroidal antiinflammatories, lipid regulators, psychiatric drugs, anti-histamines, anti-ulcer agents, antibiotics and β-blockers. As result, ibuprofen, diclofenac and fibrates (clofibric, gemfibrozil) have influence in the three bioassays (algae, daphnia and fish), others, like erythromycin for Daphnia, or sulfamethoxazole for algae, show much more specificity. In general, hazard quotients tend to increase when going downstream. Only those points located most upstream of the two rivers can be qualified under low risk for the three bioassays. The most sensitive bioassay seems to be algae, followed by Daphnia and fish.

Gros et al. [67] evaluated the hazard posed by pharmaceuticals in both surface and effluent wastewaters in different aquatic organisms, (algae, daphnids and fish) in the Ebro Basin. The overall relative order of susceptibility was estimated to be algae > daphnia > fish. RQs higher than one in these matrices were associated to erythromycin, clofibric acid and fluoxetine for daphnia and sulfamethoxazole for algae. As expected, RQs in effluent wastewater were higher than those found in river water. Regarding wastewaters, only atorvastatin to fish, erythromycin to daphnia and sulfamethoxazole and tetracycline to algae posed an ecotoxicological hazard. Some substances presented values close to one, indicating that the margin of safety in these types of waters is narrow. However, the results indicated that no significant risks could be associated to the presence of pharmaceuticals in those matrices, indicating that reduction of compound concentration after wastewater treatment as well as dilution factor once pharmaceuticals are discharged in receiving river water efficiently mitigate possible environmental hazards.

Kosma et al. [68] provided an assessment of the environmental risk posed by eighteen pharmaceuticals and personal care products presence in wastewaters by means of the risk quotient (RQ) in Aliakmon Basin. RQs were more than unity for various compounds in the effluents expressing possible threat for the aquatic environment. The results in three trophic levels showed that three of the analyzed compounds (triclosan, trimethroprim and sulfamethoxazole) pose high acute risk and two (diclofenac and triclosan) high chronic risk (RQ > 1), respectively. Algae seemed to be the most sensitive species, since PPCPs posed high acute and chronic ecotoxicological risk to them. Triclosan was found as the most critical compound in terms of contribution and environmental risk.

Table 1. Mediterranean rivers and emerging pollutants

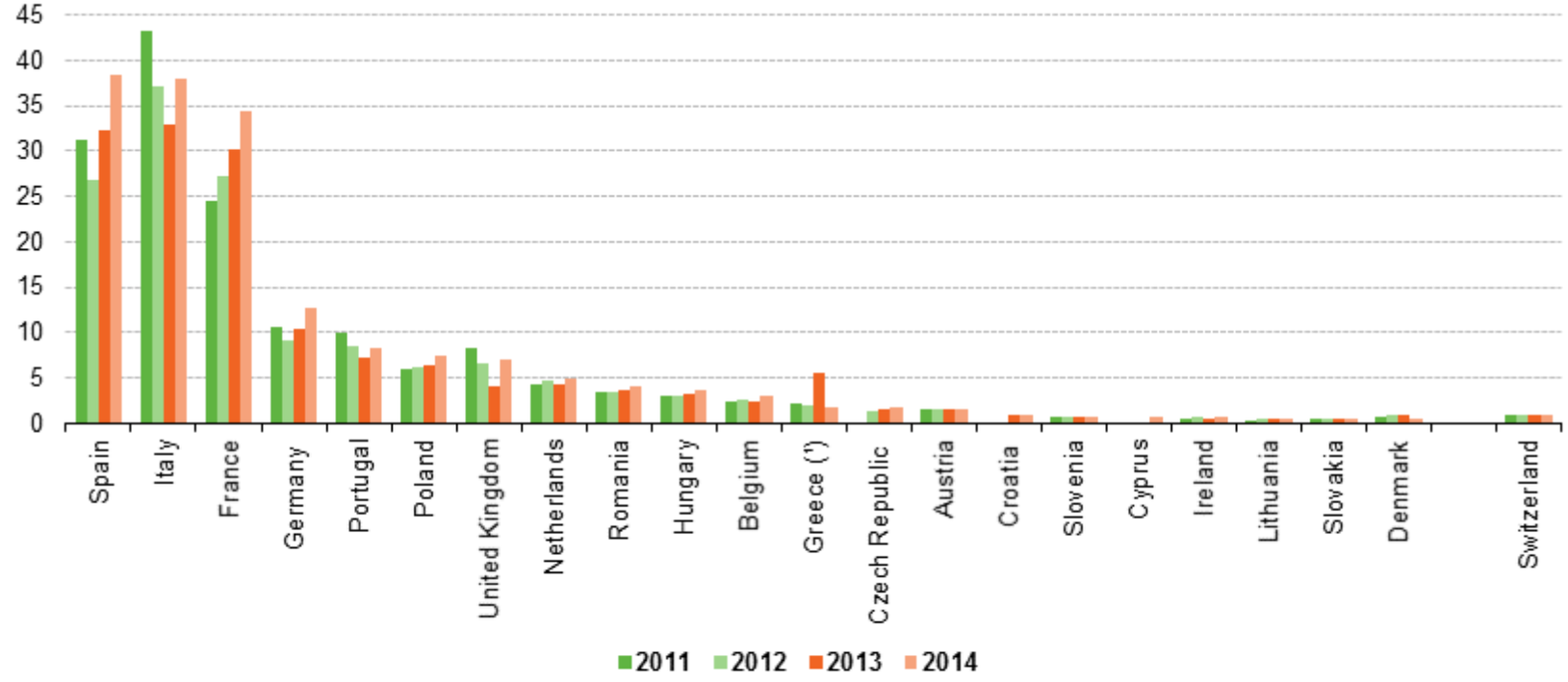
River	Country	Mean flow (m^3/s)	Maximum and minimum range (m^3/s)	Km^2	Length of river (Km)	Pollutant Pesticides (Ps) Pharmaceutical (Ph)	Concentration ($ng\ L^{-1}$) $Min^{(1)}/Max^{(2)}/Mean^{(3)}$	Bibliography
Ebro	Spain	600	1500 - 50	86.100	950	Imazalil (Ps)	$4^{(1)}$ - $409^{(2)}$	[1]
						Chlorfenvinphos (Ps)	$2^{(1)}$ - $41^{(2)}$	
						Deethylatrazine (Ps)	$6^{(1)}$ - $58^{(2)}$	
						Sulfamethoxazole (Ph)	$11^{(1)}$ - $112^{(2)}$	[2]
						Carbamazapine (Ph)	$2^{(1)}$ - $90^{(2)}$	[3]
						Clarithromycin (Ph)	$8^{(1)}$ - $141^{(2)}$	
Llogregat	Spain	19	175 – 3.52	4.948	175	Carbendazim (Ps)	$10^{(1)}$ - $697^{(2)}$	[4]
						Diuron (Ps)	$43^{(1)}$ - $159^{(2)}$	
						Malathion (Ps)	$3^{(1)}$ - $320^{(2)}$	
						Ibuprofen (Ph)	$29^{(1)}$ - $490^{(2)}$	[5]

River	Country	Mean flow (m^3/s)	Maximum and minimum range (m^3/s)	Km^2	Length of river (Km)	Pollutant Pesticides (Ps) Pharmaceutical (Ph)	Concentration ($ng\ L^{-1}$) $Min^{(1)}/Max^{(2)}/Mean^{(3)}$	Bibliography
						Diclofenac (Ph)	$17^{(1)}$ - $358^{(2)}$	
						Ofloxacin (Ph)	$8^{(1)}$ - $1903^{(2)}$	
Turia	Spain	14		6.394	280	Prochloraz (Ps)	$18^{(1)}$ - $486^{(2)}$	[6]
						Imidacloprid (Ps)	$23^{(1)}$ - $206^{(2)}$	
						Imazalil (Ps)	$43^{(1)}$ - $750^{(2)}$	
						Ibuprofen (Ph)	$6^{(1)}$ - $366^{(2)}$	[7]
						Naproxen (Ph)	$50^{(1)}$ - $1797^{(2)}$	
						Diclofenac (Ph)	$6^{(1)}$ - $865^{(2)}$	
Po	Italy	1.54	10300 - 275	71.000	652	Ciprofloxacin (Ph)	2200^{3}	[8]
						Azithromycin (Ph)	150^{3}	
						Ofloxacin (Ph)	980^{3}	
						Carbamazepine (Ph)	570^{3}	
Tiber	Italy	230		17.375	409	Dimethoate (Ps)	$1.09^{(1)}$ - $88^{(2)}$	[9]
						Diazinon (Ps)	$1.04^{(1)}$ - $42^{(2)}$	

River	Country	Mean flow (m^3/s)	Maximum and minimum range (m^3/s)	Km^2	Length of river (Km)	Pollutant Pesticides (Ps) Pharmaceutical (Ph)	Concentration ($ng\ L^{-1}$) $Min^{(1)}/Max^{(2)}/Mean^{(3)}$	Bibliography
						Malathion (pS)	$1.08^{(1)}$ - $32^{(2)}$	
						Tapazole (Ph)	$0.83^{(1)}$ - 5-$15^{(2)}$	[10]
						Mercaptobenzimidazole (Ph)	$1.95^{(1)}$ – $18.5^{(2)}$	
						Thiouracil (Ph)	$0.75^{(1)}$ – $0.89^{(2)}$	
Guadiana	Portugal	70	1500 - 20	60.361	742	Terbuthylazine (Ps)	$254^{(3)}$ – $531^{(2)}$	[11]
						Molinate (Ps)	$39^{(3)}$ - $352^{(2)}$	
						Metolachlor (Ps)	$91^{(3)}$ - $290^{(2)}$	
Rhône	France	1.710	13000 - 360	100.200	812	4-chloro-2-methylphenol (CMP) (Ps)	$0.04^{(1)}$ – $1.12^{(2)}$	[12]
						4-chloro-2-methyl-6-nitrophenol (CMNP) (Ps)	$0.02^{(1)}$ – $1.12^{(2)}$	
						(4-chloro-2-methylphenoxy)-acetic acid (MCPA) (Ps)	$0.08^{(1)}$ – $4^{(2)}$	
Aliakmon	Greece	73	137 - 21	9.210	322	Salicylic acid (Ph)	$349^{(1)}$ - $3875^{(2)}$	[13]
						Triclosan (Ph)	$149^{(1)}$ –$1742^{(2)}$	

River	Country	Mean flow (m^3/s)	Maximum and minimum range (m^3/s)	Km^2	Length of river (Km)	Pollutant Pesticides (Ps) Pharmaceutical (Ph)	Concentration ($ng\ L^{-1}$) $Min^{(1)}/Max^{(2)}/Mean^{(3)}$	Bibliography
						Carbamazepine (Ph)	$16^{(1)}$ –$54^{(2)}$	
Axios	Greece	158	279 - 49	24.000	388	Alachlor (Ps)	$260^{(2)}$	
						Metolachlor (Ps)	$520^{(2)}$	
						Trifluralin (Ps)	$312^{(2)}$	

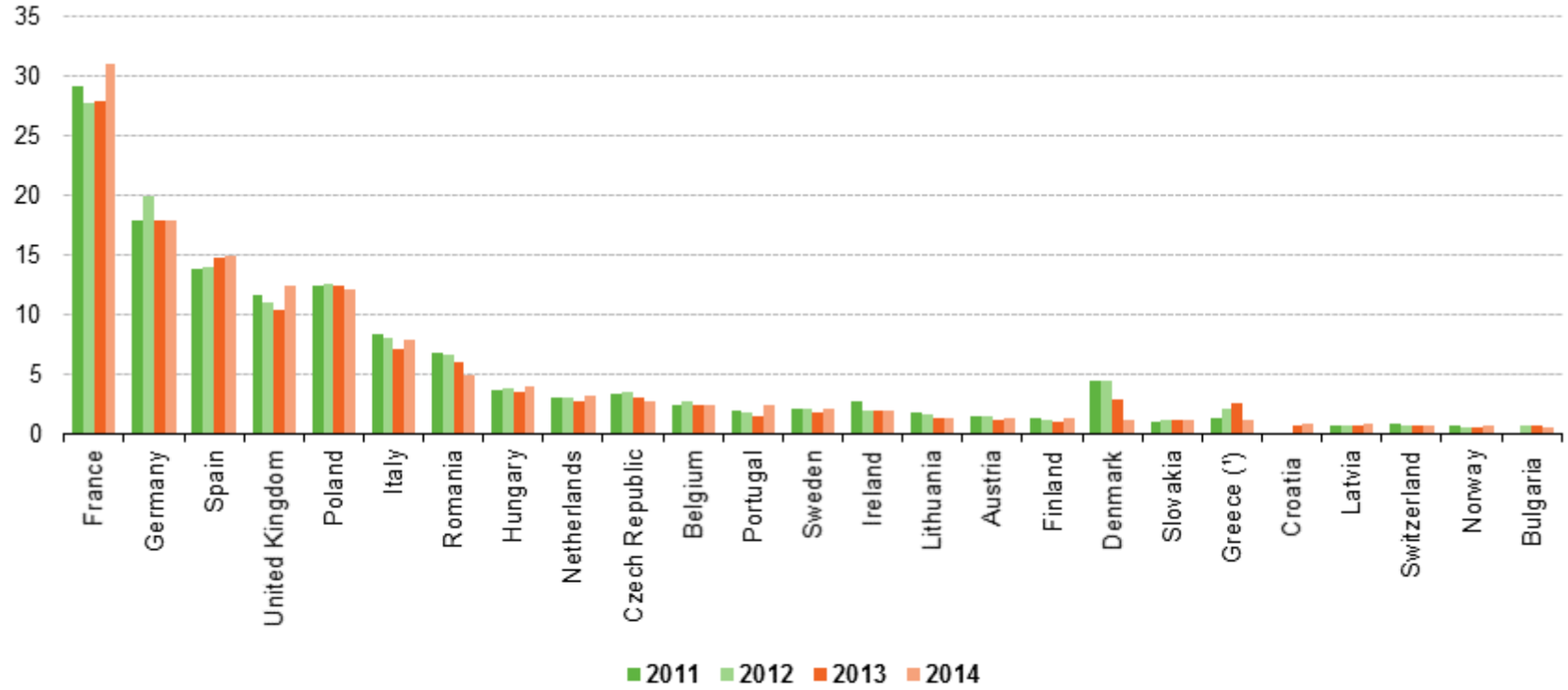
Figure 1. Sales of fungicides in Mediterranean countries



Note: Sales under 0.5 thousand tonnes in Bulgaria, Estonia, Malta, Latvia, Luxembourg, Finland, Sweden and Norway.
(*) Break in the series in 2013.

Consulted April 23, 2017 Base data Eurosat

Figure 2. Sales of herbicides in Mediterranean countries



Note: Sales under 0.5 million tonnes in Cyprus, Estonia, Malta, Luxembourg and Slovenia.
(1) Break in the series in 2013.

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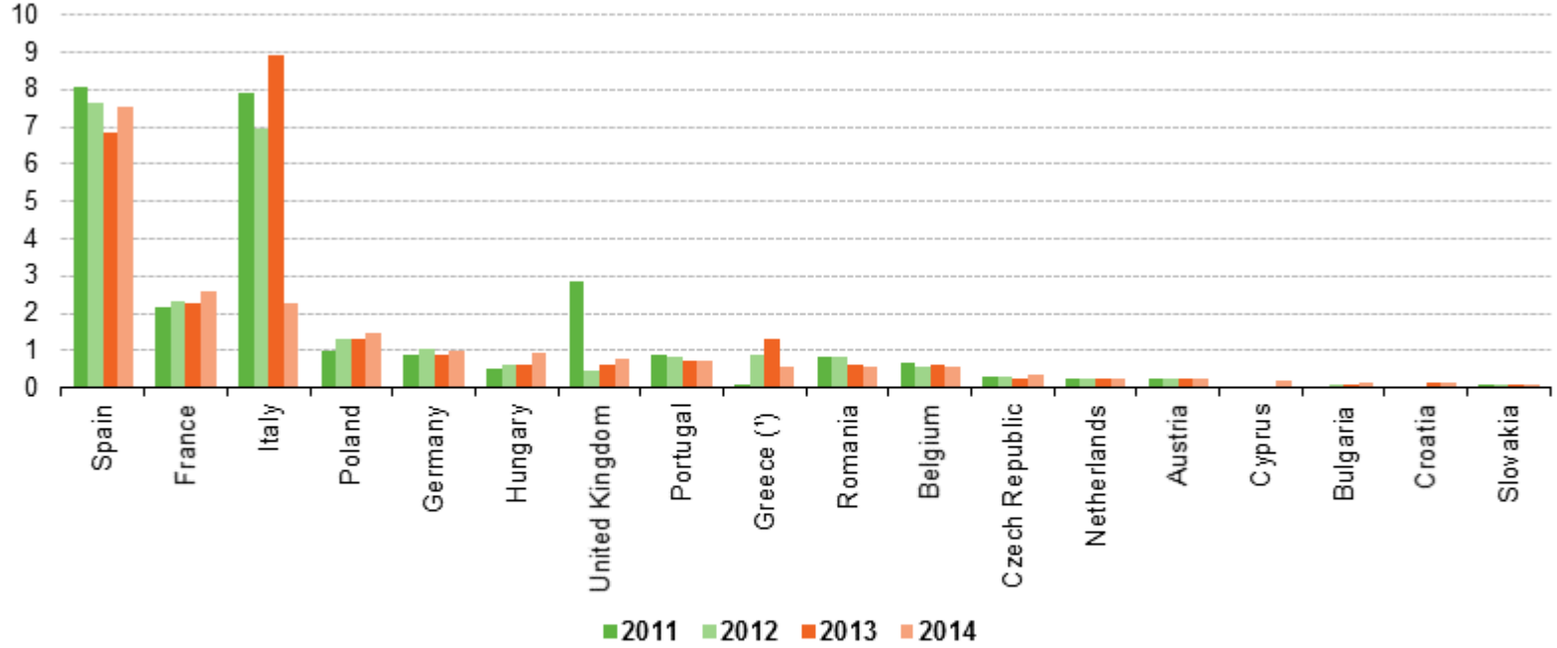
Figure 3. Sales of insecticides in Mediterranean countries



Note: Sales under 0.1 million tonnes in Denmark, Estonia, Ireland, Malta, Latvia, Lithuania, Slovenia, Finland, Sweden, Norway and Switzerland. Luxembourg: not available.
(') Break in the series in 2013.

Consulted April 23, 2017 Base data Eurosat

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SEGUNDA PARTE

V.

DESARROLLO DE UN MÉTODO ANALÍTICO Y DE EXTRACCIÓN PARA PIRETRINAS Y PIRETROIDES

Debido a su creciente utilización en los últimos años, se presenta el desarrollo de un método para el análisis simultaneo de piretrinas naturales y sintéticas basado en la microextracción líquido-líquido dispersiva (DLLME) y cromatografía liquida y espectrometría de masas optimizado para su aplicación en aguas y sedimentos. Se demuestra la eficacia de estos métodos basados en la DLLME para la extracción de piretrinas naturales y sintéticas mediante la aplicación del mismo a muestras de aguas del humedal La Albufera y sedimentos del río Turia.

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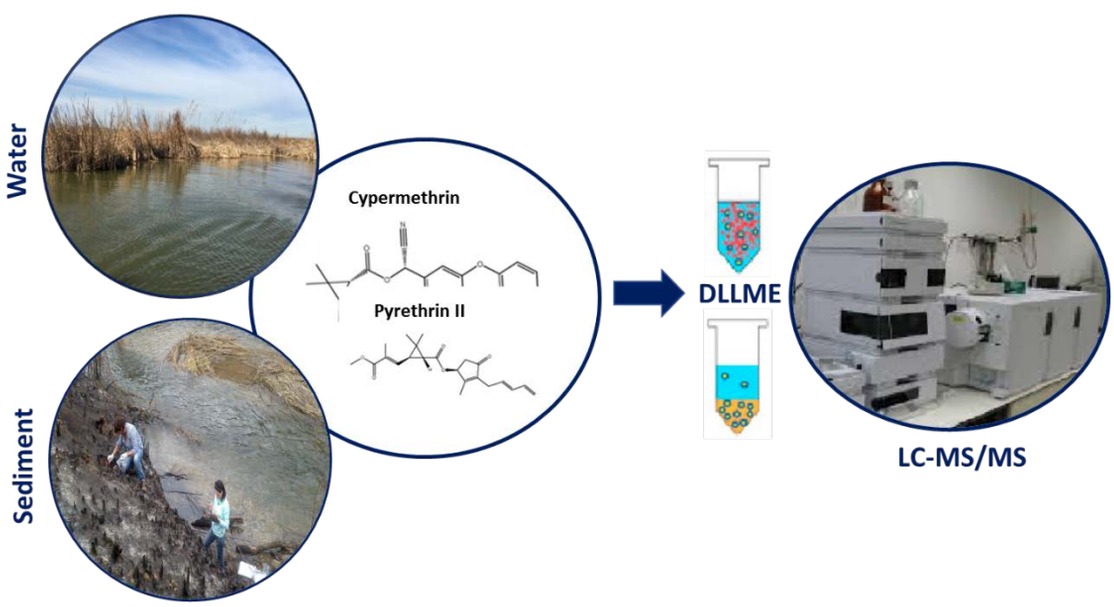
Simultaneous determination of pyrethroids and pyrethrins by dispersive liquid-liquid microextraction and liquid chromatography triple quadrupole mass spectrometry in environmental samples

Alexander Ccanccapa[*,1], Ana Masiá[1], Yolanda Picó[1],

[1]Food and Environmental Safety Research Group (SAMA-UV), Facultat de Farmàcia, Universitat de València, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain.

[*] Corresponding autor: Alexander Ccanccapa
Tel: +34 963543092; Fax: +34 963544954
E-mail: Alexander.Ccanccapa@uv.es


Water
Sediment
Cypermethrin
Pyrethrin II
DLLME
LC-MS/MS

Abstract

A simple and environmentally friendly dispersive liquid-liquid microextraction (DLLME) method coupled with electrospray ionization liquid chromatography triple quadrupole mass spectrometry (LC-QqQ-MS/MS) was developed for the simultaneous determination of seventeen synthetic and natural pyrethroids. A comparison of solid-phase extraction (SPE) vs DLLME for water samples and only `Dilute and Shoot´ vs the additional extract clean-up by DLLME for sediment samples was reported. Chloroform was used as the extraction solvent in the DLLME technique in both water and sediment samples. Ultrasonic energy was applied to make the analytes fully extracted into fine droplets, providing high recoveries in short times. Method detection limits (MDLs) ranged from 0.12 to 0.62 ng L^{-1} and recoveries from 70 to 119 % with RSDs values 2–15% (n=5) for water samples. In sediment samples, MDLs ranged from 0.50 to 2.50 ng g^{-1} and recoveries from 71 to 112 % with RSDs 2–16% (n=5). The proposed method showed a good linearity within the range of 10 ng mL^{1} – 500 ng mL^{-1}, with coefficients of determination (R^2) higher than 0.99. Matrix effects were observed for most compounds in water and sediment (ME% < –10%). The proposed methodology was applied for the analysis of water and sediment samples of the Albufera Wetland and Turia River. Acrinathrin (48 ng g^{-1}) and etofenprox (16 ng g^{-1}) were detected in sediment samples.

Key words: Liquid chromatography; Triple quadrupole mass spectrometry; Dispersive Liquid-liquid microextraction; Pyrethroids and pyrethrins; Water; Sediment

1. Introduction

Pyrethroids are organic synthetic insecticides that have been designed based on the structures of the pyrethrins, which are natural insecticides derived from chrysanthemum flowers. Natural pyrethrins consists of six esters identified as pyrethrin I and II, cinerin I and II, and jasmolin I and II, obtained from the combination of chrisanthemic or pyrethric acid with three alcohols: cinerolone, pyrethrolone, and jasmolone. They are known for their rapid knock down and lethal action against a broad range of insect pests, which don't persist nor biodegrade within the native enzyme system of mammals [1,2]. Recently, pyrethroids have increasingly replaced organophosphate, carbamate, and organochlorine pesticides [3,4]. Owing to their broad-spectrum of insecticidal activity, they are used indoors to control household pests (flies, mosquitoes, cockroaches, termites, and other harmful insects), and outdoors to protect livestock, as post-harvest insecticides on stored grain and as an agricultural pre-harvest treatment on fruit orchards and vegetables crops [5,6]. The widespread use of these compounds has resulted in contamination of environmental compartments, such as water, soil and air [7-10]. Moreover, Pyrethrins are allowed in Europe for organic production according to Regulation (EC) No. 889/2008 [11]. Drinking water quality (CE) No. 98/83 [12] establishes 0.10 μg L^{-1} as MRL (Maximum Residue Level) for individual pesticides and 0.50 μg L^{-1} for total pesticides. Pyrethroids are persistent compounds with high hydrophobicity (log K_{ow} in the range 5.7–7.6) and very low water solubility (of a few μg L^{-1}). Therefore, they rapidly dissipate from the water column and readily bind to sediment [13,7].

At present, analytical procedures developed for pyrethroid residue determination in environmental samples involved mainly gas chromatography with electron capture detection

(GC-ECD) and gas chromatography mass spectrometry (GC-MS) [14,15,7,16-22]. Reversed phase liquid chromatography (RP-LC) and UV detection were used to analyze pyrethroids in water samples [23-26,3,27-29]. Regarding sediment samples, kadethrin, cypermethrin and permethrin were already determined by RP-LC-UV [9] but most studies used GC-ECD, GC-MS and GC-MS/MS [30,31,18,17,6,15,7,16,22]. However, in food and vegetables, LC-UV was widely used for pyrethroids [32,5,33,23,34,29,20,28,21]; and LC-mass spectrometry (LC-MS) was also reported, even that in less extend, to determine both, pyrethrins [1] and pyrethroids [34]. There is just one report that analyzed pyrethrins and pyrethroids, together, by GC-MS in fish tissues [2]. To our knowledge, no other reports on natural and synthetic pyrethrins analyzed them by LC-MS/MS in environmental samples.

Most common extraction procedures for water and sediments are liquid–liquid extraction (LLE), solid-phase extraction (SPE), magnetic solid phase extraction (MSPE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE) and QuEChERS. [6,3,24,25,27,31,18,34]. However, disadvantages such as, time consuming, large organic solvent volumes, and secondary wastes, limit their application. Recently, a new micro extraction method, namely dispersive liquid-liquid micro extraction (DLLME), has been developed as an efficient sample preparation and pre-concentration method [35-38,20,28]. The advantages of DLLME are the small volume of organic solvents used, ease of operation, rapidity, low cost, high recovery, high enrichment factor, and environmentally friendly nature. DLLME is based on the formation of a cloudy solution containing fine droplets of the extraction solvent formed when the dispersive solvent and the extraction solvent are rapidly injected into the aqueous sample (ternary component solvent system consisted of aqueous sample, dispersive solvent —water and extraction solvent miscible— and extraction solvent

—water immiscible is used) [39,40]. Improvements in this method were reported using ultrasonic energy that accelerated formation of the dispersive mixture, reduced the equilibrium time and markedly increased the extraction efficiency [41,17,42-44].

The main objective of this study was to develop a simple and sensitive analytical method that simultaneously determines pyrethroid and pyrethrin residues in environmental samples. DLLME was used as a concentration and/or clean-up technique for both, water and sediment extracts, before these pesticides determination by LC-MS/MS. Special attention was given to the optimization of LC-MS/MS method to determine synthetic and natural pyrethrins and to adjust DLLME parameters to maximize the extraction efficiency. The method was validated and applied to water and sediment samples. To our knowledge, this work reports for the first time the determination of both group of pesticides together by LC-MS/MS previous DLLME in environmental samples.

2. Experimental

2.1. Chemicals and reagents

Standards of acrinathrin, bifenthrin, cyhalothrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, etofenprox, fluvalinate, flumethrin, tefluthrin and internal standard (I.S.) etofenprox-D5 were obtained from Dr. Ehrenstorfer (Augsburg, Germany) with a purity of 99%. Pyrethrins standard (pyrethrins technical mixture, CAS No.8003-34-7) was also purchased from Dr Ehrenstorfer and contains cinerin I (4.8%), cinerin II (4.7%), jasmolin I (2.9%), jasmolin II (1.8%), pyrethrin I (51.7%) and pyrethrin II (33.7%). Physico-chemical properties of pyrethroids and pyrethrins are shown on **Table S-1**. A standard solution of pyrethrins was prepared by dissolving100 mg of liquid technical mixture in 10 mL of

methanol. The individual pyrethrins percentage was taken into account to calculate the concentrations. Individual standard solutions of the solid standards of pyrethroids were prepared in methanol at the concentration of 1000 mg L^{-1}. The working standard solution was prepared by mixing the appropriate volumes of each standard solution and diluting them with methanol. The final concentration was 500 ng mL^{-1} for each pyrethroid, 240 ng mL^{-1} for cinerin I, 235 ng mL^{-1} for cinerin II, 148 for jasmolin I, 91.1 ng mL^{-1} for jasmolin II, 2585 ng mL^{-1} for pyrethrin I and 1685 ng mL^{-1} for pyrethrin II. Standard and working standard solutions were stored at -20 °C in the dark. Solutions were kept for six months and checked monthly for signs of degradation. Standards were deemed acceptable if the peak area remained within ±15% of the area obtained in the initial analysis of the standard. Working mixtures, at appropriate concentrations, were made daily by diluting aliquots of the working standard solution in methanol or in matrix extract.

Carbon tetrachloride and chloroform were purchased from VWR International (Radnor, Pensilvania, Estados Unidos); acetic acid and ammonium formate from Sigma-Aldrich (Steinheim, Germany). Deionized water was prepared from a Milli-Q system (Millipore, Bedford, MA, USA). HPLC-grade acetonitrile and methanol from Merck (Darmstadt, Germany) and OASIS HLB SPE cartridge 200 mg sorbent/6 mL cartridge from Waters (Milford, MA, USA).

2.2. Water and sediment sampling

Ten water samples were taken from the Albufera wetland and 10 sediment samples from the Turia River. Water samples were collected randomly from midstream at 0.3 m deep below the water surface using 1 L polypropylene bottles. Top layer of sediments up to 10 cm deep were

collected in the month of the river using a Van Veen grab sampler (0.5 L capacity, ca. 250 g of sample) and transferred into aluminum foil (previously washed with methanol and dried in oven at 100◦C) that was put inside an aluminum box. All samples were transported in an ice filled cooler to the laboratory where water samples were kept refrigerated at 4.5 ◦C and extracted within 48 h and sediment samples were frozen (-20 °C) and freeze-dried with a Virtis SP Scientific Lyophilizer (Gardiner, NY, USA) at -65 °C and vacuum of 1-4 mT for 48 h.

2.3. Instrumentation

The chromatographic instrument was an HP1200 series LC- an automatic injector, a degasser, a quaternary pump and a column oven – combined with an Agilent 6410 triple quadrupole (QQQ) mass spectrometer, equipped with an electrospray ionization (ESI) interface (Agilent Technologies, Waldbronn, Germany).

The chromatographic column was a Luna C18 (150 × 2.1 mm) with a 3 μm particle size (Phenomenex, Torrance, USA). The column temperature was kept at 30 °C and the volume injected was 5 μL. Flow rate was 0.3 mL min^{-1} and injection volume was 5 μL. Mobile phases consisted of 10 mM ammonium formate in Milli-Q water (A) and 10 mM ammonium formate in methanol (B). Separation was carried out in 25 min under the following conditions: 0 min, 50% B; 10 min, 83% B; 12 min, 83% B; 12 min, 98% B; and 25 min, 98% B. Then, the mobile phase returns to the initial conditions with an equilibration time of 15 min.

The ESI conditions were: capillary voltage 4000 V, nebulizer 25 psi, source temperature 300 °C and gas flow 11 L min^{-1} **(see Table S-2)**. Data were processed using a MassHunter Workstation Software for qualitative and quantitative analysis (A GL Sciences, Tokyo,

Japan). The data acquisition parameters were adjusted for each individual compound in multiple selected-reaction monitoring (MRM) mode using the Mass Hunter Optimizer software. Specifically, this software automatically selects the most intense precursor ions, the best fragmentor voltage for each of them, the finest product ions, and the optimal collision energy. Nitrogen was used as collision, nebulizing and desolvation gas. The most abundant precursor-to-product ion transition (SMR1) was monitored for the quantification and the second one (SMR2), less intense, was used as a qualifier for each compound.

2.4. DLLME extraction procedure

2.4.1. Water samples

A volume of 8 mL of water sample was placed in a 50 mL conical glass tube. The optimum mixture of 2 mL of acetonitrile, milli-Q water and acetic acid (79:20:1) (v/v) (as dispersive solvent) and 200 µL of chloroform (as extraction solvent) was quickly injected into the sample solution with a syringe and vortex 30 s. Then, the mixture was immersed in an ultrasonic water bath for 3 min. At this step, the analytes were extracted into the organic solvent droplets. After that, the mixture was centrifuged at 3500 rpm and 15 ºC for 10 min. The upper aqueous phase was removed with a Pasteur pipette and the bottom phase (chloroform) was collected using a syringe of 100 µL, model 1710 RN SYR, Hamilton (Bonaduz, Switzerland), placed in a small vial, and evaporated to dryness at 40 ºC under a stream of nitrogen. The residue was reconstituted in 200 µL of methanol and injected into the LC-MS.

2.4.2. Solid-phase extraction (SPE)

For the first test (Test 1) Oasis HLB cartridges were preconditioned with 5 mL dichloromethane–methanol (50:50) (v/v) followed by 10 mL of deionized water. Water samples (200 mL) were passed through the SPE column (flow rate ca. 10 mL min^{-1}) using a vacuum manifold that maintains a constant pressure differential between the inlet and the outlet of the cartridge (the resistance to flow of the SPE varied through the extraction by the clogging of the sorbent, consequently, the flow rate was somewhat variable). The cartridges were then, dried under vacuum for 10 min to remove residual water and analytes eluted with 10 mL of dichloromethane-methanol (50:50, v/v) drop by drop (flow rate ca. 1 mL min^{-1}). Extracts were evaporated to dryness at 40 °C under a stream of nitrogen and reconstituted with 1 mL of methanol. Then, they were filtered through 0.45 μm PTFE filters into the vials for LC–MS analysis. In the second test (Test 2), the procedure was similar but the analytes were eluted with 7 mL of n-hexane and reconstituted with 1 mL of ACN/water (70:30, v/v).

2.5. Sediment samples

Sediment (1 g) samples were accurately weighted into 50 mL polypropylene centrifuge tubes. A mixture of 4 mL of acetonitrile, milli-Q water and acetic acid (79:20:1) (v/v) was added and shaken by vortex for 30 min and centrifuged at 3500 rpm for 2 min at 15 °C. To follow Dilute and Shoot method, 500 μL of the extract was transferred into glass vial and diluted with 500 μL of acetonitrile, milli-Q water and acetic acid (20:79:1) (v/v) and injected into the LC-MS/MS.

The extraction procedure was also tested using an additional clean up step by DLLME, the extract was carefully separated from the precipitate using a Pasteur pipette and placed in 15 mL polypropylene centrifuge tubes. The aqueous acetonitrile extract was added of 100 μL of

chloroform and 8 mL of deionized water. The tube was gently shaken by hand for 30 s, and then, immersed in an ultrasonic water bath for 3 min at room temperature and centrifuged at 3500 rpm for 3 min at 15 °C. Finally, solvent phase was collected in a small vial with a syringe and evaporated to dryness at 40 ºC under a gentle stream of nitrogen. The residue was reconstituted in 100 µL of methanol and injected into the LC-MS/MS.

2.6. Validation study

The fitness-of-purpose of the optimized sample preparation methods was assessed regarding the selectivity, linearity, recovery, precision and sensitivity (limits of detection and quantification) in base on the Document No. SANTE/11945/2015 [45] . Selectivity was verified by analyzing blank and naturally contaminated water and sediment samples. The linearity was evaluated using analytical standards prepared in methanol as well as in water and sediment extracts at concentrations from 10 to 500 ng mL^{-1} of each compound in the final extract (except for pyrethins that depends on the initial ones in the working mixture). The IS was added at the fix concentration of 100 ng mL^{-1}. The calibration curve was constructed as plot of X = concentration of analyte versus Y = ratio of the area of analyte to IS.

Recovery was determined using fortified blank matrices with mutually independent replicates at the three concentration levels (1.25, 3.12 and 12.5 µg L^{-1} for water and 10, 25 and 100 ng g^{-1} for sediments) of pyrethroids and the corresponding concentrations considering their initial ones in the working mixture of pyrethrins. Five determinations were carried out for each concentration. Prior to the extraction step, the fortified samples were allowed to settle for 30 min and then processed according to the above procedure. Precision was assessed under repeatability and reproducibility (3 days) conditions and it was expressed in terms of relative

standard deviation (RSD). Finally, the limit of detection (LOD) was evaluated using a signal to noise (S/N) ratio of 3:1 and limit of quantification (LOQ) was S/N of 10:1(Instrument detection limit IDL and method detection limit MDL).

Matrix effect was calculated by comparison of the slopes obtained from analytical curves prepared in methanol and in blank water and sediment using the following equation:

$$\text{Matrix effect } (\%) = \left[\left(\frac{\text{slope curve in matrix}}{\text{slope curve in methanol}}\right)\text{-}1\right] \text{ x } 100$$

3. Results and discussion

3.1. LC-MS/MS conditions optimization

A large number of experiments were performed to optimize the MS/MS conditions, especially for pyrethroids, which form abundant adduct ions in the mass spectra like $[M + Na]^+$, $[M + K]^+$ or $[M + NH_4]^+$ when undergoing ionization in the positive ion mode. Carboxyl or carbonyl ether or ester groups in the molecule are responsible for the binding to alkali metal ions. Using mobile phases without additives, sodium adduct that do not fragment under MS/MS conditions were by far the most intense signal for all pyrethroids. Then, the selected mobile phase contained ammonium in order to favor the formation of this adduct, which fragmented much better. Other alternatives, as negative ionization mode or addition of formic or acetic acid to favor the formation of the protonated molecule, did not work. The MS2 Scan and product ion scan were first performed manually to confirm that the ammonium adduct had the highest response, if there were other adducts, and to establish the characteristic fragmentation. The m/z scan ranged from 100 and 700 and two fragmentors 40 and 80 V were used (see **Table S-3)**. Then, in a second step, the MassHunter Acquisition optimizer software

was used to establish LC-MS/MS SRM transitions, fragmentor voltages and collision energies for 17 pesticides evaluated in this study **Table S-4**. For each synthetic and natural pyrethrin, two mass transitions with the highest abundances were selected.

The LC separation of these compounds is complicated because they are apolar (see the high log K_{ow} in Table S1). Then, they require a high percentage of methanol (> 90 %) in the mobile phase to elute. Several columns, mobile phases and gradients were tested but the separation of these compounds was always poor. Other criteria, such as sensitivity and reproducibility, were taken into account to select the chromatographic method. The use of ammonium formate helped to favor the formation of [M+H]+ ions (for pyrethrins) and $[M+NH_4]^+$ (for pyrethroids) instead of the $[M+Na]^+$. The chromatographic system works better using gradients that start with a high percentage of water if high amounts of salts are used in the mobile phase because salt precipitation is prevented and head column pressure takes longer in raising. Higher robustness and reproducibility was obtained. These results are in agreement with those previously reported for pyrethrins [1].

Table 1 shows the optimum MS/MS conditions for pyrethroids and pyrethrins in positive ionization (PI) mode. The main ion observed in the mass spectrum was the ammonium adduct $[M+NH_4]^+$ for pyrethroids and the protonated molecule $[M+H]^+$ for pyrethrins. **Fig. 1** illustrates the extracted ion chromatogram obtained.

The linearity of the MS analyzer response was investigated by performing triplicate injections standard solutions. The range tested in solvent was 10–500 ng mL^{-1}. The range in water samples were 250, 625, 1250, 1875, 2500, 6250, 12500 ng L^{-1} and 1, 2.5, 5, 7.5, 10, 25, 50 ng g^{-1} in sediment samples. A linear response was observed and determination coefficients (R^2)

ranged from 0.9903 (acrinathrin) to 0.9999 (cinerin I) for all analytes. The RSD (%) ranged between 1 to 9 and finally IDL were from 12.5 pg to 50 pg **Table S-5**.

3.2. Selection and optimization of the extraction procedure in environmental samples

SPE and DLLME for water samples and only "dilute and shoot" and the addition of DLLME clean-up of the extract for sediment samples were compared. Different solvents were tested, such as, acetonitrile, chloroform, carbon tetrachloride, dichloromethane and hexane.

3.2.1. Water samples

Initial SPE experiments were carried out with 250 mL of blank water spiked at 100 ng mL^{-1} of each compound including the IS by adding 100 µL of a standard solution of 1 µg mL^{-1} prepared in methanol. The pre-concentration applied to the water samples is based on the off-line SPE procedure described by Masiá et al. [46]. The extraction step was not optimized for pyrethrins and pyrethroids since this method uses very generic conditions (250 mg of Oasis HLB and 250 mL of water). The only problem that could arise is the analyte breakthrough, which taken into account the $\log K_{ow}$ of these compounds (see Table S1) is very difficult, not to say impossible. However, two different eluents were tested to optimize the analytes elution step. A comparison between the recoveries obtained is shown in **Fig. 2**. The recoveries ranged from 15 to 48 % using 10 mL of dichloromethane-methanol (50:50, v/v) as eluent and 1 mL of methanol to reconstitute. The recoveries for pyrethroids and pyrethrins were improved, using 7 mL of n-hexane as eluent and 1 mL of ACN/water (70:30, v/v) to reconstitute (from 25 to 75 %). However, recoveries were below of the SANTE guidance requirement (70 to 120%) for some compounds [45]. Despite SPE is widely accepted as the best technique for isolating pesticides residues in water samples, there are few studies that apply it to determine natural and synthetic pyrethroids in water samples [47,48] in comparison to those that report DLLME (see Table S7 of the SI that compiles different methods).

DLLME has been reported as an alternative extraction or clean-up method depending on whether samples are liquid or solid in environmental [23,17,27,38,37], fruit and food samples [21,19,41]. This method showed good results in different matrices and advantages as simplicity of operation, rapidity, low cost, high recovery and enrichment factor. Then, it was tested as alternative to SPE.

The study was carried out with 8 mL of blank water spiked at 100 ng mL^{-1} of each pesticide. Ultrasonic energy was applied to make the analytes fully extracted into the fine droplets, providing high recoveries. This energy causes an effect known as cavitation, which generates numerous tiny bubbles in liquid media. Sonication provides an efficient contact between the solid and the extractant, usually resulting in a good recovery of the analyte, as it shown in the results. This DLLME method required an extraction solvent, low melting point, low water solubility and high extraction capability of target compounds. In this sense, chloroform ($CHCl_3$), carbon tetrachloride (CCl_4), dichloromethane (CH_2Cl_2) and n-hexane (C_6H_{14}) were investigated. The results showed that dichloromethane and n-hexane did not lead to droplet formation due their solubility in water (1.6 and 9.5%, respectively). However, $CHCl_3$ and CCl_4 displayed the highest extraction responses (see **Fig. 3A**). Consequently, $CHCl_3$ was selected as the extraction solvent for this study for being environmentally friendly. $CHCl_3$ showed high recoveries for pyrethroids (70 to 119%) and pyrethrins (73 to 114%). Exceptionally, cypermethrin and jasmolin I had recoveries lower than 70 %.

Different extraction times 1, 2, 3, 6 and 9 min were tested. Results shown in **Fig. 3B** revealed that the time does not have greater influence in the range studied. Shaking 1 and 2 min the recoveries are already high but the variability of the results was also high. Consequently, time was set at 3 min. The recoveries with this time ranged from 64 (Jasmolin I) to 106%

(Fluvalinate). Other DLLME methods used small extracting solvent as 8, 10, 50, 60 and 100 µL [49,3,37,27,43]. However, in this work was fixed in 200 µL because of the difficulty to retrieve the resulting bottom layer. This parameter could affect the reproducibility of the process. Then, we select a volume that provided consistent results.

3.2.2. Sediment samples

In the first experiment, a dilute and shoot method similar to those reported for urine and biological samples [50-54] was applied. This method is characterized by reducing the cost and the time-consuming extraction step and minimize the matrix effect. The LODs and LOQs were high (from 10 to 20 ng g^{-1} and 30 to 60 ng g^{-1}, respectively) compared to those previously reported using other methods (0.10-3.71 ng g^{-1}, see **Table S7** for detailed information). The matrix effect reported in **Table 2** for both pyrethrins and pyrethroids was high ranging from 88 to 44 %. Then, in order to reduce matrix effect, this extraction method was also tested including the previous DLLME developed for water as clean-up step that also concentrate the analytes to achieve appropriate sensitivity.

The same extractants and extraction times tested for water were also tested for sediment extracts. Only $CHCl_3$ and CCl_4 led to droplet formation. The results of the global method including extraction showed high recoveries with both dissolvents, $CHCl_3$ from 64 to 118% and CCl_4 from 63 to 112 %. However, $CHCl_3$ was selected because it is more environmental friendly than CCl_4 and provided almost the same recoveries (**Fig 4A)**. Regarding the extraction time, 1, 2, 3, 6 and 9 min were tested. As already proved for water, the recoveries were not influence by this parameter. Then, 3 min was selected (**Fig 4B)**.

Pyrethrins and pyrethroids were successfully recovered by DLLME using $CHCl_3$ as the extracting solvent and acetonitrile as the dispersive solvent at pH slightly acid in both water and sediment extracts. Furthermore, the sediments extracts are obtained with the dispersive phase and then, the only step for the clean-up is the addition of water and the extracting solvent.

3.3. Analytical performance

Calibration curves were prepared at seven concentration levels of 10, 25, 50, 75, 100, 250 and 500 ng mL^{-1} for pyrethroids. For pyrethrins taking into account the composition of the pyrethrins technical mixture, their linearity was evaluated in different ranges, namely, cinerin I (4.8–240 µg L^{-1}), cinerin II (4.7–235 µg L^{-1}), jasmolin I (2.9–145 µg L^{-1}), jasmolin II (1.8–90 µg L^{-1}), pyrethrin I (51.7–2585 µg L^{-1}), and pyrethrin II (33.7–1685 µg l^{-1}). The characteristic calibration data are listed in **Table S-5**. A good linearity across the studied ranges was achieved with determination coefficients (R^2) from 0.9921 to 0.999 in water matrix and 0.9924 to 0.9992 in sediments for all compounds investigated **Table S-6**. Exceptionally, flumethrin showed 0.9563 in sediment matrix. The I.S. etofenprox-D5 was added for water and sediment samples at level of 100 ng mL^{-1} prior to the extraction procedure, and the pyrethrins and pyrethroids were quantified employing seven-point matrix-matched calibration curves constructed by plotting analyte/I.S. peak area ratio against concentration values

The MDLs of pyrethroids and pyrethrins were in the range from 0.12 to 0.62 µg L^{-1} for water and from 0.50 to 2.50 ng g^{-1} for sediments. The limits of quantitation (LOQ, S/N=10) were f from 0.37 to 0.75 µg L^{-1} and 1.50 to 7.50 ng g^{-1} for water and sediments, respectively.

The intra-day precision varied from 2 to 15 % for water samples and from 2 to 16 % for sediment samples. Precision showed no significant difference between inter-day and intra-day assays (**Table 3)**. The recoveries obtained for water samples ranged from 70 to 119%, with the exception of cypermethrin and jasmolin I that showed recoveries of 65 and 62%, respectively. In sediment samples, recoveries varied from 71 to 112%, with the exception of Etofenprox and jasmolin I that had recoveries of 64 and 66%, respectively (**Table 3)**. The analytical characteristics of the proposed method were compared to the other reported methods, as summarized in **Table S-7**. Albaseer et al. [3] reported higher MDLs (0.05 to 0.08 mg L^{-1}) than in our study using LLME followed by HPLC-UV to determine three synthetic pyrethroids (permethrin, resmethrin and cypermethrin) in water. However, it is well known that UV detector is not sensitive to certain compounds. Mukdasai et al. [23] reported MQLs ranging from 0.25 to 5 ng mL^{-1} —similar to our study (0.37 to 0.75 ng mL^{-1}) using DME combined with dispersive μ-solid phase extraction (D-μ-SPE) and UV detector for tetramethrin, fenpropathrin, deltamethrin and permethrin. There are already few studies that optimized DLLME in water samples providing results comparable to our study [20,3,27,49,36,38,37]. Interestingly, Ye et al. [20] developed a method for sixteen pyrethroids in water samples using DLLME based on dissolved carbon dioxide flotation. The results demonstrated good performance in term of enrichment factors, MDLs (0.87-1.39 μg L^{-1}), sensitivity and extraction time (40s). However, the extractant solvent (dodecanol) is not environmental friendly.

In sediment samples, there are reports using HPLC-UV to determine pyrethroids. Chalanyova et al. [9] obtained higher MDLs (3.6 to 4.5 ng g^{-1}) for kadethrin , cypermethrin and permethrin using on-line flow-through extraction successive on-line SPE as pre-

concentration. In food samples, there were more advances in the development of analytical and extraction methods. Chung et al. [34] developed QuEChERS method to determine 15 synthetic pyrethroids by LC-MS/MS, however the LOQs was high (10 ng g^{-1}). Peruga et al. [1] achieved LOQ in a range from 0.1 to 5.3 ng g^{-1} also using LC-MS/MS and simple solvent extraction (acetone/water 70:30) to determine pyrethrins in fruits and vegetables samples. There is only one report that developed a QuEChERS coupled to GC-MS on pyrethroids and pyrethrins in fish tissues. LODs were relatively low and ranged from 0.5 to 0.3 ng g^{-1}.

3.4. Matrix effects

It is well known that matrix components affect the detection of the analytes either suppressing or enhancing the analyte signal. The effect was considered significant if the response in solvent and matrix extract do not differ more than 25 %. Matrix effects were within this range for many compounds (**Fig. 5)**. The most common effect was signal suppression but acrinathrin in sediments, cyhalothrin in water and fluvalinate in both matrices gave signal enhancement. On the supression, deltamethrin, flumethrin and tefluthrin showed a higher signal suppression in water (up to 80 %). Similarly, cyhalothrin, cyfluthrin, flumethrin, cinerin I and jasmolin II present signal suppression up to 80 % in sediment samples. The use of only one IS is not able to correct the matrix effect because it is dependent on the compound and on the sample, then, matrix matched standards were use for quantification.

3.5. Application to the environmental samples

The water samples of this study were taken of the Albufera Wetland, a natural park located in the Spanish eastern coast. The sediment samples were taken from the mouth of the Turia River (located near the park). These samples belongs to two different monitoring carried out

in 2016 in the wetland and river basin. This area has a rice production cycle. Previous studies also showed the use of pyrethroids (permethrin, bifenthrin, cyhalothrin, fluvalinate, cypermethrin and deltamethrin) in rice fields in this area [55-57]. These two factors combined highlights the importance of testing synthetic and natural pyrethroids in these ecosystems.

Acrinathrin (48 ng g^{-1}) and etofenprox (16 ng g^{-1}) were found in sediment samples (**Fig. 6**). However, none of the pyrethroids and pyrethrins selected were found in water samples. This result could be explained by the hydrophobicity of pyrethroids that tend to accumulate in sediment compartments. The average recoveries for most analytes were in a range of 70-119% for water and 71-118% for sediment samples with RSD less than 20%, which indicated that the method was reliable and could be used for the determination.

4. Conclusion

In the present study DLLME followed by LC-MS/MS was successfully developed and applied to the extraction and determination of pyrethroids and pyrethrins residues in water and sediment samples. Chloroform was used as the extractant solvent in small quantity in water and sediment, resulting in an environmental friendly extraction method. In water and sediment samples, ultrasonic energy was critical to fully extract the analytes into the fine droplets, providing high recoveries in short time. The results demonstrated that this technique exhibits low LODs and excellent sensitivity. The optimum extraction conditions were with 100 (sediment) and 200 (water) µL of chloroform and 2 mL of acetonitrile, milli-Q water and acetic acid (79:20:1) (v/v) as the extractant solvent and dispersant, with 3 min of extraction time. The LODs ranged from 0.12 to 0.62 µg L^{-1} for water samples and from 0.50 to 2.50 ng g^{-1} for sediment samples. The recoveries in water samples were from 70 to 119 %, and in

sediments from 71 to 112% (both with RSD of less than 16%). This method is a good alternative for routine analysis due to its simplicity, sensitivity, and reliability.

Pyrethroids and pyrethrins have been intensively used in agricultural, industrial, and urban areas since they are a replacement of other banned pesticides, such as organochlorine and organophosphates. The occurrence of these compounds is of concern because, even through, they are retained in sediments due to their hydrophobicity and low water solubility, can be toxic to the aquatic life.

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Compliance with Ethical Standards

Conflict of Interest: The authors declare that they have no conflicts of interes

Table 1. SRM conditions used for LC-MS/MS determination of pesticide residues

Target Pesticide	t_R [(a)] (min)	Precursor Ion	Aduct	SRM_1 [(b)]	Frag[(c)] (V)	CE[(d)] (V)	SRM_2 [(e)]	Frag[(c)] (V)	CE[(d)] (V)	SRM_2/SRM_1 (%)(%RSD)[(f)]
Acrinathrin	18.07	559	NH_4	208	76	10	181	76	30	82.7 (12.2)
Bifenthrin	19.04	440	NH_4	181	94	6	166 (165)*	94	46, 82*	47.0 (1.6)
Cyhalothrin	17,81	467	NH_4	225	66	10	141	66	46	24.5 (24.1)
Cypermethrin	17.88	433	NH_4	191	76	10	127	76	30	32.7 (37.4)
Cyfluthrin	17.54	451	NH_4	206	66	50	191	66	10	15.1 (2.2)
Deltamethrin	18.32	523	NH_4	506	100	5	281 (181)*	100	10	38.4 (12.1)
Esfenvalerate	18.12	437	NH_4	181	66	38	167	66	10	30.8 (4.7)
Etofenprox	18.96	394	NH_4	359	66	10	177	66	10	52.7 (21.0)
Flumethrin	18.68	527	NH_4	267	66	10	239	66	18	61.2 (5.4)
Fluvalinate	18.96	503	H	181	50	26	208	50	10	26.8 (9.2)
Tefluthrin	18.27	436	NH_4	177	100	20				
Cinerin I	17,74	317	H	149	66	5	107	66	20	81.3 (14.6)

Target Pesticide	t_R [(a)] (min)	Precursor Ion	Aduct	SRM_1 [(b)]	Frag [(c)] (V)	CE [(d)] (V)	SRM_2 [(e)]	Frag [(c)] (V)	CE [(d)] (V)	SRM_2/SRM_1 (%)(%RSD) [(f)]
Cinerin II	15.79	361	H	149	76	10	107	76	14	32.8 (13.6)
Jasmolin I	18.24	331	H	163	76	10	107	76	20	82.5 (9.9)
Jasmolin II	16.78	375	H	163	25	20	107	20	20	24.4 (5.7)
Pyrethrin I	17.78	329	H	161	76	10	133	76	20	53.5 (20.8)
Pyrethrin II	15.99	373	H	161	76	10	133	76	14	48.7 (8.8)

(a) t_R = retention time; (b) SRM_1 = selected product ion for quantification; (c) Frag = fragmentor; (d) CE = collision energy; (e) SRM_2 = selected product ion for qualification; (f) (%RSD) = relative standard deviation of the ratio SRM_2/SRM_1. calculated from mean values obtained from the matrix-matched calibration curves; *3rd selected product ion for quantification

Table 2. Analytical performance data for the pyrethroids and pyrethrins by Dilute and Shoot method

Target Pesticides	LOD (ng/g)	LOQ (ng/g)	Matrix Effect (%)
Acrinathrin	10	30	22
Bifenthrin	10	30	26
Cyhalothrin	10	30	-88
Cypermethrin	10	30	-6
Cyfluthrin	10	30	-58
Deltamethrin	20	60	-31
Esfenvalerate	10	30	-16
Etofenprox	10	30	8
Flumethrin	10	30	62
Fluvalinate	10	30	44
Tefluthrin	20	60	-29
Cinerin I	10	30	-84
Cinerin II	10	30	2
Jasmolin I	10	30	-28
Jasmolin II	20	60	-62
Pyrethrin I	10	30	-23
Pyrethrin II	10	30	-8

Table 3. Analytical performance data for the pyrethroids and pyrethrins by DLLME method

Target Pesticides	Water		Sediment		Recoveries (%)		RSD (%) intraday (n = 5)		RSD (%) interday (n = 5)	
	LOD (µg/L)	LOQ (µg/L)	LOD (ng/g)	LOQ (ng/g)	Water	Sediment	Water	Sediment	Water	Sediment
Acrinathrin	0.12	0.37	0.50	1.50	112	118	12	14	10	12
Bifenthrin	0.12	0.37	0.50	1.50	101	82	2	6	2	6
Cyhalothrin	0.12	0.37	0.50	1.50	110	108	10	8	11	10
Cypermethrin	0.62	0.75	2.50	7.50	65	89	8	12	6	10
Cyfluthrin	0.62	0.75	2.50	7.50	83	88	9	2	6	4
Deltamethrin	0.12	0.37	0.50	1.50	119	96	12	12	9	10
Esfenvalerate	0.62	0.75	2.50	7.50	70	88	5	5	7	5
Etofenprox	0.12	0.37	0.50	1.50	71	64	12	16	10	12
Flumethrin	0.12	0.37	0.50	1.50	114	116	5	16	7	14
Fluvalinate	0.12	0.37	0.50	1.50	100	112	6	9	8	8
Tefluthrin	0.62	0.75	2.50	7.50	116	80	7	12	8	9
Cinerin I	0.12	0.37	0.50	1.50	73	73	15	15	13	13
Cinerin II	0.12	0.37	0.50	1.50	114	92	8	14	6	12
Jasmolin I	0.12	0.37	0.50	1.50	62	64	7	10	7	8
Jasmolin II	0.62	0.75	2.50	7.50	75	82	6	8	7	8
Pyrethrin I	0.12	0.37	0.50	1.50	70	72	9	13	11	10
Pyrethrin II	0.12	0.37	0.50	1.50	107	71	9	9	10	10

Fig. 1. Chromatographic separation of the pesticides studied using LC-ESI-MS/MS: Peak identification: (1) Etofenprox; (2) bifenthrin; (3) flumethrin; (4) fluvalinate; (5) pyrethrin I; (6) cinerin I; (7) pyrethrin II; (8) cinerin II; (9) acrinathrin; (10) cyhalothrin; (11) cyfluthrin; (12) esfenvalerate; (13) deltamethrin; (14) tefluthrin; (15) cypermethrin; (16) jasmolin II; (17) jasmolin I.

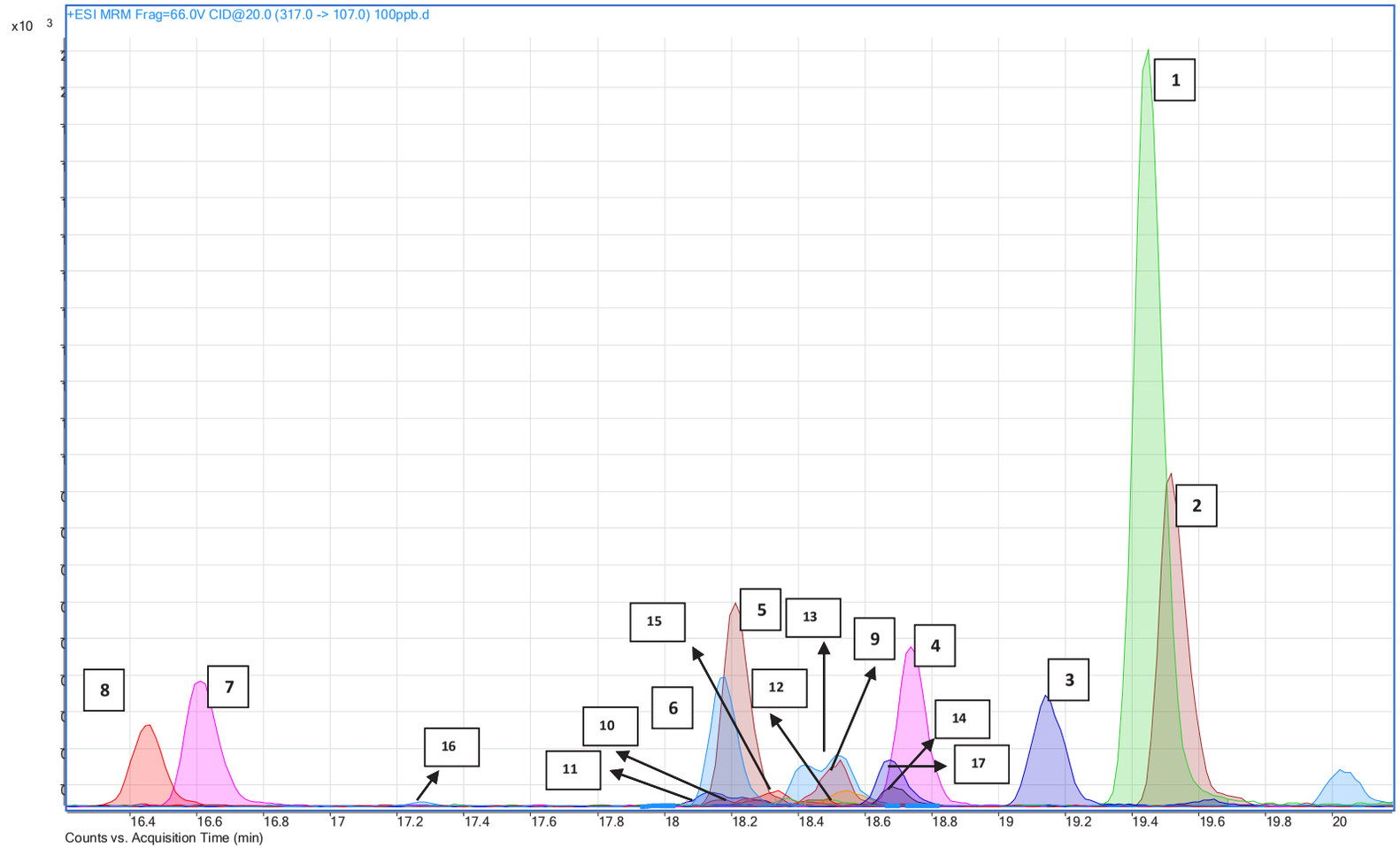


Fig. 2. Solid Phase Extraction SPE and DLLME comparison

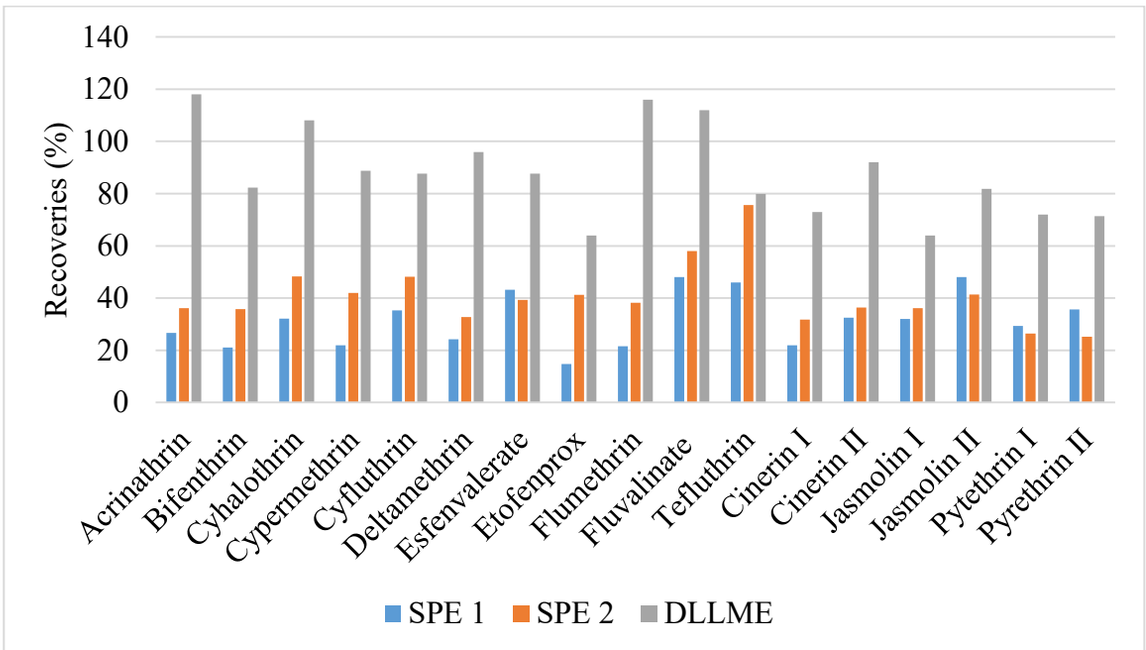
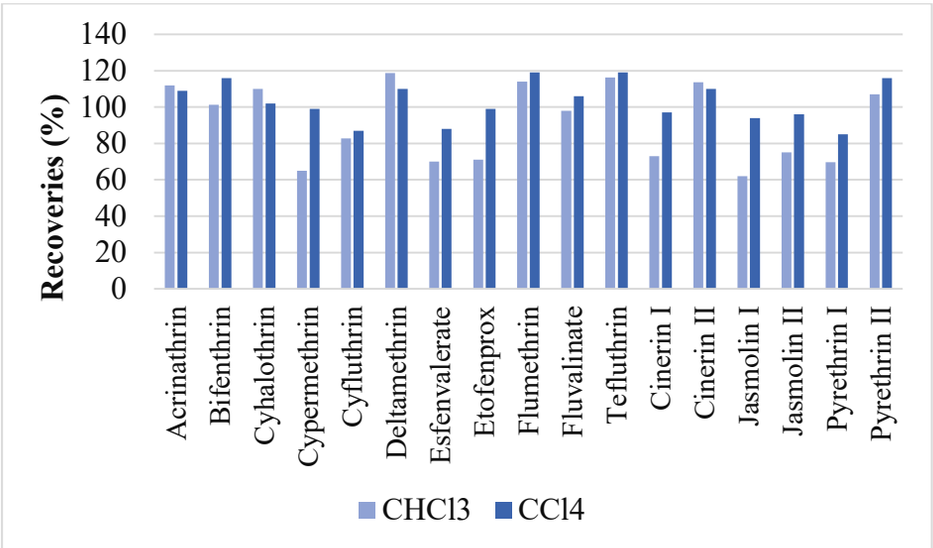


Fig. 3. Optimized variables for the DLLME procedure for water samples: (A) Effect of the extraction solvents and (B) effect of different extraction times.

A)



B)

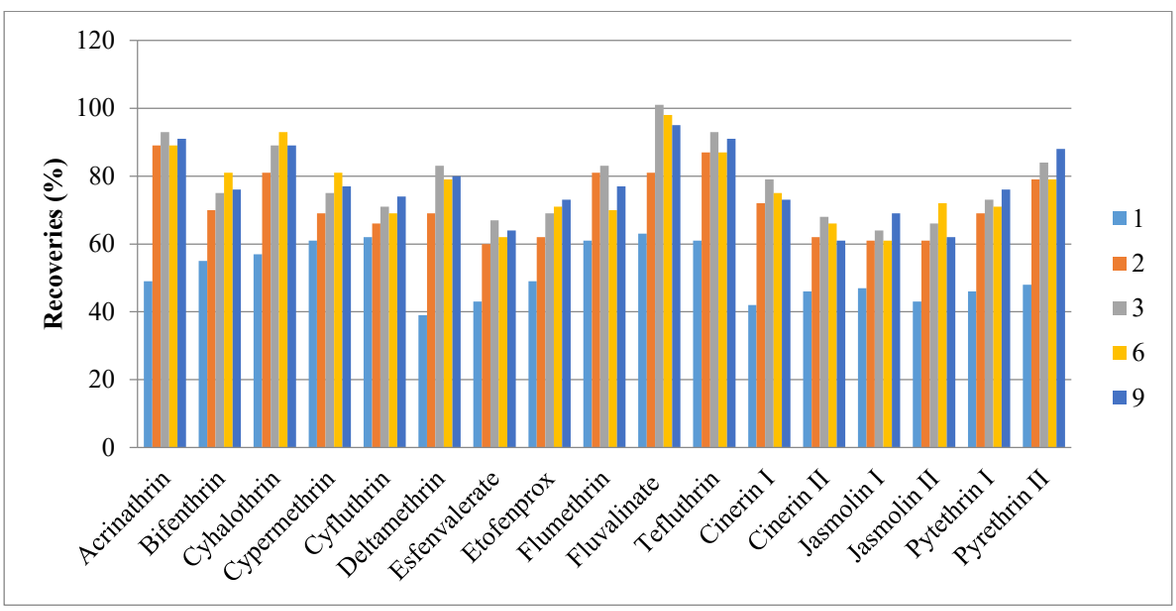
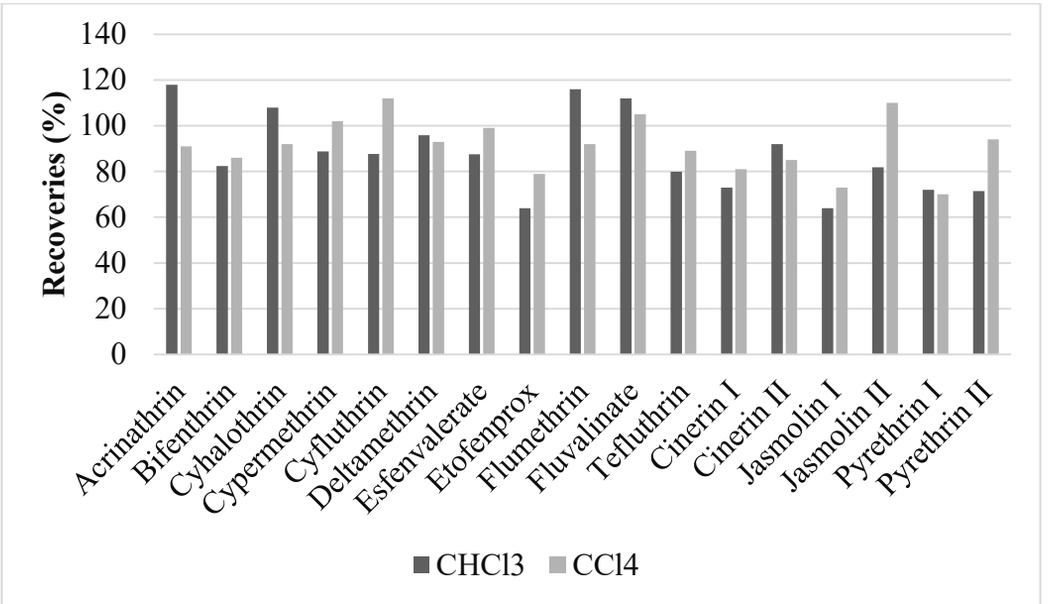


Fig. 4. Optimized variables for the DLLME procedure for sediment samples: (A) Effect of the extraction solvents and (B) effect of different extraction times.

A)



B)

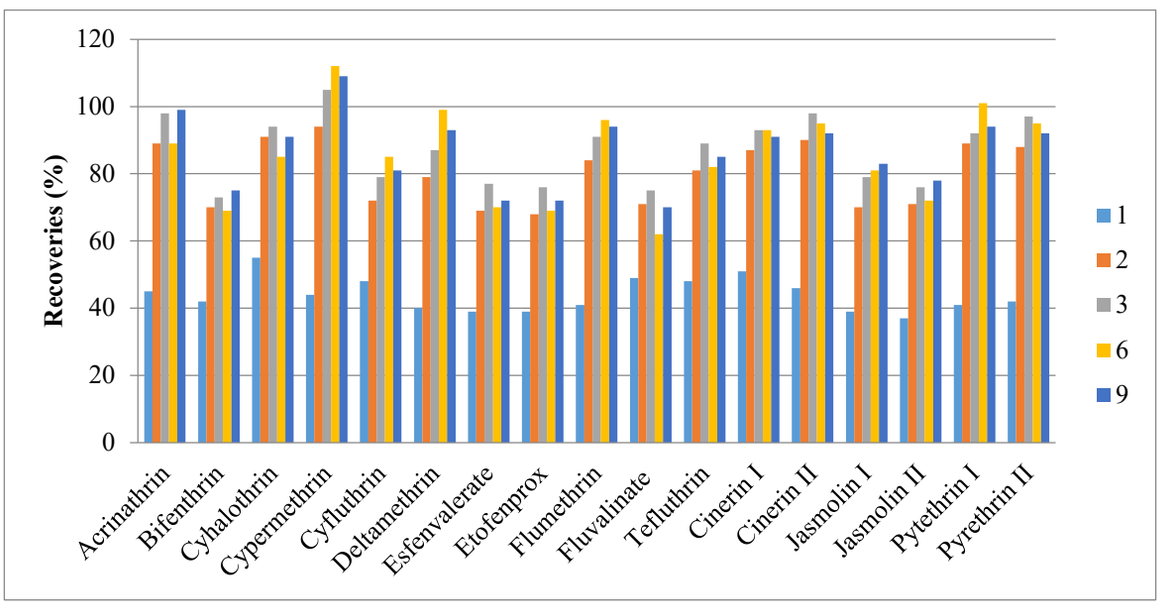


Fig. 5. Comparison of LC–MS/MS matrix effects obtained for the selected insecticides employing ultrasound-assisted dispersive liquid-liquid microextraction (UA–DLLME) in water and sediment samples.

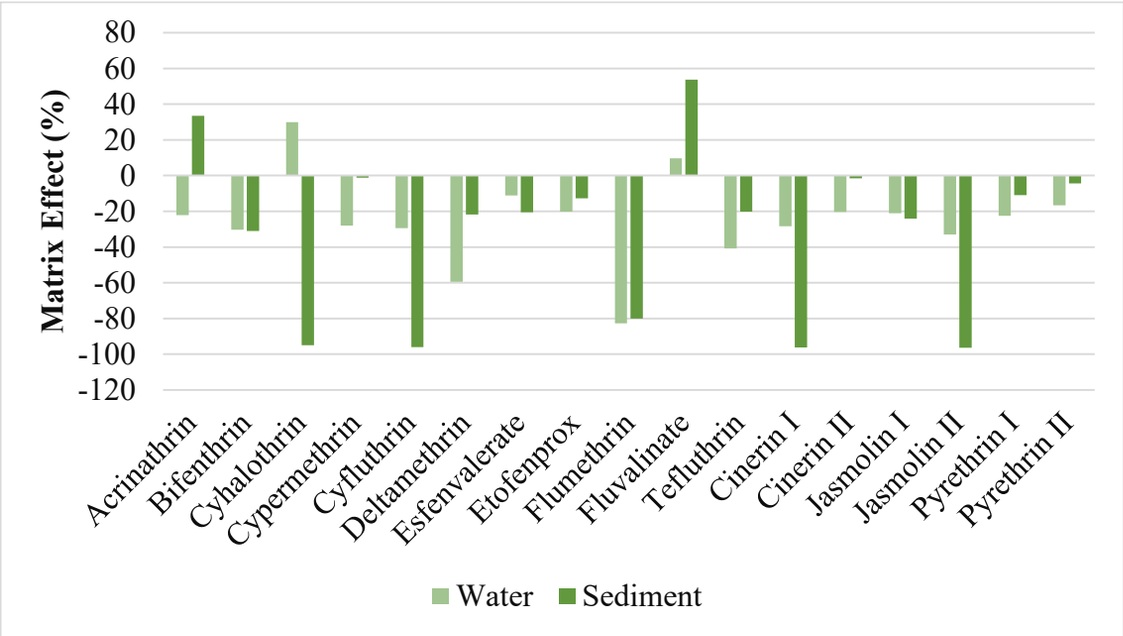
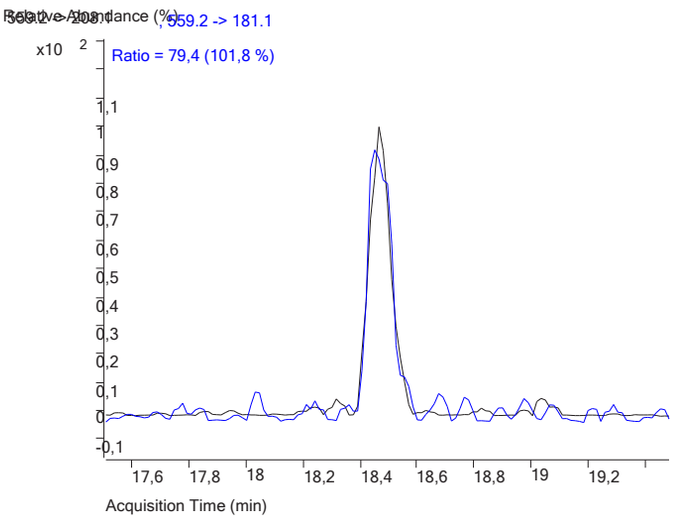
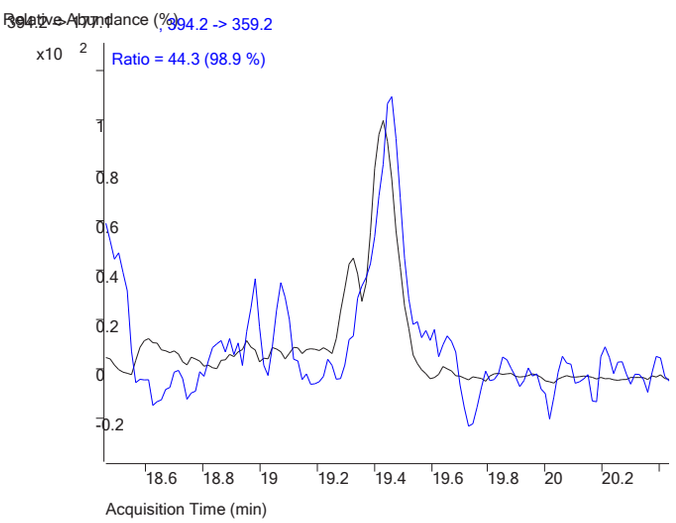


Fig. 6. Precursor ion-quantification (SMR_1) and product ion-confirmation (SMR_2) of pesticides detected in sediment samples: (A) acrinathrin and (B) etofenprox.

(A)



(B)



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SUPLEMENTARY MATERIAL

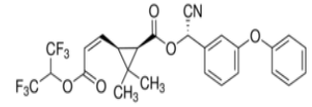
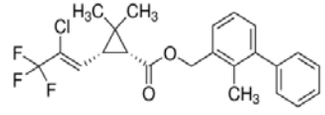
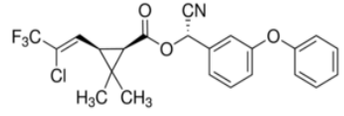
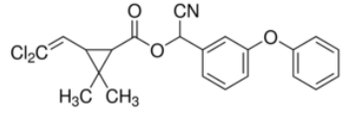
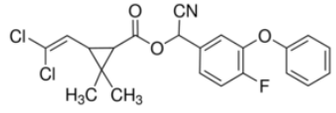
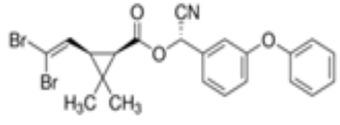
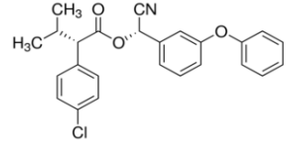
Simultaneous determination of pyrethroids and pyrethrins by dispersive liquid-liquid microextraction and liquid chromatography triple quadrupole mass spectrometry in environmental samples

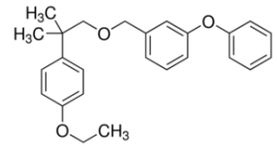
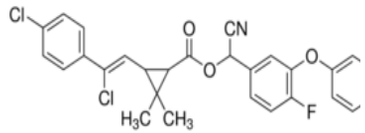
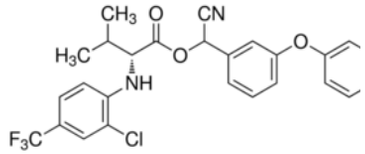
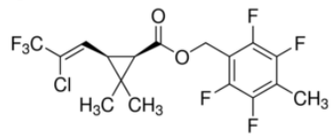
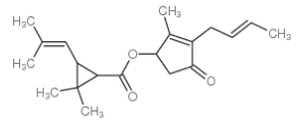
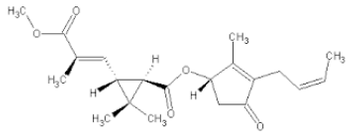
Alexander Ccanccapa[*,1], Ana Masiá[1], Yolanda Picó[1],

[1]Food and Environmental Safety Research Group (SAMA-UV), Facultat de Farmàcia, Universitat de València, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain.

[*] Corresponding autor: Alexander Ccanccapa
Tel: +34 963543092; Fax: +34 963544954
E-mail: Alexander.Ccanccapa@uv.es

Table S-1: Physico-chemical properties of pyrethroids and pyrethrins

Compound	Chemical structure	CAS Number	Chemical Formula	WM (g/mol) – Monoisotopic Mass[a]	log Kow (pH 7, 20 °C)	pKa	Water Solubility at 20° (mg/L)
Acrinathrin		101007-06-1	$C_{26}H_{21}F_6NO_5$	541.44 541.13[a]	6.3	10.62	0.0022
Bifenthrin		82657-04-3	$C_{23}H_{22}ClF_3O_2$	422.87 422.12[a]	6.6	-7.1	0.001
Cyhalothrin		91465-08-6	$C_{23}H_{19}ClF_3NO_3$	449.85 449.10[a]	6.8	10.62	0.004
Cypermethrin		52315-07-8	$C_{22}H_{19}C_{l2}NO_3$	416.30 415.07[a]	5.3	10.62	0.009
Cyfluthrin		68359-37-5	$C_{22}H_{18}C_{l2}FNO_3$	434.29 433.06[a]	6	10.31	0.0066
Deltamethrin		52918-63-5	$C_{22}H_{19}Br_2NO_3$	505.2 502.97[a]	4.6	10.62	0.0002
Esfenvalerate		66230-04-4	$C_{25}H_{22}ClNO_3$	419.90 419.12[a]	6.24	10.62	0.001

Compound	Chemical structure	CAS Number	Chemical Formula	WM (g/mol) – Monoisotopic Mass[a]	log Kow (pH 7, 20 °C)	pKa	Water Solubility at 20° (mg/L)
Etofenprox		80844-07-1	$C_{25}H_{28}O_3$	376.49 376.20[a]	6.9	10.67	0.0225
Flumethrin		69770-45-2	$C_{28}H_{22}C_{l2}FNO_3$	510.38 509.09[a]	6.2	10.31	200
Fluvalinate		69409-94-5	$C_{26}H_{22}ClF_3N_2O_3$	502.91 502.12[a]	3.85	10.62	0.002
Tefluthrin		79538-32-2	$C_{17}H_{14}ClF_7O_2$	418.73 418.05[a]	6.4	-7.1	0.016
Cinerin I		25402-06-6	$C_20H_{28}O_3$	316.4 316.2[a]	5.9	18.09	0.085 (25°)
Cinerin II		121-20-0	$C_{21}H_{28}O_5$	360.4 360.19[a]	5.9	18.09	0.30

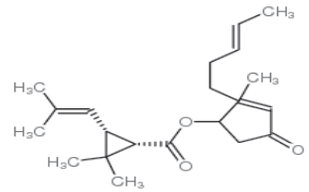
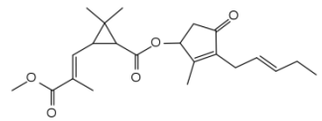
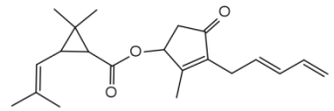
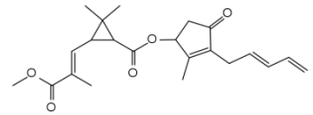
Compound	Chemical structure	CAS Number	Chemical Formula	WM (g/mol) – Monoisotopic Mass[a]	log Kow (pH 7, 20 °C)	pKa	Water Solubility at 20° (mg/L)
Jasmolin I		4466-14-2	$C_{21}H_{30}O_3$	330.4 330.21[a]	5.9	18.09	0.60
Jasmolin II		1172-63-0	$C_{22}H_{30}O_5$	374.45 374.20[a]	5.9	18.09	214.8
Pyrethrin I		121-21-1	$C_{21}H_{28}O_3$	328.4 328.20[a]	5.9	18.09	0.96
Pyrethrin II		121-29-9	$C_{22}H_{28}O_5$	372.4 372.19[a]	5.9	18.09	10.7

Table S-2. LC-MS/M condition

LC CONDITIONS	
Analytical column	Luna C18: 15.0 cm × 0.21 cm, 3 µm particle size (Phenomenex, Torrance, USA)
Column temperature	30° C
Volume injected	5 µL
Mobile phase	(A) Water – (B) methanol both with 10 mM Ammonium Formate
Flow rate	0.3 mL min^{-1}
Linear gradient	0 min (50 % B), 10 min (83 % B), 12 min (83 % B), 12 min (98 % B), 25 min (98 % B), and return to the initial conditions (equilibration time 15 min)
TRIPLE QUADRUPOLE MS/MS CONDITIONS	
Ionization characteristics and source	MS/MS performed in selected reaction monitoring mode (SRM) with electrospray ionization (ESI) in positive mode
Gas temperature	300° C
Gas flow	11 L min^{-1}
Nebulizer	25 psi
Capillary voltage	4000 V
Chamber current	1.27 µA
Scan type	Dynamic MRM, with MS1 and MS2 at unit resolution and cell acceleration voltage of 7 eV

Table S-3. Optimization MS^2 Scan and product ion scan for pyrethroid.

Acrinathrin	**m/z**	**Na**	**NH3**			
	541.4	**564.4**	**558.4**			
	MS2 Scan	**from**	**to**	**Fragmentor**	**Voltage**	Polarity
		100	**650**	**40**	**7**	+
	Result	**558**				
	Product Ion	**Precursor Ion**	**From**	**To**	**Fragmentor**	Collision Energy
		558	**100**	**650**	**40**	5-10-20
	Result	**558**	**Prod Ion 1**	**180**	**40**	20
			Prod Ion 2	**207**	**40**	10
Etofenprox	**m/z**	**Na**	**NH3**			
	376.49	**399.49**	**393.49**			
	MS2 Scan	**from**	**to**	**Fragmentor**	**Voltage**	Polarity
		100	**700**	**80**	**7**	+
	Result	**394**				
	Product Ion	**Precursor Ion**	**From**	**To**	**Fragmentor**	Collision Energy
		394	**100**	**500**	**80**	5-10-20
	Result	**394**	**Prod Ion 1**	**177**	**80**	10
			Prod Ion 2	**359**	**80**	5
Cyfluthrin	**m/z**	**Na**	**NH3**			
	433	**456**	**450**			
	MS2 Scan	**from**	**to**	**Fragmentor**	**Voltage**	Polarity
		100	**600**	**80**	**7**	+
	Result	**450.4**				

	Product Ion	**Precursor Ion**	**From**	**To**	**Fragmentor**	Collision Energy
		394	**100**	**500**	**80**	5-10-20-40-80-120
	Result	**394**	**Prod Ion 1**			
			Prod Ion 2			
Esfenvalerate	**m/z**	**Na**	**NH3**			
	419.9	**442.9**	**436.9**			
	MS2 Scan	**from**	**to**	**Fragmentor**	**Voltage**	Polarity
		100	**550**	**80**	**7**	+
	Result	**436.5**				
	Product Ion	**Precursor Ion**	**From**	**To**	**Fragmentor**	Collision Energy
		436.5	**100**	**500**	**80**	5-25-90-120
	Result	**436.5**	**Prod Ion 1**	**148.7**	**80**	25
			Prod Ion 2	**181**	**80**	25
			Prod Ion 3	**166.4**	**80**	5
Cyhalothrin	**m/z**	**Na**	**NH3**			
	449.9	**472.9**	**466.9**			
	MS2 Scan	**from**	**to**	**Fragmentor**	**Voltage**	Polarity
		100	**550**	**80**	**7**	+
	Result	**466.5**				
	Product Ion	**Precursor Ion**	**From**	**To**	**Fragmentor**	Collision Energy
		466.5	**100**	**550**	**80**	10-15-25
	Result	**466.5**	**Prod Ion 1**	**224.4**	**80**	25
			Prod Ion 2	**140.2**	**80**	25
Deltamethrin	**m/z**	**Na**	**NH3**			
	505.2	**528.2**	**522.2**			
	MS2 Scan	**from**	**to**	**Fragmentor**	**Voltage**	Polarity

		100	**650**	**80**	**7**	+
	Result	**522.3**				
	Product Ion	**Precursor Ion**	**From**	**To**	**Fragmentor**	Collision Energy
		522.3	**100**	**650**	**80**	5-10-15-25
	Result	**522.3**	**Prod Ion 1**	**278.2**	**80**	25
			Prod Ion 2	**171.3**	**80**	25
Cypermethrin	**m/z**	**Na**	**NH3**			
	416.3	**439.3**	**433.3**			
	MS2 Scan	**from**	**to**	**Fragmentor**	**Voltage**	Polarity
		100	**600**	**80**	**7**	+
	Result	**432.4**				
	Product Ion	**Precursor Ion**	**From**	**To**	**Fragmentor**	Collision Energy
		432.4	**100**	**600**	**80**	20-15
	Result	**432.4**	**Prod Ion 1**	**190.6**	**80**	20
			Prod Ion 2	**126.3**	**80**	20
Tefluthrin	**m/z**	**Na**	**NH3**			
	418.73	**441.73**	**435.73**			
	MS2 Scan	**from**	**to**	**Fragmentor**	**Voltage**	Polarity
		100	**600**	**80**	**7**	+
	Result	**435.4**				
	Product Ion	**Precursor Ion**	**From**	**To**	**Fragmentor**	Collision Energy
		435.4	**100**	**600**	**80**	5-10-15-20
	Result	**435.4**	**Prod Ion 1**	**192**	**80**	15
			Prod Ion 2	**176.3**	**80**	15
			Prod Ion 3	**127.6**	**80**	20
Flumethrin	**m/z**	**Na**	**NH3**			

	510.38	**533.38**	**527.38**			
	MS2 Scan	**from**	**to**	**Fragmentor**	**Voltage**	Polarity
		100	**650**	**80**	**7**	+
	Result	**526.4**				
	Product Ion	**Precursor Ion**	**From**	**To**	**Fragmentor**	Collision Energy
		526.4	**100**	**650**	**80**	5-10-15-20(25-35-40)
	Result	**435.4**	**Prod Ion 1**			
			Prod Ion 2			
Acrinathrin	**m/z**	**Na**	**NH_3**			
	541.4	564.4	558.4			
	MS2 Scan	**from**	**to**	**Fragmentor**	**Voltage**	**Polarity**
		100	650	40	7	+
	Result	558				
	Product Ion	**Precursor Ion**	**From**	**To**	**Fragmentor**	**Collision Energy**
		558	100	650	40	5-10-20
	Result	558	**Prod Ion 1**	180	40	20
			Prod Ion 2	207	40	10
Etofenprox	**m/z**	**Na**	**NH_3**			
	376.49	399.49	393.49			
	MS2 Scan	**from**	**to**	**Fragmentor**	**Voltage**	**Polarity**
		100	700	80	7	+
	Result	394				
	Product Ion	**Precursor Ion**	**From**	**To**	**Fragmentor**	**Collision Energy**
		394	100	500	80	5-10-20
	Result	394	**Prod Ion 1**	177	80	10
			Prod Ion 2	359	80	5

Cyfluthrin	**m/z**	**Na**	**NH_3**			
	433	456	450			
	MS2 Scan	**from**	**to**	**Fragmentor**	**Voltage**	**Polarity**
		100	600	80	7	+
	Result	450.4				
	Product Ion	**Precursor Ion**	**From**	**To**	**Fragmentor**	**Collision Energy**
		394	100	500	80	5-10-20-40-80-120
	Result	394	**Prod Ion 1**			
			Prod Ion 2			
Esfenvalerate	**m/z**	**Na**	**NH_3**			
	419.9	442.9	436.9			
	MS2 Scan	**from**	**to**	**Fragmentor**	**Voltage**	**Polarity**
		100	550	80	7	+
	Result	436.5				
	Product Ion	**Precursor Ion**	**From**	**To**	**Fragmentor**	**Collision Energy**
		436.5	100	500	80	5-25-90-120
	Result	436.5	**Prod Ion 1**	148.7	80	25
			Prod Ion 2	181	80	25
			Prod Ion 3	166.4	80	5
Cyhalothrin	**m/z**	**Na**	**NH_3**			
	449.9	472.9	466.9			
	MS2 Scan	**from**	**to**	**Fragmentor**	**Voltage**	**Polarity**
		100	550	80	7	+
	Result	466.5				
	Product Ion	**Precursor Ion**	**From**	**To**	**Fragmentor**	**Collision Energy**
		466.5	100	550	80	10-15-25
	Result	466.5	**Prod Ion 1**	224.4	80	25
			Prod Ion 2	140.2	80	25
Deltamethrin	**m/z**	**Na**	**NH_3**			

	505.2	528.2	522.2			
	MS2 Scan	**from**	**to**	**Fragmentor**	**Voltage**	**Polarity**
		100	650	80	7	+
	Result	522.3				
	Product Ion	**Precursor Ion**	**From**	**To**	**Fragmentor**	**Collision Energy**
		522.3	100	650	80	5-10-15-25
	Result	522.3	**Prod Ion 1**	278.2	80	25
			Prod Ion 2	171.3	80	25
Cypermethrin	**m/z**	**Na**	**NH_3**			
	416.3	439.3	433.3			
	MS2 Scan	**from**	**to**	**Fragmentor**	**Voltage**	**Polarity**
		100	600	80	7	+
	Result	432.4				
	Product Ion	**Precursor Ion**	**From**	**To**	**Fragmentor**	**Collision Energy**
		432.4	100	600	80	20-15
	Result	432.4	**Prod Ion 1**	190.6	80	20
			Prod Ion 2	126.3	80	20
Tefluthrin	**m/z**	**Na**	**NH_3**			
	418.73	441.73	435.73			
	MS2 Scan	**from**	**to**	**Fragmentor**	**Voltage**	**Polarity**
		100	600	80	7	+
	Result	435.4				
	Product Ion	**Precursor Ion**	**From**	**To**	**Fragmentor**	**Collision Energy**
		435.4	100	600	80	5-10-15-20
	Result	435.4	**Prod Ion 1**	192	80	15
			Prod Ion 2	176.3	80	15
			Prod Ion 3	127.6	80	20
Flumethrin	**m/z**	**Na**	**NH_3**			
	510.38	533.38	527.38			
	MS2 Scan	**from**	**to**	**Fragmentor**	**Voltage**	**Polarity**

	100	650	80	7	+
Result	526.4				
Product Ion	**Precursor Ion**	**From**	**To**	**Fragmentor**	**Collision Energy**
	526.4	100	650	80	5-10-15-20(25-35-40)
Result	435.4	**Prod Ion 1**			
		Prod Ion 2			

Table S-4. Optimized LC-MS/MS fragmentation as well as abundance of the different ions for pyretroids and pyrethrins

Pyrethroids	Precursor Ion	Fragmentor	Product Ion	Collision Energy	Abundance
Tefluthrin	436.09	100	177.1	20	3489
	436.09	100	127	70	1806
Cyfluthrin	451.09	66	191	10	16993
	451.09	66	91	54	2883
	451.09	66	206	50	1933
	451.09	66	127	30	3158
Deltamethrin	523	100	506.2	5	34492
	523	100	91.1	82	4295
	523	100	93.1	58	4191
	523	100	281.3	10	13002
Cypermethrin	433.1	76	191	10	47774
	433.1	76	91.1	50	8608
	433.1	76	127	30	9551
	433.1	76	181.1	38	3133
Cyhalothrin	467.13	66	225	10	24063
	467.13	66	141	46	3603
	467.13	66	181.1	38	2327
	467.13	66	152	100	1658
Esfenvalerato	437.16	66	125	50	5370
	437.16	66	167	10	10793
	437.16	66	181.1	38	2220
	437.16	66	104.1	98	107
Etofenprox	394.23	66	107.1	46	139891
	394.23	66	177.1	10	266760
	394.23	66	135.1	26	96407
	394.23	66	359.2	10	117295
Acrinathrin	559.16	76	181.1	30	13669
	559.16	76	208.1	10	22022
	559.16	76	83.1	14	6731
	559.16	76	152.1	100	3545
Flumethrin	527.13	66	267	10	37312
	527.13	66	239	18	18022
	527.13	66	206	50	3813
	527.13	66	203	34	5998
Bifenthrin	440.2	94	181.1	6	291002
	440.2	94	165	82	148334
	440.2	94	166	46	126414
	440.2	94	115	142	24845
Fluvalinate	503	50	208	10	241002

	503	50	181	26	178334
Pyrethrins	**Precursor Ion**	**Fragmentor**	**Product Ion**	**Collision Energy**	**Abundance**
Cinerin I	317.21	66	107.1	20	11832
	317.21	66	77.1	62	5821
	317.21	66	51.1	110	3387
	317.21	66	149.1	5	6081
Cinerin II	361.2	76	107.1	14	6964
	361.2	76	77.1	62	3650
	361.2	76	51.1	126	2192
	361.2	76	149.1	10	4008
Jasmolin I	331.23	66	77.1	66	13802
	331.23	66	79.1	38	7880
	331.23	76	107.1	20	11142
	331.23	76	163.1	10	12831
Jasmolin II	375.24	25	163	20	1225
	375.24	20	107	20	654
	375.24	20	65.1	86	559
	375.24	20	71.1	10	327
Pirethrin I	329.21	76	77.1	74	46063
	329.21	76	161.1	10	104993
	329.21	76	133	20	109539
	329.21	76	105.1	34	50539
Pirethrin II	373.2	76	161.1	10	30290
	373.2	76	77.1	90	8133
	373.2	76	51.1	138	5411
	373.2	76	133.1	14	12329

Table S-5. Instrumental parameters of the LC-MS/MS determination

	LODs (pg)	LOQs (pg)	RSDs (%) at 100 ng/mL	Linear Equation	R^2
Acrinathrin	12.5	37.5	5	y = 0.104052 x + 0.022124	0.9903
Bifenthrin	12.5	37.5	7	y = 1.664741 x - 0.038596	0.9995
Cyhalothrin	12.5	37.5	2	y = 0.159023 x + 0.005865	0.9997
Cypermethrin	12.5	37.5	3	y = 0.185489 x + 0.013841	0.992
Cyfluthrin	50	150	4	y = 0.057786 x + 0.003578	0.9944
Deltamethrin	12.5	37.5	5	y = 0.199964 x + 0.005848	0.9995
Esfenvalerate	50	150	7	y = 0.038785 x - 2.115414E-004	0.9989
Etofenprox	12.5	37.5	4	y = 3.720901 x - 0.087305	0.9996
Flumethrin	12.5	37.5	9	y = 0.480254 x + 0.015608	0.9987
Fluvalinate	12.5	37.5	4	y = 0.395655 x + 0.020309	0.9932
Tefluthrin	50	150	4	y = 0.034989 x + 6.518860E-005	0.9934
Cinerin I	12.5	37.5	1	y = 0.264266 x + 0.002710	0.9999
Cinerin II	12.5	37.5	4	y = 0.187087 x + 0.002974	0.9998
Jasmolin I	12.5	37.5	3	y = 0.090006x + 0.004711	0.9969
Jasmolin II	50	150	8	y = 0.033160 x - 3.844241E-004	0.9962
Pytethrin I	12.5	37.5	4	y = 0.857912 x + 0.010279	0.9996
Pyrethrin II	12.5	37.5	9	y = 0.549359 x + 0.010862	0.9997

Table S-6. Matrix linear equation and coefficient determination

	Linear Equation		R^2	
	Water	**Sediment**	**Water**	**Sediment**
Acrinathrin	y = 0.081936 x - 0.007565	y = 0.138947 x - 0.029354	0.9987	0.9925
Bifenthrin	y = 1.160120 x - 0.047739	y = 1.147531 x - 0.061116	0.9981	0.9972
Cyhalothrin	y = 0.206506 x + 0.011464	y = 0.296154 x - 0.017681	0.9921	0.9962
Cypermethrin	y = 0.133683 x - 3.151587E-004	y = 0.183296 x - 0.009183	0.9951	0.994
Cyfluthrin	y = 0.040751 x - 4.973209E-004	y = 0.059488 x - 0.005361	0.9961	0.9927
Deltamethrin	y = 0.081133 x + 0.036729	y = 0.156359 x - 0.009103	0.9991	0.9965
Esfenvalerate	y = 0.034456 x + 0.002962	y = 0.030976 x - 1.108923E-004	0.9973	0.9977
Etofenprox	y = 2.968606 x - 0.117110	y = 3.245050 x - 0.271173	0.9977	0.9924
Flumethrin	y = 0.083203 x + 0.006374	y = 0.608499 x - 0.051326	0.9989	0.9563
Fluvalinate	y = 0.433771 x + 0.022746	y = 0.608499 x - 0.051326	0.995	0.998
Tefluthrin	y = 0.023089 x - 8.229461E-004	y = 0.031151 x - 0.002359	0.9947	0.9992
Cinerin I	y = 0.189389 x - 0.006092	y = 0.224676 x - 0.013731	0.9989	0.9982
Cinerin II	y = 0.149081 x - 0.003607	y = 0.184114 x - 0.011299	0.9985	0.9943
Jasmolin I	y = 0.071040 x - 0.003688	y = 0.068322 x - 0.002296	0.9941	0.9959
Jasmolin II	y = 0.022246 x + 8.653277E-004	y = 0.031176 x - 0.002540	0.9937	0.9968
Pytethrin I	y = 0.665333 x - 0.024420	y = 0.735378 x - 0.044187	0.9982	0.9931
Pyrethrin II	y = 0.458206 x - 0.015531	y = 0.525334 x - 0.029859	0.9991	0.9962

Table S-7 Comparison of the proposed method and some other methods for pyrethroids and pyrethrins determination.

Pyretroids	Matrix	Extraction Method	Concentration Factor	Recoveries (*R*%)	Analytic method	LOD (1) or LOQ (2)	Ref.
Tetramethrin, fenpropathrin, deltamethrin and permethrin	Water	Dispersive liquid microextraction (DLME) combined with dispersive μ-solid phase extraction (D-μ-SPE)	166.6	91.7–104.5	HPLC with UV detection	0.05 – 2 ng mL^{-1} (1) and 0.25 – 5 ng mL^{-1} (2) (water samples)	[23]
Cypermethrin, resmethrin, permethrin	Surface water	Solid Phase Extraction (SPE)	666	94.1, 72.5, and 86.8	RP-HPLC	0.03 - 0.06 ng mL^{-1} (1) 0.11 - 0.21 ng mL^{-1} (2)	[24]
Beta-cyfluthrin, cyhalothrin, and cyphenothrin	River water	Magnetic solid phase extraction (MSPE)	400	83.2 - 95.2	HPLC	5.6 - 7.5 ng L^{-1}(1)	[25]
Beta-cyfluthrin, cyhalothrin and cyphenothrin	River water	Magnetic Separation (titanium dioxide)	1000	84.5 - 94.1	HPLC	2.8 – 6.1 ng L^{-1}(1) and 9.3 – 20.3 ng L^{-1} (2)	[26]
Cypermethrin, resmethrin, and permethrin	Ground water	Micro liquid–liquid extraction (MLLE)	5000	84.7 - 94.5	RP-HPLC	0.05 - 0.08 μg mL^{-1} (1)	[3]

Pyretroids	Matrix	Extraction Method	Concentration Factor	Recoveries (*R*%)	Analytic method	LOD (1) or LOQ (2)	Ref.
Imiprothrin, prallethrin, tetramethrin, allethrin, transfluthrin, Cypermethrin	River water	Ultrasound-assisted dispersive liquid–liquid microextraction (UA -DLLME)	10	86.2 - 109.3	HPLC	0.11 - 0.30 ng mL^{-1} (1) and 0.39 - 1.05 ng mL^{-1}(2)	[27]
Cyfluthrin, cypermethrin, deltamethrin, fenvalerate, permethrin, tetramethrin, bifenthrin, lambda-cyhalothrin, esfenvalerate, fenpropathrin, tau-fluvalinate, and resmethrin	River water	ultrasound-assisted emulsification extraction (UAEE)	200	47 - 105	GC-MS	0.03 – 35.8 ng L^{-1} (1)	[7]
bifenthrin, Lambda - cyhalothrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, permethrin, resmethrin and tefluthrin	Sediment	Accelerated solvent extraction and solid phase extraction cleanup	5	59.7 -128	GC - MS/MS	0.10 – 0.80 µg kg^{-1}(1)	[16]
Fenpropathrin, cyhalothrin and fenvalerate	Soil	Matrix solid-phase dispersion (MSPD) with ultrasound-assisted dispersive	5	83.6 - 98.5	GC-ECD	1.51 - 3.77 ng g^{-1}(2)	[17]

Pyretroids	Matrix	Extraction Method	Concentration Factor	Recoveries (*R*%)	Analytic method	LOD [(1)] or LOQ [(2)]	Ref.
		liquid–liquid microextraction (UA-DLLME)					
Bifenthrin, fenpropathrin, permethrin, cyfluthri, fenvalerate, λ-cyhalothrin, Cypermethrin, fluvalinate, deltamethrin	Soil	Microwave-assisted extraction (MAE)	1	75.7 – 119.2	GC-MS	0.0044 - 0.4225 mg L^{-1} [(1)]	[31]
Cyfluthrin, cypermethrin, deltamethrin, fenvalerate, permethrin, tetramethrin, bifenthrin, lambda-cyhalothrin, esfenvalerate, fenpropathrin, tau-fluvalinate, and resmethrin	Sediment	Sonicated and cleaned up using Florisil cartridge	10	51 - 105	GC - MS	2.6 - 62. 4 pg g^{-1} [(1)]	[7]
Bifenthrin, lambda – cyhalothrin, Cis-permethrin, trans-	Sediment	Ultrasonic extraction coupled with a heated - copper	250	88.7 - 124.2	GC - ECD	0.31 – 3.72 µg kg^{-1} [(1)]	[15]

Pyretroids	Matrix	Extraction Method	Concentration Factor	Recoveries (*R*%)	Analytic method	LOD [1] or LOQ [2]	Ref.
permethrin, cyfluthrin-1, cyfluthrin-2, cyfluthrin-3, cyfluthrin-4, fenvalerate, esfenvalerate, deltamethrin		clean-up					
Transfluthrin, prallethrin, resmethrin, bifenthrin, fenpropathrin, tetramethrin, lambda-cyhalothrin, permethrin, cypermethrin, flucythrinate, fenvalerate, deltamethrin	Soil	Pressurized liquid extraction (PLE)	10	75 - 120	GC-MS/MS	0.26 - 0.87 ng g^{-1} [1]	[18]
Kadethrin , cypermethrin and permethrin	Soil	Flow-through extraction	0.1	56 -84	RP - HPLC	27 - 32 ng g^{-1} [1]	[9]
tetramethrin, bifenthrin, lambda-cyhalothrin, permethrin, cyfluthrin, cypermethrin, flucythrinate, fenvalerate, tau-fluvalinate	Fruit juices	Dispersive liquid–liquid microextraction (DLLME)[a] and QuEChERS[b]	5	62 - 138	GC - ECD	0.5 - 2 µg L^{-1} [1] [a] and 1 - 5 µg L^{-1} [2] [b]	[19]

Pyretroids	Matrix	Extraction Method	Concentration Factor	Recoveries (*R*%)	Analytic method	LOD [1] or LOQ [2]	Ref.
and deltamethrin							
Fenpropathrin, cyhalothrin, and fenvalerate	Fruit and vegetables	Supercritical fluid extraction[(a)] and magnetic solid phase extraction[(b)]	50	91 - 99	HPLC-UV	1 ng g^{-1} [(1)] [(a)] and 0.1 mg kg^{-1} [(1)] [(b)]	[58]
Pyrethrin I, cinerin I, jasmolin I, pyrethrin II, cinerin II, and jasmolin II	fruits and vegetables	Acetone/water	0.25	70 - 110	LC-MS/MS	0.1 - 0.7 µg kg^{-1} [(1)]	[1]
etramethrin, fenpropathrin, cypermethrin, deltamethrin, fenvalerate, permethrin	Fruits and vegetables	Simple Solid-Phase Extraction (SPE)	12.5	69 - 131	HPLC-UV	0.05 - 10 µg L^{-1} [(1)]	[32]
Bifenthrin, λ-cyhalothrin, cyfluthrin, cypermethrin, fenvalerate, and deltamethrin	Orange and lettuce	Magnetic Solid Phase Extraction (MSPE)	800	90.0 - 103.7	GC–MS	0.01 - 0.02 ng g^{-1} [(1)]	[22]
Bifenthrin, cyfluthrin, cyhalothrin, cypermethrin, deltamethrin, etofenprox, fenpropathrin, fenvalerate, flucythrinate, flumethrin,	Foods	QuEChERS	1.07	88 - 110	UPLC-MS/MS	10 µg kg^{-1} [(2)]	[34]

Pyretroids	Matrix	Extraction Method	Concentration Factor	Recoveries (*R*%)	Analytic method	LOD [1] or LOQ [2]	Ref.
fluvalinate, trans-permethrin, cis-permethrin, resmethrin, tefluthrin, pyrethrin I, cinerin I, jasmolin I, pyrethrin II, cinerin II, jasmolin II							
Tetramethrin, fenpropathrin, cypermethrin, deltamethrin, fenvalerate and permethrin.	Fruit juice	Dispersive liquid–liquid microextraction (DLLME)	50	84 - 94	HPLC-UV	2 - 5 µg L^{-1} [1]	[5]

VI.

ANÁLISIS DE PATRÓN ESPACIO TEMPORAL DE RESIDUOS DE PLAGUICIDAS EN LAS CUENCAS DEL TURIA Y JÚCAR

Presenta el seguimiento de 50 plaguicidas agrupados en las siguientes familias: Anilidas, azol, benzimedazoles, carbamatos, cloroacetanilides, hormonas juvelines mimics, neonicotinoides, organofosforados, tiocarbamatos, triazinas, triazoles, ureas y otros plaguicidas en los ríos Júcar (2010-2011) y Turia (2012-2013). Además, se evaluaron los parámetros físico-químicos de las muestras ambientales y su relación con las concentraciones de plaguicidas encontrados. Asimismo, se procedio a la evaluación de riesgo a través del Coeficiente de Riesgo (RQ) para dafnias, algas y peces en ambos ríos.

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Spatio-temporal patterns of pesticide residues in the Turia and Júcar Rivers (Spain)

Alexander Ccanccapa [a,*], Ana Masiá [a], Vicente Andreu [b], Yolanda Picó [a]

[a] Food and Environmental Safety Research Group (SAMA–UV), Centro de Investigaciones sobre Desertificación (CIDE, UV–CSIC–GV) and Facultat de Farmàcia, Universitat de València, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain

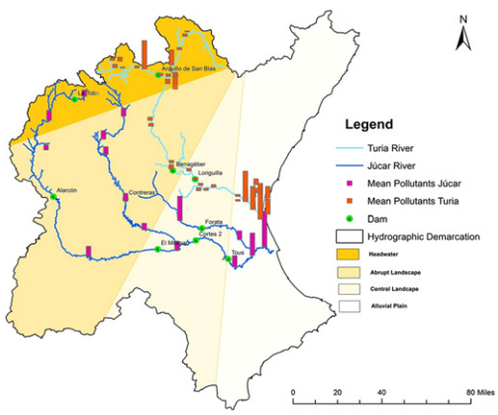
[b] Centro de Investigaciones Sobre Desertificación–CIDE (CSIC–UV–GV), Landscape Chemistry and Environmental Forensics Group, Carretera de Moncada-Náquera km 4.5, 46113 Moncada, Spain

HIGHLIGHTS

- Occurrence of the same pesticides was detected in both river basins.
- Mouth was the most contaminated area.
- Chlorpyrifos, hexythiazox and diazinon were the most frequent pesticides.
- Sediments were less contaminated than water, mostly by organophosphorus (higher K_{ow}).
- 7 pesticides were detected at concentrations higher than 100 ng L^{-1}.

GRAPHICAL ABSTRACT

Spatio-temporal patterns of pesticide residues in the Turia and Júcar Rivers 2010–2013 (Spain).



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ABSTRACT

A study was conducted on the occurrence of 50 pesticides in water and sediments of Turia and Júcar Rivers (Valencian Community, Eastern Spain) for a period of two consecutive years each, 2010/2011 and 2012/2013, respectively to assess the contribution of agriculture and urban activities on pesticide pollution. The results showed that mean concentrations of pesticides ranged from <LOQ up to 200 ng/L. Chlorpyrifos was the most frequent pesticide whereas imazalil, thiabendazole, tolclofos methyl, ethion and carbofuran were those found at higher concentrations. Ubiquitous pesticides are those with long half-lives. The most polluted parts of the rivers were the headwaters and the mouth, which could be related to the agricultural practices and rainfall. Contrarily, in the abrupt part of the rivers of difficult access the contamination is low. Other quality parameters monitored in this study also corroborate the worst water quality in the alluvial plains that coincides with higher anthropic pressure. The temporal variations also indicated a strong relation of pesticide concentrations with hydrology, the higher the river flow, the higher number and frequency of pesticides but at lower concentrations. On the contrary, at lower river flows higher pesticide concentrations were detected. The risk assessment for aquatic biota pointed out that organophosphorus and fungicides are a threat to fish and daphnia and herbicides and fungicides are hazards for algae. Thus, the strict control of pesticide concentrations is important to preserve the aquatic ecosystems health.

* Corresponding author.
E-mail address: Alexander.Ccanccapa@uv.es (A. Ccanccapa).

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1. Introduction

Pesticide is a broad term that includes insecticides, herbicides, fungicides and other compounds considered by both, the EU Water Framework Directive 2000/60/EC and the US Environmental Protection Agency (EPA) as priority pollutants (EC, 2000; EPA, 2012). As a result of massive global uses, pesticides and their degradation products spread through the environment and contaminate water, soil and atmosphere, leading to a consequent potential risk to humans and the environment. Their presence in the aquatic ecosystems is actually linked to diffuse pollution from run-off of agricultural fields and urban gardening areas. In addition, due to the input of urban run-off into the sewer lines, their presence in wastewaters cannot be underestimated (Campo et al., 2013; Giordano et al., 2009; Köck-Schulmeyer et al., 2013; Singer et al., 2010). Surface waters located in intensive agriculture areas, densely populated, are more vulnerable to pesticides (Köck-Schulmeyer et al., 2014; Masiá et al., 2015a). Apart from the toxicity data, the risk assessment is based on the concentrations determined by chemical analysis (Kuzmanović et al., 2015; Rose et al., 2015).

The presence of pesticides and their metabolites in surface water, sediments, biota and groundwater has been reported at low ng/L to several μg/L range in areas of intensive agricultural activity (De Gerónimo et al., 2014; Masiá et al., 2015a, 2013a; Page et al., 2014). These studies are mainly based on the collection and the analysis of samples for one punctual campaign because only this implies the analysis of a relatively large number of samples from different locations (Bonansea et al., 2013; De Gerónimo et al., 2014; Gómez et al., 2012; Phong et al., 2012). Several characteristics that change over the time, such as proximity of crop fields to surface waters, water body characteristics (surface area, depth and flow), and climatic conditions (temperature, humidity, wind and precipitation) affect the transport of pesticides and their transfer to other environmental compartments. These pesticide variations over the time are not taken into account when only one punctual campaign is carried out. However, they are of utmost importance in cases, such as the Mediterranean area, severely affected by water scarcity and characterized by alternative periods of torrential floods and severe droughts (Köck-Schulmeyer et al., 2014; Masiá et al., 2013a; Palma et al., 2014). The collection of long-term data (survey monitoring) is essential for the assessment of global changes in fluvial systems.

The Turia and Júcar rivers — located in the SE of Spain — are two important rivers draining their waters in the Mediterranean Sea. Both belong to the Júcar Hydrographic Demarcation. The human population living in the basin (5,162,163 inhabitants in 2009) makes an intensive use of the available water. In fact, the demand of water exceeds supply. The irrigated and rainfed agriculture is the economic activity that occupies about half of the territorial scope of the Júcar Hydrographic Demarcation with an approximate total irrigated area of 350,000 ha, mainly concentrated in the lower part of both rivers (CHJ, 2014). Nowadays, agriculture accounts for nearly 80% of water demand (1394 $hm^3\ y^{-1}$), but this appears to be stabilized or reduced, whereas urban/industrial demand is forecasted to rise.

This study is aimed at monitoring 50 currently used pesticides in water and sediments of the Júcar and Turia Rivers in two consecutive periods of time 2010–2011 and 2012–2013, respectively (see Supplementary material, Table S-1 for detailed list, physico-chemical properties and half-lives). The relation among physico-chemical water parameters, temperature, flow and the occurrence concentrations of pesticides was studied along both rivers. This is the first extensive pilot study undertaken (44 sampling points) in the Júcar Hydrographic Demarcation. It intends to improve the knowledge of these pesticides' occurrence in the aquatic environment. The selection of the target pesticides and metabolites was based on the extent of use, water solubility and amenability to LC–MS analysis.

2. Experimental

2.1. Site description

The two rivers flow into the Mediterranean Sea near the city of Valencia, Spain. The Júcar River Basin has a total drainage area of 22,123 km^2 and its main river, the Júcar, is 498 km long and has an average flow of 49.22 $m^3\ s^{-1}$. The most important tributaries are the Cabriel (with 220 km length, 4754 km^2 of drainage area and 20.92 $m^3\ s^{-1}$ of average flow) and Magro (130 km, 1544 km^2 and 0.96 $m^3\ s^{-1}$). The Turia River is a 280 km length with an average flow rate of 10.43 $m^3\ s^{-1}$ and its basin has a total drainage area of 6393.6 km^2 and receives as the most important tributary the river Alfambra (with 60 km, 1398 km^2 and 1.5 $m^3\ s^{-1}$).

In both rivers, the climate is typically Mediterranean with warm and dry summers (from July to August) and relatively wet and mild winters. The rainy season is from September to May, with maximum rainfall in October (autumn) and April (spring), and a mean annual precipitation of 500 mm, but there is a large spatial variation. The annual thermal oscillation presents continental characteristics, with cold and long winters not surpassing 4.5 °C as average temperature, and short and mild summers with an average of 21.2 °C. Periodically, the hydrological regime is affected by flood and drought events. Hydrology has been deeply altered due to human activities, and water resources are under increasing pressure (Hooke, 2006). The Júcar and Turia flows are regulated by large dams, smaller structures and weirs, and the channelization of some reaches (Lobera et al., 2015). Fig. 1 shows the location of the different sampling points (geo-references are in Table S-2). Methodologically, the area was classified into four zones according to their morphology, landscape, land use and degree of human pressure (some photos illustrating the landscape in the different zones are in Fig. S-1). These four zones are:

(i) Zone 1 or headwaters cover the headwaters of the Turia and Júcar Rivers. In the case of the Turia River, it comprises from the Sierra de Albarracín to the limits of the Teruel province, including the area and surroundings of Teruel city. The upper part of the river Júcar covers the eastern flank of the Montes Universales in the province of Cuenca where river crosses mountainous lands with a north–south direction in an area that crosses the city of Cuenca. This area shows hilly landscapes, with a very low population density.
(ii) Zone 2 or abrupt landscapes include the Rincon of Ademuz and the region of the Serranos, which are characterized by a rough and steep morphology that makes difficult the use of the river waters for agriculture. This zone also supports a low density of population, although slightly higher than the previous one.
(iii) Zone 3 or central landscapes are areas of hills and plains that involve the transition from the mountainous part of the basin to the more populated and exploited one. It is characterized by a gradual increase in the urban areas along with extensive agriculture, and
(iv) Zona 4 or alluvial plains comprise the regions of L'horta and Valencia city that make up the coastal area, which corresponds to the final phase of the Turia and Júcar River Basins. This final area includes the Natural Park of the Albufera of Valencia surrounded by the Turia River in the North and the Júcar in the South. Paradoxically, this flat area displays the highest density of growing areas, industries, infrastructure, and urban areas.

2.2. Sampling and sample analysis

The sampling was designed to perform large-scale and long-term (both complete basins, two consecutive years) monitoring to assess temporal trends of pollutants. The influence of seasonal variability was

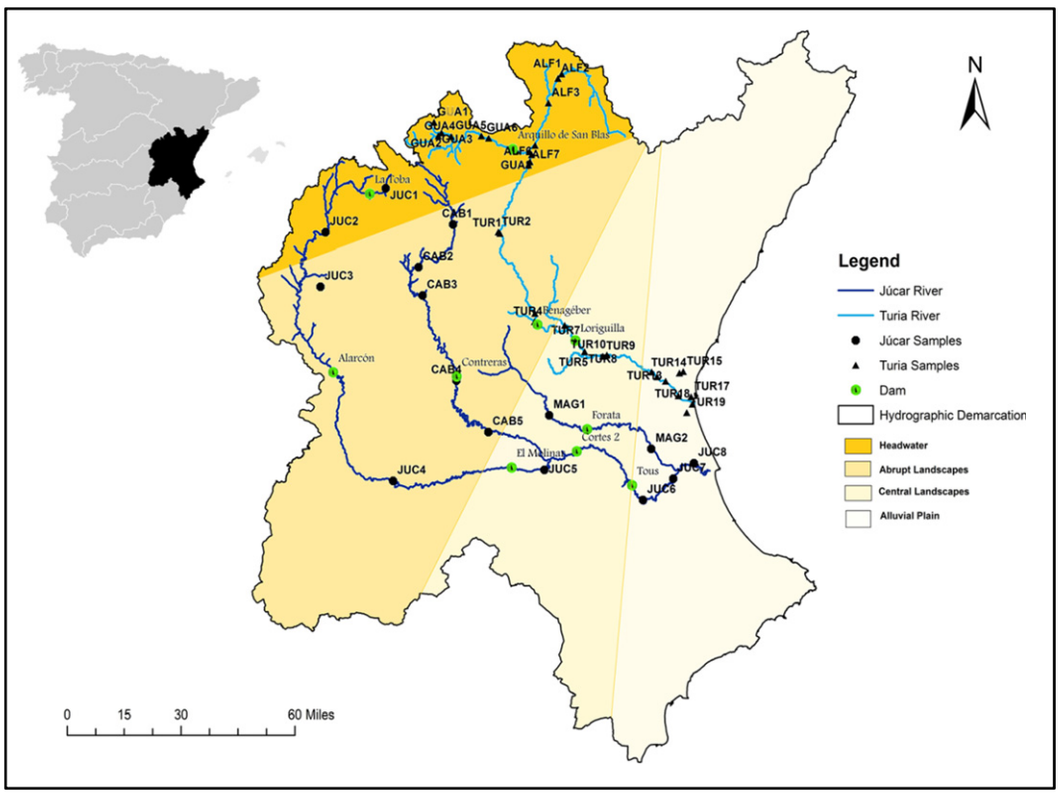


Fig. 1. Location of the sampling sites in the Turia and Júcar Rivers (Eastern Spain).

avoided monitoring the same period both years. September–November period was selected because there were not many applications of pesticides, which allow to establish what pesticides are constantly present in the environment because of their capacity of accumulation and/or persistence.

This campaign was carried out during 15 days in September/October 2010 and October/November 2011 in the Júcar River case and during 15 days in September/October 2012 and October/November 2013 in the Turia River. Sampling was carried out after a month without rainfall events to avoid their influence (runoff, dilution or infiltration). Water and sediments were collected at the 44 sampling points selected along Júcar River (15 sampling points each year) and the Turia River (23 in 2012 and 29 in 2013). In the Turia River, 6 new sampling points were added for the second monitoring year to confirm the results observed in the low part of the river corresponding to TUR14 to TUR19. In each sampling point one sample of water and one of sediment were taken. Grab water samples (2 L) were collected in clean amber glass bottles, from the middle of the river width. Sediment samples were taken in the same point as the water samples using a Van Veen grab sampler (0.5 L capacity). They were composite samples of the upper layer horizon (0–5 cm; about 500 g), made up of at least five randomly chosen subsamples collected in the sampling point. They were transferred and wrapped into an aluminum foil (previously washed with methanol and dried in oven at 100 °C) that was put inside an aluminum box.

Physical and chemical characteristics of water (temperature, pH, total soluble salts, dissolved O_2 and redox potential) were recorded at the sampling sites using a Multiparameter Eutech Instrument CyberScan PCD 650 (Thermo Fisher Scientific, Basel, Switzerland). Then, all samples were transported in hermetic boxes refrigerated with ice upon arrival at the laboratory (located in Valencia, Spain). Water samples were stored at 4 °C within 24 h to avoid any degradation, and pre-treated in the 5 subsequent days. Before the analysis, water samples were vacuum filtered through 1 μm glass fiber filters followed by 0.45 μm nylon membrane filters (VWR, Barcelona, Spain). Sediment samples were frozen, lyophilized (Hetosicc CD4, Birkerod, Denmark), pulverized, thoroughly mixed and then passed through a 2 mm Ø sieve. Intrinsic sediment characteristics were measured according to the standard laboratory techniques.

2.3. Sample preparation and analysis

The pesticides selected as target compounds are listed in the supplementary (Table S-1). Pesticides were determined by liquid chromatography tandem mass spectrometry (LC–MS/MS) using an Agilent 1260 Infinity system (Agilent technologies, Palo Alto, California, USA) equipped with a binary pump, an automatic injector, a mass spectrophotometer Agilent 6410 triple Quad LC/MS System connected by an ESI source and software Mass Hunter Workstation version B.04.00/built 4.0.225.19. Detailed conditions of the determination are provided in the supplementary material (see also text S-1 and Table S-3).

Water samples were extracted by solid-phase extraction (SPE) with Oasis HLB cartridge (Masiá et al., 2013b). The limits of detection (LODs) and quantification (LOQ) ranged from 0.1 to 2 ng/L and from 0.3 to 6 ng/L, respectively, depending on the pesticides. Calibrations curves were linear in the concentration range of 10 ng/L to 10 μg/L and the matrix effect was always ≤20%. Recoveries varied from 48% to 70% and precision was below 20% for all pesticides (details in text S-2 and Table S-5).

QuEChERS method was applied to 1 g lyophilized sediments as previously described (Masiá et al., 2015b). LODs and LOQs of the method ranged from 0.03 to 1.67 ng g^{-1} d.w. and 0.23 to 11.25 ng g^{-1} d.w. The matrix effect was <130% and recoveries were higher than 40%. The precision was below 20% (details in text S-2 and Table S-4).

2.4. Quality assurance and quality control

Pesticide concentrations were validated against a comprehensive set of quality control parameters including: laboratory and field blanks,

matrix spikes and triplicate samples. Blank contamination is the most common problem observed in the determination of pesticides at trace levels. Thus, precautions were taken to prevent contamination from personnel, organic solvents, equipment and glassware. Blank assays were performed employing MilliQ water samples, to check for laboratory background levels of the studied compounds. Though the detected amounts of the target compounds were low (below 5 ng/L), it was considered necessary to subtract the quantitative values of the compounds found in the blanks (only ethion and pyriproxyfen). In order to assure the quality of the results, field blanks were processed with the samples. It consisted of deionized water put down in the same conditions than samples during sampling process. For each batch of 10 samples analyzed, including the water field blank, a procedural blank and a recovery sample obtained by spiking at the lower level, were routinely extracted and analyzed under the same conditions as the ordinary samples. Triplicate samples analyzed were within 25% agreement for all pesticides detected above the analytical LOQ.

2.5. Statistical analysis

IBM SPSS version 22.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses including principal component analysis (PCA). Analysis of variance (ANOVA) and Tukey's multiple range test at $\alpha = 0.05$ were performed to detect differences in the variables between treatments. In the cases where the homogeneity and/or normality of the data could not be assumed, the Kruskal–Wallis and Mann–Whitney non-parametric test ($P \leq 0.05$) were applied.

Pearson statistical bivariate correlation analyses were applied, at 95% and 99% significance levels, between pesticide concentrations and water intrinsic parameters to determine possible relationships among them. When the values of a variable showed a non-normal distribution, Spearman bivariate correlations were applied at the same significance levels. Multiple stepwise linear regression analysis, discriminant analysis and categorical PCA were used to confirm the weight and dependence between variables, differences and identifying patterns in them.

2.6. Risk assessment calculation

The Risk Quotient (RQ) was calculated according to the European guidelines (EC, 2003) for each pesticide using the following equation:

$$RQ = EC/PNEC$$

where, EC is the mean or maximum concentration of pesticides detected in the water samples and PNEC is the predicted no-effect concentration. PNEC was calculated for chronic toxicity, dividing the lowest short-term $L(E)C_{50}$ or long-term non-observable effect concentration (NOEC), respectively, by an assessment factor (AF) of 1000. If the value of RQ index is higher than one ($RQ > 1$), harmful effects could be expected due to the presence of the pollutant in water. On the contrary, if the value of RQ index is less than zero point one ($RQ < 0.1$), the environmental risk is low. The intermediate situation in which the RQ index is between 0.1 and 1 ($RQ = 0.1–1$) involves medium risk.

3. Results and discussion

3.1. Water quality parameters

The levels of pesticides in different rivers and sampling period can be related to differences in environmental conditions such as pH and temperature. Although these conditions may have an effect, other variables like the type, season and number of pesticide applications in the nearby fields as well as the occurrence and magnitude of rainfall events particularly if they took place shortly after pesticide application can also influence pesticide concentrations. However, there are no data available on the types and number of pesticide applications. There were no rainfall events in the previous month to any of the sampling campaigns. Then, this parameter did not influence the sampling and, as the most intense applications of pesticides in crops are from January to March, the influence of other factors was minimized.

The temperature, pH, conductivity, resistivity, total dissolved solids (TDS) and dissolved oxygen (DO) and sodium chlorine results are presented as median values in Table 1 (minimum, maximum and mean values are shown in Table S-5). The pesticide degradation rate depends on its specific chemical properties, the pH of the water and the length of time the pesticide is in contact with the water. The half-lives of some commonly-used insecticides are presented in Table S-1.

The average pH for all sampling points was from neutral to a slightly alkaline. The pH is important because some pesticides, particularly carbamate and organophosphate insecticides, undergo in waters with a pH value greater than 7. The reaction is known as alkaline hydrolysis, and it reduces the effectiveness of the pesticide's active ingredient. However, in the Júcar River the pH median was around 7.9 in both years, whereas the organophosphorus concentration was 17.44 ng/L in 2010 and 1.71 ng/L in 2011. In Turia River the average pH were 8.3 and 8.6 (2012 and 2013, respectively) and the averages of organophosphorus were 11.4 ng/L (2012) and 4.89 ng/L (2013). This shows that other factors probably influence the variability in concentrations. Temperature also affects in the same way higher temperature favors more rapid degradations of some pesticides. It commonly ranges from 9 to 30 °C during the sampling period.

TDS (<LOD–2291 ppm), conductivity (<0–1.68 μS), resistivity (218–3973 Ω) and sodium chloride (<LOD–1520 ppm) are connected parameters to establish salinity and inversely related to the purity of water. In the case of the Turia and Júcar rivers the maximum values were recorded in the sampling points located in the low part of the basin, this means that it could be related to the presence of increased pollution and higher salt concentration. The low course of these rivers is where major percentage of urbanized, industrial, and agricultural pressures are located, therefore water quality decreases. As expected DO (2.91 mg/L in 2011) showed the lowest levels or the absence of oxygen in the mouth indicating high contamination, septic conditions of organic matter or an intense bacterial activity.

Sediments had in general sandy texture, basic pH and very variable organic matter content. The basic pH can be a point that enhance the degradation of organophosphorous pesticides and justify the relatively low concentration and the few pesticides found. However, further analysis of other properties that can affect pesticide degradation such as redox potential would be very interesting. Fate of pesticides in sediment is still an unexplored area.

Table 1
Median value of physico-chemical parameters of the rivers in water and sediments.

	Júcar river		Turia river	
	Median			
	2010	2011	2012	2013
Water				
Temp. (°C)	14.00	17.60	13.55	18.90
pH	7.89	7.99	8.27	8.64
mV	−64.10	−62.90	−63.60	−89.30
Cond (dS/m)	0.01	0.01	0.97	1.05
TDS (ppm)	509.90	573.20	618.30	772.00
NaCl (ppm)	646.10	723.60	1040.00	–
Res (Ω)	977.10	845.40	819.95	644.60
DO (mg/L)	8.89	9.35	8.50	9.73
Sediment				
MO (%)	2.54	2.78	1.62	2.37
Silt + Clay (%)	42.44	4.82	24.17	9.90
Sand (%)	57.56	95.18	75.83	89.30

3.2. Occurrence of pesticides

Results obtained for water and sediment in Júcar River 2010 and 2011 and Turia River 2012 and 2013 expressed as median and frequency of detection over the LODs are summarized in Tables 2 and 3 (maximum, minimum and mean are outlined in Tables S-6 and S-7). Pesticide residues were detected in water and sediments. The majority of pesticides were found in water at higher frequency than in sediment. The low frequency of pesticides in sediments could be related to their polarity since most of them are highly polar and consequently the tendency to accumulate in the most apolar sediments is low.

Table 2
Median and frequency (Freq) of detection of pesticides in water.

Class/pesticide	Júcar River				Turia River			
	2010		2011		2012		2013	
	Median	Freq (%)[a]	Median	Freq (%)[a]	Median	Freq (%)[a]	Median	Freq (%)[a]
Anilide								
Propanil	n.d	n.d	n.d	n.d	n.d	n.d	<0.01	3 (10)
Azol								
Imazalil	152.49	14 (93)	3.02	10 (66)	<0.01	10 (45)	3.75	21 (72)
Prochloraz	72.24	15 (100)	<0.01	1	<0.01	9 (41)	<0.01	10 (34)
Benzimidazole								
Carbendazim	n.d	n.d	2.04	8 (53)	6.25	17 (77)	1.48	25 (86)
Thiabendazole	n.d	n.d	<0.01	3 (20)	21.75	21 (95)	3.39	27 (93)
Carbamates								
3-Hydroxycarbofuran	n.d	n.d	n.d	n.d	n.d	n.d	<0.01	1 (3)
Carbofuran	n.d	n.d	n.d	n.d	<0.01	1 (5)	<0.01	10 (34)
Methiocarb	<0.01	1(6)	n.d	n.d	n.d	n.d	<0.01	2 (6)
Chloroacetanilide								
Acetochlor	n.d	n.d	n.d	n.d	n.d	n.d	<0.01	2 (6)
Alachlor	n.d	n.d	n.d	n.d	n.d	n.d	<0.01	1 (3)
Metolachlor	<0.01	1 (6)	n.d	n.d	<0.01	2 (9)	2.20	23 (79)
Juvenile hormone mimics								
Pyriproxyfen	83.21	15 (100)	n.d	n.d	n.d	n.d	0.35	15 (51)
Neonicotinoid								
Imidacloprid	<0.01	2 (13)	<0.01	3 (20)	8.04	18 (82)	3.54	21 (72)
Organophosphorus								
Azinphos ethyl	n.d	n.d	n.d	n.d	n.d	n.d	<0.01	2 (6)
Azinphos methyl	n.d	n.d	n.d	n.d	<0.01	1 (5)	<0.01	1 (3)
Chlorfenvinphos	83.07	11 (73)	1.11	10 (66)	<0.01	8 (36)	0.79	25 (86)
Chlorpyrifos	10.24	15 (100)	3.73	15 (100)	18.85	18 (81)	3.04	21 (72)
Diazinon	8.87	15 (100)	0.30	9 (60)	13.37	18 (81)	4.19	27 (93)
Dichlofenthion	38.19	15 (100)	n.d	n.d	n.d	n.d	n.d	n.d
Dimethoate	<0.01	3 (20)	<0.01	1 (6)	<0.01	1 (5)	<0.01	12 (41)
Ethion	<0.01	5 (33)	3.04	12 (80)	6.53	13 (59)	0.52	29 (100)
Fenitrothion	n.d	n.d	n.d	n.d	n.d	n.d	<0.01	2 (6)
Fenoxon	n.d	n.d	n.d	n.d	n.d	n.d	<0.01	1 (3)
Fenoxon sulfone	n.d	n.d	n.d	n.d	<0.01	3 (13)	<0.01	3 (10)
Fenoxon sulfoxide	<0.01	7 (46)	n.d	n.d	n.d	n.d	<0.01	1 (3)
Fenthion	n.d	n.d	n.d	n.d	<0.01	1 (5)	<0.01	1 (3)
Fenthion sulfone	n.d	n.d	n.d	n.d	<0.01	1 (5)	n.d	n.d
Fenthion sulfoxide	n.d	n.d	n.d	n.d	<0.01	3 (13)	<0.01	3 (10)
Malathion	<0.01	3 (20)	n.d	n.d	n.d	n.d	n.d	n.d
Parathion-ethyl	<0.01	6 (40)	n.d	n.d	n.d	n.d	<0.01	1 (3)
Tolclofos Methyl	<0.01	4 (26)	<0.01	1 (6)	<0.01	2 (9)	<0.01	1 (3)
Other pesticides								
Buprofezin	12.11	15 (100)	n.d	n.d	13.22	15 (30)	<0.01	1 (3)
Hexythiazox	17.37	15 (100)	n.d	n.d	10.22	13 (26)	0.44	27 (93)
Thiocarbamates								
Molinate	<0.01	1 (6)	n.d	n.d	<0.01	1 (5)	n.d	n.d
Triazine								
Atrazine	<0.01	3 (20)	n.d	n.d	<0.01	1 (5)	0.18	15 (51)
Deisopropylatrazine	<0.01	2 (13)	n.d	n.d	<0.01	1 (5)	<0.01	1 (3)
Desethylatrazine	<0.01	5 (33)	<0.01	1 (6)	8.68	17 (77)	<0.01	5 (17)
Propazine	n.d	n.d	n.d	n.d	n.d	n.d	<0.01	3 (9)
Simazine	n.d	n.d	n.d	n.d	<0.01	2 (9)	<0.01	4 (13)
Terbumeton	n.a	n.a	<0.01	2 (13)	<0.01	1 (5)	0.17	20 (68)
Terbumeton-desethyl	n.a	n.a	<0.01	4 (26)	<0.01	6 (27)	0.33	19 (65)
Terbuthylazine	n.a	n.a	<0.01	5 (33)	16.95	16 (72)	1.57	25 (86)
Terbuthylazine desethyl	n.a	n.a	<0.01	6 (40)	<0.01	6 (27)	4.92	22 (75)
Terbuthylazine-2 hydroxy	n.a	n.a	<0.01	8 (53)	7.65	14 (63)	1.51	26 (86)
Terbutryn	n.d	n.d	n.d	n.d	<0.01	3 (13)	0.28	19 (65)
Triazole								
Tebuconazole	n.d	n.d	n.d	n.d	<0.01	1 (5)	<0.01	13 (44)
Urea								
Diuron	n.d	n.d	<0.01	2 (13)	n.d	n.d	<0.01	6 (20)
Isoproturon	n.d	n.d	n.d	n.d	n.d	n.d	<0.01	8 (27)

n.d = non-detected.
n.a = not analyzed.
[a] Number of finding (percentage of positive samples).

Of the 50 compounds analyzed, 22 and 18 (approximately 44% and 36% of analytes) were detected at the concentration over the LOD in Júcar river (2010 and 2011) while 33 and 44 (approximately 66% and 88%) were detected in the Turia River (2012 and 2013).

Organophosphorus (chlorfenvinphos, chlorpyrifos, diazinon, dimethoate, ethion and tolclophos methyl), triazines (deethylatrazine) and azoles (imazalil and prochloraz) were detected in all samplings. In the period 2010–2011, chlorpyrifos was present in 100% of the samples. In the period 2012 and 2013, chlorpyrifos (82%, and 72% of the samples, respectively), hexythiazox and diazinon (93% in 2013) were the most frequent. Carbendazim, thiabendazole, terbumeton, terbumeton deethyl, terbuthylazine, terbuthylazine desethyl and terbuthylazine-2 hydroxy were not analyzed in 2010 because there was no information on the extent of use. However, their presence was detected by the non-target analysis of some samples (data not shown) and then, they were included in the analytical method for the next years. These compounds appear in all the other campaigns in high frequency. Júcar Hydrographic Demarcation has 42,832 km^2 and 376,896 ha under irrigation, both rivers are geographically very near, separated only by 25 km at its narrowest part. The constant frequency of these pesticides in both rivers can be due to their generalized agriculture and urban uses (Masiá et al., 2015a). Omethoate, malathion, diuron and tebuconazole were detected sporadically, even though few of them are at high concentrations.

Chlorfenvinphos, terbutryn and metolachlor (Regulation EC No 2002/2076) (EC, 2002), atrazine (Decision 2004/248/EC) (EC, 2004a), and simazine (Decision 2004/247/EC) (EC, 2004b), were detected in both sampling campaigns, despite being withdrawn from the EU (Regulation EC No 2009/1107) (EC, 2009). These compounds are resistant to hydrolysis and persistent as environmental deposits; their occurrence keeps up with the agricultural activity in the area. Their presence in surface water can be justified by runoff from soils.

The last Directive 2013/39/EU (EU, 2013) regards priority substances in the field of water policy establishes 45 priority substances of which seven substances are target compounds of this study. The maximum allowable concentration were for atrazine (2.0 μg/L), chlorfenvinphos (0.3 μg/L), chlorpyrifos (0.1 μg/L), diuron (1.8 μg/L), isoproturon (1.0 μg/L), simazine (4 μg/L) and terbutryn (0.34 μg/L). The concentrations detected for these compounds in this study are below the established limit (see Table S-6).

Samples can contain several pesticides (see Fig. S-2). In 2010–2011, 78% and 84% of the water samples contained at least 5 pesticides. Furthermore, 18% in 2010 and 4% in 2011 of the samples contained more than 11 pesticides. In 2012–2013, 68% and 52% of the water samples were contaminated with 5 pesticides and 14% and 34% of the samples, presented more than 16 pesticides. This indicates that even though individual concentrations were low and did not exceed the European threshold for drinking water, the number of pesticides in each sample was high.

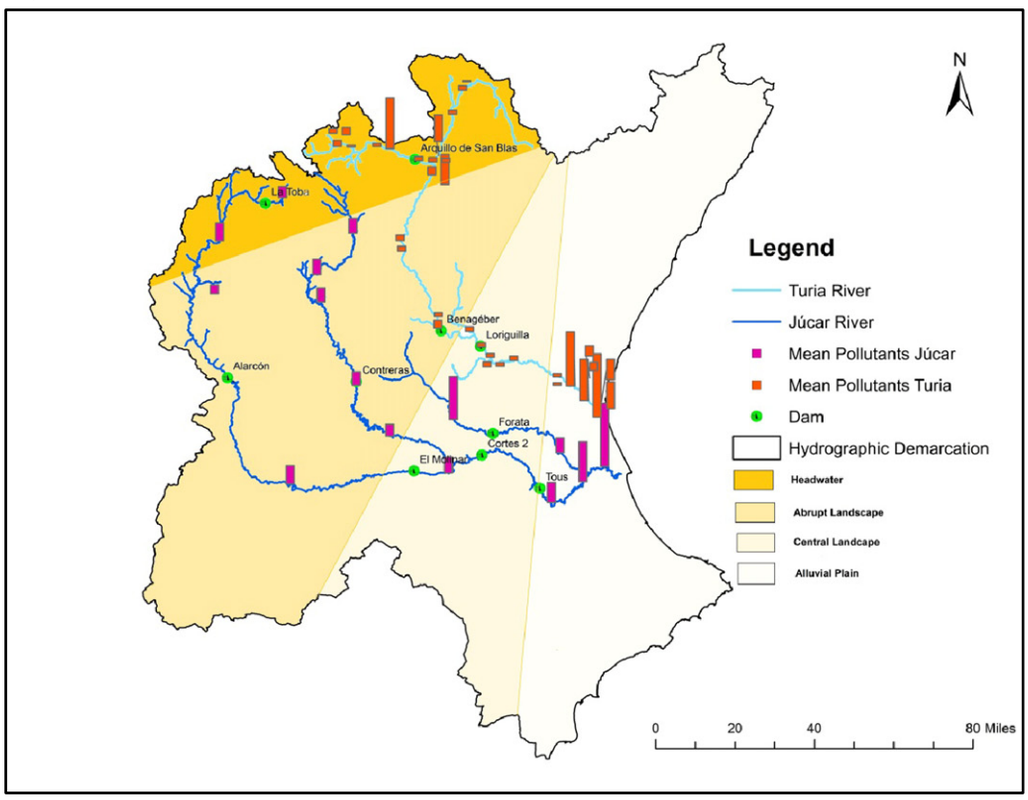
The levels of the analytes detected varied considerably, showing the maximum concentrations for fungicides such as imazalil (750 ng/L in 2012), thiabendazole (187 ng/L in 2011), tolclophos methyl (382 ng/L in 2012), prochloraz (486 ng/L), carbendazim (382 ng/L) and for the insecticides, chlorfenvinphos (106 ng/L in 2010), azinphos-methyl (148 ng/L in 2012), ethion (349 ng/L in 2013), metolachlor (446 ng/L) and imidacloprid (206 ng/L) (see Table S-6). Carbofuran (6844.5 ng/L

Table 3
Median and frequency detection of pesticides in sediment.

Class/pesticide	Júcar River				Turia River			
	2010		2011		2012		2013	
	Median	Freq (%)[a]	Median	Freq (%)[a]	Median	Freq (%)[a]	Median	Freq (%)[a]
Azol								
Imazalil	<0.01	2 (13)	<0.01	2 (13)	<0.01	2 (9)	n.d	n.d
Prochloraz	n.d	n.d	<0.01	5 (33)	n.d	n.d	n.d	n.d
Benzimidazole								
Carbendazim	n.d	n.d	n.d	n.d	<0.01	3 (14)	n.d	n.d
Thiabendazole	n.d	n.d	<0.01	3 (20)	<0.01	6 (27)	n.d	n.d
Carbamates								
Carbofuran	n.d	n.d	n.d	n.d	n.d.	n.d.	<0.01	2 (6)
Methiocarb	n.d	n.d	<0.01	1 (7)	n.d	n.d	n.d	n.d
Juvenile hormone mimics								
Pyriproxyfen	1.00	14 (93)	n.d	n.d	n.d	n.d	n.d	n.d
Neonicotinoid								
Imidacloprid	n.d	n.d	n.d	n.d	<0.01	1 (5)	n.d	n.d
Organophosphorus								
Chlorfenvinphos	<0.01	1 (7)	<0.01	1 (7)	n.d	n.d	n.d	n.d
Chlorpyrifos	2.00	15 (100)	3.15	15 (100)	63.75	22 (100)	<0.01	11 (37)
Diazinon	<0.01	2 (13)	1.37	12 (80)	<0.01	1 (5)	n.d	n.d
Dimethoate	n.d	n.d	n.d	n.d	<0.01	2 (9)	n.d	n.d
Ethion	n.d	n.d	n.d.	n.d.	<0.01	1 (5)	n.d	n.d
Fenoxon Sulfone	<0.01	n.d	<0.01	1 (7)	n.d	n.d	n.d	n.d
Malathion	<0.01	5 (33)	<0.01	1 (7)	n.d	n.d	n.d	n.d
Omethoate	<0.01	6 (40)	n.d	n.d	n.d	n.d	n.d	n.d
Parathion-ethyl	<0.01	7 (46)	n.d	n.d	n.d	n.d	n.d	n.d
Parathion-methyl	<0.01	8 (53)	n.d	n.d	n.d	n.d	n.d	n.d
Tolclofos methyl	<0.01	9 (60)	n.d	n.d	n.d	n.d	n.d	n.d
Other pesticides								
Buprofezin	1.28	15 (100)	n.d	n.d	n.d	n.d	n.d	n.d
Hexythiazox	1.21	15 (100)	<0.01	3 (20)	n.d	n.d	n.d	n.d
Triazine								
Terbumeton-desethyl	n.a	n.a	<0.01	2 (13)	<0.01	1 (5)	<0.01	2 (6)
Terbuthylazine desethyl	n.a	n.a	n.d	n.d	n.d	n.d	<0.01	2 (6)
Terbuthylazine-2 hydroxy	n.a	n.a	n.d	n.d	<0.01	1 (5)	n.d	n.d
Urea								
Isoproturon	n.d	n.d	n.d	n.d	n.d	n.d	<0.01	2 (6)

n.d = non-detected
n.a = not analyzed
[a] Number of finding (percentage of positive samples).

A)



B)

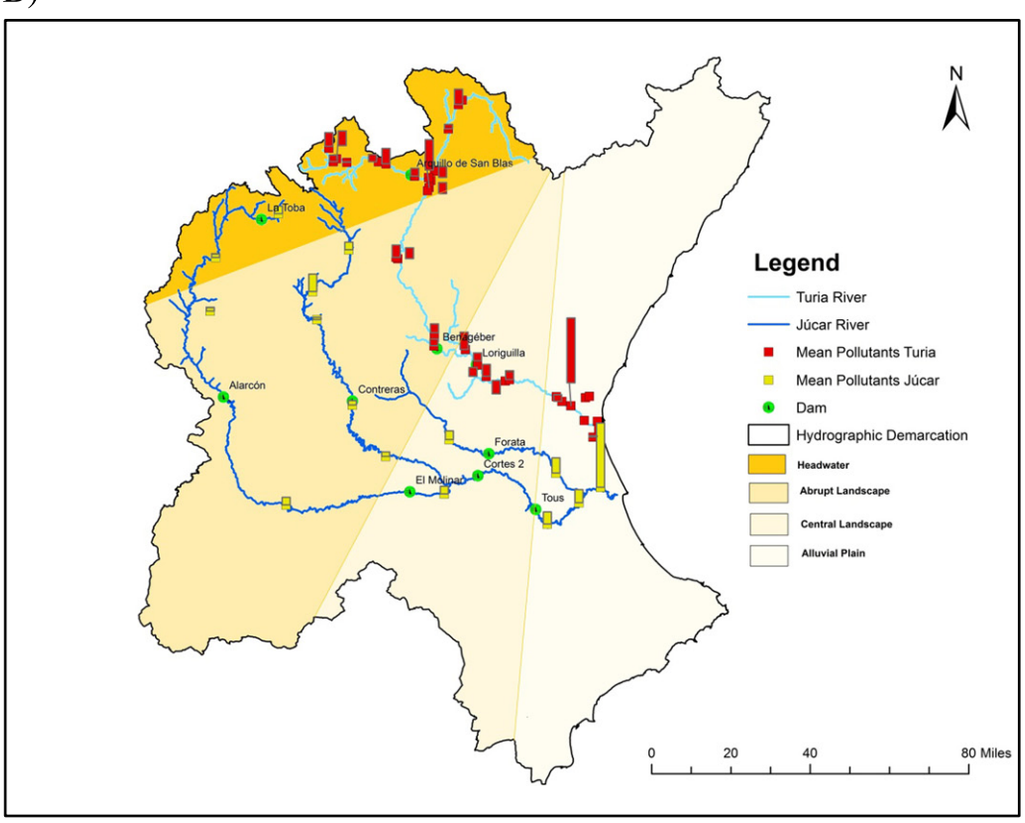


Fig. 2. Spatial distribution of pesticides in Turia and Júcar rivers. A) 2010–2013 water samples and B) 2010–2013 sediment samples.

in 2013) and azinphos methyl (148 ng/L in 2012) were isolated cases. In 2010–2011 carbofuran was not detected and in 2012 the frequency was only 5% and in 2013 was 34%, appearing mostly in the station GUA 6 (6845 ng/L), which is at the head of the basin. Azinphos methyl was not detected in 2010–2011 and in 2012 and 2013 appeared in only two samples. Concentrations of these pesticides exceed 100 ng/L, limit established for individual concentrations in drinking water according to EU legislation (EC, 1998).

Fig. 2A offers a general view of the distribution of pesticides in water samples in both campaigns. In JUC8 (2010), ALF6 (2013), TUR13 (2012–2013), TUR16 (2013), and TUR19 (2013) sites (see Fig. S-3), the sum of the compounds detected surpassed the tolerance threshold of 0.5 μg/L for the sum of pesticide residues established by the European Union Commission in order to guarantee water quality (EC, 1998). The use of treated surface water is a common practice in the area. Waters that surpassed these levels require costly treatment to eliminate these pesticides.

Regarding the correlation between the physico-chemical properties of pesticides and their occurrence, there are two parameters — the Field Half Life and Water Solubility (see Table S-1) — that can explain the observed course. The pesticides detected at high frequency are those that present long half lives and the pesticides detected at high concentration are those water soluble. Then, some pesticides, such as chlorpyrifos were frequently detected (100% of the in 2010–2011 and more than 70% in 2012–2013) but at concentrations lower than 40 ng/L (persistent with half-life up to 50 days and little water soluble ca. 1.05 mg/L). Others, such as imazalil (stable and water soluble) were also frequent (between 43% and 90% of the samples in 2010–2013) and at high concentration up to 682 ng/L in 2010, (long Field Half Life – 120 to 190 days – and high water solubility — 180 mg/L).

Finally, pesticides as propanil, malathion, methiocarb and fenitrothion that have short Field Half Lives (<6 days) and variable water solubility (ranging from 21 to 225 mg/L) were always detected at low frequency and concentrations. For example, propanil was only detect in 2013 in 10% of the samples (half life = 3 days) and malathion only in 2010 in 20% of the samples (half-life = 4 to 6 days).

The most polluted sites in the Júcar and Turia Rivers were located in the mouth area of the rivers where increased concentration gradients in water (e.g. from JUC6 to JUC8 and from TUR10 to TUR13) were identified. In this area, concentrations of imazalil and chlorpyrifos were high in the different sampling periods. The highest concentrations detected were for imazalil in 2010 and 2011 (682.72 ng/L, 222.45 ng/L respectively) in the Júcar river and in 2012 (750.29 ng/L) in the Turia River. This pesticide is widely used as antifungal in the post-harvest treatment of oranges and other fruits. Prochloraz (486.21 ng/L) and chlorfenvinphos (148.07 ng/L) were also detected at high concentrations. Chlorfenvinphos is an insecticide used for pest control in cereal, citrus, vine, fruit tree and prochloraz a fungicide applied in citrus groves. In 2013, 6 new stations were installed in the low course of Turia River to confirm the higher contamination levels in the lower part of the river. In these stations, 66% the selected pesticides were detected. Fig. S-3 shows distribution of each family of pesticide in both rivers. The principal pollutants are organophosphates, triazines, and neonicotinoids, which is consistent with the main crops of the area, orange and rice fields.

High concentrations were also observed in some points located in the western sector of the Turia headwater, which has an extension of 850 irrigated hectares. Irrigation was carried out using traditional systems formed mainly by waterwheels directly taking the waters of the River Turia. Predominant crops in the area are poplars and industrial crops, followed by maize and other cereals and finally, small orchards of own cultivation. Pesticides were mostly insecticides, particularly, carbamates with the highest concentration for carbofuran in 2013 (6.844, 50 ng/L) (withdrawal by the Regulation No. 1107/2009 CE). This pesticide was soil applied at drilling on maize, sugar beet and sunflowers. Other pesticides with the same main applications, such as carbosulfan, benfuracarb and furathiocarb are also rapidly degraded to carbofuran.

The lowest concentrations were in Zones 2 and 3. The former is characterized by abrupt landscapes that prevent the use of water because both rivers run encased by deep gorges that are becoming difficult to access. In the latter, the rivers still run between mountains and their flows have been regulated in a succession of dams. The exception in the Zone 2 is the point corresponding to MAG1 located in an area of vineyards and cereals. Both crops required some pesticide treatment during autumn period.

Table 3 shows median concentration values and frequency of detection of pesticides in sediment (maximum and mean concentrations and frequency are summarized in Table S-7). In contrast with water, sediment samples of 2010–2011 showed lower frequency of pesticides. In 2010; chlorpyrifos, buprofezin and hexythiazox were detected in all sample points nevertheless the other target compounds were found occasionally. In 2011 and 2012 chlorpyrifos was detected in 100% of the sampled points and in 2013 it was in 37% of the samples.

The highest concentrations were in the Júcar River (2010–2011) for imazalil 32 and 37 ng/g dry weight (d.w.) and in the Turia River (2012–2013) for chlorpyrifos (141 and 55 ng/g d.w.) (also frequent in water and with high log K_{ow}). Fig. S-4 shows distribution of all pesticide families studied in sediment samples in both campaigns. The main contaminants are organophosphorus, azoles, triazines, ureas and carbamates.

Fig. 2B offers a general view of the distribution of pesticides in sediment samples in both campaigns. Regarding co-occurrence of sediments (see Fig. S-4B), in 2010 and 2011 in the Júcar basin 76% and 78% samples do not present pesticides while 18% and 10% had at least 3 pesticides. The Turia basin had the same profile in 2012–2013, 80% and 90% did not present pesticides whereas 4% and 8% of the samples presented 3 or more pesticides.

A concentration gradient was detected in both campaigns for the Júcar River sediment samples (see Fig. S-3 A from JUC6 to JUC8 and MAG1 to MAG2). In the Turia River concentrations are variable between the two periods (2012 and 2013) and between sampling points without any defined pattern. Fig. S-3 B shows heterogeneous concentrations along the river, nevertheless in the year 2012 in the mouth a specific point (TUR13) exists a concentration above 800 ng/g. Oppositely, in the year 2013 the headwater has a specific point of contamination (GUA1) with a concentration above 90 ng/g.

On the temporal distribution of pesticides, in the Júcar River Basin, more pesticides at higher frequency and concentrations were detected in 2010 than in 2011. Contrarily, in the Turia River Basin, more pesticides at higher frequency and concentrations were detected in 2013 than in 2012. These behaviors can be influenced by the river flow (detailed in Table S-8). The flow of the river in each point was classified as high, medium or low by comparing its value during the sampling to the flow measurements in the last fifty years in each point where there are data available and normalizing them to 100. In the Júcar river these values ranged from the percentile 53 (in JUC2) to percentile 73 (CAB4) in 2010 and from percentile 36 (JUC9) to percentile 53 (in CAB2) in 2011 that can be considered as high and medium–low flows, respectively. In the Turia River, these percentiles were between 14 (TUR7) and 43 (TUR9) in 2012 and between 33 (TUR12) and 69 (TUR7) in 2013, classified as medium–low and high flows, respectively. This agrees partly with those already reported (Masiá et al., 2015a, 2013a) because the higher the flow, the greater the frequency and number of co-occurring pesticides, probably because higher flows are related with increased runoff with more pesticide dragged into the water. Turia and Júcar Rivers show higher concentrations at low and high flows. This can be explained because the flow of the rivers in the Valencian Community (severely affected by droughts) are very regulated by dams and channels and runoff can be not only from heavy rains but also from agricultural flooding that can contribute higher concentrations.

The different rivers of the world (including the five continents) polluted by varied class of pesticides can be seen in Table S-9. Asia still

shows organochlorine contaminants in their rivers. However organochlorine concentrations decrease against the organophosphate pollutants that increase (Gao et al., 2009; Gfrerer et al., 2002; Leong et al., 2007; Sankararamakrishnan et al., 2005; Varca, 2012). Africa shows the same pattern as Asia, organochlorine pesticides can still be detected but there is an increase of organophosphate pesticides (Darko et al., 2008; Kuranchie-Mensah et al., 2012).

Europe, North and South America and Oceania present mostly organophosphate and other less polar pesticides in their rivers (Bonansea et al., 2013; Lambropoulou et al., 2002; Palma et al., 2004; Pinheiro et al., 2011; Rebich et al., 2004; Steen et al., 2001). Regarding the concentration of pesticides, Asia and Africa present higher concentrations of organophosphates and organochlorine pesticides than the other continents whereas Europe and America present lower concentrations. Europe, Asia, North America, South America, Oceania and Africa are respectively hugely diverse regions, culturally and geographically, the studies summarized could not be fully-representative of the differences especially in the case of Africa, South America and Oceania where the number of studies is still restricted. However, the studies selected show that with small differences between developed and developing countries, there is a globalization in the use of pesticides. The rivers of the Mediterranean area present similar concentrations and types of pesticides, probably because there are similar cultivations in all the Mediterranean.

3.3. Environmental risk characterization

RQ were calculated for mean and maximum concentrations in both sampling campaigns for algae, Daphnia, and fish (2010–2013) (see Table S-10).

Carbendazim, chlorfenvinphos, chlorpyrifos, ethion, fenitrothion, hexythiazox, imazalil, metolachlor, pyriproxyfen, prochloraz and azinphos methyl presented RQ $\gg 1$ for daphnia at both, mean and maximum concentrations, demonstrating a high potential to cause negative effects in this aquatic organism. For algae, the RQ of hexythiazox, imazalil, metolachlor and prochloraz were >1 for both, mean and maximum concentrations, while ethion showed no acceptable risk only at maximum concentrations. Azinphos methyl, chlorpyrifos, carbofuran, chlorfenvinphos, dichlofenthion, ethion, pyriproxyfen carbendazim, prochloraz and imazalil showed also as a hazard for fish at both mean and maximum concentration. Insecticides showed the highest RQ values for daphnia and fish whereas herbicides for algae. Fungicides showed high RQ for all types of biota (daphnia, algae and fish). The additive effect of the many co-occurring pesticides might project a larger hazard on the aquatic ecosystem. This situation is particularly worrisome in the case of organophosphorus pesticides acting by the same toxicity mechanism because there are concentration additions or synergy. There are a number of pesticides belonging to this class that present RQ > 1 for daphnia and fish and co-occurred in water making the situation worst. Furthermore, there is a cocktail effect produced by the simultaneous presence of the different types of pesticides that are likely to induce synergistic interactions. Cholinesterase inhibitors (organophosphates and carbamates) and azole fungicides (imazalil and carbendazim) have been involved in 95% of the described synergistic cases (Cedergreen, 2014). The synergistic cases most likely all involve interactions on metabolism. The results demonstrated the interest of these studies to protect aquatic ecosystems, even though the risk assessment carried out — calculation of HQ — is very simplistic and could underestimate real toxic effects.

3.4. Magnitude of the Júcar and Turia Rivers' loads

Management of the sensitive Mediterranean coastal system requires the determination of river contaminant loads. Precipitation in the basin produces a mean annual runoff about 75 mm, which represents approximately 15% of the precipitation. However, there are other sources as a network of irrigation channels used for agricultural purposes mostly in the lower part of the rivers. The concentration of pesticides detected in water at the different sampling sites was multiplied by the flow rate to obtain environmental loads expressed in milligrams of compounds per second (see Fig. S-5). Pesticide inputs mainly occurred in the final part of the river by diffuse surface or subsurface hydrological pathways or due to bad agricultural practices. The lower part of both rivers shares an alluvial plain, of great economic importance, due to the agriculture, being the most densely populated area. The main cultivations of the area are orange orchards and rice crops. However, pesticide entrance into surface water through WWTP should not be discarded because the flow of many of these irrigation channels is maintained using the effluents of the WWTPs.

The highest loads appear in the low part of the two rivers. In case of the Júcar River the highest load is up to 13.33 mg/S and the Turia River is up to 2.08 mg/S (see Fig. S-5). The total load of pesticides released by the Júcar River to the Mediterranean Sea was estimated to be at 539 kg $year^{-1}$ in 2010 and 226 kg $year^{-1}$ in 2011. The Turia River discharges lower amount of pesticides 156 kg $year^{-1}$ in 2012 and 98 kg $year^{-1}$ in 2013. These are minimum values of pesticide discharges because the seasonal variations have not been taken into account. Previous studies pointed out that loads of pesticides are higher in spring that in any other season. These high loads of pesticides, even in periods of low pesticide concentration, could have impact on the biota and marine ecosystems (Mai et al., 2013; Soubaneh et al., 2015).

3.5. Statistical analysis

Pesticide concentration in water can be mainly influenced by: (i) degradation (half-life) (see Table S-1), (ii) drainage area and land uses that affect the quantity of pesticides from non-point sources, such as air, runoff or infiltration, (iii) river flow and water physicochemical parameters (e.g. water temperature and pH) that affect dilution and degradation and (iv) reservoirs along the river (see Table S-2), (Belenguer et al., 2014).

Significant statistical differences between river catchments were observed for pH and TDS, and for the pesticides: imazalil, diazinon, pyriproxyfen, imidacloprid, chlorfenvinphos, fenoxon sulfoxide, hexythiazox, and terbuthylazine-2-hydroxy. TDS and pH are higher in the Turia (ca. 700 mV and 8.2) than in the Júcar River (ca. 500 and 7.8). The pesticides are at low concentration and frequency in the Turia than in the Júcar River. This can be explained because the pH conditions in the Turia River favor pesticide degradation more than those of the Júcar River, particularly organophosphorous and carbamates. Table 4 outlines the results of the multiple step-wise linear regression models between the studied pesticides through water characteristics. Dissolved oxygen, electric potential (mV) and pH are the water factors that showed more influence on the pesticide dynamics in both rivers.

In general, the river's mouth covering the alluvial plains shows a different behavior regarding pesticides and particular water characteristics (conductivity, TDS, temperature or resistivity) than the other landscape units or zones. As it was shown by the results, the highest levels of pesticides in the studied matrices appeared in these mouth zones that, in the same way, support the major population density and the most intense human activities.

Analyzing the results between sampling years, 2010 showed statistical significant differences with respect to the other years about the majority of the target pesticides and water conditions (temperature, pH, dissolved oxygen, TDS, conductivity).

In the case of the Júcar River, a strong influence of the water conductivity and its NaCl content on the distribution of pesticides was observed. Among landscape units/zones, imidacloprid showed the highest statistical differences, showing Zone 3 differences regarding the others. In the same way, marked differences between zones 1–2 and 3–4 were observed, regarding water characteristics, mainly TDS,

Table 4
Multiple step-wise linear regression models between the studied pesticides through water characteristics ($Y = B_0 + B_1X_1 + B_2X_2 + \ldots$) at 99% significance.

Y	B_0	Bj	Xj	R^2	Sig.*
Global					
Imazalil	837.821	$B_1 = -95.240$	X_1 = [pH]	0.201	0.004
Pyriproxyfen	597.663	$B_1 = -80.979$	X_1 = [pH]	0.261	0.000
		$B_2 = -1.206$	X_2 = [mV]		
Chlorfenvinphos	230.885	$B_1 = -26.245$	X_1 = [pH]	0.239	0.000
Buprofezin	24.410	$B_1 = -0.720$	X_1 = [T]	0.248	0.000
		$B_2 = -0.074$	X_2 = [DO]		
Hexythiazox	57.159	$B_1 = -5.350$	X_1 = [pH]	0.212	0.000
		$B_2 = -0.471$	X2 = [T]		
Deethylatrazine	11.127	$B_1 = 0.109$	X_1 = [mV]	0.112	0.002
Júcar River					
Terbuthylazine-2-hydroxy	12.010	$B_1 = 0.179$	X_1 = [mV]	0.357	0.000
Turia River					
Metolachlor	−25.583	$B_1 = 0.356$	X_1 = [DO]	0.174	0.002
Buprofezin	35.227	$B_1 = -0.761$	X_1 = [T]	0.353	0.000
		$B_2 = -0.170$	X_2 = [DO]		
Deethylatrazine	13.227	$B_1 = 0.125$	X_1 = [mV]	0.215	0.001
Hexythiazox	16.483	$B_1 = -0.370$	X_1 = [T]	0.238	0.001
		$B_2 = -0.070$	X_2 = [DO]		

* Sig: Significance.

NaCl and resistivity. These water parameters clearly increase towards the end of the river.

Turia River presented similarities with Júcar River about the influencing water parameters on the pesticide distribution, such as TDS and resistivity, but the first showed lower influence of NaCl and higher electric conductivity. In this river, the zone that showed significant differences regarding the others was the alluvial plain (Zone 4) for the majority of pesticides.

The PCA analyses did not give significance enough to clearly determine the influence of spatial or environmental factors on the pesticide behavior in both rivers. Only in the case that we take both catchments together the explained variance reach the 73.47%, (see Fig. S-6) indicating a patent spatial influence on the pesticides behavior.

4. Conclusions

Currently used pesticides are detected in river even in periods where they are, at least in theory, not applied to surrounding crops. More pesticides were found in water and at higher concentrations than in sediments. Organophosphorus pesticides, triazines and azoles were detected in the four campaigns (2010/2013). The presence of some banned pesticides in the EU can be justified by their capacity to form deposits. Higher levels appear together with worse water quality parameters related to anthropic pressures and salinization. The spatial distribution, consistent for the four years of the study, showed the highest concentrations in the mouth of the river and corroborates significant loads of pesticides to the sea. The final area of both rivers is densely populated in comparison with the other characteristic landscapes. However, some highly polluted points are observed headwaters, even though contamination appears only punctually in one sampling.

After a large-scale monitoring of pesticides for four years in 44 different sampling points an important relation appears to exist between the flows of both rivers and the pollutants' concentrations that they present. A high–medium flow seems to be improving co-occurrence and frequency of pesticides and a low–medium flow seems to be improving concentrations.

The statistical tests pinpointed a possible relation between physico-chemical parameters of the water and pesticide concentrations. However, the three statistical tests did not provide enough statistical significance to establish clearly the influence of environmental or spatial factors in the behavior of the insecticides in both rivers.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.06.063.

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SUPLEMENTARY MATERIAL

Spatio-temporal patterns of pesticide residues in the Turia and Júcar Rivers (Spain)

Alexander Ccanccapa[1*]. Ana Masia[1]. Vicente Andreu[2]. Yolanda Picó[1]

[1]Food and Environmental Safety Research Group (SAMA-UV), Centro de Investigaciones sobre Desertificación (CIDE, UV-GV-CSIC) y Facultat de Farmàcia. Universitat de València. Av. Vicent Andrés Estellés s/n. 46100 Burjassot. Valencia. Spain.

[2] Centro de Investigaciones Sobre Desertificación-CIDE (CSIC-UV-GV). Landscape Chemistry and Environmental Forensics Group. Carretera de Moncada-Náquera km 4.5. 46113 Moncada. Spain

[*] Corresponding autor: Alexander Ccanccapa
Tel: +34 963543092; Fax: +34 963544954
E-mail: Alexander.Ccanccapa@uv.es

Table S-1: Physico-chemical properties

	CAS NUMBER	MOLECULAR FORMULA	PM (g/mol)	log Kow (pH 7. 20 ºC)	Field Half Life (Days) (a.b.c.d)	Water Solubility (mg/L) (a.b.c.d)	BIOCIDE ACTION	CHEMICAL FAMILY	STATUS
3-Hydroxycarbofuran	16655-82-6	$C_{12}H_{15}NO_4$	237.25	1.45 (low)			Metabolite	Carbamates	
Acetochlor	34256-82-1	$C_{14}H_{20}ClNO_2$	269.77	4.14 (high)	14	282	Herbicide	Chloroacetanilide	Not approved Reg. (EU) No 1372/2011 (2008/934)
Alachlor	15972-60-8	$C_{14}H_{20}ClNO_2$	269.77	3.09 (high)			Herbicide	Chloroacetanilide	Not approved 06/966/EC
Atrazine	1912-24-9	$C_8H_{14}ClN_5$	215.68	2.7 (moderate)	60	33	Herbicide	Triazine	Not Approved 2004/248/EC
Azinphos-ethyl	2642-71-9	$C_{12}H_{16}N_3O_3PS_2$	345.38	3.18 (high)			Insecticide. Acaricide	Organophosphorus	
Azinphos-methyl	86-50-0	$C_{10}H_{12}N_3O_3PS_2$	317.32	2.96 (moderate)	10	29	Insecticide. Acaricide	Organophosphorus	Not approved Reg 1335/2005

	CAS NUMBER	MOLECULAR FORMULA	PM (g/mol)	log Kow (pH 7. 20 ºC)	Field Half Life (Days) (a.b.c.d)	Water Solubility (mg/L) (a.b.c.d)	BIOCIDE ACTION	CHEMICAL FAMILY	STATUS
Buprofezin	69327-76-0	$C_{16}H_{23}N_3OS$	305.44	4.93 (high)			Insecticide. Acaricide	Other pesticides	Approved (2008/771/EC)
Carbendazim	10605-21-7	$C_9H_9N_3O_2$	191.21	1.48 (low)	30 – 150	29	Fungicide	Benzimidazole	Not approved (2006/135/EC .2010/70/EC. 2011/58/EU)
Carbofuran	1563-66-2	$C_{12}H_{15}NO_3$	221.26	1.8 (low)	30 - 117	320	Insecticide. Acaricide. Nematicide	Carbamates	Not approved (2007/416)
Chlofenvinphos	470-90-6	$C_{12}H_{14}Cl_3O_4P$	359.6	3.80 (high)	10 – 45	145	Insecticide. Acaricide	Organophosphorus	Not Approved 2002/2076
Chlorpyrifos	5598-13-0	$C_7H_7Cl_3NO_3PS$	322.53	4.00 (high)	50	1.05	Insecticide. Acaricide	Organophosphorus	Approved EC 1107/2009
Deisopropylatrazine	1007-28-9	$C_5H_8ClN_5$	173.6	1.15 (low)			Metabolite	Triazine	
Deethylatrazine	6190-65-4	$C_6H_{10}ClN_5$	187.63	1.51 (low)			Metabolite	Triazine	

	CAS NUMBER	MOLECULAR FORMULA	PM (g/mol)	log Kow (pH 7. 20 ºC)	Field Half Life (Days) (a.b.c.d)	Water Solubility (mg/L) (a.b.c.d)	BIOCIDE ACTION	CHEMICAL FAMILY	STATUS
Diazinon	333-41-5	$C_{12}H_{21}N_2O_3PS$	304.35	3.69 (high)	10 to 35	40	Insecticide. Acaricide. Repelent	Organophosphorus	Not approved 2007/393
Diclofenthion	97-17-6	$C_{10}H_{13}Cl_2O_3PS$	315.15	5.14 (high)			Insecticide	Organophosphorus	
Dimethoate	60-51-5	$C_5H_{12}NO_3PS_2$	229.26	0.70 (low)	4 – 122	25	Insecticide. Acaricide	Organophosphorus	Approved 07/25/ECReg. (EU) No 540/2011
Diuron	330-54-1	$C_9H_{10}Cl_2N_2O$	233.09	2.87 (moderate)	68	22 to 42	Herbicide	Urea	Approved 08/91/ECReg. (EU) No 540/2011
Ethion	563-12-2	$C_9H_{22}O_4P_2S_4$	384.48	5.07 (high)	56	2	Insecticide. Acaricide	Organophosphorus	Not approved 2002/2076
Fenitrothion	122-14-5	$C_9H_{12}NO_5PS$	277.23	3.32 (high)	4 – 54	21	Insecticide	Organophosphorus	Not approved 2007/379
Fenoxon	3254-63-5	$C_{10}H_{15}O_4PS$	262.26				Insecticide	Organoph	

	CAS NUMBER	MOLECULAR FORMULA	PM (g/mol)	log Kow (pH 7. 20 ºC)	Field Half Life (Days) (a.b.c.d)	Water Solubility (mg/L) (a.b.c.d)	BIOCIDE ACTION	CHEMICAL FAMILY	STATUS
								osphorus	
Fenoxon sulfone	14086-35-2	$C_{10}H_{15}O_6PS$	294.26		16.5		Insecticide (Metabolite)	Organophosphorus	
Fenoxon sulfoxide	6552-13-2	$C_{10}H_{15}O_5PS$	278.26				Insecticide (Metabolite)	Organophosphorus	
Fenthion	55-38-9	$C_{10}H_{15}O_3PS_2$	278.33	4.84 (high)	34	2	Insecticide	Organophosphorus	Not approved 04/140/EC
Fenthion sulfone	3761-42-0	$C_{10}H_{15}O_5PS_2$	310.1	2.25 (low)			Metabolite	Organophosphorus	
Fenthion sulfoxide	3761-41-9	$C_{10}H_{15}O_4PS_2$	294.1	1.92 (low)			Metabolite	Organophosphorus	
Hexythiazox	78587-05-0	$C_{17}H_{21}ClN_2O_2S$	352.88	2.67 (low)	24.6	0.5	Acaricide	Acaricide	Approved 2011/46/EUReg. (EU) No 540/2011

	CAS NUMBER	MOLECULAR FORMULA	PM (g/mol)	log Kow (pH 7. 20 ºC)	Field Half Life (Days) (a.b.c.d)	Water Solubility (mg/L) (a.b.c.d)	BIOCIDE ACTION	CHEMICAL FAMILY	STATUS
Imazalil	35554-44-0	$C_{14}H_{14}Cl_2N_2O$	297.18	2.56 (low)	120 to 190	180	Fungicide	Azol	Approved (1997/73/EC. 2007/21/EC.2010/57/EU.Reg. (EU) No 540/2011)
Imidacloprid	138261-41-3	$C_9H_{10}ClN_5O_2$	255.66	0.57 (low)	40	610	Insecticide	Neonicotinoid	Approved(2008/116/EC.2010/21/EU)
Isoproturon	34123-59-6	$C_{12}H_{18}N_2O$	206.3	2.50(low)	30	65	Herbicide	Urea	Approved 02/18/ECReg. (EU) No 540/2011
Malathion	121-75-5	$C_{10}H_{19}O_6PS_2$	330.36	2.75 (moderate)	4 – 6	145	Insecticide. Acaricide	Organophosphorus	Approved 2010/17/EUReg. (EU) No 540/2011

	CAS NUMBER	MOLECULAR FORMULA	PM (g/mol)	log Kow (pH 7. 20 ºC)	Field Half Life (Days) (a.b.c.d)	Water Solubility (mg/L) (a.b.c.d)	BIOCIDE ACTION	CHEMICAL FAMILY	STATUS
Methiocarb	2032-65-7	$C_{11}H_{15}NO_2S$	225.31	3.18 (high)	4 – 64	27	Insecticide	Carbamates	Approved 07/5/ECReg. (EU) No 187/2014Reg. (EU) No 540/2011
Metolachlor	51218-45-2	$C_{15}H_{22}ClNO_2$	283.8	3.40 (high)	39	530	Herbicide	Chloroacetanilide	Not approved 2002/2076
Molinate	2212-67-1	$C_9H_{17}NOS$	187.3	2.86 (moderate)	21	970	Herbicide	Carbamates	Approved 03/81/ECReg. (EU) No 540/2011
Omethoate	1113-02-6	$C_5H_{12}NO_4PS$	213.2	(-) 0.74 (low)			Insecticide. Acaricide	Organophosphorus	Not approved 2002/2076
Parathion-ethyl	56-38-2	$C_{10}H_{14}NO_5PS$	291.26	3.83 (high)		50 - 60	Insecticide. Acaricide	Organophosphorus	
Parathion-methyl	298-00-0	$C_8H_{10}NO_5PS$	263.21	3.00 (moderate)	10 – 60	50 - 60	Insecticide	Organophosphorus	Not approved 03/166/EC

	CAS NUMBER	MOLECULAR FORMULA	PM (g/mol)	log Kow (pH 7. 20 ºC)	Field Half Life (Days) (a.b.c.d)	Water Solubility (mg/L) (a.b.c.d)	BIOCIDE ACTION	CHEMICAL FAMILY	STATUS
Prochloraz	67747-09-5	$C_{15}H_{16}Cl_3N_3O_2$	376.7	3.5 (high)	60	27	Fungicide	Azol	Approved Reg. (EU) No 1143/2011 (2008/934)
Propanil	709-98-8	$C_9H_9Cl_2NO$	218.08	2.29 (low)	3	225	Herbicide	Anilide	Not approved Reg. (EU) No 1078/2011 (2008/769)
Propazine	139-40-2	$C_9H_{16}ClN_5$	229.71	3.95 (high)	35 to 231	5	Herbicide	Triazine	Not approved 2002/2076
Pyriproxyphen	95737-68-1	$C_{20}H_{19}NO_3$	321.37	5.37 (high)	3 to 16	0.36	Insecticide	Juvenile Hormone Mimics	
Simazine	122-34-9	$C_7H_{12}ClN_5$	201.66	2.30 (low)	57	5	Herbicide	Triazine	Not approved 04/247/EC

	CAS NUMBER	MOLECULAR FORMULA	PM (g/mol)	log Kow (pH 7. 20 ºC)	Field Half Life (Days) (a.b.c.d)	Water Solubility (mg/L) (a.b.c.d)	BIOCIDE ACTION	CHEMICAL FAMILY	STATUS
Tebuconazole	107534-96-3	$C_{16}H_{22}ClN_3O$	307.82	3.70 (high)	62	36	Fungicide	Triazole	Approved 2008/125Reg. (EU) No 540/2011Reg. (EU) No 921/2014
Terbumeton	33693-04-8	$C_{10}H_{19}N_5O$	225.29	3.04 (high)			Herbicide	Triazine	Not approved 2002/2076
Terbumeton-deethyl	30125-64-5	$C_8H_{15}N_5O$	197.24	-			Herbicide	Triazine	
Terbuthylazine	5915-41-3	$C_9H_{16}ClN_5$	229.71	3.40 (high)	18		Herbicide. Microbiocide. Algicide	Triazine	Approved (2008/934/EC)
Terbuthylazine-2-hydroxy	66753-07-9	$C_9H_{17}N_5O$	211.33				Metabolite	Triazine	
Terbutryn	886-50-0			3.34	14 to 28	25		Triazine	Not approved 2002/2076

	CAS NUMBER	MOLECULAR FORMULA	PM (g/mol)	log Kow (pH 7. 20 ºC)	Field Half Life (Days) (a.b.c.d)	Water Solubility (mg/L) (a.b.c.d)	BIOCIDE ACTION	CHEMICAL FAMILY	STATUS
Terbuthylazine-deethyl	30125-63-4	$C_7H_{12}ClN_5$	201.68	2.30 (low)			Metabolite	Triazine	
Tolclophos-methyl	57018-04-9	$C_9H_{11}Cl_2O_3PS$	301.13	4.56 (high)			Fungicide	Organophosphorus	01/21/ECReg. (EU) No 540/2011

(a) (EPA, 2012) (b) (Blanchoud et al., 2007) (c) (Lazartigues et al., 2011) (d) (Huber et al., 2000)

Table S-2: Sampling points in the Júcar and Turia rivers

RIVER OR TRIBUTARY	ABREV	LOCATION	COORDINATES UTM-ETRS-89		
	Júcar river		ZONE	X	Y
Júcar	JUC1	Huélamo	30	598583	4453975
Júcar	JUC2	Cuenca	30	573092	4436231
Júcar	JUC3	Fresneda de Altarejos	30	570913	4414489
Júcar	JUC4	Quasiermas	30	601713	4336028
Júcar	JUC5	Jalance	30	665927	4340496
Cabriel	CAB1	Salvacañete	30	627162	4439354
Cabriel	CAB2	Pajaroncillo	30	612534	4422400
Cabriel	CAB3	Villar del Humo	30	614272	4410987
Cabriel	CAB4	Venta del Moro	30	628595	4376586
Cabriel	CAB5	Villatoya	30	642129	4355758
Júcar	JUC6	Cotes	30	707741	4328283
Júcar	JUC7	Alzira	30	720584	4336933
Magro	MAG1	Requena	30	667953	4362542
Magro	MAG2	Carlet	30	711245	4348964
Júcar	JUC8	Sueca	30	729360	4343192
	Turia river				
Guadalaviar	GUA1	Tramacastilla-Noguera de A.	30	619076	4480720
Guadalaviar	GUA2	Tramacastilla	30	622260	4476432
Guadalaviar	GUA3	Tramacastilla	30	620888	4475214
Guadalaviar	GUA4	Tramacastilla-Torres de A	30	626379	4474966
Guadalaviar	GUA5	Gea de Albarracín	30	639198	4475192
Guadalaviar	GUA6	Gea de Albarracín	30	642259	4474336
Guadalaviar	GUA7	Vega de Teruel-Arquillo de San Blas	30	653932	4469355

Guadalaviar	GUA8	Vega de Teruel-Teruel	30	660519	4467637
Alfambra	ALF1	Villalba Alta-Alfambra	30	673254	4500252
Alfambra	ALF2	Villalba Alta-Alfambra	30	671693	4498425
Alfambra	ALF3	Villalba Alta-Alfambra	30	667609	4488546
Alfambra	ALF4	Vega de Teruel-Teruel	30	661934	4471408
Alfambra	ALF5	Vega de Teruel-Teruel	30	659555	4468805
Alfambra	ALF6	Vega de Teruel-Teruel	30	659792	4464765
Alfambra	ALF7	Vega de Teruel-Teruel	30	659152	4463449
Turia	TUR1	Ademuz	30	647048	4435825
Turia	TUR2	Ademuz	30	646380	4436383
Embalse Benagéber	TUR3	Embalse Benagéber	30	661930	4403832
Embalse Benagéber	TUR4	Embalse Benagéber	30	661732	4400434
Turia	TUR5	Calles	30	682987	4388327
Turia	TUR6	Calles	30	674513	4398960
Turia	TUR7	Chulilla	30	679408	4392645
Turia	TUR8	Chulilla	30	682987	4388327
Turia	TUR9	Bugarra	30	690694	4386474
Turia	TUR10	Bugarra	30	692418	4386992
Turia	TUR11	Ribarroja-Huerta Valencia	30	711436	4380129
Turia	TUR12	Parque Turia-Huerta Valencia	30	713695	4378176
Turia	TUR13	La Presa-Huerta Valencia	30	717356	4376399
Turia	TUR14	Massarojos-Huerta Valencia	30	723241	4379704
Turia	TUR15	Alfara Patriarca-Huerta Valencia	30	724835	4380286
Turia	TUR16	Quart Benáger-Huerta Valencia	30	722748	4370462
Turia	TUR18	El Brosquil-Huerta Valencia	30	728692	4367111
Turia	TUR19	Port Catarroja-Huerta Valencia	30	726311	4363694

S.1. Liquid chromatography-mass spectrometry (LC-MS/MS) determination

The analytical column was a Luna 18 (150 mm x 2.0 mm. 3 µm) from fenomenex (Paris. France). The mobile phase (A) was10 mM of formic acid in methanol and the mobile phase (B) was10 mM of formic acid in water. The initial conditions were 50% B. increased to 83% B in 10 min. and then. increased to 98% B in 2.5 min and maintained for 3 min. The stabilization time was 12 min. therefore the total run time was 27.5 min. The temperature of the column was 30 °C. flow-rate. 0.4 ml/min and injection volume. 5 µl. The source parameters were ionization voltage of 4000 V; nebulizer gas15 psi; and source temperature 300◦C.

The ionization and fragmentation of the study compounds was optimized by injecting the solutions of each analyte without column using the Optimizer program. Optimum fragmentor voltages were between 10 and 150 V. and collision energy between 10 and100 V (Table S-3 Supplementary material).

Table S-3. Dynamic MRM conditions used for LC-MS/MS determination of pesticide residues

Target Pesticide	t_R [(a)] (min)	Δt_R [(b)]	Precursor Ion	SRM_1 [c]	Frag[(d)] (V)	CE[(e)] (V)	SRM_2 [(f)]	Frag[(d)] (V)	CE[(e)] (V)	SRM_2 /SRM_1 (%)(%RSD)[(g)]
Acetochlor	13.1	3	270	224	120	10	148	120	10	32.2 (31)
Alachlor	13.09	3	270	238	80	10	162	80	15	85.7 (79)
Atrazine	9.06	2.5	216	174	120	15	132	120	20	16.6 (3)
Atrazine-desethyl	3.82	2.2	188	146	120	15	104	121	24	29.8 (1)
Atrazine-desisopropyl	2.62	1.5	174	132	120	15	96	120	15	117.9 (13)
Azinphos-ethyl	12.9	2	346	137	80	20	97	80	32	80.7 (5)
Azinphos-methyl	10.03	2	318	132	80	8	125	80	12	57.3 (24)
Buprofezin	16.83	1.8	306	201	120	10	116	120	15	61.3 (4)
Carbendazim	3.91	3.5	192	160	95	17	132	95	25	10.3 (2)
Carbofuran	6.53	2	222	165	120	10	123	120	15	61.3 (4)
Carbofuran-3-hydroxy	2.75	2	255	220	70	5	163	70	15	80 (11)
Chlorfenvinphos	14.53	1.8	359	155	120	10	127	120	15	82.4 (28)
Chlorpyrifos	17.02	2	350	198	92	13	97	92	33	88.5 (0)
Diazinon	14.57	1.5	305	169	128	21	153	128	17	86.9 (74)
Dichlofenthion	17.02	1.5	315	287	120	5	259	120	10	46.7 (8)
Dimethoate	3.06	2.1	230	199	80	5	171	80	10	37.5 (12)
Diuron	9.82	2.5	233	160	120	20	72	120	20	4.0 (2)
Ethion	17.01	2	385	199	80	5	171	80	15	38.5 (3)
Fenitrothion	12.45	1.5	278	125	140	15	109	121	12	61.6 (55)

Target Pesticide	t_R [(a)] (min)	Δt_R [(b)]	Precursor Ion	SRM_1 [c]	Frag[(d)] (V)	CE[(e)] (V)	SRM_2 [(f)]	Frag[(d)] (V)	CE[(e)] (V)	SRM_2/SRM_1 (%)(%RSD)[(g)]
Fenoxon-Sulfone	7.13	2.5	295	280	136	13	109	136	33	71.6 (23)
Fenoxon-Sulfoxide	14.33	2	279	247	114	5	169	114	13	70.7 (27)
Fenthion	14.33	2	279	247	114	5	169	114	13	70.7 (27)
Fenthion Oxon	16.51	2	263	231	128	9	216	128	21	34.5 (6)
Fenthion-Sulfone	7.89	2	311	125	146	17	109	146	21	59.4 (2)
Fenthion-Sulfoxide	7.13	3	295	280	136	13	109	136	33	71.6 (23)
Hexythiazox	17.24	1.8	353	228	120	10	168	120	20	60.7 (4)
Imazalil	14.31	2	297	201	120	15	159	120	20	57.2 (3)
Imidacloprid	2.37	1.8	256	209	80	10	175	80	10	60.2 (19)
Isoproturon	9.45	2.5	207	165	120	10	72	120	20	16.7 (1)
Malathion	12.08	2	331	127	80	5	99	80	10	78.7 (37)
Methiocarb	11.45	2	226	169	80	5	121	80	10	75.4 (9)
Methoalachlor	13.01	2	284	252	120	10	176	120	15	10.2 (1)
Molinate	11.89	1.02	188	126	80	10	55	80	20	56.0 (9)
Omethoate	1.68	1.5	214	183	80	5	125	80	20	75.6 (3)
Parathion-ethyl	13.93	1.5	292	264	88	4	236	88	8	40.9 (5)
Parathion-methyl	10.77	1.5	264	232	110	5	125	120	20	14.30
Prochloraz	14.95	2	376	308	80	10	266	80	10	21.1 (12)
Propanil	11.48	2	218	162	120	15	127	120	20	64.6 (40)
Propazine	11.16	2	230	188	120	15	146	120	20	90.5 (9)

Target Pesticide	t_R [(a)] (min)	Δt_R [(b)]	Precursor Ion	SRM_1 [c]	Frag [(d)] (V)	CE [(e)] (V)	SRM_2 [(f)]	Frag [(d)] (V)	CE [(e)] (V)	SRM_2/SRM_1 (%)(%RSD) [(g)]
Pyriproxifen	17.01	1.5	322	227	120	10	185	120	10	30.1 (4)
Simazine	6.61	2	202	132	120	20	124	120	20	81.8 (15)
Tebuconazole	14.31	2	308	125	95	25	70	95	21	5.1 (1)
Terbumeton	11.46	2	226	170	95	17	114	95	25	13.0 (0)
Terbumeton-desethyl	7.2	2	198	142	90	13	86	90	25	28.5 (2)
Terbuthylazine	11.51	1.5	230	174	95	13	96	95	25	13.3 (6)
Terbuthylazine-2-hidroxy	7.5	3	212	156	95	13	86	95	25	27.1 (1)
Terbuthylazine-deethyl	7.51	2	202	146	95	13	79	95	25	9.7 (4)
Terbutryn	13.22	2	242	186	120	15	71	120	20	4.4 (1)
Thiabendazole	5.3	3	202	175	95	25	131	95	25	34.7 (1)
Tolclofos-methyl	15.03	2	301	269	120	15	125	115	12	112.0 (49)

(a) t_R = retention time; (b) Δt_R = delta retention time. that is the centred retention time window; (c) SRM_1 = selected product ion for quantification; (d) Frag = fragmentor; (e) CE = collision energy; (f) SRM_2 = selected product ion for qualification; (g) (%RSD) = relative standard deviation of the ratio SRM_2/SRM_1. calculated from mean values obtained from the matrix-matched calibration curves

S.2. Quality assurance and quality control

For method validation. parameters such as linearity. sensitivity. recoveries. precision and matrix effects were evaluated in the three studied matrices according to the Guidelines on Method Validation and Quality Control (QC) procedures for pesticide residues (see Table S-5). The limits of detection (MLDs) and quantification (MLQs) of the method. both calculated using spiked matrices. were defined as the minimum amount of analyte whose qualified transition(SRM2) present a signal-to-noise ratio (S/N) ≥ 3 and ≥ 10. respectively. MLDs ranged from 0.01 to 2 ng/L for water and from 0.03 to 1.67 ng/g for sediment. Recovery tests were carried out by spiking a pure water sample at 10 ng/L (low spike) and 100 ng/L (high spike) of each pesticide. For sediment the spiked levels were of 25 ng/L (low spike) and 100 ng/L (high spike) of each pesticide. Five replicates were done in order to evaluate the precision of the method. In water samples. recoveries varied from 48% to 70% and precision was below20% for all pesticide. In sediment samples. recoveries were higher than 40% (see Table

S5-). Pesticide concentrations were validated against a comprehensive set of quality control parameters including: laboratory and field blanks. matrix spikes and triplicate samples. Blank contamination is the most common problem observed in the determination of pesticides at trace levels. Thus. precautions were taken to prevent contamination from personnel. organic solvents. equipment and glassware. Blank assays were performed employing MilliQ water samples. to check for laboratory background levels of the studied compounds. Though the detected amounts of the target compounds were low (below 5 ng/L). it was considered necessary to subtract the quantitative values of the compounds found in the blanks (only ethion and pyriproxyfen). In order to assure the quality of the results. field blanks were processed with the samples. It consisted of deionized water put down in the same conditions than samples during sampling process. For each batch of 10 samples analyzed including the water field blanks. a procedural blank and a spiked recovery sample obtained by spiking at the

low level. were routinely extracted and analyzed under the same conditions as the ordinary samples. Triplicate samples analyzed were within 25% agreement for all pesticides detected above the analytical method detection limit.

Linearity was established preparing increasing concentration calibration curves for each compound. ranging between 0.01 to 50 ng/L in methanol and matrix-matched standard. The calibration curves were linear with correlation coefficients (r^2) higher than 0.99 for all target compounds.

The comparison between the slopes of methanol and matrix-matched standard calibration curves was used to evaluate matrix effects. The results (data not shown) revealed matrix-induced suppression (< 20 %) in river water samples and enhancement of the signal for waste water. sediment and biota (112%. 130% and 140%. respectively). In all cases. matrix effect was eliminated by the use of matrix-matched calibration for quantitation. even though for river water was unnecessary since matrix effect was considered negligible.

Table S-4. Recoveries of the selected pesticides and Relative Standard Deviations (RSD %) at a concentration of 10 ng/L in water and 25 ng/g for sediments; LODs and LOQs obtained for the two matrices tested.

	Water				Sediment			
Target pesticide	Recovery	RSD (%)	LOD (ng/L)	LOQ (ng/L)	Recovery	RSD (%)	LOD (ng/g)	LOQ (ng/g)
Acetochlor	66	4	2.00	6.00	61	12	1.67	5.00
Alachlor	58	11	2.00	6.00	75	13	1.67	5.00
Atrazine	65	17	1.30	4.00	40	10	1.08	3.25
Atrazine-desethyl	56	6	2.00	6.00	40	14	1.67	5.00
Atrazine-desisopropyl	54	3	2.00	6.00	99	12	1.67	5.00
Azinphos-ethyl	58	17	0.50	1.50	98	12	0.42	1.25
Azinphos-methyl	51	7	0.50	1.50	98	14	0.42	1.25
Buprofezin	52	19	0.50	1.50	62	11	0.42	1.25
Carbendazim	65	16	0.01	0.04	40	10	0.03	0.10
Carbofuran	58	13	0.20	0.60	77	11	0.17	0.50
Carbofuran-3-hydroxy	67	5	0.20	0.60	42	13	0.17	0.50
Chlorfenvinphos	61	18	0.20	0.60	42	20	0.17	0.50
Chlorpyrifos	52	4	0.20	0.60	44	11	0.17	0.50
Diazinon	49	6	0.04	0.20	60	15	0.03	0.10
Dichlofenthion	65	15	0.50	1.50	62	12	0.42	1.25
Dimethoate	57	4	1.00	3.00	64	11	0.83	2.50
Diuron	49	11	1.00	5.00	43	11	0.83	2.50
Ethion	54	4	0.50	1.50	42	14	0.42	1.25
Fenitrothion	67	12	2.00	6.00	78	9	1.67	5.00
Fenoxon	78	7	0.40	2.00	78	13	0.34	1.00
Fenoxon-Sulfone	57	12	0.20	1.00	105	10	0.17	0.50
Fenoxon-Sulfoxide	53	4	0.20	1.00	91	14	0.17	0.50
Fenthion	51	10	0.20	1.00	62	13	0.17	0.50

Fenthion-Sulfone	58	3	0.20	1.00	63	12	0.17	0.50
Fenthion-Sulfoxide	61	11	0.20	1.00	44	12	0.17	0.50
Hexythiazox	70	6	0.20	1.00	78	12	0.17	0.50
Imazalil	65	20	0.30	1.00	64	13	0.25	0.75
Imidacloprid	60	9	0.04	0.20	64	12	0.03	0.10
Isoproturon	56	7	0.30	1.00	64	10	0.25	0.75
Malathion	51	9	0.30	1.00	43	13	0.25	0.75
Methiocarb	66	5	0.30	1.00	41	11	0.25	0.75
Methoalachlor	52	8	0.30	1.00	61	12	0.25	0.75
Molinate	61	17	0.50	1.50	63	14	0.42	1.25
Omethoate	54	6	0.30	1.00	92	10	0.25	0.75
Parathion-ethyl	61	7	2.00	6.00	42	11	1.67	5.00
Parathion-methyl	68	17	2.00	6.00	40	11	1.67	5.00
Prochloraz	53	14	0.80	6.00	78	11	0.67	2.00
Propanil	57	3	0.30	1.00	44	12	0.25	0.75
Propazine	62	14	0.30	1.00	63	14	0.25	0.75
Pyriproxifen	67	14	0.50	1.50	43	15	0.42	1.25
Simazine	58	8	2.00	6.00	63	13	1.67	5.00
Tebuconazole	49	11	0.13	0.40	43	12	0.33	1.00
Terbumeton	70	7	0.01	0.04	78	12	0.03	0.10
Terbumeton-desethyl	66	4	0.13	0.40	61	12	0.33	1.00
Terbuthylazine	67	13	0.01	0.04	78	9	0.03	0.10
Terbuthylazine-2-hidroxy	65	17	0.01	0.04	40	9	0.03	0.10
Terbuthylazine-deethyl	52	5	0.01	0.04	44	10	0.03	0.10
Terbutryn	65	12	0.13	0.40	80	10	0.33	1.00
Thiabendazole	54	4	0.13	0.40	42	14	0.33	1.00
Tolclofos-methyl	66	12	0.50	1.50	41	12	0.42	1.25

Table S-5: Physico-chemical parameters of the rivers water

	Júcar river						Turia river					
	2010			2011			2012			2013		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Temp. (ºC)	9.00	19.00	14.35	8.70	20.10	15.69	10.50	20.20	14.55	12.80	30.00	19.46
pH	7.5	8.1	7.9	7.4	8.1	7.9	7.9	8.8	8.3	7.5	9.1	8.6
mV	-72.70	-37.00	-61.45	-72.80	-29.50	-58.02	-82.90	-47.80	-65.55	-117.00	-29.60	-88.20
Cond (dS/m)	0.00	0.07	0.01	0.01	0.02	0.01	0.68	1.38	0.99	0.17	1.68	0.92
TDS (ppm)	321.70	865.10	544.43	0.00	994.00	545.21	366.30	1045.00	667.79	124.80	2291.00	850.44
NaCl (ppm)	0.00	914.70	456.09	0.00	968.00	577.02	710.10	1520.00	1078.09	--	--	--
Res (Ω)	571.40	1532.00	990.48	419.60	1752.00	935.51	480.20	1365.00	847.50	218.20	3973.00	825.14
DO (mg/L)	7.12	9.81	8.47	2.91	26.01	9.66	6.28	10.22	8.60	5.14	12.78	9.70

Table S-6: Maximum (Max) and mean concentrations and frequency (Freq) of detection of pesticides in water.

Class/Pesticide	2010 Concentration ng/L			2011 Concentration ng/L			2012 Concentration ng/L			2013 Concentration ng/L		
	Mean	Max	Freq (%)[a]	Mean	Max	Freq (%)[a]	Mean	Max	Freq (%)[a]	Mean	Max	Freq (%)[a]
ANILIDE												
PROPANIL	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	2.33	45.94	3 (10)
AZOL												
IMAZALIL	172.19	682.72	14 (93)	24.09	222.45	10 (66)	43.35	750.29	10 (45)	8.03	38.94	21 (72)
PROCHLORAZ	65.37	83.62	15 (100)	1.00	14.96	1	10.76	33.26	9 (41)	18.30	486.21	10 (34)
BENZIMIDAZOLE												
CARBENDAZIM	n.a	n.a	n.a	12.66	79.39	8 (53)	23.37	382.12	17 (77)	8.83	101.68	25 (86)
THIABENDAZOLE	n.a	n.a	n.a	23.45	187.36	3 (20)	25.30	107.62	21 (95)	10.33	109.94	27 (93)
CARBAMATES												
3-HYDROXYCARBOFURAN	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.09	1 (3)
CARBOFURAN	n.d	n.d	n.d	n.d	n.d	n.d	1.15	25.23	1 (5)	282.71	6844.50	10 (34)
METHIOCARB	0.19	2.79	1(6)	n.d	n.d	n.d	n.d	n.d	n.d	0.18	5.14	2 (6)
CHLOROACETANILIDE												
ACETOCHLOR	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	3.50	71.15	2 (6)
ALACHLOR	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.61	17.73	1 (3)
METOLACHLOR	29.80	446.99	1 (6)	n.d	n.d	n.d	1.28	15.59	2 (9)	12.45	58.39	23 (79)
JUVENILE HORMONE MIMICS												
PYRIPROXYFEN	79.83	99.59	15 (100)	n.d	n.d	n.d	n.d	n.d	n.d	0.45	3.27	15 (51)
NEONICOTINOID												
IMIDACLOPRID	3.53	51.84	2 (13)	2.19	14.31	3 (20)	23.12	206.96	18 (82)	20.93	95.00	21 (72)
ORGANOPHOSPHORUS												
AZINPHOS ETHYL	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.02	0.48	2 (6)

Class/Pesticide	2010 Concentration ng/L			2011 Concentration ng/L			2012 Concentration ng/L			2013 Concentration ng/L		
	Mean	Max	Freq (%)[a]	Mean	Max	Freq (%)[a]	Mean	Max	Freq (%)[a]	Mean	Max	Freq (%)[a]
AZINPHOS METHYL	n.d	n.d	n.d	n.d	n.d	n.d	6.73	148.07	1 (5)	0.07	2.15	1 (3)
CHLORFENVINPHOS	62.97	106.85	11 (73)	1.64	7.82	10 (66)	8.23	46.84	8 (36)	2.43	11.60	25 (86)
CHLORPYRIFOS	16.31	41.22	15 (100)	4.28	8.68	15 (100)	17.23	34.45	18 (81)	6.07	30.75	21 (72)
DIAZINON	8.05	12.42	15 (100)	0.72	2.00	9 (60)	12.13	29.40	18 (81)	6.57	37.00	27 (93)
DICHLOFENTHION	38.61	54.81	15 (100)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
DIMETHOATE	1.21	8.74	3 (20)	0.37	5.48	1 (6)	1.24	27.23	1 (5)	6.15	97.39	12 (41)
ETHION	2.96	24.28	5 (33)	2.79	5.30	12 (80)	8.06	83.68	13 (59)	12.67	349.93	29 (100)
FENITROTHION	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.63	17.96	2 (6)
FENOXON	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.01	0.30	1 (3)
FENOXON SULFONE	n.d	n.d	n.d	n.d	n.d	n.d	0.88	10.17	3 (13)	0.09	1.34	3 (10)
FENOXON SULFOXIDE	11.24	50.66	7 (46)	n.d	n.d	n.d	n.d	n.d	n.d	0.05	1.44	1 (3)
FENTHION	n.d	n.d	n.d	n.d	n.d	n.d	0.83	18.27	1 (5)	0.05	1.45	1 (3)
FENTHION SULFONE	n.d	n.d	n.d	n.d	n.d	n.d	3.14	69.09	1 (5)	n.d	n.d	n.d
FENTHION SULFOXIDE	n.d	n.d	n.d	n.d	n.d	n.d	0.83	8.08	3 (13)	0.09	1.34	3 (10)
MALATHION	2.14	12.62	3 (20)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
PARATHION-ETHYL	13.15	41.92	6 (40)	n.d	n.d	n.d	n.d	n.d	n.d	0.43	12.38	1 (3)
TOLCLOFOS METHYL	7.30	36.12	4 (26)	0.49	7.36	1 (6)	23.66	382.61	2 (9)	0.27	7.76	1 (3)
OTHER PESTICIDES												
BUPROFEZIN	11.86	14.07	15 (100)	n.d	n.d	n.d	11.82	24.93	15 (30)	0.01	0.19	1 (3)
HEXYTHIAZOX	17.45	24.34	15 (100)	n.d	n.d	n.d	6.53	15.21	13 (26)	0.54	4.20	27 (93)
THIOCARBAMATES												
MOLINATE	0.61	9.09	1 (6)	n.d	n.d	n.d	0.65	14.29	1 (5)	n.d	n.d	n.d
TRIAZINE												
ATRAZINE	2.71	19.80	3 (20)	n.d	n.d	n.d	0.34	7.49	1 (5)	0.69	7.33	15 (51)
DEISOPROPYLATRAZINE	3.42	26.37	2 (13)	n.d	n.d	n.d	0.11	2.48	1 (5)	0.30	5.44	1 (3)

Class/Pesticide	2010 Concentration ng/L			2011 Concentration ng/L			2012 Concentration ng/L			2013 Concentration ng/L		
	Mean	Max	Freq (%)[a]	Mean	Max	Freq (%)[a]	Mean	Max	Freq (%)[a]	Mean	Max	Freq (%)[a]
DEETHYLATRAZINE	5.36	29.14	5 (33)	0.80	11.97	1 (6)	7.55	15.81	17 (77)	0.15	4.36	5 (17)
PROPAZINE	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.05	0.61	3 (9)
SIMAZINE	n.d	n.d	n.d	n.d	n.d	n.d	3.57	55.57	2 (9)	1.32	14.51	4 (13)
TERBUMETON	n.a	n.a	n.a	1.38	10.89	2 (13)	0.76	16.74	1 (5)	0.80	7.63	20 (68)
TERBUMETON-DESETHYL	n.a	n.a	n.a	7.45	52.01	4 (26)	2.56	27.05	6 (27)	1.71	9.93	19 (65)
TERBUTILAZINE	n.a	n.a	n.a	4.45	16.40	5 (33)	17.14	57.33	16 (72)	2.91	20.26	25 (86)
TERBUTYLAZINE DEETHYL	n.a	n.a	n.a	4.65	31.10	6 (40)	10.32	59.45	6 (27)	6.15	29.54	22 (75)
TERBUTILAZINE-2 HIDROXY	n.a	n.a	n.a	2.62	14.24	8 (53)	8.59	31.30	14 (63)	2.34	10.69	26 (86)
TERBUTRYN	n.d	n.d	n.d	n.d	n.d	n.d	2.12	19.94	3 (13)	0.66	4.18	19 (65)
TRIAZOLE												
TEBUCONAZOLE	n.d	n.d	n.d	n.d	n.d	n.d	3.27	72.00	1 (5)	2.98	21.06	13 (44)
UREA												
DIURON	n.d	n.d	n.d	1.23	9.37	2 (13)	n.d	n.d	n.d	0.94	8.95	6 (20)
ISOPROTURON	n.d	n.d	n.d	n.d	n.d	n.d	3.02	14.11	5 (22)	1.78	13.07	8 (27)

[a] Number of finding (percentage of positive samples).

n.d = non-detected

n.a = not analyzed

Table S-7: Maximum and mean concentrations and frequency detection of pesticides in sediment.

	2010			2011			2012			2013		
	Concentration ng/L			Concentration ng/L			Concentration ng/L			Concentration ng/L		
	Mean	Max	Freq (%)[a]	Mean	Max	Freq (%)[a]	Mean	Max	Freq (%)[a]	Mean	Max	Freq (%)[a]
Azol												
IMAZALIL	2.33	32.87	2 (13)	2.94	37.28	2 (13)	1.39	25.76	2 (9)	n.d	n.d	n.d
PROCHLORAZ	n.d	n.d	n.d	3.09	13.12	5 (33)	n.d	n.d	n.d	n.d	n.d	n.d
Bencimidazole												
CARBENDAZIM	n.d	n.d	n.d	n.d	n.d	n.d	0.59	4.94	3 (14)	n.d	n.d	n.d
THIABENDAZOLE	n.d	n.d	n.d	2.53	16.52	3 (20)	8.16	70.25	6 (27)	n.d	n.d	n.d
Carbamates												
CARBOFURAN	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d.	1.54	34.09	2 (6)
METHIOCARB	n.d	n.d	n.d	0.52	7.84	1 (7)	n.d	n.d	n.d	n.d	n.d	n.d
Juvenile Hormone Mimics												
PYRIPROXYPHEN	1.41	5.54	14 (93)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Neonicotinoid												
IMIDACLOPRID	n.d	n.d	n.d	n.d	n.d	n.d	0.12	2.60	1 (5)	n.d	n.d	n.d
Organophosphorus												
CHLORFENVINPHOS	0.13	2.00	1 (7)	0.07	1.03	1 (7)	n.d	n.d	n.d	n.d	n.d	n.d
CHLORPYRIFOS	3.28	16.47	15 (100)	3.48	9.90	15 (100)	61.28	141.40	22 (100)	4.51	55.95	11 (37)
DIAZINON	0.38	3.79	2 (13)	1.57	3.48	12 (80)	0.05	1.00	1 (5)	n.d	n.d	n.d
DIMETHOATE	n.d	n.d	n.d	n.d	n.d	n.d	0.28	4.30	2 (9)	n.d	n.d	n.d
ETHION	n.d	n.d	n.d	0.09	1.31	1 (7)	1.85	40.60	1 (5)	n.d	n.d	n.d
FENOXON SULFONE	n.d	n.d	n.d	0.11	1.61	1 (7)	n.d	n.d	n.d	n.d	n.d	n.d
MALATHION	1.52	11.24	5 (33)	0.10	1.57	1 (7)	n.d	n.d	n.d	n.d	n.d	n.d
Other pesticides												

	2010			2011			2012			2013		
	Concentration ng/L			Concentration ng/L			Concentration ng/L			Concentration ng/L		
	Mean	Max	Freq (%)[a]	Mean	Max	Freq (%)[a]	Mean	Max	Freq (%)[a]	Mean	Max	Freq (%)[a]
BUPROFEZIN	1.49	3.31	15 (100)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
HEXYTHIAZOX	1.28	3.63	15 (100)	0.28	2.01	3 (20)	n.d	n.d	n.d	n.d	n.d	n.d
Triazines												
TERBUMETON-DEETHYL	n.a	n.a	n.a	0.38	3.38	2 (13)	0.06	1.38	1 (5)	0.76	16.28	2 (6)
TERBUTHYLAZINE DEETHYL	n.a	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	1.97	44.22	2 (6)
TERBUTHYLAZINE-2 HYDROXY	n.a	n.a	n.a	n.d	n.d	n.d	4.80	105.50	1 (5)	n.d	n.d	n.d
Ureas												
ISOPROTURON	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.11	2.35	2 (6)

[a] Number of finding (percentage of positive samples).
n.d = non-detected
n.a = not analyzed

Table S-8: Summary of the main hydrological factors influencing the distribution of pesticides in the study sites

Sampling sites	Date	River flow (m3/s)[a]	Percentile[b]	% Variation	River flow prev. month (m^3/s)	Mean river flow (m^3/s)[c]	Elevation (m asl)	Drainage basin (km^2)	Reservoir upstream of sitew	Storage volume (hm^3)	Residence time (days)
JUCAR RIVER-1st CAMPAIGN											
JUC1	15/10/2010	1.02	-	-	1.07	2.53	-	-	-	-	-
JUC2	15/10/2010	3.66	53%	-	3.89	6.98	916	1839	La Toba	4.12	34.06
JUC3	15/10/2010	6.5	-	-	5.96	10.43	-	-	-	-	-
JUC4	18/10/2010	2.87	32%	-	3.28	6.11	692	21579	Alarcón	566.48	2815
JUC5	19/10/2010	2.84	34%	-	2.76	4.42	520	21579	El Molinar	0.5	1.63
CAB2	18/10/2010	3.01	61%	-	21.7	4.11	-	-	-	-	-
CAB3	14/10/2010	0.31	-	-	0.29	0.29	-	-	-	-	-
CAB4	14/10/2010	7.73	73%	-	4.05	5.03	560	4754	Contreras	420.8	649
JUC6	19/10/2010	2.46	57%	-	14.6	12.19	54	21579	Tous	77.85	114
MAG1	20/10/2010	0.12	-	-	0.13	0.37	-	-	-	-	-
MAG2	20/10/2010	0.17	-	-	0.16	0.06	290	1544	Forata	17.19	1898
JUC8	20/10/2010	4.4	32%	-	-	-	-	-	-	-	-
JUCAR RIVER- 2nd CAMPAIGN											
JUC1	05/10/2011	0.73	-	-	0.92	2.58			-	-	-
JUC2	04/10/2011	2.85	45%	8%	2.3	6.43	916	1839	La Toba	3.84	74
JUC3	04/10/2011	5.01	-		4.68	8.96					
JUC4	06/10/2011	3	36%	-4%	3.66	5.47	692	21579	Alarcón	672.02	2592
JUC5	24/10/2011	3.01	39%	-5%	4.43	4.1	520	21579	El Molinar	0.52	1.23
CAB2	05/10/2011	2.36	53%	8%	2.36	4.02	-	-	-	-	-

Sampling sites	Date	River flow (m3/s)[a]	Percentile[b]	% Variation	River flow prev. month (m^3/s)	Mean river flow (m^3/s)[c]	Elevation (m asl)	Drainage basin (km^2)	Reservoir upstream of sitew	Storage volume (hm^3)	Residence time (days)
CAB3	05/10/2011	0.12	-		2.36	0.31	-	-	-	-	-
CAB4	04/10/2011	0.66	46%	27%	1.41	5.17	560	4754	Contreras	392.94	9095
JUC6	07/10/2011	1.3	30%	27%	12.6	12.04	54	21579	Tous	77.32	79
MAG1	07/10/2011	0.05	-	-	0.03	0.32			-	-	-
MAG2	07/10/2011	0.05	-	-	0.04	0.07	290	1544	Forata	13.25	613
JUC8	11/10/2011	4.52	36%	-4%	-	-	-	-	-	-	-
TURIA RIVER-1st CAMPAIGN											
GUA3	15/10/2012	0.13	10.7%	-	0.62	1.56	-	-	-	-	-
GUA6	16/10/2012	0.39	-	-	0.27	1.61	-	-	-	-	-
GUA7	16/10/2012	0.09	7.6%	-	0.09	0.92	-	-	-	-	-
GUA8	16/10/2012	0.59	0.8%	-	0.13	0.6	-	-	-	-	-
ALF2	16/10/2012	0.03	4.7%	-	0.03	0.51	-	-	-	-	-
ALF4	17/10/2012	-	-	-	-	-	851	6394	Arquillo de San Blas	7.59	82
ALF5	17/10/2012	0.12	0.0%	-	0.72	2.25	-	-	-	-	-
TUR1	20/10/2012	2.45	-	-	1.13	2.97	-	-	-	-	-
TUR5	22/10/2012	6.35	-	-	3.79	5.72	540	6394	Benagéber	73.36	140
TUR7	22/10/2012	0.53	14.3%	-	2.7	3.94	-	-	-	-	-
TUR8	22/10/2012	-		-	-	-	252	6394	Loriguilla	20.27	47
TUR9	22/10/2012	2.77	43.0%	-	4.26	6.34	-	-	-	-	-
TUR12	18/10/2012	4.33	21.6%	-	4.34	6.12	-	-	-	-	-
TURIA RIVER-2nd CAMPAIGN											
GUA3	05/10/2013	0.26	28.2%	-17.5%	0.25	1.56	-	-	-	-	-

Sampling sites	Date	River flow (m3/s)[a]	Percentile[b]	% Variation	River flow prev. month (m^3/s)	Mean river flow (m^3/s)[c]	Elevation (m asl)	Drainage basin (km^2)	Reservoir upstream of sitew	Storage volume (hm^3)	Residence time (days)
GUA6	04/10/2013	0.95	-	-	0.87	2.15	-	-	-	-	-
GUA7	07/10/2013	0.17	13.3%	-5.7%	0.25	1.71	-	-	-	-	-
GUA8	07/10/2013	1.03	1.2%	-0.4%	0.3	1.11	-	-	-	-	-
ALF2	06/10/2013	0.23	26.1%	-21.4%	0.23	1.9	-	-	-	-	-
ALF4	07/10/2013	-	-	-	-	-	851	6394	Arquillo de San Blas	16.89	395
ALF5	07/10/2013	0.39	1.0%	-1.0%	1.04	2.3	-	-	-	-	-
TUR1	09/10/2013	1.65	-	-	1.62	2.79	-	-	-	-	-
TUR5	09/10/2013	4.85	-	-	4.5	5.83	540	6394	Benagéber	95.04	157
TUR7	09/10/2013	5.88	69.6%	-55.3%	5.71	4.16	-	-	-	-	-
TUR8	09/10/2013	-	-	-	-	-	252	6394	Loriguilla	16.12	41
TUR9	09/10/2013	7.02	56.1%	-13.1%	6.92	6.12	-	-	-	-	-
TUR12	10/10/2013	5.71	33.2%	-11.6%	5.7	6.05	-	-	-	-	-

[a] Date corresponding to the sampling day [b] Date corresponding 50 last years [c] Date corresponding to the average of the last 10 years

Table S-9: Comparative concentrations of pesticides in rivers of the world

Continent	Country	River	Sampling period	Pollutant	Method	Concentration (ng/L) min - max	Ref.
Asia	Malaysia	Selangor River	2002 - 2003	Diazinon	GC-MS	106.7	(Leong et al., 2007)
				Heptachlor		96.7	
				Chlorpyrifos		101.5	
				Endosulfan		98.1	
	India	Ganger river	2007 - 2009	Methyl parathion	LLE - GC - ECD	65280	(Sankararamakrishnan et al., 2005)
				Malathion		62500	
				Ethion		75810	
	China	Yangtze. Yellow. Pearl. Songhua. Liaohe. Haihe and Huaihe rivers	2003 - 2004	Dimethoate	GC - MS	< 1.3 - 2660	(Gao et al., 2009)
				Malathion		< 0.8 - 1290	
				Parathion methyl		< 0.9 - 480	
				Parathion		< 0.9 - 150	
	Philippines	Lucban river	2007 - 2009	Malathion	GC - ECD	100 - 3330	(Varca, 2012)
	China	Liao-He River	2002	Atrazine	SPE - GC - MS	350	(Gfrerer et al., 2002)
				Propazine		< 50	
				Simazine		< 50	
				Dieethylatrazine			
				Deisopropilatrazin e		< 50 200	

Continent	Country	River	Sampling period	Pollutant	Method	Concentration (ng/L) min - max	Ref.
America	Chile	Traiguén river	2001 - 2003	Picloram	SPE-HPLC-DAD	300 700 - 3000	(Palma et al., 2004)
	Brazil	San Francisco River	2011	Simazine Hexazinone Carbendazim Dimethoate Methyl Parathion Ethion	SDME-GC-MS	300 200 - 4500 2380 150 78	(Pinheiro et al., 2011)
	EEUU	Mississippi River Basin	1999 - 2001	Permethrin Alachlor Dimethenamid Metolachlor Atrazine Cyanazine Metribuzin Propazine Simazine Fluometuron	SPE-LC-MS	1165 180 530 2020 9840 810 1400 120 610 190	(Rebich et al., 2004)
	Argentina	Suquia River	2010-2011	Atrazine Endosulfan-sulfate	SPE-SPEM-GC-MS	0.6 - 433.9 1.6 - 106.7	(Bonansea et al., 2013)
Africa	Ghana	Lake Bosomtwi	2004 - 2006	Alpha-cypermethrin	ECD-GC	28.4 - 112.4 0.75 - 1.61	(Darko et al., 2008)

Continent	Country	River	Sampling period	Pollutant	Method	Concentration (ng/L) min - max	Ref.
				Lindane		0.33 - 0.42	
				Endosulfan		0.1 - 0.9	
				p.p-DDE			
				p.p-DDT		0.13 - 0.86	
Europa	Portugal	Guadiana River	2011 - 2012	Atrazine	SPE–LC–MS/MS	18.61	(Palma et al., 2014)
				Terbuthylazine		531.92	
				DIA		108	
				Chlortoluron		94.12	
				Metolachlor		290.94	
				Dimethoate		49.81	
				Molinate		352.12	
				2.4-D		967.47	
				Bentazone		1769.02	
				MCPA		579.65	

Continent	Country	River	Sampling period	Pollutant	Method	Concentration (ng/L) min - max	Ref.
	Greece	Kalamas River	2000	EPTC	SPEM-GC-FDT-MS	120	(Lambropoulou et al., 2002)
				DEA		30 - 90	
				Trifluralin		20 - 300	
				Atrazine			
				Alachlor		20 - 230	
				Carbofuran		40 - 130 30 - 150	
				Diazinon		40 - 250	
				Parathion methyl		50 - 90	
	Netherlands	Scheldt river	1996 - 1998	Atrazine	SPE-GC-MS	60 - 750	(Steen et al., 2001)
				Simazine		50 – 570	
				Alachlor		2 - 100	
				Metolachlor		25 - 1000	

Mediterranean cases	Spain	Guadadalquivir river	2010 - 2011	Diuron Imazalil	SPE-LC-MS/MS	1350 0.59 - 8.05	(Masiá et al., 2013)
				Imidacloprid		2.34 - 19.2	
				Chlorfenvinphos		0.07 - 26.48	
				Chlorpyrifos		0.67 - 14.8	
				Diazinon		0.66 - 456.72	
				Dimethoate		2.7 - 69.26	
				Omethoate		2.23 - 11.71	
				Atrazine		4.61 - 18.63	
				Deisopropylatrazine		7.67 - 43.4	
				Deethylatrazine		4.35 - 97.21	
				Terbuthylazine		1.84 - 728	
				Terbuthylazine deethyl		3.61 - 23.8	
				Terbuthylazine-2 hydroxy		6.7 - 64.4	
				Diuron		23.05 - 67.69	
	Spain	Ebro river	2005	Malathion	SPE-LC–ESI-MS/MS	0.21	(Kuster et al., 2008)
				Simazine		0.36	
				Fenitrothion		0.68	
				Molinate		0.84	
				Atrazine		0.90	
				MCPA		13.90	
				Propanil		16.82	
				Alachlor		62.90	
				Bentazone		126.80	
	Spain	Llobregat and Anoia rivers	2005 - 2006	Simazine	SPE-LC-ESI–MS/MS	0.14 - 53.6	(Ricart et al., 2010)
				Desethylatrazine		27.1 - 27.1	

Mediterranean cases	Spain	Guadadalquivir river	2010 - 2011	Diuron Imazalil	SPE-LC-MS/MS	1350 0.59 - 8.05	(Masiá et al., 2013)
	Spain	Júcar	2010	Terbuthylazine	SPE-LC-MS	0.13 - 21.9	(Belenguer et al., 2014)
				Diazinon		0.83 - 785	
				Dimethoate		0.65 - 87.8	
				Diuron		0.4 - 99.7	
				Linuron		0.22 - 327	
				MCPA		0.11 - 67.4	
				Alachlor		2.17 - 17.1	
				Metolachlor		7.37	
				Chlorfenvinphos		96.68	
				Chlorpyrifos		36.23	
				Dichlofenthion		50.85	
				Fenoxon sulfoxide		50.66	
				Hexythiazox		20.65	
				Imazalil		171.5	
				Parathion ethyl		34.25	
				Prochloraz		82.79	
				Pyriproxyfen		99.59	
				Tolclofos methyl		28.64	

Table S-10. RQ for Algae (A). Daphnia (B) and Fish (C) in Júcar and Turia River 2010 - 2013

A)

Class/Pesticide	Chronic 96/72 h NOEC in Algae								
		2010		2011		2012		2013	
	PNEC	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max
3-Hydroxycarbofuran									
Acetochlor									
Alachlor									
Atrazine	100	< 0.1	0.2			< 0.1	< 0.1	< 0.1	< 0.1
Azinphos Ethyl	446								
Azinphos Methyl	1000					< 0.1	0.1	< 0.1	< 0.1
Buprofezin	1146	< 0.1	< 0.1			< 0.1	< 0.1		
Carbendazim	302			< 0.1	0.3	0.1	1.3	0.0	0.3
Carbofuran	3200					< 0.1	< 0.1	0.1	2.1
Chlorfenvinphos	1000	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Chlorpyrifos	43	0.4	1	0.1	0.2	0.4	0.8	0.1	0.7
Deethylatrazine									
Deisopropylatrazine									
Diazinon	10000	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Dichlofenthion	204	0.2	0.3						
Dimethoate	32000	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Diuron	93			< 0.1	0.1			< 0.1	< 0.1
Ethion	129	< 0.1	0.2	< 0.1	< 0.1	0.1	0.6	0.1	2.7
Fenitrothion	100							< 0.1	< 0.1
Fenoxon									
Fenoxon Sulfone	81113								

Class/Pesticide	Chronic 96/72 h NOEC in Algae								
		2010		2011		2012		2013	
	PNEC	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max
Fenoxon Sulfoxide									
Fenthion									
Fenthion Sulfone									
Fenthion Sulfoxide									
Hexythiazox	7	2.5	3.5			0.9	2.2	< 0.1	< 0.1
Imazalil	92	1.9	7.4	0.3	2.4	0.5	8.2	0.1	0.4
Imidacloprid	10000	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Isoproturon	52					0.1	0.3	< 0.1	0.3
Malathion	14993	< 0.1	< 0.1						
Methiocarb	3200	< 0.1	< 0.1					< 0.1	< 0.1
Metolachlor	0.59	50.5	757.6			2.2	26.4	21.1	99.0
Molinate									
Parathion-Ethyl									
Prochloraz	10	6.5	8.4	0.1	1.5	1.1	3.3	1.8	48.6
Propanil									
Propazine	40							< 0.1	< 0.1
Pyriproxyfen	213	0.4	0.5					< 0.1	< 0.1
Simazine	600					< 0.1	< 0.1	< 0.1	< 0.1
Tebuconazole									
Terbumeton									
Terbumeton-Desethyl									
Terbutilazine									
Terbutilazine-2									

Class/Pesticide	Chronic 96/72 h NOEC in Algae								
		2010		2011		2012		2013	
	PNEC	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max
Hidroxy Terbutryn	28					0.1	0.7	< 0.1	0.1
Terbutylazine Deethyl									
Thiabendazole	3200			< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Tolclofos Methyl									

B)

Class/Pesticide	Chronic 96/72 h NOEC in Aquatic invertebrates (Daphnia magna)								
		2010		2011		2012		2013	
	PNEC	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max
3-Hydroxycarbofuran									
Acetochlor									
Alachlor									
Atrazine	250	< 0.1	< 0.1			< 0.1	< 0.1	< 0.1	< 0.1
Azinphos Ethyl	0.418							< 0.1	1.1
Azinphos Methyl	0.4					16.8	370.2	0.2	5.4
Buprofezin	80	0.1	0.2			0.1	0.3	< 0.1	< 0.1
Carbendazim	1.5			8.4	52.9	15.6	254.7	5.9	67.8
Carbofuran	8					0.1	3.2	35.3	855.6
Chlorfenvinphos	0.1	629.7	1068.5	16.4	78.2	82.3	468.4	24.3	116
Chlorpyrifos	4.6	3.5	9.0	0.9	1.9	3.7	7.5	1.3	6.7
Deethylatrazine									

Class/Pesticide	Chronic 96/72 h NOEC in Aquatic invertebrates (Daphnia magna)								
		2010		2011		2012		2013	
	PNEC	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max
Deisopropylatrazine									
Diazinon	0.56	14.4	22.2	1.3	3.6	21.7	52.5	11.7	66.1
Dichlofenthion	0.0417	925.9	1314.4						
Dimethoate	40	< 0.1	< 0.1	< 0.1	0.1	< 0.1	0.7	0.2	2.4
Diuron	96			< 0.1	< 0.1			< 0.1	< 0.1
Ethion	0.12	24.7	202.3	23.3	44.2	67.2	697.3	105.6	2916.1
Fenitrothion	0.087							7.2	206.4
Fenoxon									
Fenoxon Sulfone	256022								
Fenoxon Sulfoxide									
Fenthion									
Fenthion Sulfone									
Fenthion Sulfoxide									
Hexythiazox	6.1	2.9	4.0			1.1	2.5	0.1	0.7
Imazalil	15	11.5	45.5	1.6	14.8	2.9	50	0.5	2.6
Imidacloprid	1800	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1
Isoproturon	120					< 0.1	0.1	< 0.1	0.1
Malathion	0.06	35.7	210.3						
Methiocarb	0.1	1.9	27.9					1.8	51.4
Metolachlor	707	< 0.1	0.6			< 0.1	< 0.1	< 0.1	< 0.1
Molinate									
Parathion-Ethyl									
Prochloraz	18	3.6	4.6	0.1	0.8	0.6	1.8	1.0	27.0
Propanil									

Class/Pesticide	Chronic 96/72 h NOEC in Aquatic invertebrates (Daphnia magna)								
		2010		2011		2012		2013	
	PNEC	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max
Propazine	420							< 0.1	< 0.1
Pyriproxyfen	0.015	5322.0	6639.3					30.0	218.0
Simazine	2500					< 0.1	< 0.1	< 0.1	< 0.1
Tebuconazole									
Terbumeton									
Terbumeton-Desethyl									
Terbutilazine									
Terbutilazine-2 Hidroxy									
Terbutryn	205					< 0.1	< 0.1	< 0.1	< 0.1
Terbutylazine Deethyl									
Thiabendazole	42			0.6	4.5	0.6	2.6	0.2	2.6
Tolclofos Methyl									

C)

Class/Pesticide	Chronic 21 days NOEC in Fish								
		2010		2011		2012		2013	
	PNEC	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max
3-Hydroxycarbofuran									
Acetochlor									
Alachlor									
Atrazine	2000	< 0.1	< 0.1			< 0.1	< 0.1	< 0.1	< 0.1
Azinphos Ethyl	21							< 0.1	< 0.1
Azinphos Methyl	0.17					39.6	871.0	0.4	12.6
Buprofezin	52	0.2	0.3			0.2	0.5	< 0.1	< 0.1
Carbendazim	3.2			4.0	24.8	7.3	119.4	2.8	31.8
Carbofuran	2.2					0.5	11.5	128.5	3111.1
Chlorfenvinphos	30	2.1	3.6	0.1	0.3	0.3	1.6	0.1	0.4
Chlorpyrifos	0.14	116.5	294.4	30.6	62.0	123.1	246.1	43.4	219.6
Deethylatrazine									
Deisopropylatrazine									
Diazinon	700	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Dichlofenthion	4	9.7	13.7						
Dimethoate	400	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Diuron	410			< 0.1	< 0.1			< 0.1	< 0.1
Ethion	12	0.2	2.0	0.2	0.4	0.7	7.0	1.1	29.2
Fenitrothion	88							< 0.1	0.2
Fenoxon									
Fenoxon Sulfone	23					< 0.1	0.4	< 0.1	< 0.1
Fenoxon Sulfoxide									
Fenthion									
Fenthion Sulfone									

Class/Pesticide	Chronic 21 days NOEC in Fish								
		2010		2011		2012		2013	
	PNEC	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max
Fenthion Sulfoxide									
Hexythiazox	40	0.4	0.6			0.2	0.4	< 0.1	0.1
Imazalil	43	4.0	15.9	0.6	5.2	1.0	17.4	0.2	0.9
Imidacloprid	9020	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Isoproturon	1000					< 0.1	< 0.1	< 0.1	< 0.1
Malathion	91	< 0.1	0.1						
Methiocarb	50	< 0.1	< 0.1						
Metolachlor	373	0.1	1.2			< 0.1	< 0.1	< 0.1	0.2
Molinate									
Parathion-Ethyl									
Prochloraz	49	1.3	1.7	< 0.1	0.3	0.2	0.7	0.4	9.9
Propanil									
Propazine	277							< 0.1	< 0.1
Pyriproxyfen	4.3	18.6	23.2					0.1	0.8
Simazine	700					< 0.1	< 0.1	< 0.1	< 0.1
Tebuconazole									
Terbumeton									
Terbumeton-Desethyl									
Terbutilazine									
Terbutilazine-2 Hidroxy									
Terbutryn	104					< 0.1	0.2	< 0.1	< 0.1
Terbutylazine Deethyl									

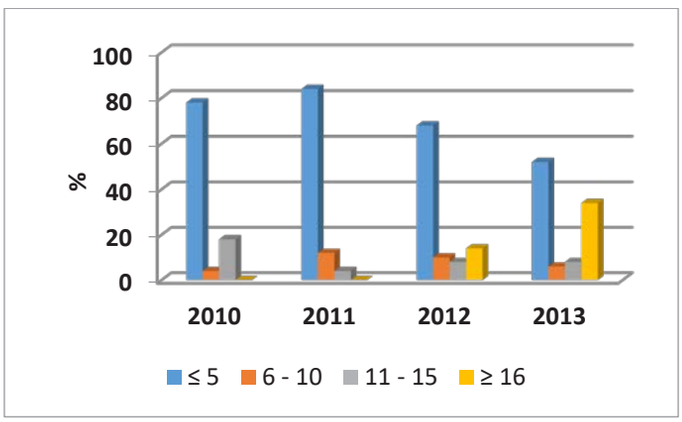
Class/Pesticide	Chronic 21 days NOEC in Fish								
		2010		2011		2012		2013	
	PNEC	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max	RQ_Mean	RQ_Max
Thiabendazole	12			2.0	15.6	2.1	9.0	0.9	9.2
Tolclofos Methyl									

Figure S-1: Pictures of the landscape units in the Júcar and Turia rivers

Júcar River
Turia river
Central landscapes
Alluvial plain
MINISTERIO
DE MEDIO AMBIENTE
SECRETARIA
GENERAL PARA
EL TERRITORIO Y
LA BIODIVERSIDAD
CONFEDERACION
HIDROGRAFICA
DEL JUCAR
Presa de Forata
CARACTERÍSTICAS:
Término Municipal: Yátova
Río: Magro
Superficie de Cuenca: 1058 Km²
Tipología de Presa: Gravedad
Volumen Máximo: 373 Hm³
Altura Sobre Cimientos: 68.40 m
Longitud de Coronación: 225 m
Capacidad Aliviaderos: 1110 m³/s
Capacidad de la toma: 24.50 m³/s
Año de entrada en Servicio: 1965
GENERALITAT VALENCIANA
ACOTADO DE PESCA -
TRAMO CON MUERTE
ZONA DE PESCA CONTROLADA
BUGARRA-GESTALGAR

Figure S-2. Co-occurrence of pesticides in A) waters and B) sediments in 2010-2011 (Júcar river) and 2012-2013 (Turia river)

A)



B)

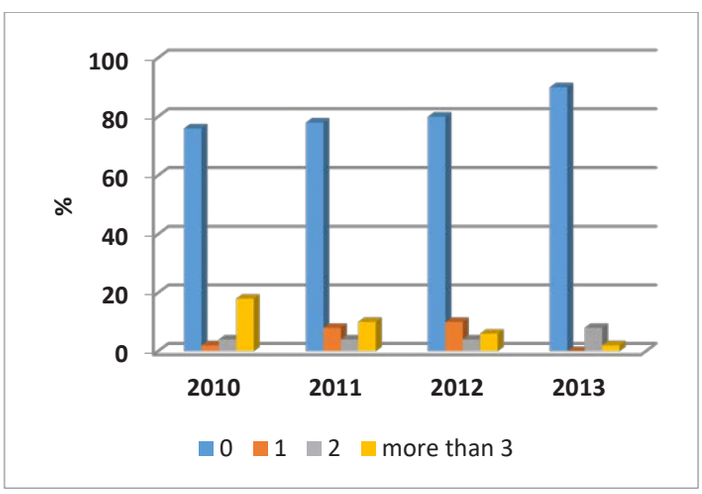
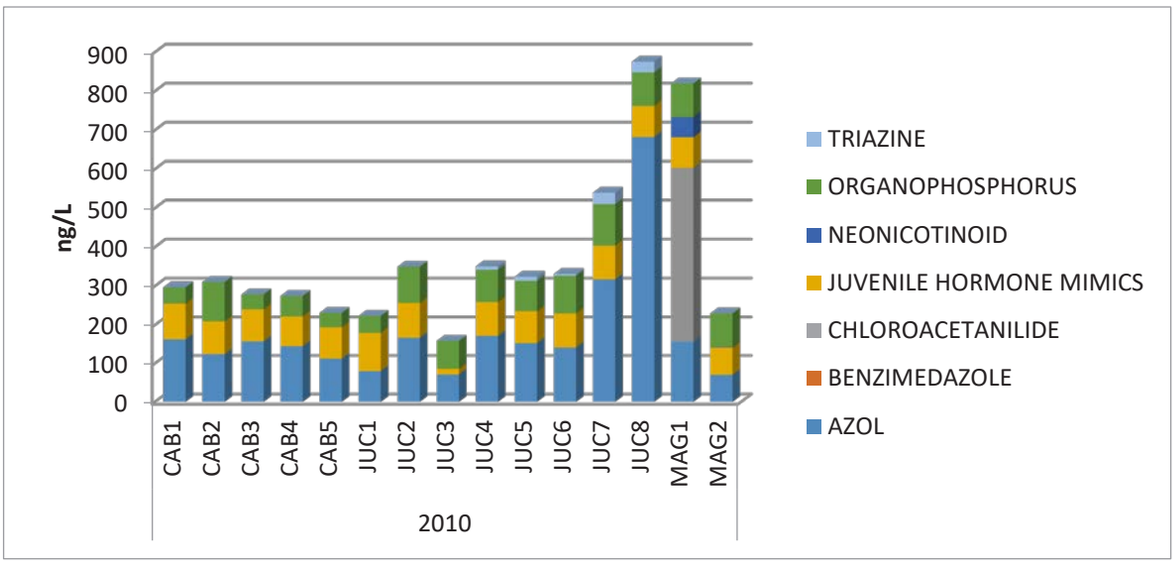
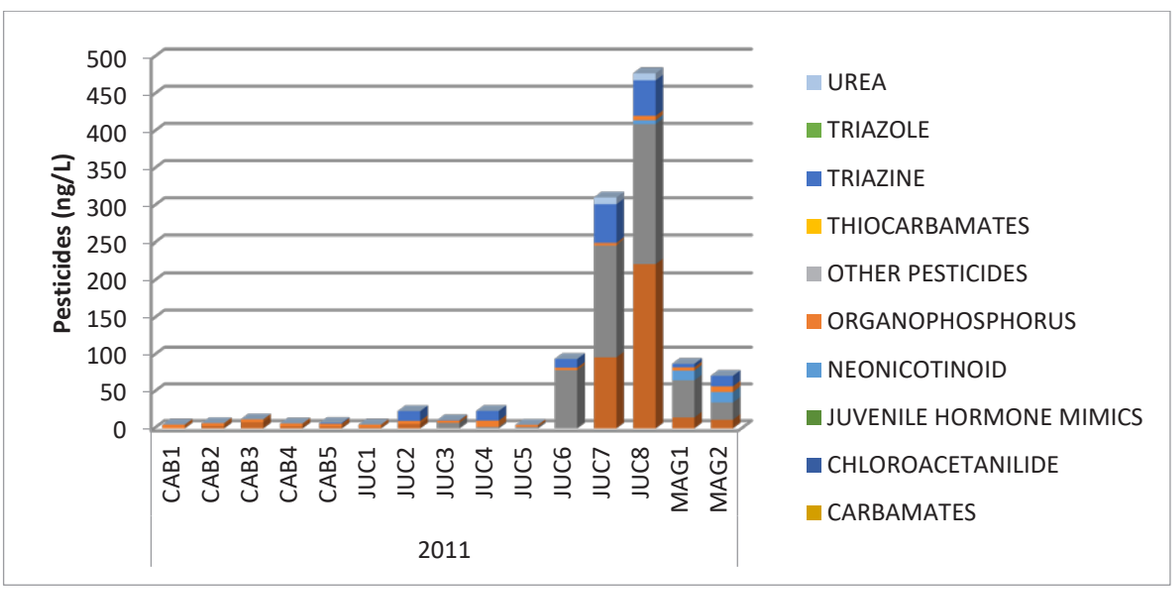


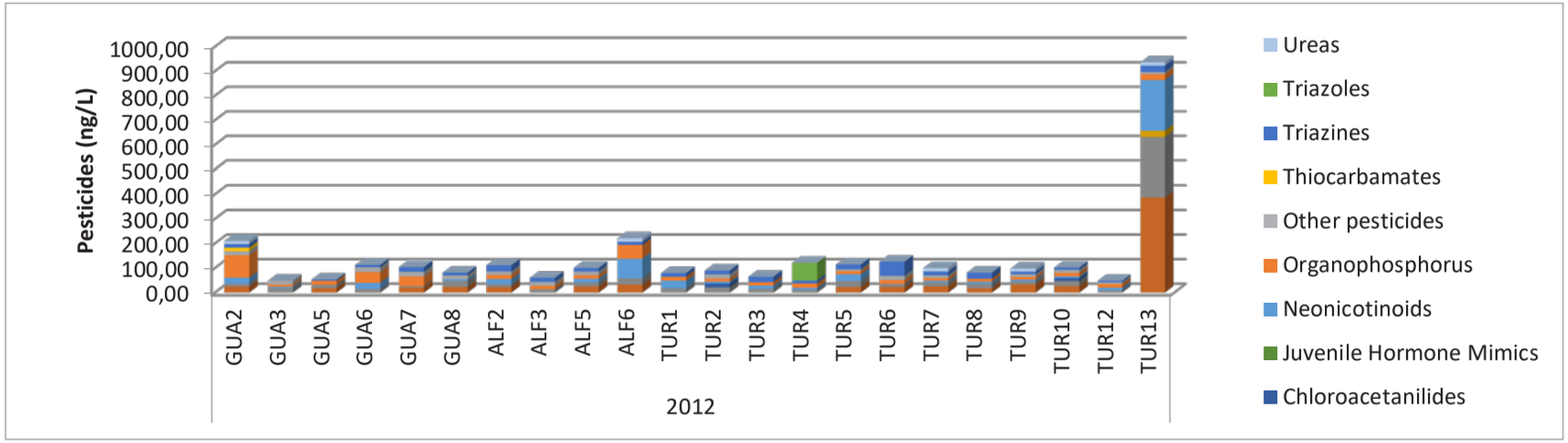
Figure S–3: Cummulative distribution of each family pesticide in waters at each sampling site
A) 2010 – 2011 Júcar river and B) 2012 – 2013 Turia river

A)





B)


Pesticides (ng/L)
1000,00
900,00
800,00
700,00
600,00
500,00
400,00
300,00
200,00
100,00
0,00
GUA2 GUA3 GUA5 GUA6 GUA7 GUA8 ALF2 ALF3 ALF5 ALF6 TUR1 TUR2 TUR3 TUR4 TUR5 TUR6 TUR7 TUR8 TUR9 TUR10 TUR12 TUR13
2012
Ureas
Triazoles
Triazines
Thiocarbamates
Other pesticides
Organophosphorus
Neonicotinoids
Juvenile Hormone Mimics
Chloroacetanilides

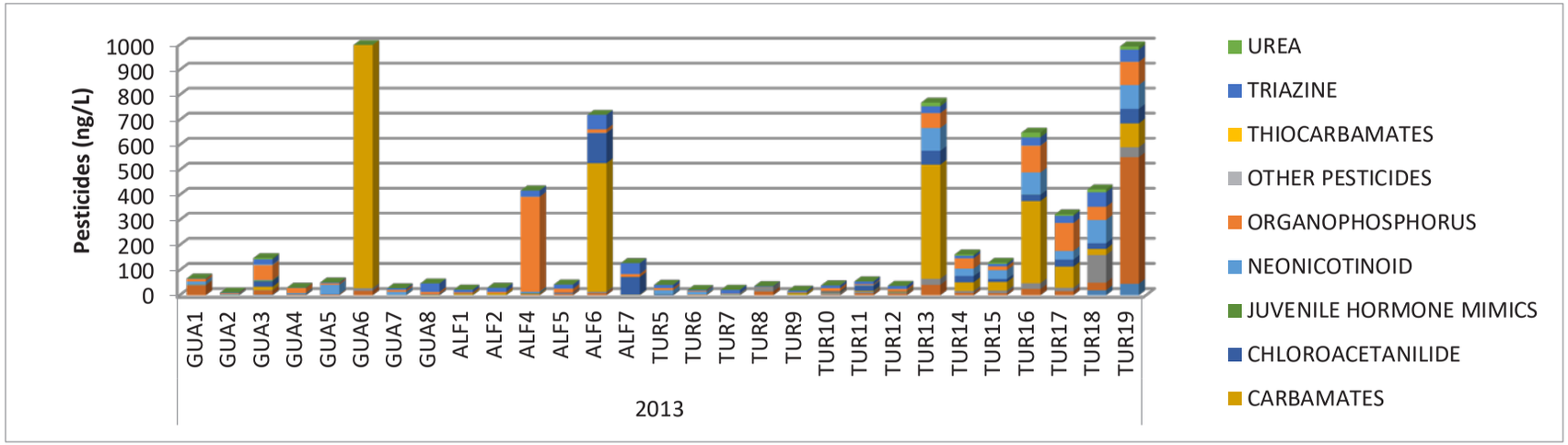
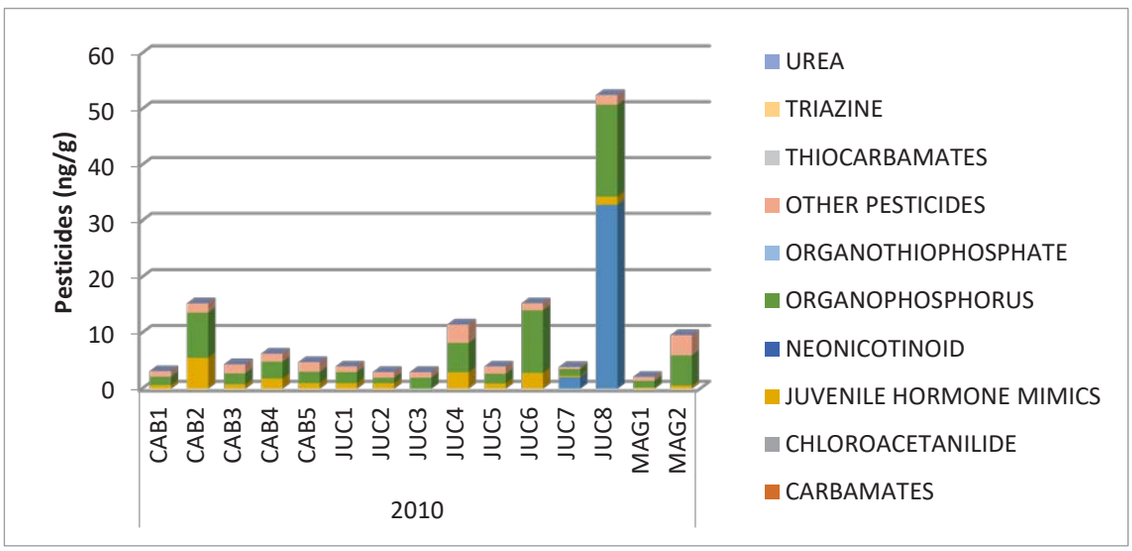
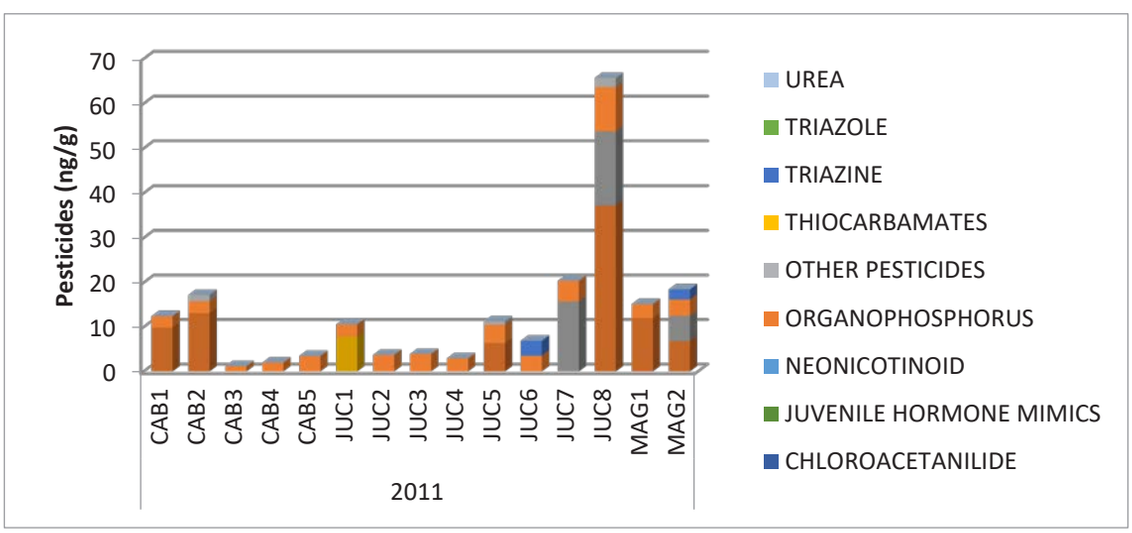

Pesticides (ng/L)
1000
900
800
700
600
500
400
300
200
100
0
GUA1 GUA2 GUA3 GUA4 GUA5 GUA6 GUA7 GUA8 ALF1 ALF2 ALF4 ALF5 ALF6 ALF7 TUR5 TUR6 TUR7 TUR8 TUR9 TUR10 TUR11 TUR12 TUR13 TUR14 TUR15 TUR16 TUR17 TUR18 TUR19
2013
UREA
TRIAZINE
THIOCARBAMATES
OTHER PESTICIDES
ORGANOPHOSPHORUS
NEONICOTINOID
JUVENILE HORMONE MIMICS
CHLOROACETANILIDE
CARBAMATES

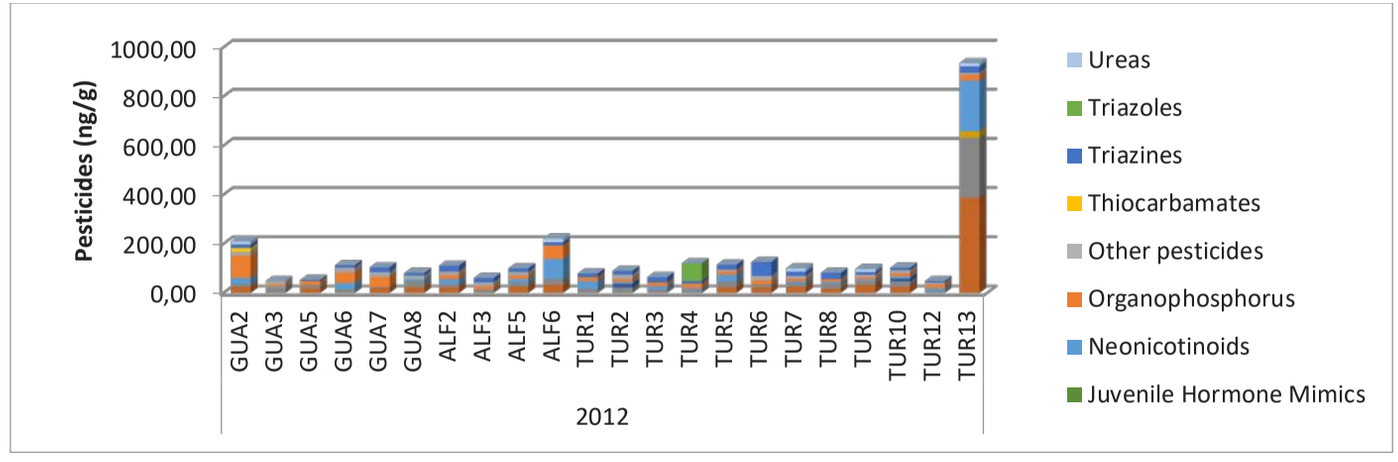
Figure S-4: Cumulative distribution of each family pesticide in sediments at each sampling site (A) 2010- 2011 Júcar river and (B) 2012 – 2013 Turia river.

A)





B)


1000,00
800,00
600,00
400,00
200,00
0,00
Pesticides (ng/g)
GUA2
GUA3
GUA5
GUA6
GUA7
GUA8
ALF2
ALF3
ALF5
ALF6
TUR1
TUR2
TUR3
TUR4
TUR5
TUR6
TUR7
TUR8
TUR9
TUR10
TUR12
TUR13
2012
Ureas
Triazoles
Triazines
Thiocarbamates
Other pesticides
Organophosphorus
Neonicotinoids
Juvenile Hormone Mimics

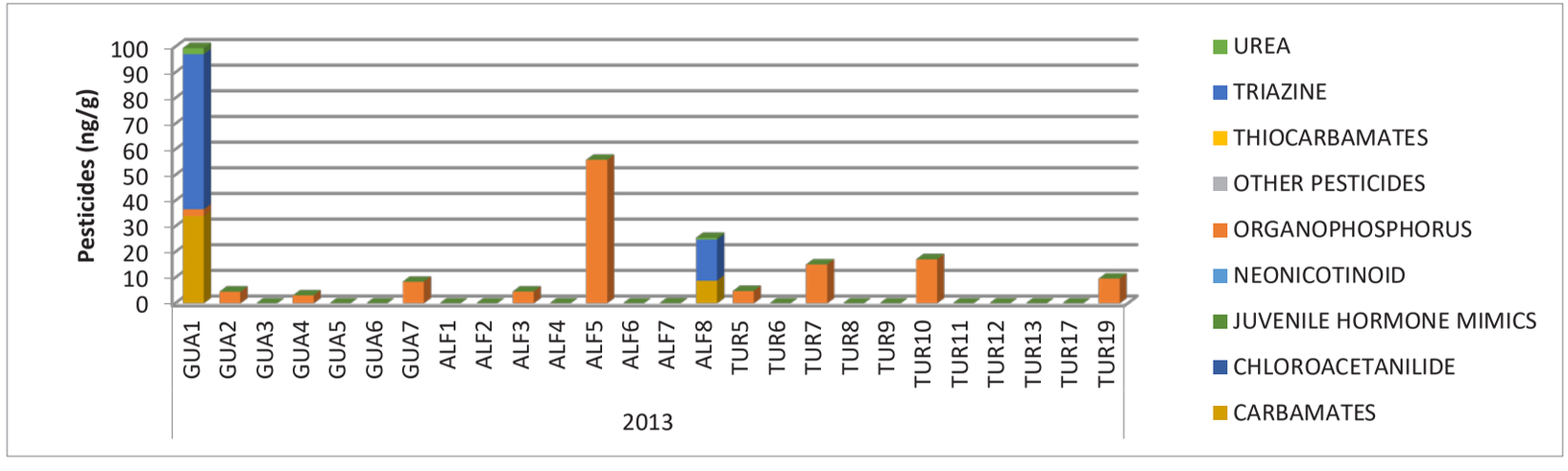
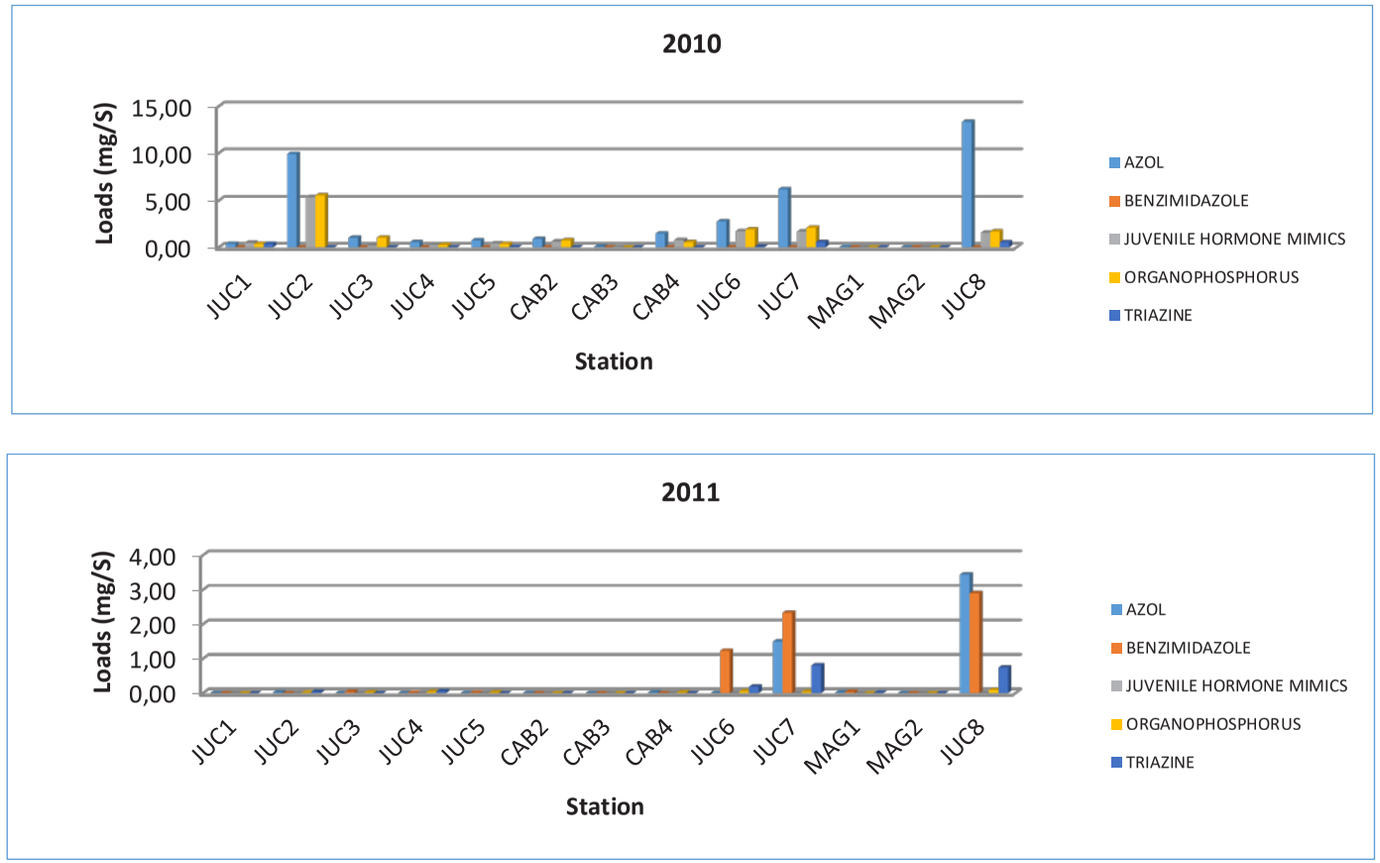

100
90
80
70
60
50
40
30
20
10
0
Pesticides (ng/g)
GUA1
GUA2
GUA3
GUA4
GUA5
GUA6
GUA7
ALF1
ALF2
ALF3
ALF4
ALF5
ALF6
ALF7
ALF8
TUR5
TUR6
TUR7
TUR8
TUR9
TUR10
TUR11
TUR12
TUR13
TUR17
TUR19
2013
UREA
TRIAZINE
THIOCARBAMATES
OTHER PESTICIDES
ORGANOPHOSPHORUS
NEONICOTINOID
JUVENILE HORMONE MIMICS
CHLOROACETANILIDE
CARBAMATES

Figure S-5. Loads of pesticides in water samples for the two consecutive years A) Júcar river 2010 – 2011 and B) Turia river 2012 – 2013

A)



B)

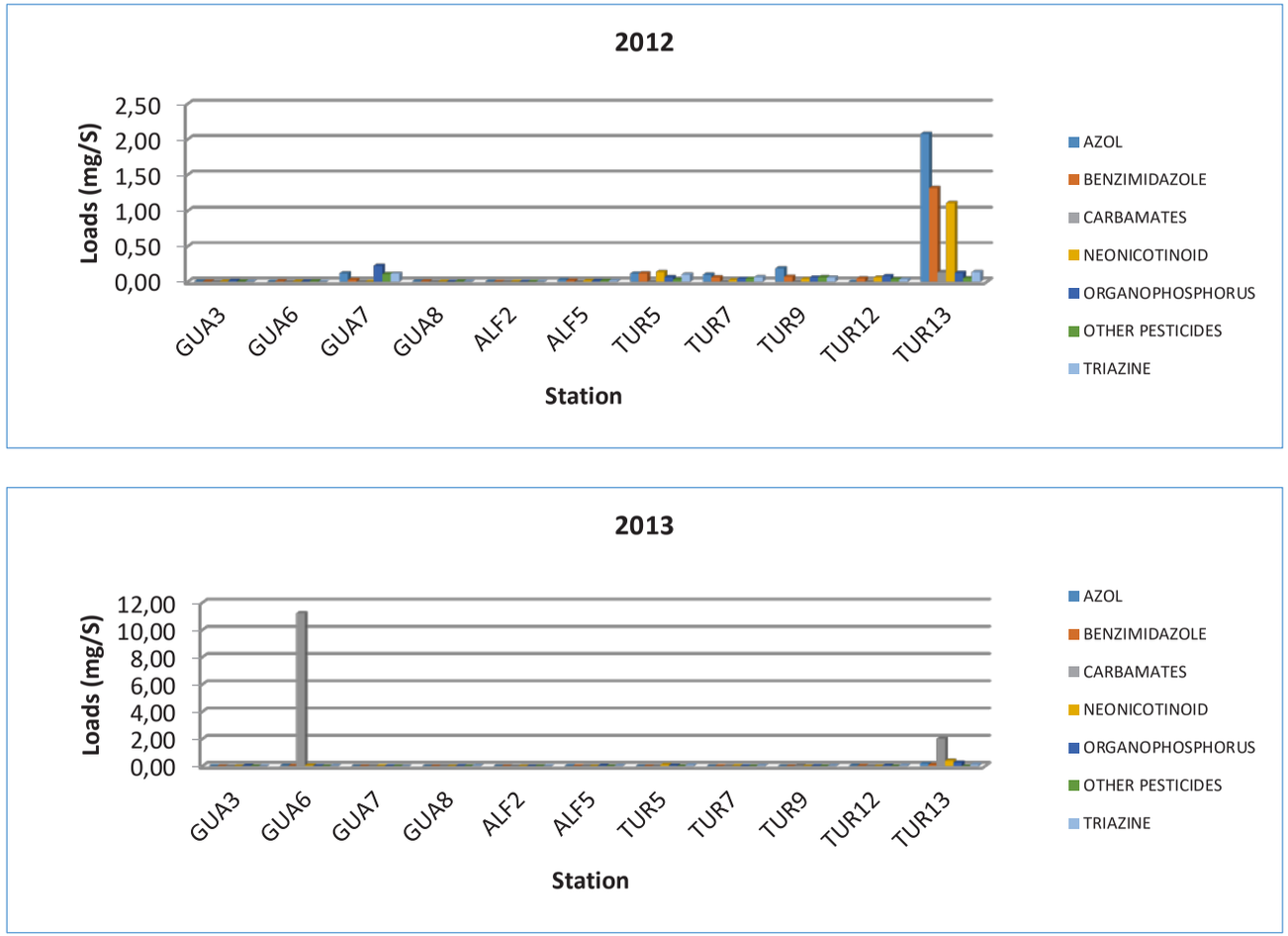
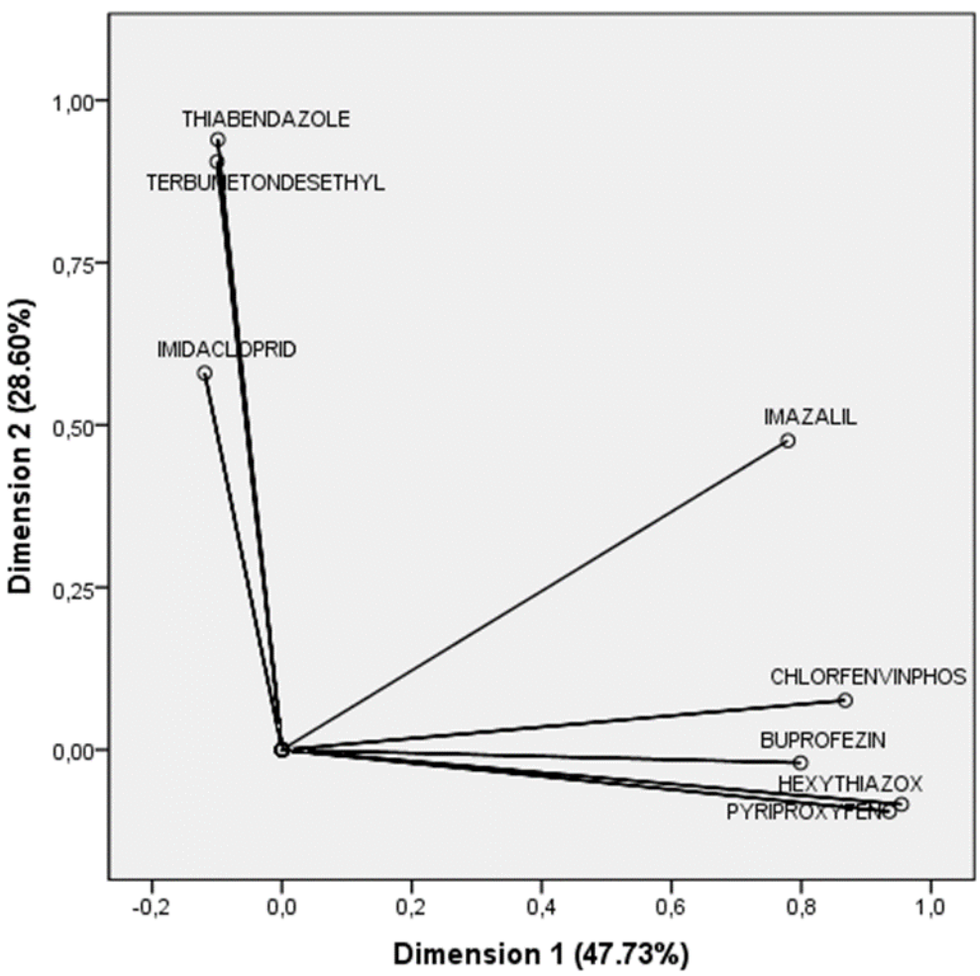

2012
Loads (mg/S)
2,50
2,00
1,50
1,00
0,50
0,00
GUA3
GUA6
GUA7
GUA8
ALF2
ALF5
TUR5
TUR7
TUR9
TUR12
TUR13
Station
AZOL
BENZIMIDAZOLE
CARBAMATES
NEONICOTINOID
ORGANOPHOSPHORUS
OTHER PESTICIDES
TRIAZINE
2013
Loads (mg/S)
12,00
10,00
8,00
6,00
4,00
2,00
0,00
GUA3
GUA6
GUA7
GUA8
ALF2
ALF5
TUR5
TUR7
TUR9
TUR12
TUR13
Station
AZOL
BENZIMIDAZOLE
CARBAMATES
NEONICOTINOID
ORGANOPHOSPHORUS
OTHER PESTICIDES
TRIAZINE

Figure S-6. PCA analysis of the data of both river basins.



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VII.

PRESENCIA Y VALORACIÓN DE RIESGOS DE PLAGUICIDAS EN LA CUENCA DEL EBRO

Se realizó el mismo seguimiento de plaguicidas en la cuenca del Ebro como estudio comparativo y complementario. En este estudio se evalúo de riesgo para la biota por medio de las Unidades Toxicas (TU), un concepto más integral de la toxicidad de los contaminantes debido a que considera el "cocktail" de concentraciones y su impacto en los distintos niveles tróficos como las dafnias, algas y peces. También se analizó el coeficiente de riesgo (RQ) en los mismos bioindicadores ambientales.

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Pesticides in the Ebro River basin: Occurrence and risk assessment☆

Alexander Ccanccapa [a, *], Ana Masiá [a], Alícia Navarro-Ortega [b], Yolanda Picó [a], Damià Barceló [b, c]

[a] Food and Environmental Safety Research Group (SAMA-UV), Facultat de Farmàcia, Universitat de València, Av. Vicent Andrés Estellés s/n, 46100, Burjassot, Valencia, Spain
[b] Water and Soil Quality Research Group, Dep. of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain
[c] Catalan Institute for Water Research (ICRA), H2O Building, Scientific and Technological Park of the University of Girona, Emili Grahit 101, 17003 Girona, Spain

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Daphnia
Fish

ABSTRACT

In this study, 50 pesticides were analyzed in the Ebro River basin in 2010 and 2011 to assess their impact in water, sediment and biota. A special emphasis was placed on the potential effects of both, individual pesticides and their mixtures, in three trophic levels (algae, daphnia and fish) using Risk Quotients (RQs) and Toxic Units (TUs) for water and sediments. Chlorpyrifos, diazinon and carbendazim were the most frequent in water (95, 95 and 70% of the samples, respectively). Imazalil (409.73 ng/L) and diuron (150 ng/L) were at the highest concentrations. Sediment and biota were less contaminated. Chlorpyrifos, diazinon and diclofenthion were the most frequent in sediments (82, 45 and 21% of the samples, respectively). The only pesticide detected in biota was chlorpyrifos (up to 840.2 ng g^{-1}). Ecotoxicological risk assessment through RQs showed that organophosphorus and azol presented high risk for algae; organophosphorus, benzimidazoles, carbamates, juvenile hormone mimic and other pesticides for daphnia, and organophosphorus, azol and juvenile hormone mimics for fish. The sum TU_{site} for water and sediments showed values < 1 for the three bioassays. In both matrices, daphnia and fish were more sensitive to the mixture of pesticide residues present.

1. Introduction

Pesticides are a widespread group of chemical substances used to improve agricultural production. However, these substances could be persistent in water, accumulative in sediment or bioaccumulative in biota, depending on their solubility and Log K_{ow}. They are hazardous for living organisms, human health or environment, even at low concentrations (Campo et al., 2013; Claver et al., 2006; Damásio et al., 2011; Giordano et al., 2009; Masiá et al., 2015a). Furthermore, physical, chemical and biological processes degrade pesticides into one or more transformation products that could be more toxic or persistent than the parent one. There is a need of data on the real occurrence of pesticide residues in environmental matrices (De Gerónimo et al., 2014; Köck-Schulmeyer et al., 2014; Palma et al., 2014a; Bruzzoniti et al., 2014; Martínez-Domínguez et al., 2015; Masiá et al., 2014, 2015b; Wei et al., 2015).

The potential ecotoxicological risks associated with pesticide residue contamination are addressed through toxic units (TUs) and/or risk quotients (RQs) (EC, 2003; Ginebreda et al., 2014; Kökc et al., 2010). Their application in most studies is restricted to water samples (Ginebreda et al., 2014; Kuzmanović et al., 2016). However, pesticide residues can also be adsorbed into sediments (Masiá et al., 2015b). WFD (EC, 2000) and environmental quality standards (EQS) (EC, 2008; EU, 2013) unquestionably support to include sediments in the risk assessment. A variety of methods were proposed but only scarcely applied to evaluate the potential toxicity of sediments (e.g., toxic equivalent factor approach, TUs summation, hazard index) (Schwarzenbach and Westall, 1981; Booij et al., 2015; de Castro-Catalá et al., 2016).

Another problem caused by pesticides contamination is the simultaneous occurrence of several of them and the need to establish the real impact of these mixtures on biota (Cedergreen,

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* Corresponding author.
E-mail address: Alexander.Ccanccapa@uv.es (A. Ccanccapa).

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2014; Roig et al., 2015), which can be predicted by independent action (IA) or concentration addition (CA). The former assumes that the components have different mechanisms of action —ignoring synergies/antagonisms and effect summation and therefore underestimating the effect— and the latter that have a similar one —overestimating the effects. (Cedergreen, 2014; Ginebreda et al., 2014; Kuzmanović et al., 2016). CA is often the recommended first step on a tiered process because presents the worst case scenario (even that synergies are not considered) (de Castro-Catalá et al., 2016).

Mediterranean area is one of the most affected by climatic fluctuations that alter hydrological conditions and originate the great wavering on concentrations of the cocktail of pesticide residues present in water (Batalla et al., 2004). Ebro River is the second largest river of the Iberian Peninsula and the first one that flows into the Mediterranean area of Spain. Previous studies performed in the Ebro River linking occurrence of pollutants, concentrations and toxicity, but most of them have focused on a single chemical family or select one environmental matrix (water, soils, sediments or biota) (Claver et al., 2006; Damásio et al., 2010; Köck-Schulmeyer et al., 2013; Köck et al., 2010; Navarro et al., 2010; Silva et al., 2011).

The objective of this study was to establish pesticide's occurrence, spatial distribution and transport and to evaluate the ecotoxicological risk in three trophic levels (Algae, daphnia and fish), using RQs for each pesticide and sumTUs for each sampling site. The partial objectives of this study were to (i) monitor the concentration of 50 pesticides and transformation products in the surface waters, sediments and biota of the Ebro River basin in two consecutive campaigns (2010–2011) (ii) compare the concentration of the pesticides found in the present study with those detected since 2001 and with the EQS values of the pesticides included in the Directive 2013/39/EU (EU, 2013), and (iii) perform an environmental risk assessment not only for water concentrations but also sediments based on the RQs and TUs methods.

2. Experimental design

2.1. Physical setting and sampling

The Ebro River is located at the northeast of Spain and drains an area of approximately 85,000 km^2. It has 928 km in length and receives waters from several tributaries, which altogether represent 12,000 km of waterway network, ending into Mediterranean Sea and forms a delta of more than 300 km^2 (Lacorte et al., 2006; Navarro et al., 2010; Roig et al., 2015). The basin is characterized by a Mediterranean valley, which forms a triangular morphological unit, surrounded by mountains. Mean annual precipitation and temperature vary with altitude, ranging respectively from 1800 mm to 8 °C in the Pyrenees to 320 mm and 18 °C in the Ebro valley. Traditionally, the Ebro River basin is agricultural land, but lately industry has been a growing sector. In 2008, one third of the total surface of the basin was agricultural and it is still the most irrigated area in Spain (906.000 ha) (Herrero-Hernández et al., 2013), the most important crops are herbaceous plants (all over the basin), grapes for wine production (La Rioja), fruit trees (Lleida) and rice (Ebro Delta) (Silva et al., 2011). The Spanish statistics estimated that ca. 14,000 T of pesticides were used in 2010 and ca. 13,500 T in 2011. The monitoring in this study comprised two sampling campaigns, 2010 and 2011, including 24 sampling stations for water and sediments covering the whole River Basin (see Fig. S-1 and S-2) and finally five for biota sampling in 2010. These sites are representative of the whole basin (geo – references are in Table S-2).

Samples were taken in October in both years. Grab water samples (2 L) were collected in clean amber glass bottles, from the middle of the river width. Each bottle was thoroughly rinsed with MilliQ water at the laboratory and with the river water at the sampling point before collection. Sediment samples (approx. 250 g) were taken in the same point as the water ones using a Van Veen grab sampler (0.5 L capacity). They were transferred and wrapped into an aluminum foil (previously washed with methanol and dried in oven at 100 °C) that was put inside an aluminum box. Fish samples were only collected in 2010 at five selected sites of the River course: EBR2, EBR3, EBR4, EBR5 and OCA using electro-fishing because the complexity of the basin, the difficulties to perform electrofishing and the small sample sizes obtained.

All samples were transported in hermetic boxes refrigerated with ice upon arrival at the laboratory. There, the water samples were kept at 4°°C and pre-treated and processed in a period not exceeding 5 days. Before the analysis, water samples were vacuum filtered through 1 μm glass fiber filters followed by 0.45 μm nylon membrane filters (VWR, Barcelona, Spain). Sediment and fish samples were frozen, lyophilized (Hetosicc CD4, Birkerod, Denmark), pulverized, thoroughly mixed, passed through a 2 mm Ø sieve and kept at −20 °C until the analysis that was performed within 3 months.

2.2. Extraction procedures and instrumental analysis: water, sediment and fish samples

For this study, 42 pesticides including some of their transformation products were determined in the 2010 campaign. Carbendazim, thiabendazole, terbumeton, terbumeton deethyl, terbuthylazine, terbuthylazine deethyl, terbuthylazine-2-hydroxy and tebuconazole were added in the next year. These pesticides belong to different chemical families, with a variety of uses as well as different physicochemical characteristics and toxicity (see Table S-1).

The water extraction was carried out according to Masiá et al. (2013b). Very briefly, water samples (200 mL) were extracted using an Oasis HLB solid-phase extraction (SPE) cartridge (200 mg sorbent/6 mL cartridge, Waters, Milford, MA, USA). The cartridge was dried under vacuum for 10 min and the analytes eluted with 10 mL of dichloromethane–methanol (50:50, v/v). The extract was evaporated to dryness and reconstituted with 1 mL of methanol. The fish and sediment samples were extracted using the QuEChERS method as described by Masiá et al. (2015b). Lyophilized sediment (1 g) or fish (2 g) were extracted with 8 ml of H_2O MilliQ, 15 ml of acetonitrile, 6 g of $MgSO_4$ and 1.5 g of NaCl. Then, 2 mL of the resulting supernatant were cleaned-up by dispersive SPE with 0.3 g of $MgSO_4$, 0.1 g of PSA, 0.1 g of C_{18} and 0.015 g of GCB. All samples were analyzed in triplicate. The results presented are the average of the three values.

The chromatographic instrument was an HP1200 series LC – automatic injector, degasser, quaternary pump and column oven – combined with an Agilent 6410 triple quadrupole (QQQ) mass spectrometer, equipped with an electrospray ionization interface (Agilent Technologies, Waldbronn, Germany). Data were processed using a MassHunter Workstation Software for qualitative and quantitative analysis (A GL Sciences, Tokyo, Japan). The detailed conditions are in the Supplementary material Tables S-3 and S-4).

2.3. Quality assurance and quality control

The analytical methods validation was detailed in the SM Table S-5. The method's limits of detection (MLDs) and quantification (MLQs) ranged from 0.01 to 2 ng L^{-1} for water, from 0.03 to 1.67 ng g^{-1} for sediment and from 0.08 to 3.75 ng g^{-1} for biota. Recovery tests were carried out in quintuplicate in order to evaluate the precision of the method. In water samples, recoveries varied

from 48% to 70% and precision was below 20% for all pesticides. In sediment and biota samples, recoveries were higher than 40% and precision ≤22%.

Pesticide concentrations were assessed though a comprehensive quality control scheme that included: laboratory and field blanks, matrix spikes and triplicate samples. Blank contamination is the most common problem observed in the determination of pesticides at trace levels. Thus, precautions were taken to prevent contamination from personnel, organic solvents, equipment and glassware. Blank assays were performed employing MilliQ water samples, to check for laboratory background levels of the studied compounds.

2.4. Risk assessment

The Toxic Units (TUs) and Risk Quotient (RQ) were calculated according to the European guidelines for each pesticide (EC, 2003) in at least three representative taxons (algae, *Daphnia magna*, and fish) of three trophic levels in the ecosystem. Acute 48 h EC50 for *D. magna,* 72 h EC50 for algae and 96 h LC50 for fish, as well as Chronic 96 h NOEC data for algae and 21 days NOEC for fish and *D. magna* of each chemical was collected from the website http://sitem.herts.ac.uk/aeru/ppdb/en/atoz.htm. In this database the EC50 for *D. magna* is refereed to immobilization, for algae (unknown species) to growth inhibition and for fish (*Oncorhynchus mykiss* mostly) to survival. Values of any compound not available in this site were calculated using the ECOSAR™ v. 1.11 (ECOlogical Structure Activity Relationship), in which the lowest toxicity prediction for each taxon was chosen to set in the worst-case scenario.

The toxic unit (TU_i) (Sprague, 1971) is used for the ecotoxicological risk assessment of measured concentrations of compounds (C_i). The TU of each compound was based on acute toxicity values. The following equation was applied for water and sediment samples.

$$TU_i\ (algae,\ daphnia,\ fish) = \frac{C_i}{EC50_i}$$

where TUi is the toxic unit of a compound i; C_i measured concentration (ng L^{-1}) in the water samples; $EC50_i$ (ng L^{-1}) is the effective concentration of 50% of individuals when exposed to the substance concerned.

Site specific toxic stress (TUsite) was calculated by summing all the individual TUi of each detected compound at all of the 24 studied sites.

$$Sum\ TU_{site} = \sum_{i=1}^{n} TU_i$$

Sediment-associated pesticide concentrations were converted to pore-water concentrations according to the equilibrium-partitioning approach to comply with the sediment benchmark toxicity tests that are based on dissolved phase pesticides in pore water. Pore water concentrations from sediments were calculated according to Di Toro et al. (1991) as:

$$C_{pw} = \frac{C_s}{K_d}$$

where K_d is the partitioning coefficient, C_S is the sediment concentration and C_{PW} the pore water concentration of the pesticide. K_d was calculated as:

$$K_d = K_{oc} \times f_{oc}$$

where K_{OC} is the dimensionless organic carbon–water partitioning coefficient for the pesticide and f_{OC} is the fraction of total organic carbon measured in the sediment samples. The K_{OC} was calculated as:

$$\log K_{OC} = a \times \log K_{OW} + b$$

where K_{OW} is the octanol–water partitioning coefficient. The constants a and b were set to 0.72 and 0.49, respectively (Schwarzenbach and Westall, 1981). TUs > 1 indicates environmental concern.

RQ was calculated using the following equation:

$$RQ = EC/PNEC$$

where, EC is the mean or maximum concentration of pesticides detected in the water samples and PNEC is the predicted no-effect concentration. PNEC can be calculated for acute or chronic toxicity, dividing the lowest short-term EC50 or long-term NOEC respectively by an assessment factor (AF), in this case 1000. The AF is an arbitrary factor to consider the inherent uncertainty in the obtained laboratory toxicity data. If RQ > 1, harmful effects could be expected due to the presence of the pollutant in water. On the contrary, if RQ < 0.1, the environmental risk is low. The intermediate situation in which the RQ is between 0.1 and 1 involves medium risk.

3. Results and discussion

Pollutants were more frequent in water than in sediment and biota (more apolar matrices). The low frequency can be explained because of the 50 target pesticides, only 21 had values Log K_{ow}> 3 (high), 6 between 2.5 and 3 (moderate) and 17 had values < 2.5 (low). Tables 1–3 show the minimum, maximum, mean and frequency of detection of the studied pesticides in the water, sediment and biota samples, respectively.

3.1. Residues of pesticides in water samples

The frequency was higher in 2010 than 2011. Organophosphorus, juvenile hormone mimics, azols, triazines, ureas and other pesticides were detected in both campaigns (See Table 1). In 2010, pyriproxyphen, chlorpyrifos, diazinon, buprofezin and hexythiazox were the most frequents (>90% of the samples) followed by imazalil and prochloraz (70% of the samples). In 2011, carbendazim was the most frequent (70% of the samples), whereas, diazinon, terbuthylazine and terbutryn frequency was >45% of the samples. Chlorpyrifos (95% of the samples in 2010) was already reported as the most commonly detected pesticide in the Ebro River (Claver et al., 2006; Navarro et al., 2010) even though is not usually persistent in water systems. Diazinon had a high frequency in 2010 (95% of the samples) but a medium-low one in 2011 (45%). This compound is stable in water, moderately soluble and slightly volatile (Table S-1). In 2011, carbendazim (not analyzed in 2010) was present in 70% of the sampling points. This fungicide has a low water solubility, can be persistent in water under certain conditions and is moderately persistent in soil. Herbicides terbuthylazine and terbutryn not analyzed in 2010 were detected in 50% of the samples in 2011. On the legal or illegal use of pesticides, of 50 target compounds analyzed, 14 —withdrawn by the European Union— were detected in both campaigns including carbendazim, metolachlor, azinphos methyl, chlorfenvinphos, diazinon, fenitrothion, fenthion, omethoate, parathion-methyl, atrazine, propazine, simazine, terbumeton and terbutryn (See Table S-1).

The pollution profile in both campaigns was marked by azoles, organophosphorus and triazines (detailed concentration at each

Table 1
Minimum, maximum and mean concentrations and frequency of detection of the studied pesticides in water samples.

Pollutants	2010				2011			
	Concentration (ng L^{-1})				Concentration (ng L^{-1})			
	Min	Max	Mean	Freq (%)[a]	Min	Max	Mean	Freq (%)[a]
Azol								
Imazalil	4.91	409.76	61.01	17 (70)	1.28	121.70	7.50	8 (33)
Prochloraz	2.24	34.47	15.59	17 (70)	2.14	2.14	0.09	1 (4)
Benzimidazole								
Carbendazim	n.a	n.a	n.a	n.a	0.04	11.63	2.78	17 (70)
Thiabendazole	n.a	n.a	n.a	n.a	0.43	48.77	3.58	5 (20)
Carbamates								
3-Hydroxycarbofuran	8.47	8.47	0.35	1 (4)	0.20	0.20	0.01	1 (4)
Methiocarb	n.d	n.d	n.d	n.d	1.24	2.52	0.30	4 (16)
Chloroacetanilide								
Metoalachlor	n.d	n.d	n.d	n.d	1.10	4.86	0.55	7 (29)
Juvenile Hormone Mimics								
Pyriproxyphen	0.89	37.74	24.38	23 (95)	4.76	4.76	0.20	1 (4)
Neonicotinoid								
Imidacloprid	1.84	2.77	1.06	11 (45)	1.64	14.96	1.66	9 (37)
Organophosphorus								
Azinphos Methyl	n.d	n.d	n.d	n.d	2.31	2.31	0.10	1 (4)
Chlorfenvinphos	2.54	41.24	17.97	18 (75)	1.57	1.57	0.07	1 (4)
Chlorpyrifos	2.64	16.40	5.97	23 (95)	1.01	2.86	0.32	5 (20)
Diazinon	0.12	13.58	5.65	23 (95)	0.53	20.39	1.35	11 (45)
Diclofenthion	13.62	22.73	12.86	18 (75)	n.d	n.d	n.d	n.d
Dimethoate	2.33	3.19	0.47	4 (16)	61.56	61.56	2.57	1 (4)
Fenitrothion	2.64	2.64	0.11	1 (4)	36.49	36.49	1.52	1 (4)
Fenoxon	2.64	2.64	0.11	1 (4)	n.d	n.d	n.d	n.d
Fenoxon Sulfone	2.64	2.64	0.11	1 (4)	n.d	n.d	n.d	n.d
Fenoxon Sulfoxide	2.64	20.84	4.43	9 (37)	n.d	n.d	n.d	n.d
Fenthion	2.64	2.64	0.11	1 (4)	0.33	0.33	0.01	1 (4)
Fenthion Sulfone	2.64	2.64	0.11	1 (4)	n.d	n.d	n.d	n.d
Fenthion Sulfoxide	2.64	2.64	0.11	1 (4)	n.d	n.d	n.d	n.d
Malathion	n.d	n.d	n.d	n.d	7.93	7.93	0.33	1 (4)
Omethoate	n.d	n.d	n.d	n.d	3.47	3.47	0.14	1 (4)
Parathion-Ethyl	14.01	14.45	1.19	2 (8)	n.d	n.d	n.d	n.d
Parathion-Methyl	n.d	n.d	n.d	n.d	2.00	2.00	0.08	1 (4)
Tolclophos-Methyl	8.30	16.07	3.50	7 (29)	0.50	0.50	0.02	1 (4)
Other Pesticides								
Buprofezin	2.32	8.25	5.82	22 (91)	n.d	n.d	n.d	n.d
Hexythiazox	1.90	10.57	7.41	22 (91)	1.21	1.21	0.05	1 (4)
Triazines								
Atrazine	8.13	12.22	1.99	5 (20)	n.d	n.d	n.d	n.d
Deisopropylatrazine	4.35	13.15	1.30	4 (16)	6.96	19.16	2.72	6 (25)
Deethylatrazine	6.57	58.82	7.67	7 (29)	4.99	4.99	0.21	1 (4)
Propazine	3.26	3.26	0.14	1 (4)	n.d	n.d	n.d	n.d
Simazine	30.71	47.95	3.28	2 (8)	n.d	n.d	n.d	n.d
Terbumeton	n.a	n.a	n.a	n.a	5.22	5.22	0.22	1 (4)
Terbumeton-Deethyl	n.a	n.a	n.a	n.a	0.42	9.72	0.89	8 (33)
Terbuthylazine	n.a	n.a	n.a	n.a	0.11	10.10	2.21	12 (50)
Terbuthylazine Deethyl	n.a	n.a	n.a	n.a	0.77	2.41	0.29	4 (16)
Terbuthylazine-2 Hydroxy	n.a	n.a	n.a	n.a	0.23	11.59	1.41	6 (25)
Terbutryn	14.85	14.85	0.65	1 (4)	0.92	30.54	7.66	12 (50)
Triazole								
Tebuconazole	n.a	n.a	n.a	n.a	1.66	15.38	2.36	8 (33)
Ureas								
Diuron	2.64	150.96	6.40	2 (8)	7.52	24.47	1.95	3 (12)
Isoproturon	2.58	25.48	1.60	4 (16)	2.41	2.41	0.10	1 (4)

n.d = Not detected.
n.a = Not analyzed.
[a] Number of findings (percentage of positive samples).

point is shown in Fig S3A), Samples of 2010 were more contaminated than those of 2011. The annual pesticide loads from the Ebro River to the Mediterranean Sea were estimated to be 4359 kg in 2010 and 1606 kg in 2011. These estimations correspond to the October–November period, which is characterized by lower pesticide discharge compared to spring time. These annual pesticide loads released to the sea could affect the Ebro Delta, biota and marine ecosystems. There are several estimations in different Mediterranean rivers of the pesticide loads that arrives yearly to the Sea: Jucar River 539 kg and Turía River 156 kg (Ccanccapa et al., 2016; Mai et al., 2013; Soubaneh et al., 2015). Mediterranean Sea receives already 2301 kg of pesticides yearly just from these three rivers. Tables 4 and 5 outline concentration of pesticides in water samples of the Ebro River and of other Mediterranean Rivers from 2001 to present. Regarding pollutants found in the Ebro River organophosphorus, carbamates, triazine, azol and ureas were always the most detected compounds. The concentrations were within the range from 3 to 12,597 ng L^{-1}. The main pesticides found were atrazine, molinate, propanil, diazinon, diuron, malathion, terbuthylazine, imidacloprid, tebuconalezole and dimethoate in

Table 2
Minimum, maximum and mean concentrations and frequency of detection of the studied pesticides in sediment samples.

Pollutants	2010				2011			
	Concentration (ng g^{-1} dw)				Concentration (ng g^{-1} dw)			
	Min	Max	Mean	Freq (%)[a]	Min	Max	Mean	Freq (%)[a]
Azol								
Imazalil	7.35	7.35	0.33	1 (4)	4.20	4.20	0.18	1 (4)
Prochloraz	4.60	4.60	0.21	1 (4)	n.d	n.d	n.d	n.d
Chlorpyrifos	0.18	9.59	1.06	10 (45)	0.88	36.17	7.66	19 (82)
Diazinon	0.28	8.85	0.63	10 (45)	0.62	3.30	0.20	3 (13)
Diclofenthion	n.d	n.d	n.d	n.d	1.44	28.82	1.73	5 (21)
Ethion	n.d	n.d	n.d	n.d	5.10	5.10	0.22	1 (4)
Malathion	1.84	1.84	0.08	1 (4)	n.d	n.d	n.d	n.d
Other Pesticides								
Hexythiazox	n.d	n.d	n.d	n.d	0.50	0.50	0.02	1 (4)
Triazines								
Terbutryn	3.97	21.61	1.16	2 (9)	0.10	0.10	0.00	1 (4)

[a] Number of findings (percentage of positive samples).**n.d** = Not detected. **n.a** = Not analyzed.

agreement with this study. Although the profile of contamination is variable, since 2005 the pesticide residue concentration increased from 4680 ng L^{-1} to 12,597 ng L^{-1} in 2011.

The spatial distribution (See Fig. 1A) of pesticides along the Ebro River and its tributaries could be related to the land use (Belenguer et al., 2014; Ccanccapa et al., 2016; Vryzas et al., 2009). Pesticide concentrations were moderate to low in most of the river course. The most polluted sites are Zadorra (ZAD) in the head and Segre (SEG) as well as the Ebro Delta in the mouth. Station ZAD —located in Alava (Basque Country)— is part of the Natura 2000 Network but surrounding by cereals, sugar beets and potatoes crops and influenced by the Crispijana wastewater treatment plant. In this point, diuron exceed 100 ng L^{-1}, limit established for individual concentrations in drinking water according to EU legislation (EC, 1998). The sampling point of the Segre River (SEG) had the highest concentrations of all tributaries. In 2010, this point exceed 500 ng L^{-1}, limit established for group pesticides in drinking water, and imazalil exceed 100 ng L^{-1}, individual limit established (EC, 1998) and in 2011 the total concentration was 233.33 ng L^{-1}. These high concentrations are only punctual. Fruit trees, corn, wheat and barley crops are characteristics of this area. The high concentrations of fungicide imazalil in both campaigns could be related to the post-harvest treatments of apples and pears. The Ebro Delta receives a high load of pesticides because of the intensive agricultural activities that are carried out upstream and in the Delta itself (rice cultivation) (Kuster et al., 2008). The spatial distribution showed clearly the increasing concentration gradient for both campaigns in the points sampled EBR-7, EBR-8 and EBR-9 (see Fig. 1 -A). In 2010, the concentrations go up from 2.32 ng L^{-1} to 109.24 ng L^{-1} and in 2011 from 1.11 ng L^{-1} to 30.54 ng L^{-1} (Kuster et al., 2008; Ochoa et al., 2012).

The co-occurrence of different pesticides in the water samples are shown in Fig. S-4A. In 2010, 38% of the samples contained less than 5 pesticides and 22% of the samples contained more than 16 pesticides. This means that even though concentrations were low, and there was one point (SEG) that exceed the European threshold for drinking water, the number of pesticides in each sample was high. In 2011, 42% of the samples present less than 5 pesticides but 22% of the samples present among 6 to 16 pesticides. In 2011 the co-occurrence was lower than in 2010.

The differences between both sampling campaigns could be related to the river flow (see Table S-6). Considering all the flow measurements in the last ten years in each point where there are data available and normalizing them to 100, the water flow in the first campaign ranged from 0.03 m^3 s^{-1} (MAT) to 213.40 m^3 s^{-1} (EBR-7), these values represent percentiles 18% and 50% that could be considered medium–high. On the contrary, in 2011 the flows ranged from 0.01 m^3 s^{-1} (MAT) to 155.43 m^3 s^{-1} (EBR-7), percentiles 5% and 20%, respectively. These values are below of 50% percentile and could be considered low. Apparently, the higher flow, the greater frequency and co-occurrence of pesticides, and consequently in 2010 the frequency and co-occurrence was higher than 2011 (Table 1 and Fig. S-4A). Regarding the low flow, there are reports that point out that lower flows are related with higher concentrations (Masiá et al., 2015a). However, this work shows low concentrations also at low flows. The concentration could vary taking into account the physico-chemical properties of pesticides but also other environmental conditions as precipitation or temperatures (see Table S-1) (Ccanccapa et al., 2016).

3.2. Residues of pesticides in sediment samples

Pesticides detected in sediment samples in both campaigns are outlined in Table 2. Out of the 42 pesticides analyzed in 2010 and 50 pesticides in 2011, 6 and 7 respectively, were detected at the concentrations over the MLODs. In 2010, 14% of the analytes —imazalil, prochloraz, chlorpyrifos, diazinon, malathion and terbutryn— were found. The concentrations detected ranged from 1.84 to 21.61 ng g^{-1} of dry weight (d.w). In 2011, pesticides detected were imazalil, chlorpyrifos, diazinon, diclofenthion, ethion, hexythiazox and terbutryn, and their concentrations ranged from 0.10 to 36.17 ng g^{-1} of d.w.

Regarding the frequency, diazinon and chlorpyrifos were the most prevalent compounds in 2010, which appeared in 45% of the samples. In 2011, chlorpyrifos (82%) and diclofenthion (21%) were the most frequently detected compounds. These pesticides had high octanol/water partition coefficient (log k_{ow}) (see Table S-1), consequently, are hydrophobic, low water soluble and tend to accumulate in sediment. However, other factors influence pesticides accumulation such as the application moment and the time elapses before the next major storm event. Chlorpyrifos was detected at high frequency in both campaigns and there are other reports that also remark their presence in the Mediterranean area (Ccanccapa et al., 2016; Masiá et al., 2015a, 2013a).

The spatial distribution of pesticides in sediment is shown in Fig. 1B and the contribution of each family of pesticides is detailed in Fig. S-4B. In 2010, the most ubiquitous pesticides were organophosphorus (38.99 ng L^{-1}), triazine (25.57 ng L^{-1}) and azol (11.94 ng L^{-1}). However, in 2011 only organophosphorus (225.62 ng L^{-1}) were found in all points sampled. Regarding the highest concentrations, in 2010 were for terbutryn (21.61 ng L^{-1}) and chlorpyrifos (9.59 ng L^{-1}) in points sampled ZAD and EBR-9

Table 3
Minimum, maximum and mean concentrations and frequency of detection of the studied pesticides in biota samples.

Pollutants	2010			
	Concentration ng g^{-1} dw			
	Min	Max	Mean	Freq %
ORGANOPHOSPHORUS				
CHLORPYRIFOS	n.d	n.d	n.d	n.d
Barbus (*Barbus guiraonis*)	n.d	n.d	n.d	n.d
Barbus (*Barbus guiraonis*): Adult	n.d	n.d	n.d	n.d
Barbus (*Barbus guiraonis*): Young	n.d	n.d	n.d	n.d
Carp (*Cyprinus carpus*)	840.25	840.25	420.13	1 (20)
Carp (*Cyprinus carpus*): Adult	n.d	n.d	n.d	n.d
European catfish (*Silurus glanis*): Adult	168.62	168.62	84.31	1 (20)

n.d = Not detected.

Table 4
Historical data of the pesticides concentrations in the Ebro Basin.

Year	Location	Family	Pesticide	Concentration (ng L^{-1})		Ref.
				Max	Mean	
2001–2004	Ebro River	Urea	Diuron	–	105	(Claver et al., 2006)
		Carbamates	Molinate	–	751	
		Triazine	Atrazine	–	451	
		Chloroacetanilide	Metolachlor	–	200	
		Organophosphates	Chlorpyrifos	–	312	
2004–2006	Ebro River	Triazine	Atrazine	825	62	(Navarro et al., 2010)
		Organophosphates	Dimethoate	259	115	
		Chloroacetanilide	Alachlor	272	32	
		Carbamate	Molinate	344	107	
		Anilide	Propanil	156	34	
2005	Ebro Delta	Triazine	Triazines	935	697	(Damásio et al., 2010)
		Anilide	Propanil	4680	1757	
		Carbamate	Molinate	485	318	
2007–2009	Ebro Basin	Chloroacetanilide	Alachlor	3	3	(Köck-Schulmeyer et al., 2013)
		Anilide	Propanil	36	9	
		Organophosphates	Diazinon	684	133	
		Urea	Diuron	452	93	
		Triazine	Terbuthylazine	71	21	
2008	Ebro Delta	Organophosphates	Malathion	5825	1072	(Köck et al., 2010)
		Urea	Diuron	408	72	
		Carbamates	Molinate	3590	526	
		Triazine	Terbuthylazine	1550	250	
2011	Ebro River	Triazine	Terbuthylazine	12,597	–	(Herrero-Hernández et al., 2013)
		Urea	Diuron	8551	–	
		Neonicotinoid	Imidacloprid	656	–	
		Chloroacetanilide	Acetochlor	314	–	
		Triazole	Tebuconazole	3236	–	
		Organophosphates	Dimethoate	7549	–	

Table 5
Historical data of pesticides concentration in the Mediterranean area.

Year	Location	Family	Pesticides	Concentration (ng L^{-1})		Ref.
				Max	Mean	
2010	Jucar River	Triazine	Atrazine-desethyl	11	–	(Belenguer et al., 2014)
		Organophosphorus	Chlorfenvinphos	93	–	
		Azol	Imazalil	172	–	
		Other Pesticides	Hexythiazox	21	–	
		Juvenile Hormone Mimics	Pyriproxyfen	100	–	
2010–2011	Guadalquivir River	Azole	Carbendazim	11	1	(Masiá et al., 2013a)
		Juvenile hormone mimics	Imidacloprid	19	2	
		Organophosphorus	Diazinon	457	19	
2010–2011	Llobregat River	Triazine	Terbuthylazine-2-hydroxy	50	13	(Masiá et al., 2015a)
		Organophosphorus	Malathion	320	58	
		Benzimidazole	Carbendazim	697	273	
		Carbamates	Carbofuran	7	3	
		Azol	Prochloraz	10	10	
		Other Pesticides	Hexythiazox	24	13	
		Neonicotinoid	Imidacloprid	67	25	
		Urea	Diuron	160	109	
		Chloroacetanilide	Metolachlor	13	11	
		Juvenile Hormone Mimics	Pyriproxyphen	2	2	
2012–2013	Turia River	Anilide	Propanil	46	2	(Ccanccapa et al., 2016)
		Azol	Imazalil	750	43	
		Benzimidazole	Carbendazim	382	23	
		Carbamates	Carbofuran	6845	283	
		Chloroacetanilide	Metolachlor	58	12	
		Juvenile Hormone Mimics	Pyriproxyfen	3	0	
		Neonicotinoid	Imidacloprid	207	23	
		Organophosphorus	Ethion	350	13	
		Other Pesticides	Buprofezin	25	12	
		Thiocarbamates	Molinate	14	1	
		Triazine	Terbutylazine Deethyl	59	10	
		Triazole	Tebuconazole	21	3	
		Urea	Isoproturon	13	2	

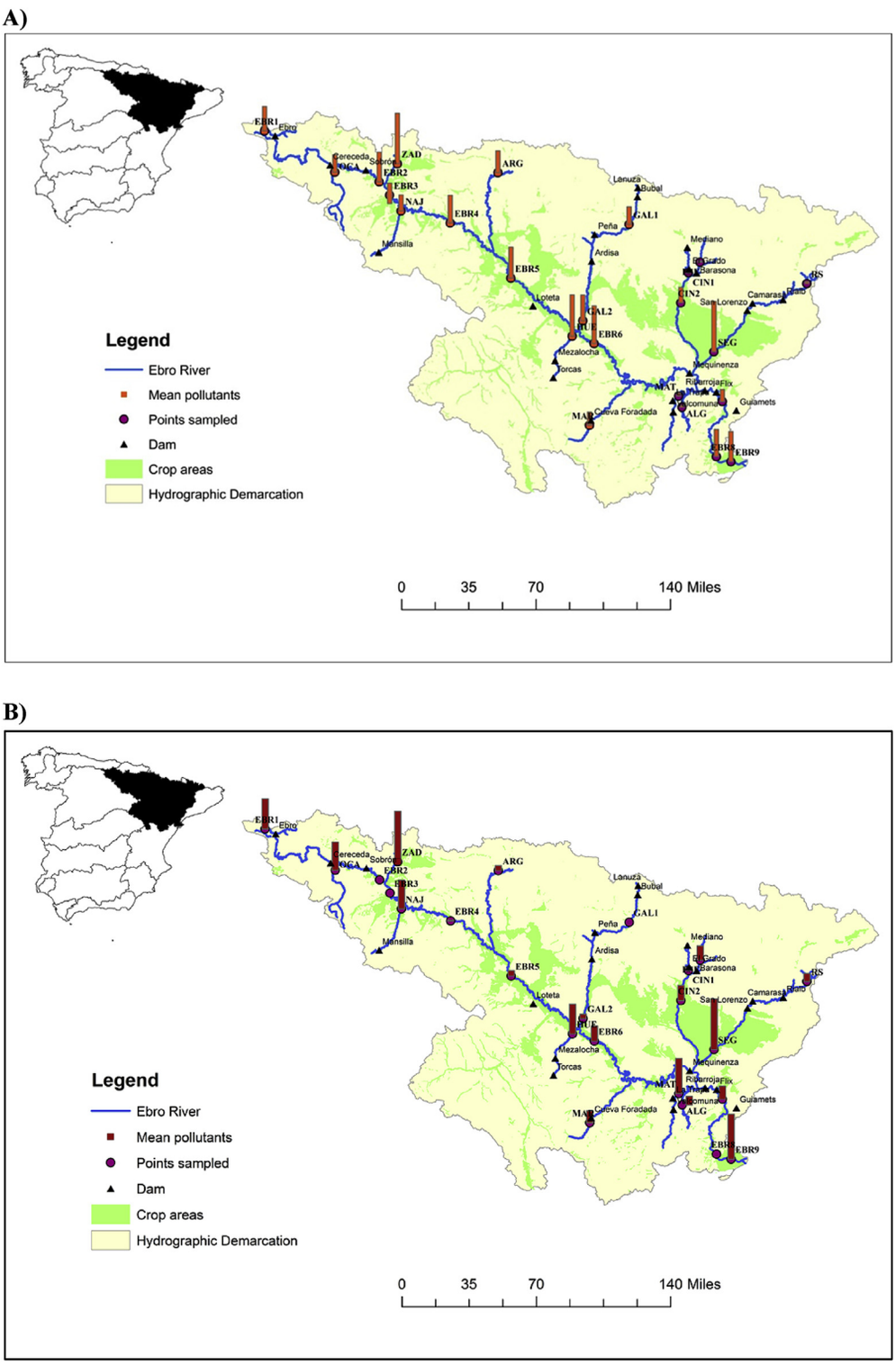

Fig. 1. Spatial distribution of pesticides in Ebro basin. A) 2010–2011 water samples and B) 2010–2011 sediment samples.

respectively. In 2011, chlorpyrifos (36.17 ng L^{-1} in EBR-1) and diclofenthion (28.82 ng L^{-1} in OCA) had the highest concentrations.

The co-occurrence of pesticides in sediments can be seen in the Fig. S-4B. In both campaigns, 86% of the sediment samples did not present pesticides. In 2010, 9% and 2011, 12% had at least 5 pesticides. Only 5% and 2% samples, consecutively, presented up to 10 pesticides.

3.3. Residues of pesticides in biota samples

Fish samples were taken at five points (EBR-2, EBR-3, EBR-4, EBR-5 and OCA) in one campaign (2010). The collected fish species

include, barbus (*Barbus guiraonis*), carp (*Cyprinus carpus*) and european catfish (*Silurus glanis*) (see Table 3). Chlorpyrifos ($K_{ow} = 4$) was the only pesticide detected in two fish species (Carp and European catfish). The concentrations were high, carp presented 840.25 ng g^{-1} dw and European catfish 168.62 ng g^{-1} dw. These data indicated possible bioaccumulation of these pesticides in fish. There are studies carried out in Mediterranean Rivers that pointed out chlorpyrifos bioaccumulation's in different fish species (Belenguer et al., 2014; Masiá et al., 2015a). Chlorpyrifos is considered as highly toxic to aquatic organisms.

4. Toxic units and risk quotient for water and sediments

The Sum TU_{site} could help to estimate the toxic effects of the mixture of pollutants per monitoring area by summing single compound TU for each sampling point as well as to study toxicity due to the contaminant present in sediments. However, the obtained Sum TU_{site} for water (Table 6) and sediment (Table 7) were <1 in all sites, evidencing that there is no acute risk associated with pollution either in water or sediments. Among the studied sites EBR-6 (0.26), ARG (0.24), ZAD (0.21), SEG (0.12), HUE (0.21), EBR-5 (0.21) and EBR-2 (0.23) showed the highest Sum TU_{site} values always for daphnia and water (See Fig. 2A). These sites reflected a dispersed pollution along the basin and a corresponding loss of ecological quality. The values do not reach the unit but are indicative of the sensitivity of *D. magna* to the mixture of pesticide residues in comparison with the other trophic levels. In 2011 the values were very low. These results clearly pointed out that there are not acute effects due to the mixtures of contaminants. However, complex chronic effects and interactions can not be discharged.

Subsequently, to evaluate the impact of the pesticides on the Ebro River basin ecosystems, the risk quotient (RQ) method was used employing, whenever is possible, the NOEC values obtained from chronic toxicity tests for producing the corresponding PNECs. Table 8 (Detailed Table S-7) shows the results obtained for the pesticides exhibiting low to high risk at either average or extreme condition, as calculated from their corresponding mean and maximum concentrations (Masiá et al., 2015a; Palma et al., 2014b; Sánchez-Bayo et al., 2002). Hexythiazox and prochloraz were present in some samples at levels that involved a risk, mean and maximum concentrations (RQ values > 1) for algae. Carbendazim, chlorfenvinphos, chlorpyrifos, diazinon, dichlofenthion, fenitrothion, hexythiazox, imazalil, malathion, methiocarb, and pyriproxyfen showed also as a hazard for daphnia at mean and maximum concentrations. Finally, Chlorpyrifos, dichlofenthion, imazalil, and pyriproxyfen presented RQ > 1 for fish at both, mean and maximum concentrations. Chronic toxicity test showed the high risk caused by pesticides in three trophic levels (algae, daphnia and fish); this could cause changes in fish and invertebrate communities and the decrease of the most sensitive species or increase of the more resistant ones, with a consequent loss of biodiversity. On the other hand, out of the 6 pesticides found with values above RQ > 1 for algae, all those are herbicides and fungicides. These compounds affect photosynthesis in microalgae and its reduction in aquatic ecosystems (Booij et al., 2015). For daphnia, 16 pollutants (RQ > 1) —mostly insecticides and fungicides— could produce seriously effect in this trophic level. Finally, for fish, 8 pesticides exceed RQ > 1. Mixtures of organophosphate, azoles and carbamates pesticides were commonly found in water samples. These pesticides inhibit the activity of acetylcholinesterase and have potential to interfere with behaviors that may be essential for the survival of species. There are reports of the Carps exposed to mixtures containing some of the organophosphorus, azoles and carbamates showed concentration additive or synergistic neurotoxicity (Cedergreen, 2014; Wang et al., 2015). This implies that single-chemical assessments systematically underestimate actual risks to aquatic species in watersheds where insecticides mixtures occur. RQ and TU are important indexes to estimate the risk in different trophic levels and for the protection of aquatic ecosystems.

Table 6
Toxic units for the different sites and trophic levels for water samples.

	Algae		Daphnia		Fish	
	2010	2011	2010	2011	2010	2011
MAR	E–	E–	0.190	E–	0.028	E–
ALG	E–	E–	0.073	0.001	0.004	E–
ARG	0.002	0.006	0.240	0.001	0.020	E–
CIN1	E	E–	0.053	E–	0.003	E–
CIN2	0.001	E–	0.182	E–	0.017	E–
EBR1	0.001	E–	0.139	E–	0.019	E–
EBR2	0.001	E–	0.232	0.051	0.017	0.003
EBR3	0.001	E–	0.172	E–	0.017	E–
EBR4	0.001	E–	0.221	E–	0.019	E–
EBR5	0.001	0.003	0.210	0.011	0.019	0.001
EBR6	0.001	E–	0.263	0.001	0.020	E–
EBR7	E–	0.004	E–	0.013	E–	0.001
EBR8	0.001	0.004	0.204	E–	0.017	E–
EBR9	0.001	0.004	0.167	E–	0.023	E–
ESE	E–	E–	0.041	E–	0.003	E–
GAL1	0.001	E–	0.126	0.001	0.015	E–
GAL2	0.002	E–	0.153	0.001	0.015	E–
HUE	0.001	0.004	0.215	0.036	0.021	0.001
MAT	0.001	E–	0.029	0.000	0.002	E–
NAJ	E–	0.001	0.149	0.001	0.019	E–
OCA	E–	E–	0.102	E–	0.024	E–
RS	E–	E–	0.077	E–	0.003	E–
SEG	0.001	0.002	0.122	0.016	0.019	0.001
ZAD	0.064	0.012	0.217	0.004	0.019	E–

E– More than four decimals.

Table 7
Toxic units for the different sites and trophic levels for sediment samples.

	Algae		Daphnia		Fish	
	2010	2011	2010	2011	2010	2011
MAR	E–	E–	0.003	E–	E–	E–
ALG	E–	E–	0.004	E–	E–	E–
ARG	0.001	0.001	0.008	E–	E–	E–
CIN1	E–	n.d	0.001	n.d	E–	n.d
CIN2	E–	E–	0.006	E–	E–	E–
EBR1	E–	E–	0.003	E–	E–	E–
EBR2	E–	E–	0.012	0.002	E–	E–
EBR3	E–	E–	0.004	E–	E–	E–
EBR4	E–	E–	0.022	n.d	0.001	n.d
EBR5	n.a	E–	n.a	E–	n.d	E–
EBR6	E–	E–	0.005	E–	E–	E–
EBR7	E–	E–	E–	E–	E–	E–
EBR8	n.a	n.a	n.a	n.a	n.a	n.a
EBR9	E–	0.001	0.004	E–	E–	E–
ESE	E–	E–	0.006	n.d	E–	n.d
GAL1	E–	E–	0.003	E–	E–	E–
GAL2	0.002	E–	0.009	E–	E–	E–
HUE	E–	0.001	0.004	0.002	E–	E–
MAT	0.001	E–	0.003	E–	E–	E–
NAJ	E–	E–	0.002	E–	E–	E–
OCA	E–	E–	0.002	E–	E–	E–
RS	E–	n.d	0.006	n.d	E–	n.d
SEG	0.001	E–	0.008	E–	E–	E–
ZAD	0.003	0.001	0.002	E–	E–	E–

E– More than four decimals.
n.d: Not detected.
n.a: Not analyzed.

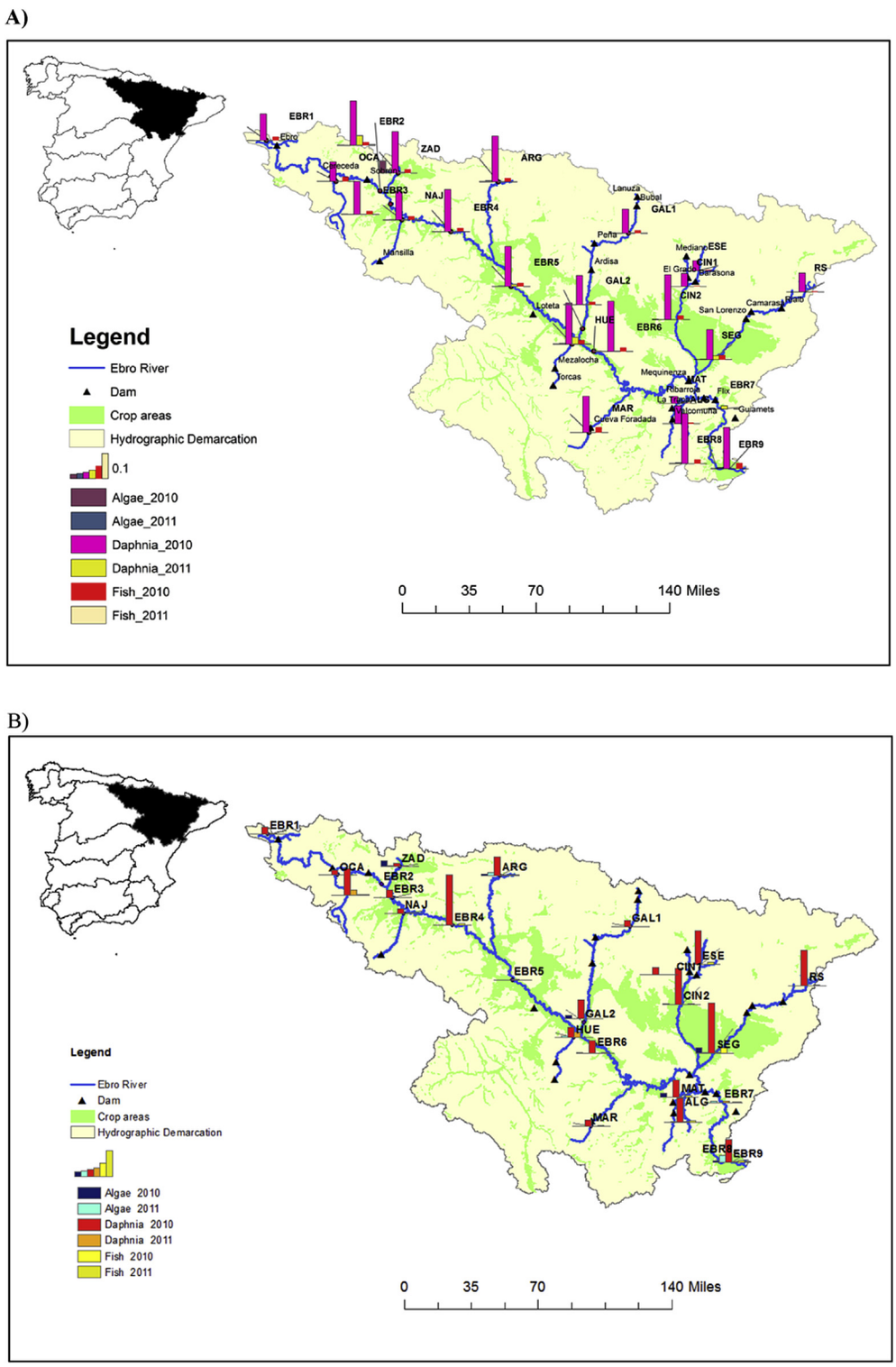

Fig. 2. Sum TU_{site} in sampling site for algae, daphnia and fish 2010–2011 A) Water samples and B) Sediment samples.

5. Conclusions

The survey carried out in 2010 and 2011 in the Ebro River and its tributaries regarding determination, distribution and ecotoxicological effects of 50 pesticides showed a dispersed pattern of concentration and risk on the different trophic levels (algae, daphnia and fish) along the basin. Water samples were the most frequently contaminated in both campaigns and in lesser extent sediment and biota samples. The most ubiquitous pesticides were azoles, organophosphorus and triazines in both years. The annual loads of pesticides for the Ebro basin were estimated in 4359 kg in 2010 and 1606 kg in 2011. This estimation was made in October and

Table 8
RQ for algae, daphnia and fish.

Pollutants	PNEC Ng L-1	2010		2011	
		RQ-Mean	RQ-Max	RQ-Mean	RQ-Max
Chronic 96/72 h NOEC in Algae					
Alachlor					
Atrazine	100	<0.1	0.1		
Chlorpyrifos	43	0.1	0.4	<0.1	<0.1
Dichlofenthion	204	0.1	0.1		
Diuron	93	0.1	1.6	<0.1	0.3
Fenitrothion	100	<0.1	<0.1	<0.1	0.4
Hexythiazox	7	1.1	1.5	<0.1	0.2
Imazalil	92	0.7	4.5	0.1	1.3
Isoproturon	52	<0.1	0.5	<0.1	<0.1
Metolachlor	1			0.9	8.2
Prochloraz	10	1.6	3.4	<0.1	0.2
Propazine	40	<0.1	0.1		
Pyriproxyfen	213	0.1	0.2	<0.1	<0.1
Tebuconazole	100			<0.1	0.2
Terbutryn	28	<0.1	0.5	0.3	1.1
Chronic 96/72 h NOEC in Aquatic invertebrates (*Daphnia magna*)					
Azinphos Methyl	0.4			0.2	5.8
Buprofezin	80	0.1	0.1		
Carbendazim	1.5			1.9	7.8
Chlorfenvinphos	0.1	179.7	412.4	0.7	15.7
Chlorpyrifos	4.6	1.3	3.6	0.1	0.6
Diazinon	0.56	10.1	24.2	2.4	36.4
Dimethoate	40	<0.1	0.1	0.1	1.5
Diuron	96	0.1	1.6	<0.1	0.3
Fenitrothion	0.09	1.3	30.3	17.5	419.4
Hexythiazox	6.1	1.2	1.7	<0.1	0.2
Imazalil	15	4.1	27.3	0.5	8.1
Isoproturon	120	<0.1	0.2	<0.1	<0.1
Malathion	0.06			5.5	132.1
Methiocarb	0.1			3	25.2
Prochloraz	18	0.9	1.9	<0.1	0.1
Pyriproxyfen	0.02	1625.6	2515.7	13.2	317
Tebuconazole	10			0.2	1.5
Terbutryn	205	<0.1	0.1	<0.1	0.1
Thiabendazole	42			0.1	1.2
Chronic 21 days NOEC in Fish					
Azinphos Methyl	0.17			0.6	13.6
Buprofezin	52	0.1	0.2		
Carbendazim	3.2			0.9	3.6
Chlorfenvinphos	30	0.6	1.4	<0.1	0.1
Chlorpyrifos	0.14	42.6	117.2	2.3	20.4
Dichlofenthion	4	3.2	5.7		
Dimethoate	400	<0.1	<0.1	<0.1	0.2
Diuron	410	<0.1	0.4	<0.1	0.1
Fenitrothion	88	<0.1	<0.1	<0.1	0.4
Fenoxon Sulfone	23	<0.1	0.1		
Hexythiazox	40	0.2	0.3	<0.1	<0.1
Imazalil	43	1.4	9.5	0.2	2.8
Malathion	91			<0.1	0.1
Methiocarb	50			<0.1	0.1
Prochloraz	49	0.3	0.7	<0.1	<0.1
Pyriproxyfen	4.3	5.7	8.8	<0.1	1.1
Simazine	700	<0.1	0.1		
Tebuconazole	12			0.2	1.3
Terbutryn	104	<0.1	0.1	0.1	0.3
Thiabendazole	12			0.3	4.1

November; a period characterized by lower pesticide discharge, and in 24 points sampled, demonstrating a high impact in the delta and marine ecosystems. The ecotoxicological assessment point out that exist a chronic toxicity (RQ index) caused by pesticides (organophosphorus, azol, carbamates and juvenile hormone mimics) in three trophic levels (algae, daphnia and fish), specially in *Daphnia magna*. The Toxic unit for water and sediments, calculated to assess the effects of the cocktail of pesticide residues and know the specific sites impacted, showed the daphnia as the most sensitive in 2010 along the basin. According to the TUs, there are not acute effects due to pesticide concentrations either in water or sediments. However, several pesticides showed a RQ > 1 indicating that pesticide risk to the aquatic communities needs further study. A long-term chronic study on assessment of these mixtures is highly required.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2015.12.059.

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SUPLEMENTARY MATERIAL

Pesticides in the Ebro River Basin: Occurrence and risk assessment

Alexander Ccanccapa[*,1], Ana Masiá[1], Alicia Navarro[2], Yolanda Picó[1], Damià Barceló[2,3]

[1]Food and Environmental Safety Research Group (SAMA-UV), Facultat de Farmàcia, Universitat de València, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain.

[2] Water and Soil Quality Research Group, Dep. of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain

[3] Catalan Institute for Water Research (ICRA), H2O Building, Scientific and Technological Park of the University of Girona, Emili Grahit 101, 17003 Girona, Spain

[*] Corresponding autor: Alexander Ccanccapa
Tel: +34 963543092; Fax: +34 963544954
E-mail: Alexander.Ccanccapa@uv.es

Table S-1: Physico-chemical properties

	CAS Number	Chemical Formula	PM (g/mol)	log Kow (pH 7, 20 °C)	Field Half Life (Days)	Water Solubility (mg/L)	Biocide Action	Chemical Family	Status
Hexythiazox	78587-05-0	$C_{17}H_{21}ClN_2O_2S$	352.88	2.67 (low)	24.6	0.5	Acaricide	Other Pesticides	Approved 2011/46/EUReg. (EU) No 540/2011
Propanil	709-98-8	$C_9H_9Cl_2NO$	218.08	2.29 (low)	3	225	Herbicide	Anilide	Not Approved Reg. (EU) No 1078/2011 (2008/769)
Imazalil	35554-44-0	$C_{14}H_{14}Cl_2N_2O$	297.18	2.56 (low)	120 to 190	180	Fungicide	Azol	Approved Reg. (EU) No 705/2011 (1997/73/EC.2007/21/EC.2010/57/EU.Reg. (EU) No 540/2011)
Prochloraz	67747-09-5	$C_{15}H_{16}Cl_3N_3O_2$	376.7	3.5 (high)	60	27	Fungicide	Azol	Approved Reg. (EU) No 1143/2011 (2008/934)
Thiabendazole	148-79-8	$C_{10}H_7N_3S$	201.25	2.39 (low)			Fungicide	Bencimidazole	Approved 01/21/ECReg. (EU) No 540/2011
Carbendazim	10605-21-7	$C_9H_9N_3O_2$	191.21	1.48 (low)	8	22	Fungicide	Benzimidazole	Not Approved Reg. (EU) No 540/2011Reg. (EU) No 542/2011 (2006/135/EC.2010/70/EC.2011/58/EU)
3-Hydroxycarbofuran	16655-82-6	$C_{12}H_{15}NO_4$	237.25	1.45 (low)			Metabolite	Carbamates	

	CAS Number	Chemical Formula	PM (g/mol)	log Kow (pH 7, 20 ºC)	Field Half Life (Days)	Water Solubility (mg/L)	Biocide Action	Chemical Family	Status
Carbofuran	1563-66-2	$C_{12}H_{15}NO_3$	221.26	1.8 (low)	30 - 117	320	Insecticide. Acaricide. Nematicide	Carbamates	Not approved 2007/416
Methiocarb	2032-65-7	$C_{11}H_{15}NO_2S$	225.31	3.18 (high)	4 – 64	27	Insecticide	Carbamates	Approved 07/5/ECReg. (EU) No 187/2014Reg. (EU) No 540/2011
Molinate	2212-67-1	$C_9H_{17}NOS$	187.3	2.86 (moderate)	21	970	Herbicide	Carbamates	Not approved 03/81/EC Reg. (EU) No 540/2011
Acetochlor	34256-82-1	$C_{14}H_{20}ClNO_2$	269.77	4.14 (high)	14	282	Herbicide	Chloroacetanilide	Nor Approved Reg. (EU) No 1372/2011 (2008/934)
Alachlor	15972-60-8	$C_{14}H_{20}ClNO_2$	269.77	3.09 (high)			Herbicide	Chloroacetanilide	Not Approved 06/966/EC
Metolachlor	51218-45-2	$C_{15}H_{22}ClNO_2$	283.8	3.4 (high)	39	530	Herbicide	Chloroacetanilide	Not Approved 2002/2076
Pyriproxyphen	95737-68-1	$C_{20}H_{19}NO_3$	321.37	5.37 (high)	3 to 16	0.36	Insecticide	Juvenile Hormone Mimics	
Imidacloprid	138261-41-3	$C_9H_{10}ClN_5O_2$	255.66	0.57 (low)	40	610	Insecticide	Neonicotinoid	Approved Reg. (EU) No 485/2013Reg. (EU) No 540/2011 (2008/116/EC.2010/21/EU)
Azinphos-ethyl	2642-71-9	$C_{12}H_{16}N_3O_3PS_2$	345.38	3.18 (high)			Insecticide. Acaricide	Organophosphorus	

	CAS Number	Chemical Formula	PM (g/mol)	log Kow (pH 7, 20 °C)	Field Half Life (Days)	Water Solubility (mg/L)	Biocide Action	Chemical Family	Status
Azinphos-methyl	86-50-0	$C_{10}H_{12}N_3O_3PS_2$	317.32	2.96 (moderate)	10	29	Insecticide. Acaricide	Organophosphorus	Not Approved Reg 1335/2005
Chlorfenvinphos	470-90-6	$C_{12}H_{14}Cl_3O_4P$	359.6	3.8 (high)	10 – 45	145	Insecticide. Acaricide	Organophosphorus	Not Approved 2002/2076
Chlorpyrifos	5598-13-0	$C_7H_7Cl_3NO_3PS$	322.53	4 (high)	21	8	Insecticide. Acaricide	Organophosphorus	Approved EC 1107/2009
Diazinon	333-41-5	$C_{12}H_{21}N_2O_3PS$	304.35	3.69 (high)	60	18.4	Insecticide. Acaricide. Repellent	Organophosphorus	Not Approved 2007/393
Diclofenthion	97-17-6	$C_{10}H_{13}Cl_2O_3PS$	315.15	5.14 (high)			Insecticide	Organophosphorus	
Dimethoate	60-51-5	$C_5H_{12}NO_3PS_2$	229.26	0.704 (low)	4 – 122	25	Insecticide. Acaricide	Organophosphorus	Approved 07/25/EC Reg. (EU) No 540/2011
Ethion	563-12-2	$C_9H_{22}O_4P_2S_4$	384.48	5.07 (high)	56	2	Insecticide. Acaricide	Organophosphorus	Not Approved 2002/2076
Fenitrothion	122-14-5	$C_9H_{12}NO_5PS$	277.23	3.32 (high)	4 – 54	21	Insecticide	Organophosphorus	Not approved 2007/379
Fenoxon	3254-63-5	$C_{10}H_{15}O_4PS$	262.26	-			Insecticide	Organophosphorus	
Fenoxon sulfone	14086-35-2	$C_{10}H_{15}O_6PS$	294.26	-	16.5		Insecticide (Metabolite)	Organophosphorus	
Fenoxon sulfoxide	6552-13-2	$C_{10}H_{15}O_5PS$	278.26	-			Insecticide (Metabolite)	Organophosphorus	
Fenthion	55-38-9	$C_{10}H_{15}O_3PS_2$	278.33	4.84 (high)	34	2	Insecticide	Organophosphorus	Not Approved 04/140/EC

	CAS Number	Chemical Formula	PM (g/mol)	log Kow (pH 7, 20 ºC)	Field Half Life (Days)	Water Solubility (mg/L)	Biocide Action	Chemical Family	Status
Fenthion sulfone	3761-42-0	$C_{10}H_{15}O_5PS_2$	310.1	2.25 (low)			Metabolite	Organophosphorus	
Fenthion sulfoxide	3761-41-9	$C_{10}H_{15}O_4PS_2$	294.1	1.92 (low)			Metabolite	Organophosphorus	
Malathion	121-75-5	$C_{10}H_{19}O_6PS_2$	330.36	2.75 (moderate)	4 – 6	145	Insecticide. Acaricide	Organophosphorus	Approved 2010/17/EU Reg. (EU) No 540/2011
Parathion-ethyl	56-38-2	$C_{10}H_{14}NO_5PS$	291.26	3.83 (high)		50 - 60	Insecticide. Acaricide	Organophosphorus	
Tolclophos-methyl	57018-04-9	$C_9H_{11}Cl_2O_3PS$	301.13	4.56 (high)			Fungicide	Organophosphorus	
Omethoate	1113-02-6	$C_5H_{12}NO_4PS$	213.2	(-) 0.74 (low)			Insecticide. Acaricide	Organophosphorus	Not Approved 2002/2076
Parathion-methyl	298-00-0	$C_8H_{10}NO_5PS$	263.21	3 (moderate)	10 – 60	50 - 60	Insecticide	Organophosphorus	Not Approved 03/166/EC
Buprofezin	69327-76-0	$C_{16}H_{23}N_3OS$	305.44	4.93 (high)			Insecticide. Acaricide	Other pesticides	Approved 2011/6/EUReg. (EU) No 540/2011 (2008/771/EC)
Atrazine	1912-24-9	$C_8H_{14}ClN_5$	215.68	2.7 (moderate)	60	33	Herbicide	Triazine	Not Approved 2004/248/EC
Deisopropylatrazine	1007-28-9	$C_5H_8ClN_5$	173.6	1.15 (low)			Metabolite	Triazine	
Deethylatrazine	6190-65-4	$C_6H_{10}ClN_5$	187.63	1.51 (low)			Metabolite	Triazine	
Propazine	139-40-2	$C_9H_{16}ClN_5$	229.71	3.95 (high)	35 to 231	5	Herbicide	Triazine	Not Approved 2002/2076

	CAS Number	Chemical Formula	PM (g/mol)	log Kow (pH 7, 20 ºC)	Field Half Life (Days)	Water Solubility (mg/L)	Biocide Action	Chemical Family	Status
Simazine	122-34-9	$C_7H_{12}ClN_5$	201.66	2.3 (low)	57	5	Herbicide	Triazine	Not Approved 04/247/EC
Terbumeton-deethyl	30125-64-5	$C_8H_{15}N_5O$	197.24				Herbicide	Triazine	
Terbuthylazine	5915-41-3	$C_9H_{16}ClN_5$	229.71	3.4 (high)	18		Herbicide. Microbiocide. Algaecide	Triazine	Approved Reg. (EU) No 820/2011 (2008/934/EC)
Terbuthylazine-2-hydroxy	66753-07-9	$C_9H_{17}N_5O$	211.33	-	14 to 28	25	Metabolite	Triazine	
Terbutryn	886-50-0	C10H19N5S	241.36	3.34	14 to 28	25		Triazine	Not Approved 2002/2076
Terbuthylazine-deethyl	30125-63-4	$C_7H_{12}ClN_5$	201.68	2.3 (low)			Metabolite	Triazine	
Tebuconazole	107534-96-3	$C_{16}H_{22}ClN_3O$	307.82	3.7 (high)	62	36	Fungicide	Triazole	Approved 2008/125Reg. (EU) No 540/2011Reg. (EU) No 921/2014
Diuron	330-54-1	$C_9H_{10}Cl_2N_2O$	233.09	2.87 (moderate)	68	22 to 42	Herbicide	Urea	Approved 08/91/EC Reg. (EU) No 540/2011
Isoproturon	34123-59-6	$C_{12}H_{18}N_2O$	206.3	2.5 (low)			Herbicide	Urea	A proved 02/18/EC Reg. (EU) No 540/2011

Table S-2: Sampling points in the Ebro Basin

CODE	RIVER	LOCATION	COORDINATES UTM		
			ZONE	X	Y
EBR1	Ebro	Nestares	30	405193	4761644
OCA	Oca	Oña	30	466118	4731520
EBR2	Ebro	Miranda de Ebro	30	503672	4726140
ZAD	Zadorra	Villodas	30	517732	4742302
EBR3	Ebro	Haro	30	513141	4715725
NAJ	Nájerilla	San Asensio	30	523620	4703281
ARG	Arga	Echauri	30	602161	4740847
EBR4	Ebro	Mendavia	30	565335	4696194
EBR5	Ebro	El Bocal (Tudela)	30	619147	4653811
GAL1	Gállego	Jabarrella	30	714638	4705571
GAL2	Gállego	Villanueva de Gállego	30	681725	4622524
HUE	Huerva	Zaragoza	30	673724	4609044
EBR6	Ebro	Presa de Pina	30	692418	4604252
MAR	Martín	Alcaine	30	693300	4535853
ESE	Ésera	Graus	30	280915	4676203
CIN1	Cinca	Graus	30	271142	4667380
CIN2	Cinca	Monzón	31	264776	4642241
RS	Ribera Salada	Inglabaga	31	370389	4658269
SEG	Segre	Torres de Segre	31	292482	4601301
MAT	Matarranya	Nonaspe	31	262933	4564305
ALG	Algars	Batea	31	265824	4554895
EBR7	Ebro	Ascó	31	299521	4559714
EBR8	Ebro	Tortosa	31	294619	4513636
EBR9	Ebro	Delta del Ebre	31	306788	4509432

Table S-3. Instrumental determination characteristics

LC CONDITIONS	
Analytical column	Luna C18: 15.0 cm × 0.21 cm. 3 μm particle size (Phenomenex. Torrance. USA)
Column temperature	30° C
Volume injected	5 μL
Mobile phase	(A) Water – (B) methanol both with 10 mM Ammonium Formate
Flow rate	0.4 mL min^{-1}
Linear gradient	0 min (50 % B). 10 min (83 % B). 12 min (83 % B). 12.5 min (98 % B). 15.5 min (98 % B). and return to the initial conditions (equilibration time 12 min)
TRIPLE QUADRUPOLE MS/MS CONDITIONS	
Ionization characteristics and source	MS/MS performed in selected reaction monitoring mode (SRM) with electrospray ionization (ESI) in positive mode
Gas temperature	300° C
Gas flow	10 L min^{-1}
Nebulizer	15 psi
Capillary voltage	4000 V
Chamber current	1.27 μA
Scan type	Dynamic MRM. with MS1 and MS2 at unit resolution and cell acceleration voltage of 7 eV

Table S-4. Dynamic MRM conditions used for LC-MS/MS determination of pesticide residues

Target Pesticide	t_R [(a)] (min)	Δt_R [(b)]	Precursor Ion	SRM_1 C	Frag[(d)] (V)	CE[(e)] (V)	SRM_2 [(f)]	Frag[(d)] (V)	CE[(e)] (V)	SRM_2/SRM_1 (%)(%RSD)[(g)]
Acetochlor	13.1	3	270	224	120	10	148	120	10	32.2 (31)
Alachlor	13.09	3	270	238	80	10	162	80	15	85.7 (79)
Atrazine	9.06	2.5	216	174	120	15	132	120	20	16.6 (3)
Atrazine-deethyl	3.82	2.2	188	146	120	15	104	121	24	29.8 (1)
Atrazine-deisopropyl	2.62	1.5	174	132	120	15	96	120	15	117.9 (13)
Azinphos-ethyl	12.9	2	346	137	80	20	97	80	32	80.7 (5)
Azinphos-methyl	10.03	2	318	132	80	8	125	80	12	57.3 (24)
Buprofezin	16.83	1.8	306	201	120	10	116	120	15	61.3 (4)
Carbendazim	3.91	3.5	192	160	95	17	132	95	25	10.3 (2)
Carbofuran	6.53	2	222	165	120	10	123	120	15	61.3 (4)
Carbofuran-3-hydroxy	2.75	2	255	220	70	5	163	70	15	80 (11)
Chlorfenvinphos	14.53	1.8	359	155	120	10	127	120	15	82.4 (28)
Chlorpyrifos	17.02	2	350	198	92	13	97	92	33	88.5 (0)
Diazinon	14.57	1.5	305	169	128	21	153	128	17	86.9 (74)
Dichlofenthion	17.02	1.5	315	287	120	5	259	120	10	46.7 (8)
Dimethoate	3.06	2.1	230	199	80	5	171	80	10	37.5 (12)
Diuron	9.82	2.5	233	160	120	20	72	120	20	4.0 (2)
Ethion	17.01	2	385	199	80	5	171	80	15	38.5 (3)
Fenitrothion	12.45	1.5	278	125	140	15	109	121	12	61.6 (55)
Fenoxon-Sulfone	7.13	2.5	295	280	136	13	109	136	33	71.6 (23)
Fenoxon-Sulfoxide	14.33	2	279	247	114	5	169	114	13	70.7 (27)
Fenthion	14.33	2	279	247	114	5	169	114	13	70.7 (27)
Fenoxon	16.51	2	263	231	128	9	216	128	21	34.5 (6)
Fenthion-Sulfone	7.89	2	311	125	146	17	109	146	21	59.4 (2)
Fenthion-Sulfoxide	7.13	3	295	280	136	13	109	136	33	71.6 (23)
Hexythiazox	17.24	1.8	353	228	120	10	168	120	20	60.7 (4)
Imazalil	14.31	2	297	201	120	15	159	120	20	57.2 (3)
Imidacloprid	2.37	1.8	256	209	80	10	175	80	10	60.2 (19)
Isoproturon	9.45	2.5	207	165	120	10	72	120	20	16.7 (1)
Malathion	12.08	2	331	127	80	5	99	80	10	78.7 (37)

Target Pesticide	t_R [(a)] (min)	Δt_R [(b)]	Precursor Ion	SRM_1 [(c)]	Frag [(d)] (V)	CE [(e)] (V)	SRM_2 [(f)]	Frag [(d)] (V)	CE [(e)] (V)	SRM_2/SRM_1 (%)(%RSD) [(g)]
Methiocarb	11.45	2	226	169	80	5	121	80	10	75.4 (9)
Metolachlor	13.01	2	284	252	120	10	176	120	15	10.2 (1)
Molinate	11.89	1.02	188	126	80	10	55	80	20	56.0 (9)
Omethoate	1.68	1.5	214	183	80	5	125	80	20	75.6 (3)
Parathion-ethyl	13.93	1.5	292	264	88	4	236	88	8	40.9 (5)
Parathion-methyl	10.77	1.5	264	232	110	5	125	120	20	14.30
Prochloraz	14.95	2	376	308	80	10	266	80	10	21.1 (12)
Propanil	11.48	2	218	162	120	15	127	120	20	64.6 (40)
Propazine	11.16	2	230	188	120	15	146	120	20	90.5 (9)
Pyriproxyfen	17.01	1.5	322	227	120	10	185	120	10	30.1 (4)
Simazine	6.61	2	202	132	120	20	124	120	20	81.8 (15)
Tebuconazole	14.31	2	308	125	95	25	70	95	21	5.1 (1)
Terbumeton	11.46	2	226	170	95	17	114	95	25	13.0 (0)
Terbumeton-deethyl	7.2	2	198	142	90	13	86	90	25	28.5 (2)
Terbuthylazine	11.51	1.5	230	174	95	13	96	95	25	13.3 (6)
Terbuthylazine-2-hydroxy	7.5	3	212	156	95	13	86	95	25	27.1 (1)
Terbuthylazine-deethyl	7.51	2	202	146	95	13	79	95	25	9.7 (4)
Terbutryn	13.22	2	242	186	120	15	71	120	20	4.4 (1)
Thiabendazole	5.3	3	202	175	95	25	131	95	25	34.7 (1)
Tolclofos-methyl	15.03	2	301	269	120	15	125	115	12	112.0 (49)

(a) t_R = retention time; (b) Δt_R = delta retention time —the centered retention time window; (c) SRM_1 = selected reaction monitoring transition for quantification; (d) Frag = fragmentor; (e) CE = collision energy; (f) SRM_2 = selected reaction monitoring transition for qualification; (g) (%RSD) = relative standard deviation of the ratio SRM_2/SRM_1 — calculated from mean values obtained from the matrix-matched calibration curves

Table S-5. Recoveries of the selected pesticides and Relative Standard Deviations (RSD %) at a concentration of 10 ng/L in water and 25 ng/g for sediments and biota; LODs and LOQs obtained for the three matrices tested.

	Water				Sediment				Biota			
Target pesticide	Recovery	RSD (%)	LOD (ng/L)	LOQ (ng/g)	Recovery	RSD (%)	LOD (ng/L)	LOQ (ng/g)	Recovery	RSD (%)	LOD (ng/L)	LOQ (ng/g)
Acetochlor	66	4	2,00	6,00	61	12	1,67	5,00	72	22	1,67	5,00
Alachlor	58	11	2,00	6,00	75	13	1,67	5,00	84	15	1,67	5,00
Atrazine	65	17	1,30	4,00	40	10	1,08	3,25	76	8	2,44	7,31
Atrazine-desethyl	56	6	2,00	6,00	40	14	1,67	5,00	78	5	3,75	11,25
Atrazine-desisopropyl	54	3	2,00	6,00	99	12	1,67	5,00	72	9	3,75	11,25
Azinphos-ethyl	58	17	0,50	1,50	98	12	0,42	1,25	89	10	0,94	2,81
Azinphos-methyl	51	7	0,50	1,50	98	14	0,42	1,25	91	14	0,94	2,81
Buprofezin	52	19	0,50	1,50	62	11	0,42	1,25	84	15	0,94	2,81
Carbendazim	65	16	0,01	0,04	40	10	0,03	0,10	94	15		
Carbofuran	58	13	0,20	0,60	77	11	0,17	0,50	140	18	0,28	0,90
Carbofuran-3-hydroxy	67	5	0,20	0,60	42	13	0,17	0,50	98	18	0,38	1,13
Chlorfenvinphos	61	18	0,20	0,60	42	20	0,17	0,50	88	9	0,38	1,13
Chlorpyriphos	52	4	0,20	0,60	44	11	0,17	0,50	84	10	0,38	1,13
Diazinon	49	6	0,04	0,20	60	15	0,03	0,10	84	8	0,08	0,23
Dichlofenthion	65	15	0,50	1,50	62	12	0,42	1,25	70	9	0,94	2,81
Dimethoate	57	4	1,00	3,00	64	11	0,83	2,50	80	12	0,94	2,80
Diuron	49	11	1,00	5,00	43	11	0,83	2,50	70	15	1,88	5,63
Ethion	54	4	0,50	1,50	42	14	0,42	1,25	88	11	0,94	2,81
Fenitrothion	67	12	2,00	6,00	78	9	1,67	5,00	84	10	3,75	11,25
Fenoxon	78	7	0,40	2,00	78	13	0,34	1,00	68	10	0,76	2,26
Fenoxon-	57	12	0,20	1,00	105	10	0,17	0,50	89	13	0,38	1,13

Sulfone												
Fenoxon-Sulfoxide	53	4	0,20	1,00	91	14	0,17	0,50	128	15	0,38	1,13
Fenthion	51	10	0,20	1,00	62	13	0,17	0,50	87	12	0,38	1,13
Fenthion-Sulfone	58	3	0,20	1,00	63	12	0,17	0,50	105	8	0,38	1,13
Fenthion-Sulfoxide	61	11	0,20	1,00	44	12	0,17	0,50	90	15	0,38	1,13
Hexythiazox	70	6	0,20	1,00	78	12	0,17	0,50	75	10	0,38	1,13
Imazalil	65	20	0,30	1,00	64	13	0,25	0,75	60	10	0,56	1,69
Imidacloprid	60	9	0,04	0,20	64	12	0,03	0,10	80	11	0,08	0,23
Isoproturon	56	7	0,30	1,00	64	10	0,25	0,75	80	9	0,56	1,69
Malathion	51	9	0,30	1,00	43	13	0,25	0,75	100	9	0,56	1,69
Methiocarb	66	5	0,30	1,00	41	11	0,25	0,75	85	8	0,56	1,69
Methoalachlor	52	8	0,30	1,00	61	12	0,25	0,75	96	6	0,56	1,69
Molinate	61	17	0,50	1,50	63	14	0,42	1,25	84	10	0,94	2,81
Omethoate	54	6	0,30	1,00	92	10	0,25	0,75	58	15	0,56	1,69
Parathion-ethyl	61	7	2,00	6,00	42	11	1,67	5,00	94	8	3,75	11,25
Parathion-methyl	68	17	2,00	6,00	40	11	1,67	5,00	96	10	3,75	11,25
Prochloraz	53	14	0,80	6,00	78	11	0,67	2,00	70	13	1,50	4,50
Propanil	57	3	0,30	1,00	44	12	0,25	0,75	68	11	0,60	1,69
Propazine	62	14	0,30	1,00	63	14	0,25	0,75	82	11	0,45	1,50
Pyriproxifen	67	14	0,50	1,50	43	15	0,42	1,25	92	8	0,94	2,81
Simazine	58	8	2,00	6,00	63	13	1,67	5,00	82	17	3,75	11,25
Tebuconazole	49	11	0,13	0,40	43	12	0,33	1,00	80	8	-	-
Terbumeton	70	7	0,01	0,04	78	12	0,03	0,10	90	9	-	-
Terbumeton-desethyl	66	4	0,13	0,40	61	12	0,33	1,00	85	11	-	-
Terbuthylazine	67	13	0,01	0,04	78	9	0,03	0,10	85	8	-	-
Terbuthylazine-2-hidroxy	65	17	0,01	0,04	40	9	0,03	0,10	88	13	-	-
Terbuthylazine-	52	5	0,01	0,04	44	10	0,03	0,10	87	12	-	-

deethyl												
Terbutryn	65	12	0,13	0,40	80	10	0,33	1,00	84	9		
Thiabendazole	54	4	0,13	0,40	42	14	0,33	1,00	92	7		
Tolclofos-methyl	66	12	0,50	1,50	41	12	0,42	1,25	90	8	0,94	2,81

* Both calculated using spiked matrices, were defined as the minimum amount of analyte whose qualified transition (SRM2) present a signal-to-noise ratio (S/N) ≥3 and ≥10, respectively. Out by spiking a pure water sample at 10 ng/L (low spike) and 100 ng/L (high spike) of each pesticide. For sediment and biota the spiked levels were of 25 ng/L (low spike) and 100 ng/L (high spike) of each pesticide

Table S-6. Flows in each sampling point in Ebro basin

CODE	CORRESPONDENCE		1st CAMPAIGN			2nd CAMPAIGN			% variation
	SAIH	SAICA	date	flow	% total	date	flow	% total	
EBR1	A178	-	07/10/2010	1.21	25%	20/09/2011	0.61	4%	-21%
OCA	A093	922	06/10/2010	1.38	25%	20/09/2011	0.75	2%	-23%
EBR2	A001	901	06/10/2010	27.4	-	19/09/2011	25.7	-	-
ZAD	A74 - A75	-	07/10/2010	1.86	21%	21/09/2011	1.42	7%	-14%
EBR3	-	907	06/10/2010	-	-	20/09/2011	-	-	-
NAJ	A038	925	06/10/2010	3.74	38%	20/09/2011	9.99	68%	30%
ARG	A069-A068	903	07/10/2010	3.71	44%	21/09/2011	1.53	13%	-31%
EBR4	A120	908	06/10/2010	25.78	12%	19/09/2011	30.6	21%	9%
EBR5	A284	902	05/10/2010	85.9	-	19/09/2011	57	-	-
GAL1	A123	904	29/09/2010	9.97	12%	21/09/2011	7.04	4%	-8%
GAL2	A209	919	30/09/2010	10.24	32%	22/09/2011	9.99	29%	-3%
HUE	A216	-	30/09/2010	1.29	46%	22/09/2011	2.14	66%	20%
EBR6	A011 + A089		05/10/2010	79.88	43%	22/09/2011	43.94	11%	-32%
MAR	A127	928	05/10/2010	0.22	19%	26/09/2011	0.22	19%	0%
ESE	EA13		29/09/2010	7.63	-	28/09/2011	5.57	-	-
CIN1	A047	-	29/09/2010	0.35	-	28/09/2011	0.13	-	-
CIN2	-	916	29/09/2010	-	-	28/09/2011	-	-	-
RS	-	-	28/09/2010	-	-	12/09/2011	-	-	-
SEG	A025	-	28/09/2010	27.45	12%	27/09/2011	31.4	23%	11%
MAT	A176		28/09/2010	0.03	18%	26/09/2011	0.01	5%	-13%
ALG	A177	-	28/09/2010	0	-	27/09/2011	0	-	-
EBR7	A163	906	27/09/2010	213.4	50%	27/09/2011	155.43	20%	-30%
EBR8	A027	-	27/09/2010	130.6	28%	26/09/2011	105.18	11%	-17%
EBR9	-	-	27/09/2010	-	-	26/09/2011	-	-	-

Table S-7. RQ for Algae (A). Daphnia (B) and Fish (C)

A)

Pollutants	Chronic 96/72 h NOEC in Algae				
	PNEC ng L^{-1}	2010		2011	
		RQ-Mean	RQ-Max	RQ-Mean	RQ-Max
3-Hydroxycarbofuran					
Acetochlor					
Alachlor					
Atrazine	100	< 0.1	0.1		
Azinphos Ethyl	446				
Azinphos Methyl	1000			< 0.1	< 0.1
Buprofezin	1146	< 0.1	< 0.1		
Carbendazim	302			< 0.1	< 0.1
Carbofuran	3200				
Chlorfenvinphos	1000	< 0.1	< 0.1	< 0.1	< 0.1
Chlorpyrifos	43	0.1	0.4	< 0.1	< 0.1
Deethylatrazine					
Deisopropylatrazine					
Diazinon	10000	< 0.1	< 0.1	< 0.1	< 0.1
Dichlofenthion	204	0.1	0.1		
Dimethoate	32000	< 0.1	< 0.1	< 0.1	< 0.1
Diuron	93	0.1	1.6	< 0.1	0.3
Ethion	129				
Fenitrothion	100	< 0.1	< 0.1	< 0.1	0.4
Fenoxon					
Fenoxon Sulfone	81113	< 0.1	< 0.1		
Fenoxon Sulfoxide					
Fenthion					
Fenthion Sulfone					
Fenthion Sulfoxide					
Hexythiazox	7	1.1	1.5	< 0.1	0.2
Imazalil	92	0.7	4.5	0.1	1.3
Imidacloprid	10000	< 0.1	< 0.1	< 0.1	< 0.1
Isoproturon	52	< 0.1	0.5	< 0.1	< 0.1
Malathion	14993			< 0.1	< 0.1
Methiocarb	3200			< 0.1	< 0.1
Metolachlor	1			0.9	8.2
Molinate					
Parathion-Ethyl					
Prochloraz	10	1.6	3.4	< 0.1	0.2
Propanil					

Pollutants	Chronic 96/72 h NOEC in Algae				
	PNEC ng L^{-1}	2010		2011	
		RQ-Mean	RQ-Max	RQ-Mean	RQ-Max
Propazine	40	< 0.1	0.1		
Pyriproxyfen	213	0.1	0.2	< 0.1	< 0.1
Simazine	600	< 0.1	0.1		
Tebuconazole	100			< 0.1	0.2
Terbumeton					
Terbumeton-Desethyl					
Terbuthylazine					
Terbuthylazine-2 Hydroxy					
Terbutryn	28	< 0.1	0.5	0.3	1.1
Terbuthylazine Deethyl					
Thiabendazole	3200				
Tolclofos Methyl					
Omethoate					
Parathion-Methyl					

B)

Pollutants	Chronic 96/72 h NOEC in Aquatic invertebrates (Daphnia magna)				
	PNEC ng L^{-1}	2010		2011	
		RQ-Mean	RQ-Max	RQ-Mean	RQ-Max
3-Hydroxycarbofuran					
Acetochlor	22				
Alachlor	220				
Atrazine	250	< 0.1	< 0.1		
Azinphos Ethyl	0.42				
Azinphos Methyl	0.4			0.2	5.8
Buprofezin	80	0.1	0.1		
Carbendazim	1.5			1.9	7.8
Carbofuran	8				
Chlorfenvinphos	0.1	179.7	412.4	0.7	15.7
Chlorpyrifos	4.6	1.3	3.6	0.1	0.6
Deethylatrazine					
Deisopropylatrazine					
Diazinon	0.56	10.1	24.2	2.4	36.4
Dichlofenthion	0.04	308.4	545.2		
Dimethoate	40	< 0.1	0.1	0.1	1.5
Diuron	96	0.1	1.6	< 0.1	0.3
Ethion	0.12				

Pollutants	Chronic 96/72 h NOEC in Aquatic invertebrates (Daphnia magna)				
	PNEC ng L^{-1}	2010		2011	
		RQ-Mean	RQ-Max	RQ-Mean	RQ-Max
Fenitrothion	0.09	1.3	30.3	17.5	419.4
Fenoxon					
Fenoxon Sulfone	256022	< 0.1	< 0.1		
Fenoxon Sulfoxide					
Fenthion					
Fenthion Sulfone					
Fenthion Sulfoxide					
Hexythiazox	6.1	1.2	1.7	< 0.1	0.2
Imazalil	15	4.1	27.3	0.5	8.1
Imidacloprid	1800	< 0.1	< 0.1	< 0.1	< 0.1
Isoproturon	120	< 0.1	0.2	< 0.1	< 0.1
Malathion	0.06			5.5	132.1
Methiocarb	0.1			3.0	25.2
Metolachlor	707			< 0.1	< 0.1
Molinate					
Parathion-Ethyl					
Prochloraz	18	0.9	1.9	< 0.1	0.1
Propanil					
Propazine	420	< 0.1	< 0.1		
Pyriproxyfen	0.02	1625.6	2515.7	13.2	317.0
Simazine	2500	< 0.1	< 0.1		
Tebuconazole	10			0.2	1.5
Terbumeton					
Terbumeton-Desethyl					
Terbuthylazine					
Terbuthylazine-2 Hydroxy					
Terbutryn	205	< 0.1	0.1	< 0.1	0.1
Terbuthylazine Deethyl					
Thiabendazole	42			0.1	1.2
Tolclofos Methyl					
Omethoate					
Parathion-Methyl					

C)

Pollutants	Chronic 21 days NOEC in Fish				
		2010		2011	
	PNEC ng L^{-1}	RQ-Mean	RQ-Max	RQ-Mean	RQ-Max
3-Hydroxycarbofuran					
Acetochlor	130				
Alachlor	190				
Atrazine	2000	< 0.1	< 0.1		
Azinphos Ethyl	21				
Azinphos Methyl	0.17			0.6	13.6
Buprofezin	52	0.1	0.2		
Carbendazim	3.2			0.9	3.6
Carbofuran	2.2				
Chlorfenvinphos	30	0.6	1.4	< 0.1	0.1
Chlorpyrifos	0.14	42.6	117.2	2.3	20.4
Deethylatrazine					
Deisopropylatrazine					
Diazinon	700	< 0.1	< 0.1	< 0.1	< 0.1
Dichlofenthion	4	3.2	5.7		
Dimethoate	400	< 0.1	< 0.1	< 0.1	0.2
Diuron	410	< 0.1	0.4	< 0.1	0.1
Ethion	12				
Fenitrothion	88	< 0.1	< 0.1	< 0.1	0.4
Fenoxon					
Fenoxon Sulfone	23	< 0.1	0.1		
Fenoxon Sulfoxide					
Fenthion					
Fenthion Sulfone					
Fenthion Sulfoxide					
Hexythiazox	40	0.2	0.3	< 0.1	< 0.1
Imazalil	43	1.4	9.5	0.2	2.8
Imidacloprid	9020				
Isoproturon	1000				
Malathion	91			< 0.1	0.1
Methiocarb	50			< 0.1	0.1
Metolachlor	373			< 0.1	< 0.1
Molinate					
Parathion-Ethyl					
Prochloraz	49	0.3	0.7	< 0.1	< 0.1
Propanil					
Propazine	277	< 0.1	< 0.1		

Pollutants	Chronic 21 days NOEC in Fish				
		2010		2011	
	PNEC ng L^{-1}	RQ-Mean	RQ-Max	RQ-Mean	RQ-Max
Pyriproxyfen	4.3	5.7	8.8	< 0.1	1.1
Simazine	700	< 0.1	0.1		
Tebuconazole	12			0.2	1.3
Terbumeton					
Terbumeton-Desethyl					
Terbuthylazine					
Terbuthylazine-2 Hydroxy					
Terbutryn	104	< 0.1	0.1	0.1	0.3
Terbuthylazine Deethyl					
Thiabendazole	12			0.3	4.1
Tolclofos Methyl					
Omethoate					
Parathion-Methyl					

Figure S-1. Location of the sampling sites in the Ebro basin

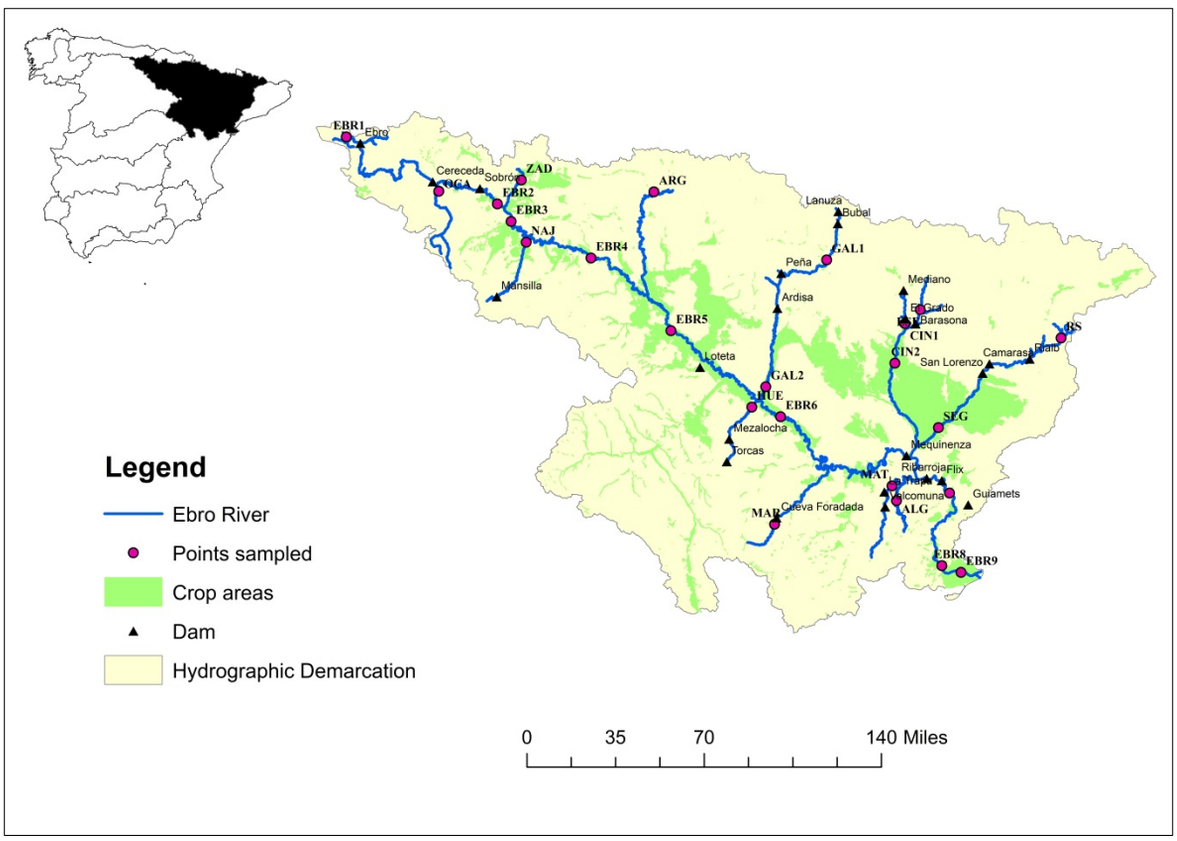


Figure S-2. Pics of the Ebro River and its Tributaries

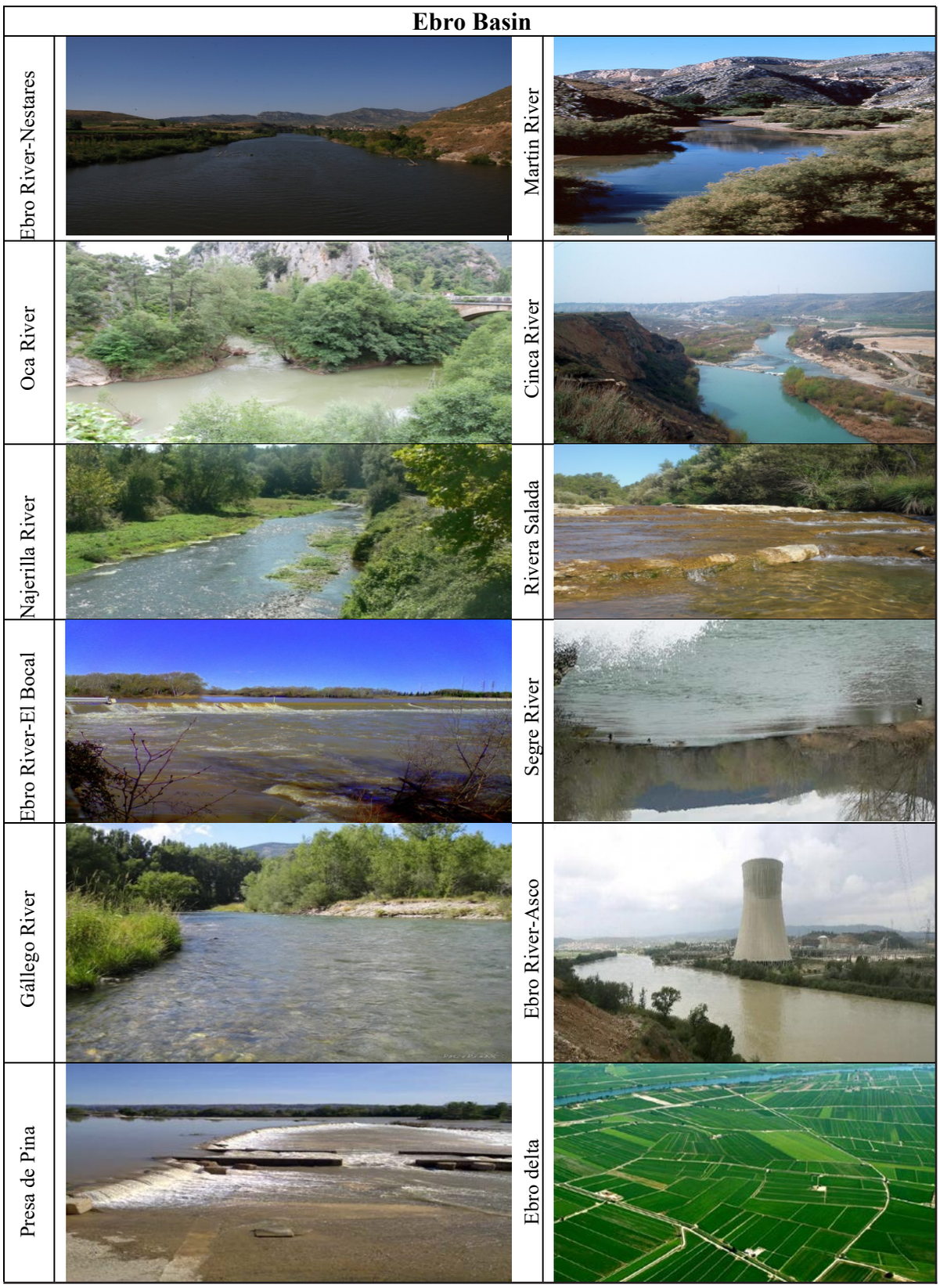
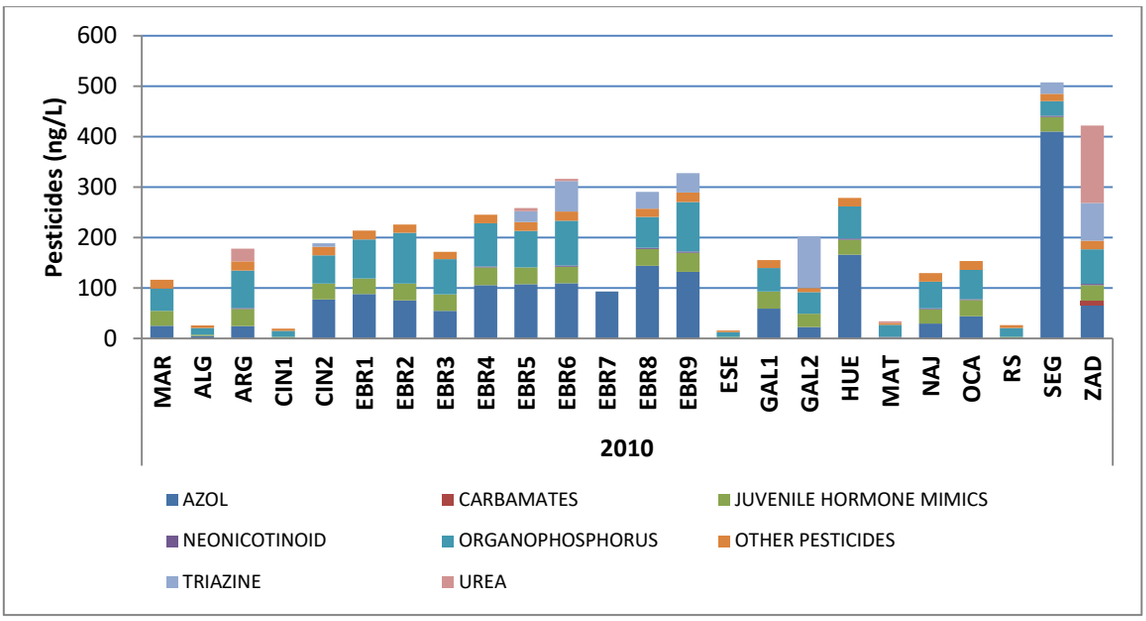
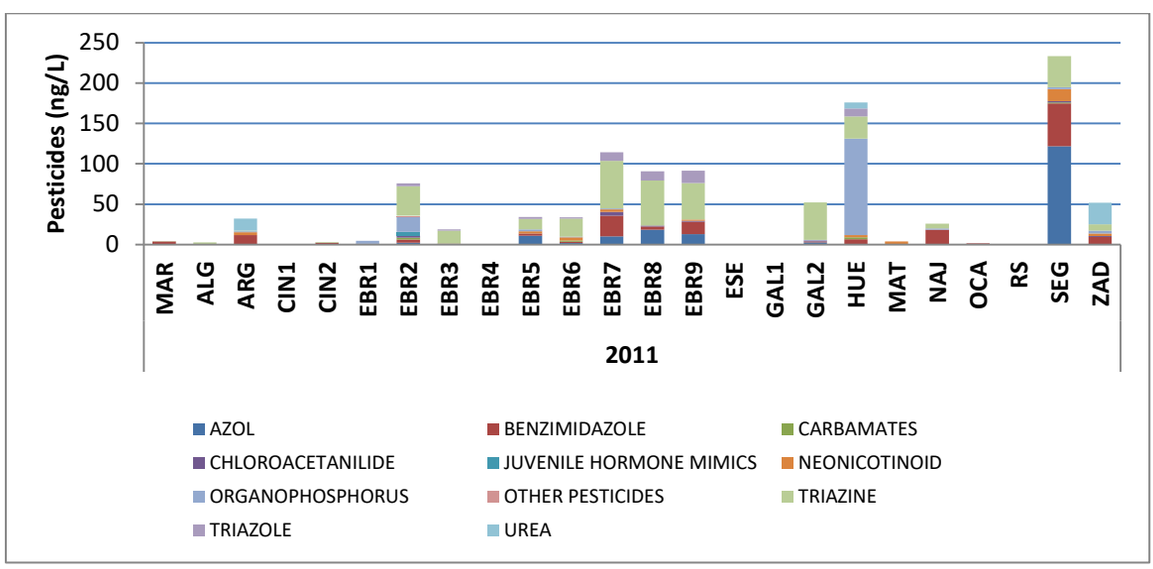


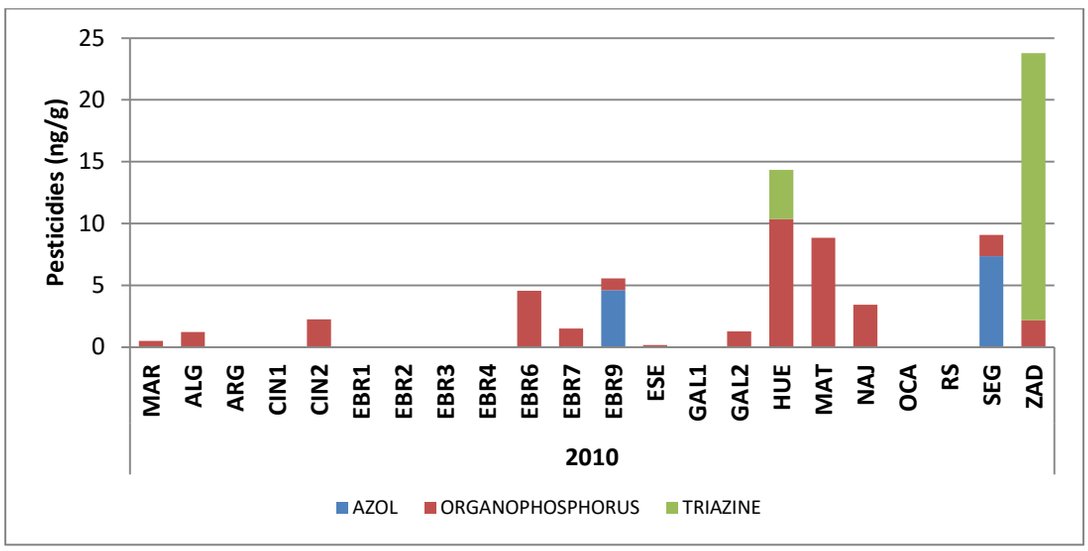
Figure S-3. Sum of pesticide families along the Ebro River in 2010 and 2011 A) Water samples B) Sediment samples and C) Biota samples

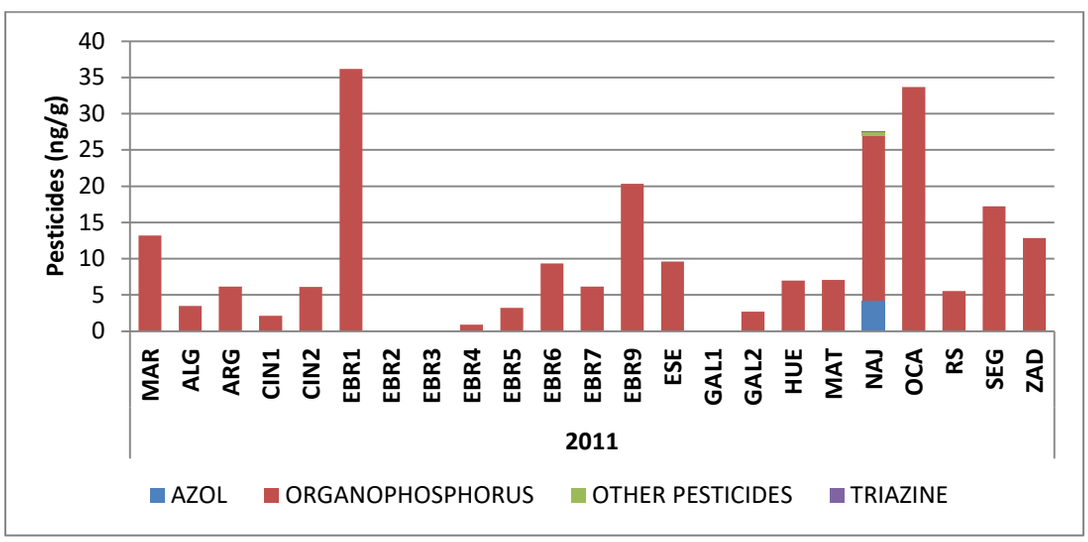
A)





B)


Pesticidies (ng/g)
25
20
15
10
5
0
MAR
ALG
ARG
CIN1
CIN2
EBR1
EBR2
EBR3
EBR4
EBR6
EBR7
EBR9
ESE
GAL1
GAL2
HUE
MAT
NAJ
OCA
RS
SEG
ZAD
2010
AZOL
ORGANOPHOSPHORUS
TRIAZINE


Pesticides (ng/g)
40
35
30
25
20
15
10
5
0
MAR
ALG
ARG
CIN1
CIN2
EBR1
EBR2
EBR3
EBR4
EBR5
EBR6
EBR7
EBR9
ESE
GAL1
GAL2
HUE
MAT
NAJ
OCA
RS
SEG
ZAD
2011
AZOL
ORGANOPHOSPHORUS
OTHER PESTICIDES
TRIAZINE

C)

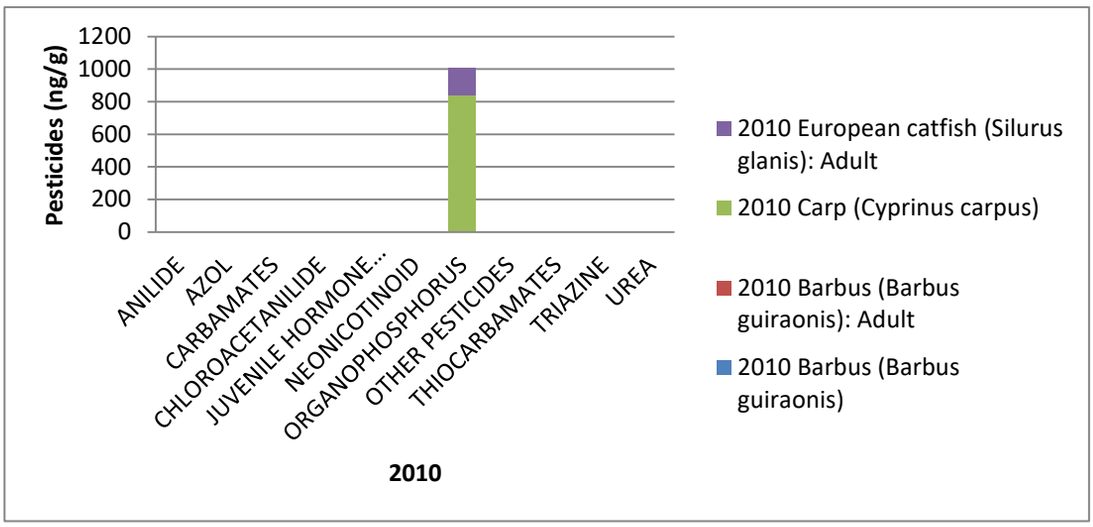
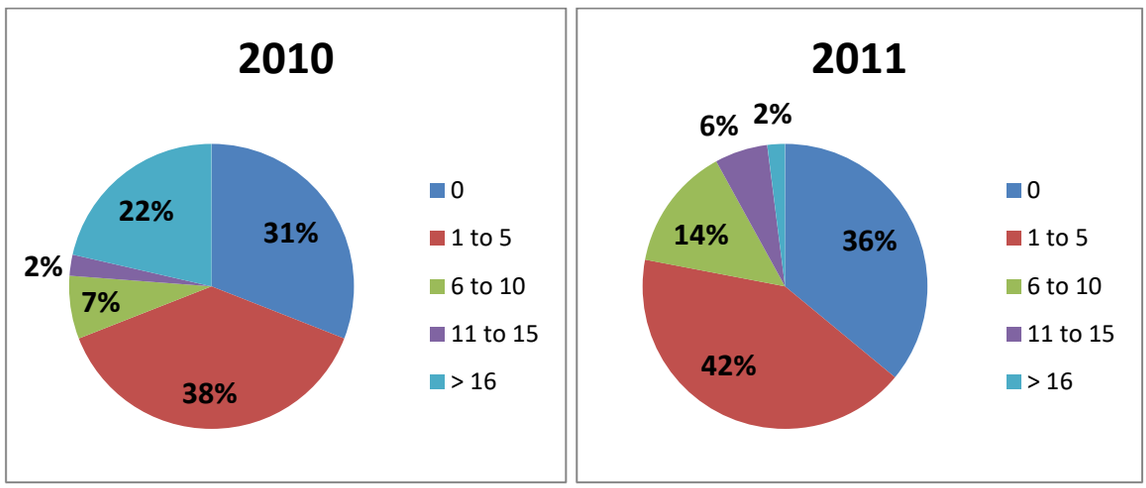
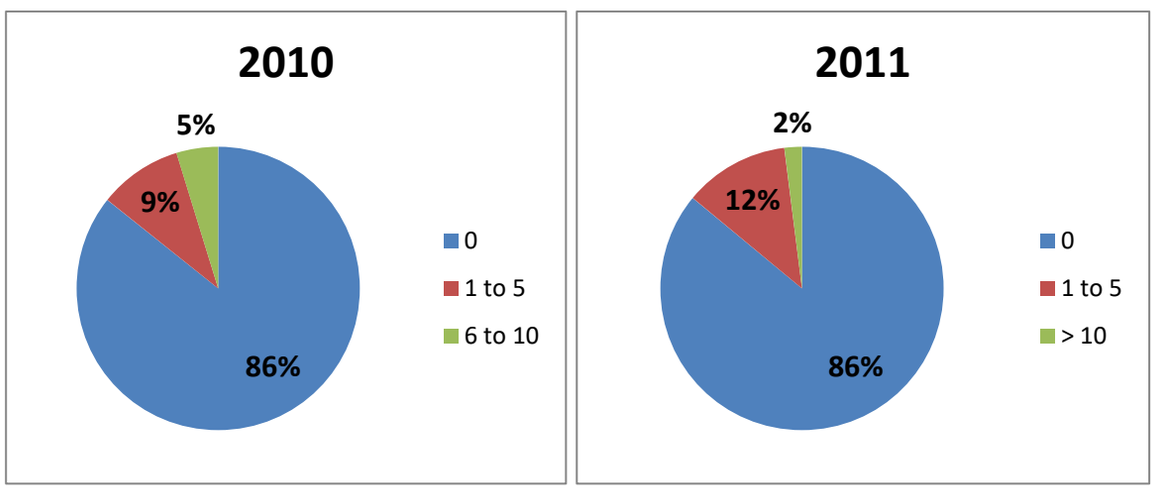

Pesticides (ng/g)
1200
1000
800
600
400
200
0
ANILIDE
AZOL
CARBAMATES
CHLOROACETANILIDE
JUVENILE HORMONE...
NEONICOTINOID
ORGANOPHOSPHORUS
OTHER PESTICIDES
THIOCARBAMATES
TRIAZINE
UREA
2010
2010 European catfish (Silurus glanis): Adult
2010 Carp (Cyprinus carpus)
2010 Barbus (Barbus guiraonis): Adult
2010 Barbus (Barbus guiraonis)

Figure S-4. Co-occurrence of pesticides in A) water samples, B) sediment samples.

A)



B)



VIII.

ANÁLISIS DIRIGIDO, DE AMPLIO ESPECTRO Y NO DIRIGIDO EN EL RÍO TURIA

Aguas y sedimentos del Turia se analizaron en 2016 utilizando un método analítico (UPLC-QqQ-TOF) de análisis dirigido, de amplio espectro, y no dirigido para identificar emergentes utilizando. El análisis de amplio espectro se basó en la utilización de una librería teórica de 2.200 componentes de Water Corporation (plaguicidas, fármacos, drogas de abuso, productos de cuidado personal y toxinas) y el modo de adquisición de datos "Data Independent Acquisition" (DIA). El análisis no dirigido se realizó con la ayuda de bases de datos como el Chem. Spider. Finalmente, se desarrolló un método de análisis dirigido a 170 plaguicidas y 33 fármacos, incluyendo los encontrados en el análisis de amplio espectro y no dirigido.

***PUBLICACIÓN # 4:* "*S*uspect, non-target and target screening of emerging pollutants using Data Independent Acquisition: Assessment of a Mediterranean River basin"** *Environ. Sci. Technol*. (enviada)

Suspect, non-target and target screening of emerging pollutants using Data Independent Acquisition: Assessment of a Mediterranean River basin

Alexander Ccanccapa[a*], Yolanda Pico[a], Xavier Ortiz[b], Eric Reiner[b]

[(a)] Environmental and Food Safety Research Group(SAMA-UV), Desertification Research Centre CIDE (CSIC-UV-GV), Faculty of Pharmacy. University of Valencia. Burjassot, Valencia, Spain

[(b)] Ontario Ministry of the Environment and Climate Change, 125 Resources Road, Toronto (ON), Canada

* Corresponding autor: Alexander Ccanccapa

Tel: +34 963543092; Fax: +34 963544954

E-mail: Alexander.Ccanccapa@uv.es

Abstract

Three approaches (target, suspected and non-target screening) in one workflow based on liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS) was developed to assess the presence of emerging pollutants (EPs) in water and sediment samples of a Mediterranean basin. Identification of the potential contaminants was based on mass accuracy, isotopic pattern, theoretical fragmentation, and retention time using UNIFI software. In suspect screening, a library containing 2,200 components was used against data independent acquisition (DIA); of which 68 compounds were identified, 6 of them confirmed and quantified with the real standard. Untargeted screening was time consuming and required additional manual processing. An online database (Chemspider) was used to identify non-target compounds. Eprosartan, contaminant recently identified close to waste water treatment plant (WWTP), was confirmed and quantified. The strategy and workflow to suspect and untargeted screening is functional. Target screening of 171 pesticides and 33 pharmaceuticals (included components identified in suspected and untargeted screening) confirmed the spatial distribution of EPs in the basin. This information is of global interest when attempting to establish climate-change influences on pollutant-concentrations. QTOF-MS screening versatility and high-resolution potential allow for a comprehensive assessment of EPs in basins.

1. Introduction

Priority and emerging pollutants (EPs), such as pesticides, pharmaceuticals and hormones (PPHs), preservatives from personal care products (PCPs), phthalates or artificial sweeteners have been detected in the aquatic environment[1-7], which can be harmful for biota.[8, 9] EPs are not yet covered by guidelines or legislative intervention that are currently available to regulate their presence in the environment.[10-13] Once EPs are released into the environment, they are subject to biotic and abiotic transformation through the hydrolysis, photolysis, oxidation, and microbial metabolism; thereby, generating transformation products that may be more persistent and toxic than their parent compounds and possibly present at relatively larger concentrations.[13-15] Usually, EPs and their TPs reach to surface and groundwater water via run-off or sub-soil tile drains.[3, 16, 17]

The most common technique to analysis EPs is liquid chromatography–mass spectrometry (LC–MS) with several mass analyzers such as ion trap, triple quadrupole (QqQ), quadrupole linear ion trap (QTRAP), time-of-flight (TOF), quadrupole time-of-flight (QTOF) or orbitrap.[5-7, 17-23] However, target screening methods, where the chemicals are selected in advance, can only cover a relatively small proportion of these organic contaminants. This can result in bias (due to the preselection) and potential chemical stressors may not be detected.[24] In recent years, there is a growing trend to analyze environmental samples beyond the intended target compound list, and a shift towards non-targeted or general unknown screening.[1, 25-27] High-resolution MS (HRMS) such as QTOF and Orbitrap instruments can provide high-quality information by combining sensitive full-spectrum data with high mass resolution (>25,000) and mass accuracy (<2 ppm). In theory, the presence of an unlimited number of compounds can be investigated at the proper sensitivity, without requiring the pre-selection of

analytes or even without having reference standards available.[13, 14, 28] HRMS can be used [14, 29-31] in data-dependent acquisition (DDA) mode, which selects those precursor ions detected in a survey scan meeting some previously defined characteristics for subsequent isolation and fragmentation in a serial manner. Data independent acquisition (DIA) avoids specific selection during LC-MS analysis by co-selection and co-fragmentation.[32-34] However, a DDA approach sometimes diminishes reproducibility (co-elution); furthermore compounds found at the trace level may not be selected during the precursor scan.

The aim of the presented work was monitoring the Turia basin (2016) through suspect screening using a HRMS-DIA against a local library (Waters corporation) that contains 2,200 compounds (pesticides, pharmaceuticals, personal care products and toxins), non-target screening against ChemSpider online database both using UNIFI platform; as well as a target screening with 164 pesticides and 29 personal care and pharmaceutical products PCPP adding the confirmed compounds (purchased standards) in the suspect and non-target steps. The present work also describes the potential and versatility of HRMS workflow as a routine tool that allows a comprehensive analysis of the pollution in the basins.

2. Materials and methods

A list of the origin of materials used, including solvents and standards, can be found in the Supporting Information (**SI-1**).

2.1. Site description and sampling

The area of study is located in the Central-East part of Spain. The Turia River is 243 km long and drains an area of approximately 6250 km^2, making it one of the most important rivers in the Mediterranean basin [2, 3, 35]. Thirty two water samples and twenty-nine

sediment samples were taken homogenously through the course of the river from its source to its mouth on June of 2016 (**Fig. S-1**). Additionally, two effluent samples collected from the Waste Water Treatment Plants (WWTP) Pinedo I and Pinedo II in Valencia (sampling points are georeferenced in **Table S-1**, supplementary material) were included. Water samples were collected at midstream and, 80 cm below the surface, in 1 L polypropylene bottles and; top layer sediment samples (approx. 250 g) were taken at the same point as water samples using a Van Veen grab sampler (0.5 L capacity). They were transferred and wrapped into aluminum foil, then put inside an aluminum box. Immediately after, samples were transported to the laboratory, where they were kept refrigerated at 4.5 ◦C and extracted within 48 h. Sediment samples were frozen (-20° C) and freeze-dried with a Virtis SP Scientific Lyophilizer (Warminster, PA, USA) at -65 °C and vacuum of 1-4 mT for 48 h. Then, lyophilized sediment was sieved through a series of sieves to collect the fraction < 125 μm.

2.2.Extraction procedure

2.2.1. Water samples

The pre-concentration applied to the water samples is based on the off-line Solid Phase Extraction (SPE) procedure described previously [27]. Water samples (200 mL) were passed through the SPE column (flow rate ca. 10 mL min^{-1}) previously conditioned with methanol-dichloromethane and water using vacuum. The cartridges (OASIS HLB 200 mg sorbent/6 mL cartridge, Waters) were then dried under vacuum for 10 min to remove residual water and analytes were eluted with 10 mL of dichloromethane-methanol (50:50, v/v). Extracts were evaporated to dryness at 40 °C under a stream of nitrogen and reconstituted with 1 mL of methanol. Then, they were filtered through 0.45 μm PTFE filters into the vials for LC–MS analysis.

2.2.2. Sediment samples

Stepwise, 5 mL of distilled water, 5 mL of methanol with 5 mL of Macllvaine-EDTA (100 mL of 0.1 M of citric acid, 62.5 mL 0.2 M Na_2HPO_4 and 6.05 g of NA_2-EDTA) buffer were added to 1 g of soil, previously lyophilized. This mix was sonicated during 30 min in a S 120 H Elma Ultrasonic (Singen, Germany) and centrifuged for 6 min at 3000 rpm with a 5810 R Centrifuge from Eppendorf (Hamburg, Germany). The supernatant was collected; 200 mL of distilled water was added, and extracted by SPE as water samples.

2.3.Ultra-high pressure liquid chromatography

The chromatography column, stationary phase and mobile phases were selected in base on a pre-established screening method[27]. The chromatographic instrument was a Waters Acquity UPLC system (Milford, MA, USA). Chromatographic separation was carried out using a column Luna C18 (15.0 cm × 0.21 cm) with a 3 μm particle size (Phenomenex, Torrance, USA). A binary mobile phase of A (10 mM formic acid in water) and B (10 mM formic acid in methanol) was applied with following program: 0-15 min, 10% A; 15-18.5 min, 95% A; 18.5-19 min, 95% A; 19-23 min, 10% A. The analytical column and the sample manager were kept at 35 °C and 7 °C respectively. An aliquot of 10 μL was injected into UPLC-QTOF-MS, flow rate of 0.4 mL/min.

2.4. Quadrupole time-of-flight mass spectrometry

A Xevo G2-S Q-TOF mass spectrometer (Waters, Milford, MA USA) was used in positive ESI for acquisition using two modes, MS for target analysis and DIA (MS^E) for suspect and untargeted analysis. MS mode was set in full scan mode between 100 to 1200 m/z; scan speed at 4 scan/sec; collision energy off; mass accuracy calibrated to

less than 2 ppm; mass resolution more than 24K and XIC tolerance, 20 mDa. DIA mode allows both precursor and product ion data to be simultaneously acquired during a single run. The DIA method consists of three functions, the first (low energy, LE) applies collision energy of 4 eV, the second function (high energy, HE) acquires through a collision energy ramp of 10 - 45 eV and the third function acquires the lock mass data for internal on-the-fly mass calibration. The MS range is 50 - 1200 m/z with a scan time of 0.25 s in continuum mode, preserving the peak shape of the exact-mass precursor and product ions. The source conditions whose maximum intensities were achieved were the following: capillary voltage 2 kV, sample cone, 80 V, source offset 80 V, source temperature 125 °C, desolvation temperature 250 °C, cone gas flow rate 50 L h^{-1}, desolvation gas (N_2) flow rate 600 L h^{-1}.

During the data acquisition the mass was corrected using an external reference (Lock-Spray™) consisting of 0.2 mg mL^{-1} solution of leucine-enkephalin (Waters, Milford, MA, USA) infused continuously at 10 mL min^{-1} via a lockspray interface. This generated a reference ion in positive mode at m/z 556.2771 that was used for real-time mass corrections in order to maintain the mass accuracy and reproducibility.

2.5. Data processing

Data acquisition and processing were performed by using UNIFI screening platform (Waters Corporation, Milford MA, USA) on suspect and untargeted screening (DIA) and Masslynx™ v4.1 (Waters Corporation, Milford MA, USA) on target screening (MS) (**Fig. S-2**).

The workflow (**Fig. 1**) and identification confidence criteria used on suspect and untargeted screening were based on those described by Schymanski et al.[36], Krauss et al.[37] and Pedersen et al (**Fig. S-3**).[38] The analysis method for DIA was performed as

follow: Firstly, all the continuum data was peak-detected using a 3D peak algorithm based on the calculation of the peak volumes by the detection of all the ion crests in a given mountain range of retention-time/m/z-pairs **(Fig. 2).** Secondly the identity of the compounds was established by setting target mass tolerance at 5.0 ppm and fragment match tolerance at 2.0 mDa. The mass defect setting was enabled selecting H^+ adduct. Halogen match setting included four Cl and three Br atoms.

Suspect screening workflow

The library used in the suspected method (Waters, Milford, MA USA) consisted of 2,200 components including pesticides, pharmaceuticals, personal care products among other toxins. Each component included mass spectra, retention time, molecular formula and structure. Suspect screening was performed by matching candidate components on the library with DIA screening results. The tentative identification was based on (filter 1-F1) accurate mass (< 5 ppm), isotope fit (>90%) and shared observed fragments with theoretical fragmentation (5 or more fragments in common, 2 mDa accuracy). Each sample was systematically evaluated following an in-house UNIFI workflow.

Non-targeted screening workflow

Unidentified candidate components on suspect screening, obtained by DIA, were used as candidate masses for the non-targeted screening compared against ChemSpider on line database (over 58 million structures including formula and structure). This step was made manually and was time consuming. The non-targeted screening was performed in seven steps. Step 1 involved selecting the top six (water and sediment) polluted samples obtained in suspect screening and steps 2–6 were performed with UNIFI.

1) Selecting: All samples and candidate components (from suspect screening results) were exported to Microsoft Excel, including observed m/z, RT values,

response, and identification status. Then, the eight samples (water and sediment) that contained more components identified and at high frequency were selected. All unidentified masses that belonged to samples selected were used to for untargeted screening.

2) Filtering: A filter (filter 2 - F2) with the following criteria was used: Chromatographic width radio less than 1; mass peak resolution greater than 7.000, response greater than 25.000 and status non-identified.
3) Elucidation tool set: All the non-identified components filtered were elucidated. (The elucidation of the molecular formula was performed by the UNIFI 'chemical composition' tool, which uses an algorithm, i-FIT™; a similar concept to the Seven Golden Rules by Kind et al.[39] Mass and isotope-intensity errors were used to further specify. Potential molecular formulas were displayed with an i-FIT confidence percentage (representing the relative certainty of the assigned molecular formulas). Any molecular formula with an i-FIT confidence greater than 90% was further evaluated, with the highest scoring molecular formula being preferred in later steps. In addition to the elemental composition containing C, H, N, O, and S, attention must be paid to selecting other elements commonly present in environmental contaminants such as F, Cl and Br.
4) *In silico* ranking: The candidate structures were in silico fragment-matched via the UNIFI software, using a combinatorial fragmentation approach. The combinatorial fragmentation approach attempts to match observed product ions to the candidate structures by disconnecting covalent bonds in the candidate structure. Disconnecting a bond gives a score, with the lowest score being the most probable. The candidate structures are then ranked based on predicted intensity (%-matched intensity of total intensity) and a number of matched

product ions, the most probable structure being the one with highest predicted intensity and highest number of matched product ions. When several structures matched equally well, the most probable candidate structure was picked based on the product ion scoring. Fragment match was set more than 5 with less than 2 mDa of mass error.

5) Matching online database: UNIFI software was used online to match with the ChemSpider Library, including PubChem (over 10,000,000 structures) and Thomson Pharma libraries (over 2,000,000).
6) Tentative identification: All those compounds that followed the filter application were ranked based on number of citations, number of match fragmentation and intensity. If a relevant compound was identified, (HR)MS spectra were sought in the literature for confirmation. The comparison was preformed manually. Reference standards were required for the final confirmation of the identification.

Target screening

Target screening was focused on all 168 pesticides and 30 pharmaceutical compounds, including the compounds identified on suspected and untargeted screening. This step included the compounds identified and confirmed on suspect and untargeted screening. Quantification was performed using external standard calibration (pesticides 0.5, 1, 5, 10, 20, 40, 80 ng mL^{-1} and pharmaceuticals 20, 200 and 2000 ng mL^{-1}).

3. Results and discussion

3.1. Application in environmental samples

3.1.1. Suspect screening

Suspect screening was done on 10 samples of water and 10 samples of sediment, out of 32 water and 29 sediment samples collected. Criteria selection was based on previous studies about the occurrence of EPs in the Turia basin.[1-3, 40-44] All these studies concluded that pollution is mostly found in the mouth of the basin, specifically at points in the headwater and areas close to the WWTP (are more polluted). Basically, the top ten are points located in the mouth and effluent of the WWTP (**Fig. S-1**).

The criteria adopted from Schymanski et al. [45] was followed to establish different levels of confirmation, such as mass exact, unequivocal molecular formula, tentative candidates, probable structure (Library from Water) and confirmed structure (buying standard).

Samples were analyzed by DIA and matched with a library from Water Corporation (2.200 components), which included the structure (as a .mol file) for theoretical fragmentation prediction. The tentative identification was based on accurate mass (< 5.0 ppm), isotope fit (>90%) and shared common fragments with theoretical fragmentation (5 or more fragments in common, 2 mDa accuracy), chromatographic with radio (< 1) and mass peak resolution (> 7.000) and response (>25.000) (F1). **Figure 3** show the interface of UNIFI platform when the data are processed and matched as follow as: (5A) template of workflow, (5B) component identification list, (5C) selected ion chromatogram of a single component corresponding to 5B, and finally (5D) the respective mass spectrum and fragmentation (example of cucurmenol identified by the library).

Following this approach, 68 compounds were identified (**Table 1**). Forty-five pollutants were identified in water samples, whereas 42 pollutants were identified in sediment. With this step, all the identified compounds were at level 2 (probable structure). A total

of 51 pesticides, 15 pharmaceuticals and 2 mycotoxins were detected. All the identified compounds had less than 5 ppm as mass error and the RT were from 3.4 (trimethoprim) and 17.6 (betamethasone).

In water samples, the pollutants identified were pesticides and pharmaceuticals (**Table S-2A**). Each water sample showed at least 7 different compounds. However, the most polluted samples were WWTP-II (17), WWTP-I (10) and ALF-5 (13). This means the effluents of the WWTP are areas mainly impacted by EPs and this workflow could detect pesticides and pharmaceuticals substances for water samples. Previous studies focusing on target screening (pesticides, pharmaceuticals, drugs, and perfluoroalkyl substances) found a wide range of concentrations of the EPs (sometime above MRLs), especially close to the WWTP.[1, 3, 6, 41, 46, 47]

Pesticides, pharmaceuticals and mycotoxins were also identified in Sediment samples (**Table S-2B**). The most polluted deposits were ALF1 (23), ALF3 (10) y ALF5 (11) and TUR4 (12). The sediments of the tributary Alfambra (ALF) in the Turia River identified further EPs; ALF5 reaching critical levels. (more EPs, especially for ALF5, which was one of the most polluted samples).

Finally, tentative structural assignments were confirmed with their standards (level 1). Standards used in the present study are described in Supplemental Information (SI-1).

Standards were injected and compared with the sampled candidates based on retention time, exact mass and fragmentation. Dexamethasone, isoprocarb, bupirimate and penoxsulam did not match with the samples candidates in term of exact mass (mass error >5 ppm) and retention time. However, imazalil, tebuconazole, nytempiram, matalxyl, thiabendazole and oxytetracycline were found in target screening (last step) as

in the library in water and sediment samples. These compounds were confirmed by standard and finally they were quantified.

Structure assignments based on exact mass, isotope pattern and fragmentation are an important step to identify non-targeted contaminants; however, its theoretical nature sometimes matched false positives (e.g. dexamethasone **Fig. 4**) in terms of mass accuracy, RT, or fragmentation.

3.1.2. Untargeted screening

Based on the suspect screening results, the top eight most contaminated samples (water, sediment and effluent) were selected screened for untargeted EPs using DIA acquisition. Like with suspected screening, DIA acquisition was employed for the untargeted screening of the selected samples). This process was manually and time consuming. The remote on-line library (ChemSpider) was used for the identification using UNIFI. This approach was applied to candidate masses with "no identified" status by the library in 4 water samples (ALF5, GUA2, GUA6, TUR8), 2 effluents (WWTP1 and WWTP2) and 2 sediment samples (ALF5 and TUR4).

Initially, there were around 15.000-20.000 features per sample. The UNIFI platform allows creating filters to reduce or remove candidate masses that are unlikely to lead to tentatively identified compounds while retaining compounds of interest. The filter designed for this step was based on chromatographic peak width (< 1 rel. chrom. resolution), mass resolution (> 7,000) and peak intensity (> 25,000 detector counts); it reduced the feature to 500-1000 per sample. Then, the parameter halogen match (containing Cl, Br, F) was added to the filter resulting in a reduction of 50-100. Finally, candidate structures were selected via the elucidation of the molecular formula and then tentatively identified via *in silico* fragmentation. Elucidation was based on accurate

mass (< 5 ppm), i-FIT (> 90%), matching fragments (< 2 mDa, N > 5) and fragmentation relative intensity. Among the different libraries included in ChemSpider, PubChem (over 10,000,000 structures) and Thomson Pharma (over 2,000,000) were selected for (the) identification; the results were ranked according the fragment matches (>5) and citation (>10).

Table 2 summarizes the compounds identified per each sample as main candidates. The potential candidates tentatively identified according to Schumansky's Level 2 criteria were alprenolol (250.1814), ampyrone (204.113), tapentadol (222.1855), safingol (302.3054), rolipram (276.1602), paroxypropione (151.0751), tinabinol (375.2353), cyclopent (320.2228), ibuverine (291.1966), tanacaine (235.181), 5-CT (204.1132), eprosartan (425.1539) and crotetamide (227.1762) (**Fig. S-3**). These candidates serve as analgesics, anti-inflammatories, anaesthetics, antidepressants and antihypertensive agents. All the samples selected proportionated information of the tentative candidates, exceptionally GUA2 and TUR8. Contrarily, WWTP-I, WWTP-II and ALF5 (water and sediment samples) gave the most important path to find new compounds.

Alprenolol, ampyrone, tepantadol, tanacaine and 5-CT were identified n WWTP-I, all having more than 5 common fragments and more than 21 citations. This means that these compounds were found in other works and were registered in scientific literature. This was an important way to rank the candidates. WWTP-II showed alprenolol, tanacaine, eprosartan and 5-CT as potential candidates with common fragments (4-218) and more than 21 citations. Alprenolol, tanacaine and 5-CT were found in both WWTP I and II. (Meanwhile, alprenolol, tanacaine and 5-CT were found in both samples). Common fragments and citations were the main criteria to select some of them and purchase the real standard. Rolipram, paraxypropine, ibuverine and cyclopent were identified in sample ALF5.

The last step was selecting the candidates (potential hits) to confirm their presence in the samples using a real standard. After analysis of precursor and products ions of the mentioned compounds on the scientific literature eprosartan and 5-CT were selected to purchase their real standards. 5-CT was selected due its presence in two important samples (WWTP-I and WWTP-II) and showed many citation (40) and common fragments (8) in ChemSpider data base. The other hand, eprosartan (WWTP-II) was selected, as 5-CT, because showed many common fragments (18) and citation (319). However, the last reports about Eprosartan showed its presence close to the WWTP, these clues were important to take a decision and purchase a real standard. Finally, both standards were injected. As result, 5-CT did not match with its corresponding sample in terms of time RT and product ions. However, eprosartan made an excellent match with its sample and could be quantified in the target screening in all water and sediment samples (**Figure 5**).

Eprosartan is an angiotensin II receptor antagonist, (which is) used in the treatment of hypertension. It has recently been detected in wastewater at moderately to high concentrations[48-50].

Shah et al.[51] reported eprosartan through TOF-MS/MS spectrum with sixteen fragments, one of them was m/z 207 corresponding neutral loss of $C_8H_6O_2$. Also, there are reports in MS/MS spectra.[50, 52]

Target screening

Under this approach, data evaluation on target screening is based on the high resolution UPLC–QTOF MS full scan. The total run time was 20 min, with pesticides eluting between 1.27 – 19.14 min and pharmaceuticals between 1.84 – 17.34 min. 171 pesticides and 33 pharmaceuticals were analyzed, including the compounds identified in

suspected and untargeted screening, mix standards in methanol at 80 and 200 ng mL^{-1}, respectively. The quantification was based on external standard calibration.

33 pesticides and 7 pharmaceutical compounds were detected in water samples; whereas 34 pesticides and 6 pharmaceuticals were detected in sediment samples. Results obtained for water and sediment samples in Turia River 2016 expressed as median and frequency of detection are summarized in **Table 3**. In water samples, the most frequent pesticides were 3-hydroxycarbofuran (97%), etaconazole (63%), fenarimol (63%), neburon (90%), propiconazole (84%) and mefenacet (60%); whereas the most frequent pharmaceutical compounds were carbamezapine (27%) and lodocaine (18%).

In water samples, pesticides with the highest concentrations were 3-hydroxycarbofuran (778 ng L^{-1}), dimethoate (203 ng L^{-1}), eprosartan (1967 ng L^{-1}), and thiabendazole (533 ng L^{-1}); whereas pharmaceutical compunds with the highest concentrations were carbamezapine (1196 ng L^{-1}), trimethropim (2569 ng L^{-1}) and lidocaine (2407 ng L^{-1}).

In sediment samples, the most frequent pesticides were 3-hydroxycarbofuran (97%), etaconazole (69%), ethofumesate (69%), fenarimol (62%), fenuron (68%) and neburon (100%); whereas the most frequent pharaceutica compounds were Carbamezapine (34%) and oxytetracycline (13%).

In sediment samples, pesticides with the highest concentrations were: ethofumesate (483 ng g^{-1}) and 3-hydroxycarbofuran (83 ng g^{-1}); whereas pharmaceutical compounds with the highest concentrations were oxytetracycline (2033 ng g^{-1}), diazepam (1085 ng g^{-1}) and doxycycline (845 ng g^{-1}).

Both, identified and confirmed compounds in suspect and untargeted screening were included in the quantification. The compounds confirmed in suspect screening as imazalil (24%) was the most frequent and thiabendazole (533 ng L^{-1}) had the highest

concentration in water samples. In sediment samples the most frequent were tebuconazole (31%) and imazalil (17%) and the highest concentration was represented for tebuconazole (13 ng L^{-1}). Regarding pharmaceutical compounds, oxytetracycline was present in 13 % of the sediment samples and had one of the highest concentrations (2033 ng g^{-1}).

Eprosartan, a pharmaceutical compound confirmed through untargeted workflow WWTP-II, was present in 18% of the samples and the maximum concentration was 1967 ng L^{-1} localized is the mouth of the basin.

Samples can contain several EPs. The monitoring of the river showed 81% of water samples and 86 % of sediment samples contained at least 5 pesticides. The pharmaceuticals showed less co-occurrence than pesticides (at least 12% of the samples contained more than 2 compounds). However, 21% of the water samples contained more than 10 pesticides.

Regarding the spatial distribution of EPs, this work confirmed the pattern of pollution of previous monitoring in the Turia River (**Fig. 6**).[1-3, 41, 43, 44, 46] The mouth and specific points localized in the head of the basin are the most polluted sites. WWTP-I and WWTP-II are the main areas impacted by EPs. From TUR12 to TUR15, pesticides and pharmaceutical were detected at high frequency. In TUR15, 20 pesticides were detected in water samples and 14 in sediment samples (**Fig. S-5)**. Another area impacted by EPs was the head of the river, specifically the Alfambra tributary, where the stretch from ALF1 to ALF6 showed high frequency of pollutants in water and sediment samples, pharmaceuticals as oxytetracycline, doxycycline, diazepam and carbamezapine showed high concentrations (>500 ng L^{-1} and ng g^{-1}).

Previous reports showed chemical pollution (EPs) as the greatest threats to freshwater ecosystems, especially in Mediterranean watersheds, which are characterized by periodical low flows that may exacerbate chemical exposure.[1, 3, 5-7, 16-21, 41, 46, 47, 53-55] Conversely, one of the consequences of climate change (that has recently attracted attention) is its potential to alter the environmental distribution and biological effects of chemical toxicants. These two factors could influence the toxicity, fate and distribution behaviours of EPs. Different groups of emerging pollutants have been detected in Mediterranean basins during the last decade. The toxicological studies in the aquatic fauna and climate change suggested that the bioavailability and toxicity of the EPs in wildlife is likely to increase in response to rising temperatures and salinity. Increases in ambient temperature alter the toxicokinetics of chemical pollutants in exposed biota, as well as its homeostasis, exacerbating the adverse effects of contaminants.[56, 57] Regarding spatial distribution, the main climate drivers for changing pesticide fate and behaviour are thought to be changes in rainfall seasonality and intensity and increased temperatures, but the effect of climate change on pesticide fate and transport is likely to be very variable and difficult to predict. In the long-term, indirect impacts, such as land-use change driven by changes in climate, may have a more significant effect on EPs in surface and ground waters than the direct impacts of climate change on EPs fate and transport.[58, 59]

4. Conclusions

A comprehensive screening combining suspect, untargeted and target approaches was performed and applied on samples taken from a Mediterranean basin. Suspect screening was based on Data Independent Acquisition against a library that contained 2.200 EPs (pesticides, pharmaceuticals, personal care products and toxins). Workflow and tentative identification was based on accurate mass (< 5ppm), isotope fit (>90%), which

shared common fragments with theoretical fragmentation (5 or more fragments in common, 2 mDa accuracy). A total of 51 pesticides, 15 pharmaceuticals and 2 mycotoxins compounds were identified with the library. Six compounds were confirmed and quantified with the real standard in water and sediment samples (imazalil, tebuconazole, nytempiram, metalaxyl, thiabendazole and oxytetracycline).

Non-target screening was a time consuming, manual process. As suspect screening, UNIFI platform - DIA against ChemSpider database online were used to perform the identification The top eight polluted samples and non-identified compounds were used for this step. The elucidation of the masses gave rich information. As result, we identified thirteen potential hits. They were selected based on accurate mass (< 5 ppm), i-FIT (> 90%), matching fragments (< 2 mDa, N > 5), fragmentation relative intensity and citations. Two of the potential candidates were choosing to buy standard (5-CT and eprosartan). Only eprosartan was confirmed and quantified in all water and sediment samples.

Target screening included 171 pesticides and 33 pharmaceuticals (also suspected and untargeted compounds confirmed). In water samples, 33 pesticides and 7 pharmaceutical compounds were detected; whereas 34 pesticides and 6 pharmaceutical compounds were detected in sediment samples. The spatial distribution of EPs in the Turia basin was confirmed with the monitoring done in 2016. The main areas affected by EPs are the mouth and headwater (Alfambra tributary) of the basin.

HRMS showed versatility and potential to analyze EPs through three approaches (suspected, untargeted and target screening) and could be used as an ad routine tool to perform a comprehensive assess of the pollution in the basins.

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SUPLEMENTARY MATERIAL

Suspect, non-target and target screening of emerging pollutants using Data Independent Acquisition: Assessment of a Mediterranean River basin

Alexander Ccanccapa[a*], Yolanda Pico[a], Xavier Ortiz[b], Eric Reiner[b]

[(a)] Environmental and Food Safety Research Group(SAMA-UV), Desertification Research Centre CIDE (CSIC-UV-GV), Faculty of Pharmacy. University of Valencia. Burjassot, Valencia, Spain

[(b)] Ontario Ministry of the Environment and Climate Change, 125 Resources Road, Toronto (ON), Canada

* Corresponding autor: Alexander Ccanccapa
Tel: +34 963543092; Fax: +34 963544954
E-mail: Alexander.Ccanccapa@uv.es

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Pesticides: 3-Hydroxycarbofuran, Acephate, Acetamiprid, Acibenzolar-S-methyl, Aldicarb sulfone, Aldicarb sulfoxide, Ametryn, Avermectin B1a, Avermectin B1b, Azoxystrobin, Benalaxyl, Benzoximate, Bitertanol, Boscalid, Bromucanozole, Bupirimate, Buprofezin, Butoxycarboxim, Carbaryl, Carbendazim, Carfentrazone-ethyl, Chlorfluazuron, Chloroxuron, Clethodim, Clofentezine, Clothianidin, Cyazofamid, Cyproconazole, Cyprodinil, Desmedipham, Diclobutrazol, Dicrotophos, Diethofencarb, Difenoconazole, Diflubenzuron, Dimethoate, Dimethomorph, Dimoxystrobin, Diniconazole, Doramectin, Emamectin-benzoate b1a, Emamectin-benzoate b1b, Epoxiconazole, Eprinomectin, Etaconazole, Ethiofencarb, Ethiprole, Ethirimol, Ethofumesate, Etoxazole, Famoxadone, Fenamidone, Fenarimol, Fenazaquin, Fenbuconazole, Fenhexamid, Fenobucarb, Fenoxycarb, Fenpropimorph, Fenpyroximate, Fenuron, Fipronil, Flonicamid, Fluazinam, Fludioxinil, Flufenacet, Flufenoxuron, Fluometuron, Fluoxastrobin, Fluquinconazole, Flusilazole, Flutolanil, Flutriafol, Fuberidazole, Furalaxyl, Furathiocarb, Hexaconazole, Hexaflumuron, Hexythiazox, Hydramethylnon, Imazalil, Imidacloprid, Indoxacarb Ipconazole, Iprovalicarb, Isoproturon, Linuron, Lufenuron, Mandipropamid, Mefenacet, Mepanipyrim, Mesotrione, Metaflumizone, Metalaxyl, Metconazole, Methabenzthiazuron, Methiocarb, Methoprotryne, Metobromuron, Metribuzin, Mevinphos, Mexacarbate, Monceren (Pencycuron), Monocrotophos, Monolinuron, Moxidectin, Myclobutanil, Neburon, Nitenpyram, Novaluron, Nuarimol, Omethoate, Oxadixyl, Paclobutrazol, Penconazole, Phenmedipham, Picoxystrobin, Pirimicarb, Prochloraz, Promecarb, Prometon, Prometryne, Propamocarb, Propargite, Propham, Propiconazole, Propoxur, Prothioconazole, Pyracarbolid, Pyraclostrobin, Pyridaben, Pyrimethanil, Pyriproxyfen, Quinoxyfen, Rotenone, Secbumeton, Siduron, Simetryn, Spinetoram, Spinosad (Spinosyn A), Spinosad (Spinosyn D), Spirodiclofen, Spiromesifen, Spiroxamine, Sulfentrazone, Tebuconazole, Tebufenpyrad, Tebuthiuron, Teflubenzuron, Temephos, Terbumeton, Terbutryn, Tetraconazole, Thiabendazole, Thiacloprid, Thiamethoxam, Thidiazuron, Thiofanox, Thiophanate-methyl, Triadimenol, Trichlorfon, Tricyclazole, Trifloxystrobin, Triflumizole, Triflumuron, Triticonazole, Vamidothion, Zoxamide, Isoprocarb, Penoxulam

Pharmaceuticals and Personal Care Products: Acetaminophen, Atenolol, Atorbastatin, Caffeine, Carbamezapine, Chlorotetracycline, Diazepam, Doxycycline, Enrofloxacin, Erythromycin, Glyburide, Hydrocortisone, Ketoprofen, Lidocaine, Lincomycin, Meclocycline, Monensin sodium, Naproxen, Norethindrone, Oxytetracycline, Progestrone, Roxithromycin, Sulfchloropyrizine, Sulfadiazine, Sulfamethazine, Sulfametthizole, Sulfamethoxazole, Sulfathiazole, Tetracycline, Trimethoprim, Dexamethasona, 5-CT, Eprosartan. All the analytes were purchased from Sigma-Aldrich (Ontario, Canada) (restek of pesticides and pharmaceutical belonging to Ontario Environmental and Climate Change Ministry)

Sample preparation

Formic acid, Na_2EDTA. Na_2HPO_4, citric acid and methanol (gradient grade for liquid chromatography) were purchased from Sigma–Aldrich (Steinheim, Germany), dichloromethane was obtained from Merck (Darmstadt, Germany). High purity water was prepared using a Milli-Q water purification system (Millipore, Milford, MA, USA).

Table S-1. Sampling points in the Turia River

RIVER OR TRIBUTARY	ABREV	LOCATION	COORDINATES UTM-ED-50		
Turia river			ZONE	X	Y
Alfambra	ALF1	Villalba Alta-Alfambra	30	673254	4500252
Alfambra	ALF2	Villalba Alta-Alfambra	30	671693	4498425
Alfambra	ALF3	Villalba Alta-Alfambra	30	667613	4488523
Alfambra	ALF4	Vega de Teruel	30	661934	4471408
Alfambra	ALF5	Vega de Teruel	30	659555	4468805
Alfambra	ALF6	Vega de Teruel	30	659792	4464765
Alfambra	ALF7	Vega de Teruel	30	659152	4463449
Guadalaviar	GUA1	Tramacastilla	30	619076	4480720
Guadalaviar	GUA2	Tramacastilla	30	622260	4476432
Guadalaviar	GUA3	Tramacastilla	30	620888	4475214
Guadalaviar	GUA4	Tramacastilla	30	626379	4474966
Guadalaviar	GUA5	Gea de Albarracín	30	639198	4475192
Guadalaviar	GUA6	Gea de Albarracín	30	642259	4474336
Guadalaviar	GUA7	Vega de Teruel	30	653932	4469355
Guadalaviar	GUA8	Vega de Teruel	30	654871	4468880
Guadalaviar	GUA9	Vega de Teruel	30	660519	4467637
Turia	TUR1	Ademuz-Torrealta	30	647048	4435825
Turia	TUR2	Ademuz-Torrealta	30	646380	4436383
Turia	TUR3	Embalse Benagéber	30	661930	4403832

RIVER OR TRIBUTARY	ABREV	LOCATION			
			COORDINATES UTM-ED-50		
Turia river			ZONE	X	Y
Turia	TUR4	Calles	30	674005	4399240
Turia	TUR5	Calles	30	674513	4398960
Turia	TUR6	Chulilla- Embalse Loriguilla	30	679408	4392645
Turia	TUR7	Chulilla- Embalse Loriguilla	30	682987	4388327
Turia	TUR8	Bugarra	30	690694	4386474
Turia	TUR9	Bugarra	30	692418	4386992
Turia	TUR10	Huerta Valencia-Riba-Roja	30	711436	4380129
Turia	TUR11	Huerta Valencia-Riba-Roja	30	713695	4378176
Turia	TUR12	Huerta Valencia-Manises	30	717356	4376399
Turia	TUR13	Huerta Valencia-Moncada	30	723241	4379704
Turia	TUR14	Huerta Valencia-Moncada	30	724835	4380286
Turia	TUR15	Huerta Valencia-Catarroja	30	726311	4363694
Turia	WWTP-I	Valencia	30	728661	4368239
Turia	WWTP-II	Valencia	30	728616	4368756

Table S -2 Intensity and frequency of compounds detected by match library in water (A) and sediment (B) samples

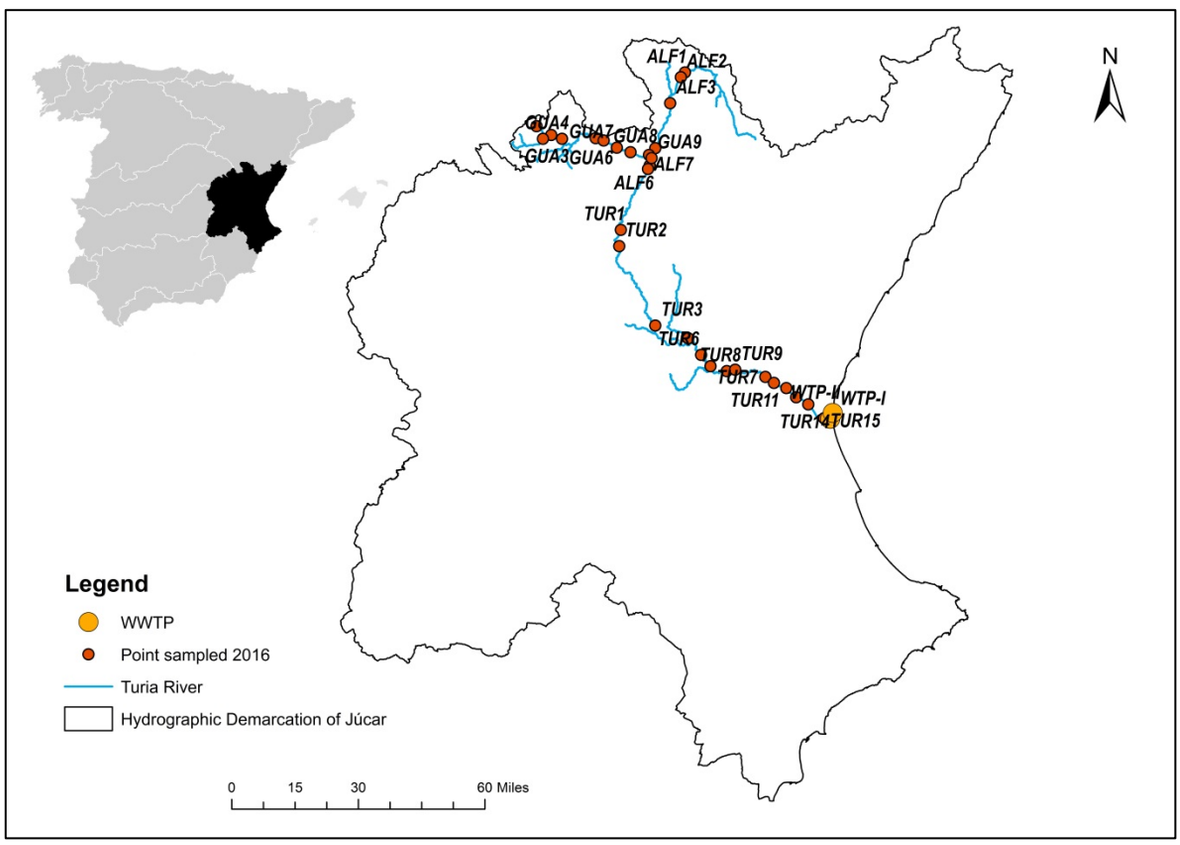
A	ALF-1	ALF-5	GUA-2	GUA-6	TUR-8	TUR-5	TUR-7	TUR-8	WTP-I	WTP-II	Frequency (%)
3,4,5-Trimethacarb										44695	10
Atenolol							31167				10
Azithromycin									38075	45494	20
Betamethasone		60431					63295				20
Brassinolide			86324		80801	74325	79089		73979		50
Bupirimate										294017	10
Cefuroxime									48962		10
Cinerin I		45070			33291	34855		188303			40
Ciobutide								36343	76710	471323	30
Climbazole		38030									10
Corticosterone				34758							10
Coumoxystrobin				214539	156635						20
Curcumenol	47869	54616	55345	105766	49750	53215	50276				70
Cycloprate	25707										10
Dexamethasone	80927	74610		93472	68272	90831	133213			126231	70
Dimefuron		30971	26626								20
Diofenolan		31397			27286			68080		41270	40
Fucaojing										117733	10
Hexalure			25497								10
Isofenphos	32976	32222	32561	31443	27773	31618	34372				70
Isoprocarb		79815	71190	70467	57771	116826	64503				60
Japothrins									44921		10
Jasmolin I								174522			10
Juvenile hormone I	44648		44216		43049	40851	42315				50

A	ALF-1	ALF-5	GUA-2	GUA-6	TUR-8	TUR-5	TUR-7	TUR-8	WTP-I	WTP-II	Frequency (%)
Kuicaoxi		37481	30475	26929	28038	27987	29637				60
Metalaxyl				50796							10
Oxpoconazole										47722	10
Penoxsulam								230454			10
Piperophos										78309	10
Precocene II	56863	44364	62425		35436	50775	50490				60
Profluthrin	158662	149199	134334	154339	107414	116483	129802	71056	106150	85960	100
Propiconazole II										47802	10
Pyrolan										1799927	10
Quizalofop-P-tefuryl		26585									10
Rifaximin									115135		10
Strychnine									40365		10
Tebuconazole								60488			10
Terallethrin				43455							10
Thiabendazole										1650308	10
Thiophanate								140731		249207	20
Trifluralin										36103	10
Trimethoprim										49813	10
Trunc-call								25895			10
Tulobuterol									96907		10
Vaniliprole									36556	60502	20

B	ALF-1	ALF-3	ALF-5	ALF-6	GUA9	TUR-1	TUR-4	TUR9	TUR11	TUR15	Frequency (%)
3,4,5-Trimethacarb	238658			115459						136061	30
6a-Methylprednisolone	144301		67526								20
Aflatoxin B2	128220										10
Aflatoxin G1								30744			10
Beta-cyfluthrin	26890	26098					25099				30
Betamethasone	55861						48117				20
Brassinolide			66708								10
Budesonide			80403				68057				20
Butocarboxim sulfoxide	74657										10
Chloreturon				30222							10
Cinerin I		41442				40411					20
Climbazole						34039					10
Clobetasol propionate	49579	73476	62170								30
Coumafuryl	43005										10
Coumoxystrobin	127368										10
Curcumenol	48797	48916	42475	31983		52420	40708	31065		26404	80
Dexamethasone	131373						59636	100511	104371		40
Dimefuron								25775			10
Diofenolan	53997	59862	65421	58998	25905	30990	97785		135581	87616	90
Empenthrin	83122										10
Flurenol	60603				30323						20
Gossyplure					27450						10
Hexalure	27039										10
Hydrocortisone			37676								10

B	ALF-1	ALF-3	ALF-5	ALF-6	GUA9	TUR-1	TUR-4	TUR9	TUR11	TUR15	Frequency (%)
Imazalil								25130			10
Isofenphos	26066	33116				28624	27545				40
Isoprocarb		105621	211362		67526	90514	202231	120429			60
Japothrins	40466			33040			27539			43435	40
Juvenile hormone I	40398	38382	37130				36835	37065			50
Juvenile hormone III				39787							10
Kinoprene				98832	45282						20
Kuicaoxi						27092					10
Methoprene	61291							112882	99756		30
Morfamquat	47622										10
Nitenpyram	210470										10
Oxytetracycline				63123							10
Piprotal										1072434	10
Profluthrin	268598	287376		219026	212136	228843	289715	162229	178293	117607	90
Profoxydim	120764										10
Spiroxamine II			27277	53383							20
Tebuconazole										47704	10
Terallethrin		55281	47374				65056				30

Figure S-1. Sampling point in the Turia Basin



IX.

RESUMEN DE RESULTADOS

En este apartado se presenta un resumen detallado de los resultados obtenidos a lo largo de la investigación de la presente tesis doctoral. Finalmente, se presentan las conclusiones generales de esta tesis.

1. Desarrollo de metodologías analíticas

1.1. Cromatografía liquida y espectrometría de masas (LC-MS/MS)

Los plaguicidas seleccionados para la presente tesis cubren un amplio rango de polaridades (K_{ow} de 057 a 514) Se seleccionó la cromatografía liquida acoplada a la espectrometría de masas en tándem, utilizando como interface la ionización por electrospray (ESI) para la determinación de los mismos El espectrómetro de masas se utilizó en modo de monitorización de reacciones seleccionadas (SRM), debido a su capacidad de obtener la máxima sensibilidad

Los métodos analíticos se validaron siguiendo la normativa de la Unión Europea (EU) No SANTE/11945/2015 [1] En esta normativa, se establece que, para identificar el compuesto, es necesario monitorizar 2 transiciones ion precursor → ion producto, al seleccionar las dos transiciones correspondientes al analito, la más abundante fue seleccionada para la cuantificación (SRM1) y la menos intensa para la confirmación (SRM2) La tabla S-3 (apartado VI) muestra las transiciones seleccionadas para cada pesticida y sus correspondientes tiempos de retención

El cromatógrafo utilizado para los trabajos de investigación que tuvieron como objetivo un análisis dirigido (target screening) fue un HP1200 serie LC- con un inyector automático, un desgasificador, una bomba cuaternaria y un horno de columna - combinado con un espectrómetro de masas Agilent 6410 triple cuadrupolo (QQQ), equipado con una interfaz de ionización por electrospray (ESI) (Agilent Technologies, Waldbronn, Alemania)

Para la investigación de todos los plaguicidas (apartados V, VI y VII) se utilizó una columna cromatográfica Luna C18 (15,0 cm x 0,21 cm) con un tamaño de partícula de 3

µm (Phenomenex, Torrance, EEUU) La temperatura de la columna se mantuvo a 30◦C y el volumen inyectado fue de 5 µL En el caso los piretroides (apartado V) la fase móvil tuvo un flujo de 0,3 ml min^{-1} con una elución de gradiente El solvente A fue Agua Milli - Q con formiato de amonio a 10 mM y el solvente B fue metanol con formiato de amonio a 10 mM La separación se llevó a cabo en 25 minutos bajo las siguientes condiciones: 0 min, 50% B; 10 min, 83% de B; 12 min, 83% de B; 12 min, 98% de B; Y 25 min, 98% de B Después, la fase móvil vuelve a las condiciones iniciales con un tiempo de equilibrio de 15 min En el caso de las investigaciones de los ríos Turia, Júcar y Ebro (apartados VI y VII), las condiciones iniciales fueron: 0 min 50% B; 10 min, 83% B; 2,5 min, 98% B; y finalmente se mantuvo durante 3 min El tiempo de estabilización fue de 12 min Por lo tanto, el tiempo total de ejecución fue de 27,5 min El flujo fue de 0,4 ml min^{-1}

Los métodos fueron evaluados y optimizados en función a su selectividad, linealidad, recuperación, precisión y sensibilidad (límites de detección y cuantificación) cumpliendo los requerimientos de la directriz SANTE respecto a la validación de métodos analíticos como se mencionó líneas arriba La selectividad se verificó analizando muestras de agua y sedimento La linealidad se evaluó a través de la respuesta del patrón del compuesto en metanol y en extractos de agua y sedimentos a una concentración de 10 a 500 ng mL^{-1} Las recuperaciones se determinaron usando matrices adicionadas y réplicas Las recuperaciones en los tres trabajos (apartados V, VI y VII) estuvieron entre un 70 y 120% La precisión se evaluó en condiciones de repetitividad (mismo día) y reproducibilidad (3 días distintos) y se expresó en términos de desviación estándar relativa (RSD) Los límites de detección y cuantificación (LODs y LOQs) pueden verse en las Tablas 3 (V), S-4 (VI) , S-5 (VII) Finalmente, el efecto de

la matriz se estimó comparando las pendientes de las rectas preparadas en metanol y las matrices ambientales blanco (agua y sedimento)

1.2. Cromatografía líquida y espectrometría de masas con analizador de cuadrupolo tiempo de vuelo (QqTOF)

Las muestras de aguas y sedimentos del último estudio del río Turia en 2016 fueron procesados a través de un UHPLC-QqQ-TOF con el objetivo de incorporar las búsquedas de amplio espectro y "no dirigida" que permitiera conocer el perfil global de la contaminación de la cuenca, que muchas veces los métodos "target" pueden distorsionar debido a sus capacidades La búsqueda de amplio espectro ó "suspected screening" se realizó usando una librería con 2200 componentes (plaguicidas, fármacos, productos de cuidado personal, drogas y toxinas)

La columna cromatográfica, fase estacionaria y fases móviles se seleccionaron en base a un método establecido por Masia A et al [2] y descrito anteriormente El cromatógrafo utilizado fue un Waters Acquity UPLC (Milford, MA, EEUU) Se inyectó una alícuota de 10 µL en UPLC-QTOF-MS / MS, y la velocidad de flujo fue de 0,4 ml min^{-1}

Se usó un espectrómetro de masas Q-TOF Xevo G2-S (Waters, Milford, MA USA) en modo ESI positivo La adquisición de datos se realizó utilizando dos programas, sólo MS para el "análisis dirigido" utilizando patrones analíticos y MS^E para el análisis "no dirigido" El programa MS se ajustó en modo de exploración completa "full scan" entre 100 y 1200 *m/z*; velocidad de escaneo, 4 scan/s; sin energía de la colisión; precisión de masa inferior a 2 ppm; resolución de más de 24K y tolerancia XIC, 20 mDa El programa de MS^E operó en modo DIA (data independent aqcuisition) que permitió que tanto los datos de los iones precursores como los de los fragmentos se adquirieran

simultáneamente durante una sola ejecución El método MS^E consta de 3 funciones, la primera (baja energía, LE) aplica energía de colisión de 4 eV, la segunda función (alta energía, HE) opera a través de una rampa de energía de colisión de 10 - 45 eV y la tercera función permite la calibración simultanea on line (lock mass) El rango de masa fue de 50 – 1200 m/z con un tiempo de exploración de 0,25 s en modo continúo Las condiciones de la fuente fueron las siguientes: voltaje capilar 2 kV, cono de muestra 80 V, fuente de offset 80 V, temperatura de fuente 125 ° C, temperatura de desolvatación 250 ° C, flujo de gas de cono 50 L h^{-1}, gas de desolvatación (N2) con un flujo de 600 L h^{-1}

Durante la adquisición de datos, la masa se corrigió usando una referencia externa (Lock-Spray^{TM}) consistente en una solución de 0,2 mg mL^{-1} de leucina-encefalina infundida continuamente a 10 mL min^{-1} a través de una interfaz de lockspray Esto generó un ión de referencia en modo positivo a m/z 5562771 que se usó para correcciones de masa en tiempo real con el fin de mantener la precisión de la masa y la reproducibilidad

2. Desarrollo y optimización de métodos de extracción

En la presente investigación se desarrollaron 3 métodos de extracción para muestras de aguas y sedimentos en función de los plaguicidas que queremos determinar El método de micro extracción liquido-líquido dispersivo (DLLME) se desarrolló para extraer piretrinas y piretroides en aguas y como etapa de purificación en el método para sedimentos La DLLME se comparó con la extracción en fase solida (SPE) para muestras de aguas, ya que este método permite extraer y concentrar simultáneamente otros plaguicidas de muy amplia polaridad como se pone de manifiesto en esta Tesis Para sedimentos, se desarrolló un método de extracción basado en el principio “dilute

and shoot" ampliamente utilizado en muestras biológicas Este método que normalmente no se acopla a etapas de purificación, se ensayó, tal cual y combinado con la DLLME Desafortunadamente, la DLLME queda restringida a compuestos apolares, ya que un requisito es utilizar muy bajos volúmenes de disolventes inmiscibles con el agua Esto lo hace un método ideal para el caso de las piretrinas que son compuestos muy apolares El QuEChERS también se utilizó para analizar un amplio espectro de plaguicidas en sedimentos

2.1. Método de micro extracción liquido-liquido dispersiva (DLLME)

Este método fue optimizado para muestras de aguas del humedal L'Albufera y sedimentos del río Turia (apartado V) para la determinación insecticidas piretroides y piretrinas (tercera generación) Este método fue comparado con la extracción en fase solida (SPE) para muestras de aguas La optimización consistió en la comparación de distintos disolventes (acetonitrilo, cloroformo, tetracloruro de carbono, diclorometano y hexano), tiempos de extracción (1, 2, 3, 6 y 9 min) y la utilización del baño de ultrasonido para mejorar la extracción

El procedimiento en el caso de las muestras de aguas fue el siguiente: se colocó un volumen de 8 ml de la muestra de agua en un tubo Falcón de 50 ml Previamente se preparó una mezcla de 2 ml de acetonitrilo, agua mili-Q y ácido acético (79: 20: 1) (v/v) (como disolvente dispersante) y 200 µl de cloroformo (como disolvente de extracción) se inyectó rápidamente en la solución de muestra con una jeringa A continuación, la mezcla se sumergió en un baño de ultrasónico durante 3 min a temperatura ambiente y se agitó 3 min en un vortex En esta etapa, los analitos se extrajeron en las gotitas de disolvente orgánico Después de eso, la mezcla se centrifugó a 3500 rpm y 15 ºC durante 10 min La fase acuosa (parte superior) se retiró con una pipeta Pasteur y la fase orgánica

(cloroformo) se recogió usando una jeringa de 100 µl, modelo 1710 RN SYR, Hamilton (Bonaduz, Suiza), se colocó en un pequeño vial y se evaporó a sequedad a 40ºC bajo una corriente de nitrógeno El residuo se reconstituyó en 200 µl de metanol y se inyectó en la LC-MS

En el caso de los sedimentos, se pesó 1 g (previa liofilización) y se colocó en tubos de centrífuga de polipropileno de 50 ml Se añadió una mezcla de 4 ml de acetonitrilo, agua mili-Q y ácido acético (79: 20: 1) (v / v) y se agitó en el vortex durante 30 min y se centrifugó a 3500 rpm durante 2 min a una temperatura de 15ºC El procedimiento de extracción prosiguió con un paso de limpieza adicional por DLLME, el extracto se separó cuidadosamente del precipitado usando una pipeta Pasteur y se colocó en tubos de centrifuga de polipropileno de 15 ml Se añadió al extracto acuoso de acetonitrilo 100 µl de cloroformo y 8 ml de agua desionizada El tubo se agitó durante 30 s, y después, se sumergió en un baño de ultrasónico durante 3 min a temperatura ambiente y se centrifugó a 3500 rpm durante 3 min a 15 ° C Finalmente, la fase disolvente se recogió en un pequeño vial con una jeringa y se evaporó a sequedad a 40 ºC bajo una corriente suave de nitrógeno El residuo se reconstituyó en 100 µl de metanol y se inyectó en el LC-MS / MS

Los límites de detección del método (MDL) de los piretroides y piretrinas estudiados estuvieron en un rango de 0,12 a 0,62 µg L^{-1} para muestras de agua y de 0,50 a 2,50 ng g^{-1} para muestras de sedimentos Los límites de cuantificación (LOQ, S/N = 10) oscilaron entre 0,37 a 0,75 µg L^{-1} para agua y entre,50 a 7,50 ng g^{-1} para sedimento Las precisión intra-día varió de 2 a 15% para muestras de agua y de 2 a 16% para muestras de sedimento La precisión inter-día no mostró diferencias significativas con respecto a la precisión intra-día en los ensayos Las recuperaciones obtenidas para muestras de agua

oscilaron entre 70 y 119 %, excepto para la cipermetrina y la jasmolina I con recuperaciones de 65 y 62%, respectivamente En muestras de sedimentos las recuperaciones variaron de 71 a 112 -%, excepto para el Etofenprox (64 %) y jasmolin I (66%) Con respecto al efecto matriz, fue considerado significativo si la diferencia obtenida entre las pendientes de la recta preparada en metanol y la preparada en un extracto de la matriz era superior al 10% Finalmente se observó efectos de matriz para la mayoría de los compuestos en agua y sedimentos (ME% <-10%)

Los resultados demostraron que esta técnica presenta bajos LODs y excelente sensibilidad, resultando en una alternativa para el análisis de rutina debido a su simplicidad, sensibilidad y confiabilidad El método propuesto se aplicó con éxito a muestras de agua y sedimentos del humedal La Albufera y del río Turia, respectivamente En muestras de sedimento se detectó acrinatrina (48 ng g^{-1}) y etofenprox (16 ng g^{-1})

2.2. Extracción en fase Solida (SPE)

La preconcentración aplicada a las muestras de agua está basada en la SPE “off line” previamente descrita por Masiá A et al [2] El siguiente procedimiento fue aplicado para las muestras de los estudios del río Turia, Júcar y Ebro (apartados V, VI y VII): los cartuchos HLB de Oasis fueron pre acondicionados con 5 ml de diclorometano-metanol (50:50) (v/v), 5 mL de metanol y 5 ml de agua desionizada Se pasaron muestras de agua (200 ml) a través de la columna SPE (caudal de aproximadamente 10 ml min^{-1}) utilizando un colector de vacío que mantuvo una diferencia de presión constante entre la entrada y la salida del cartucho (la resistencia al flujo de la SPE varió a través de la extracción por el material del sorbente, consecuentemente, el caudal es algo variable) Luego, los cartuchos se secaron bajo vacío durante 10 minutos para eliminar el agua

residual y los analitos se eluyeron con 10 ml de diclorometano-metanol (50:50, v/v) gota a gota (caudal aproximadamente 1 ml min^{-1}) Los extractos se evaporaron a sequedad a 40 ◦ C bajo una corriente de nitrógeno en un evaporador Zymark TurboVap LV (Hopkinton, MA, EUA) y se reconstituyeron con 1 ml de metanol A continuación, se filtraron a través de filtros de PTFE de 0,45 μm en los viales de auto-muestreo para análisis de LC-MS

Los límites de detección (LOD) y cuantificación (LOQ) oscilaron entre 0,1 y 2 ng L^{-1} y 0,3 y 6 ng L^{-1}, respectivamente, dependiendo de cada uno de los plaguicidas Las curvas de calibración fueron lineales en el rango de concentración de 10 ng L^{-1} a 10 μg L^{-1} y el efecto de la matriz fue siempre ≤20% Las recuperaciones variaron de 48% a 70% y la precisión fue inferior al 20% para todos los plaguicidas (Tabla S-4 y S-5 apartados VI y VII)

2.3. Método de extracción QuEChERS

Este método fue reportado por primera vez por Anastassiades et al [3] Masiá et al [4] optimizó este método para suelos, sedimentos y lodos El procedimiento que se siguió para la extracción de sedimentos de los ríos Turia, Júcar y Ebro fue la siguiente: se utilizó 1 g de muestra liofilizada, que se pesó en un tubo Falcón de 50 ml y se homogeneizó con 7,5 ml de agua y 10 ml de acetonitrilo Después, se añadió $MgSO_4$ (6 g), NaCl (1,5 g), citrato trisódico deshidratado [$Na_3C_6H_5O_7$ + $2H_2O$ (1,5 g)] y sesquihidrato de hidrógeno citrato disódico [$HOC(COOH)(CH_2COONa)_2 15H_2O$(075g)] se añadieron tanto para tamponar como para separar las fases por efecto de la fuerza iónica “salting-out” La mezcla se agitó intensamente en un vórtice durante 1 minuto y se centrifugó a 3000 rpm durante 5 min Posteriormente, se aplicó una limpieza “clean up” a una alícuota (1 ml) de la fase orgánica superior mediante extracción dispersiva en

fase sólida (d-SPE) usando PSA (50 mg), $MgSO_4$ (150 mg) y C18 (50 mg) Esta mezcla se agitó en un vórtice durante 1 minuto y se centrifugó a 3000 rpm durante 5 min El sobrenadante se filtró a través de un filtro de PTFE de 0,45 µm y se introdujo en un vial para análisis de LC-MS/MS

Los LODs y LOQs del método variaron de 0,03 a 1,67 ng g-1 dw (peso seco) y de 0,23 a 11,25 ng g^{-1} El efecto de la matriz fue de <130% y las recuperaciones fueron superiores al 40% La precisión fue inferior al 20% (Tabla S-4 y S-5 apartado VI y VII)

3. Aplicación de los métodos analíticos y de extracción para la evaluación ambiental de las cuencas mediterráneas

3.1. Presencia de plaguicidas en el río Turia

Los resultados en aguas y sedimentos del río Turia en los años 2012 y 2013 se expresan como media y frecuencia de detección (Tablas 2 y 3 apartado VI) Se detectaron residuos de plaguicidas en agua y sedimentos Los plaguicidas se detectaron con mayor frecuencia en agua que en sedimentos debido a su elevada polaridad La baja frecuencia de los plaguicidas en los sedimentos podría estar relacionada con su polaridad debido a que la mayoría de los compuestos seleccionados son polares (Kow <3) y, en consecuencia, la tendencia a acumularse en los sedimentos (apolares) es relativamente baja De los 50 compuestos analizados, 33 y 44 (aproximadamente 66% y 88%) fueron detectados (2012 y 2013)

Las principales familias de plaguicidas detectados en los dos muestreos fueron organofosforados (clorfenvinfos, clorpirifos, diazinón, dimetoato, etión y tolclofos metilo), triazinas (detilatrazina) y azoles (imazalil y procloraz) En los nuestros del año 2012 y 2013, clorpirifos (82% y 72% de las muestras, respectivamente), hexitiazox y

diazinon (93% en 2013) fueron los más frecuentes La frecuencia de estos plaguicidas podría estar vinculada a la agricultura intensiva y usos urbanos

Clorfenvinfos, terbutrina y metolacloro (Reglamento 2002/2076), atrazina (Decisión 2004/248/CE), y simazina (Decisión 2004/247/ CE), se detectaron en ambas campañas de muestreo, a pesar de haber sido retiradas de la UE (Regulation EC No 2009/1107) Estos compuestos son resistentes a la hidrólisis y persistentes, pueden formar depósitos en el medio ambiente; su incidencia principalmente se debe a la actividad agrícola a lo largo de la cuenca Su presencia en las aguas superficiales podría justificarse por escorrentía a partir de residuos acumulados en suelos tras tratamientos anteriores a su prohibición

La cantidad y el número simultáneo de plaguicidas presentes en cada muestra puede observarse en la Fig S-2 (apartado VI) En 2012 - 2013, el 68% y el 52% de las muestras de agua tenían al menos 5 plaguicidas y el 14% y 34% de las muestras más de 16 plaguicidas Esto indica que, aunque las concentraciones individuales son bajas y no superan el umbral de la legislación europea para el agua potable, el número de plaguicidas en cada muestra era alto

Los niveles de los analitos detectados variaron considerablemente, sin embargo, los contaminantes que tuvieron las concentraciones más elevadas fueron fungicidas como el imazalil (750 ng L^{-1}), tolclofos metil (382 ng L^{-1} en), procloraz (486 ng L^{-1}), carbendazima (382 ng L^{-1}) y los insecticidas azinfos metíl (148 ng L^{-1}), etión (349 ng L^{-1}) e imidacloprid (206 ng L^{-1}) (Tabla S-6 apartado VI) Las concentraciones de estos plaguicidas superan los 100 ng L^{-1}, límite establecido para las concentraciones individuales en el agua potable de acuerdo con la legislación de la UE (CE, 1998)

Los sitios más contaminados se localizaron en el área de la desembocadura de la cuenca, donde se identificaron gradientes de concentración a lo largo del río (por ejemplo TUR10 a TUR13) En esta zona la concentración del imazalil fue alta (75029 ng L^{-1}) Este compuesto es ampliamente utilizado como antifúngico en el tratamiento post-cosecha de cítricos y otras frutas La Figura S-3 (apartado VI) muestra la distribución de cada familia de plaguicidas en ambos ríos Los principales contaminantes son organofosforados, triazinas y neonicotinoides Este hallazgo está vinculado con los principales cultivos de la zona que son principalmente naranjos y arrozales

Respecto a los sedimentos analizados, la Tabla 3 (apartado VI) muestra los valores promedio y frecuencia de los plaguicidas detectados (las concentraciones máximas, media y la frecuencia se muestran en la Tabla S-7 apartado VI) En contraste con el agua, las muestras de sedimentos mostraron una menor frecuencia de plaguicidas Sin embargo, en 2012 se detectó clorpirifos (Log K_{ow} = 4) en el 100% de los puntos muestreados y en 2013 estuvo presente en el 37% de las muestras Del mismo modo, este pesticida registró las más altas concentraciones en ambas campañas (141 y 55 ng g^{-1} dw) La Figura S-4 (apartado VI) muestra la distribución de todas las familias de plaguicidas estudiados en muestras de sedimento en ambas campañas Los contaminantes principales son organofosforados, azoles, triazinas, ureas y carbamatos En cuanto a la numero de plaguicidas que aparecen simultáneamente en una misma muestra en sedimentos (Fig S-2B apartado VI), el 80% y 90% (2012 y 2013) de muestras presentaron 3 o más plaguicidas

La Figura S-3B (apartado VI) muestra concentraciones heterogéneas en las muestras de sedimentos a lo largo del río, sin embargo, en el año 2012 en un punto específico de la desembocadura (TUR13) registró una concentración por encima de 800 ng g^{-1} En

contraposición, el año 2013 la cabeza tiene un punto específico de contaminación (GUA1) con una concentración por encima de 90 ng g^{-1}

3.2. Presencia de plaguicidas en el río Júcar

Los resultados de la monitorización del río Júcar en los años 2010 y 2011 se resumen en Tablas 2 y 3 (apartado VI), lo mismos están expresados como mediana y frecuencia de detección (máximo, mínimo y media en las Tablas S-6 y S -7 apartado VI) El patrón de contaminación es muy similar al río Turia Ambos ríos pertenecen a la Demarcación Hidrográfica del Júcar, la cual tiene 42832 Km^2 de extensión y 376896 Ha bajo riego, se encuentran geográficamente muy próximos, separados sólo por 25 km en su parte más estrecha La presencia de plaguicidas en ambos ríos está vinculada principalmente a la agricultura generalizada y los usos urbanos

De los 50 compuestos analizados, 22 y 18 (aproximadamente 44% y 36% de los analitos) fueron detectados en concentraciones superiores al LOD en el río Júcar (2010 y 2011) Al igual que en el río Turia, las familias de plaguicidas con mayor presencia fueron organofosforados, triazines y azoles Clorpirifos fue uno de los plaguicidas detectado en el 100% de las muestras La presencia simultánea de varios de plaguicidas en una misma muestra en 2010 – 2011 fue de un 78% y el 84% Las muestras de agua contenían al menos 5 plaguicidas (Fig S-2 apartado VI) Las concentraciones más altas estuvieron representadas por el imazalil (682 ng L^{-1}) y metolaclor (446 ng L^{-1}) en 2010 Las concentraciones detectadas de estos plaguicidas superan los 100 ng L^{-1}, límite establecido para las concentraciones individuales en el agua potable de acuerdo con la legislación de la Unión Europea UE (CE, 1998)

Los puntos más contaminados a lo largo del río Júcar fueron JUC7, JUC8 y MAG1 (2010) ubicados en la desembocadura de la cuenca En estos puntos la suma de los plaguicidas detectados superó los límites permisibles establecidos para el agua potable (0,5 μg $L^{-1)}$ por la Unión Europea (CE, 1998)

Los resultados del análisis de las muestras de sedimentos pueden observarse en la Tabla 3 (apartado VI) y se encuentran expresados en el promedio y frecuencia de detección (las concentraciones máxima y media y la frecuencia se resumen en la Tabla S-7 apartado VI) El pesticida más frecuente fue clorpirifos, el cual fue identificado en todos los puntos muestreados en ambas campañas Las mayores concentraciones se registraron para el imazalil 32 y 37 ng g^{-1} dw (2010-2011) La Figura S-4 (apartado VI) muestra la distribución de todas las familias de plaguicidas estudiadas en muestras de sedimento en ambas campañas Las familias de contaminantes con mayor presencia son organofosforados, azoles, triazinas, ureas y carbamatos

El año 2010 y 2011, el 18% y 10% de las muestras, consecutivamente, contuvieron residuos de al menos 3 plaguicidas distintos en las muestras de sedimentos (ver Fig S-4B apartado VI) Al igual que las muestras de aguas, en los sedimentos se presentó un gradiente de concentración entre los puntos de muestreo MAG1 y MAG2, dicho gradiente podría estar vinculado a la existencia de una zona muy industrializada entre ambos puntos, y entre los sitios JUC6 y JUC8, esta área está ubicada en la desembocadura de la cuenca, con presencia de actividades agrícolas y áreas urbanas

3.3. Presencia de plaguicidas en el río Ebro

La Tabla 1 (apartado VII) muestra el promedio y la frecuencia de plaguicidas encontrados en las campañas de los años 2010 y 2011 en la cuenca del Ebro El año

2010 presentó una mayor frecuencia de plaguicidas que el 2011 En ambas campañas se detectaron organofosforados, hormonas juveniles, azoles, triazinas y ureas En 2010, piriproxifeno, clorpirifos, diazinón, buprofezina y hexythiazox fueron los más frecuentes (> 90% de las muestras) seguidos de imazalil y prochloraz (70% de las muestras) En 2011, la carbendazima fue el más frecuente (70% de las muestras), mientras que la frecuencia de diazinon, terbutilazina y terbutrina fue> 45% de las muestras El clorpirifos (95% de las muestras en 2010) es un pesticida que previamente se había observado en el río Ebro a concentraciones de ng L^{-1} [5, 6] Sin embargo, este compuesto no suele ser persistente en sistemas de agua El diazinón tuvo una alta frecuencia en 2010 (95% de las muestras), este compuesto es estable en agua, moderadamente soluble y ligeramente volátil En 2011, la carbendazima estuvo presente en el 70% de los puntos de muestreo Este fungicida es poco soluble en agua y es moderadamente persistente en los suelos De los 50 plaguicidas analizados, 14 plaguicidas (metolaclor, azinfos metilo, clorfenvinfos, diazinón, fenitrotion, fentión, ometoato, paratión-metilo, atrazina, propazina, simazina, terbumeton y terbutrina) recientemente retirados por la Unión Europea, se detectaron en las campañas de muestreo (Tabla S-1 apartado VII)

La detección simultánea de diferentes plaguicidas en la misma muestra de agua se muestra en la Fig S-4ª (apartado VII) En 2010, el 38% de las muestras contenían menos de 5 plaguicidas y el 22% de las muestras contenían más de 16 plaguicidas Esto significa que a pesar de las bajas concentraciones, el punto de muestreo (SEG) superó el umbral de los límites permisibles para el agua potable considerando el conjunto de plaguicidas en un mismo punto (05 μg L^{-1}) En 2011, el 42% de las muestras presentan menos de 5 plaguicidas, mientras que el 22% de las muestras presentaron entre 6 y 16 plaguicidas En 2011 el número de plaguicidas que contenía cada muestra fue menor que

en 2010 El perfil de contaminación en ambas campañas estuvo representado por azoles, organofosforados y triazinas (Figura S-3A apartado VII) Las muestras de 2010 estuvieron más contaminadas que las de 2011

Los resultados de los plaguicidas encontrados en muestras de sedimentos pueden observarse en la Tabla 2 (apartado VII) De los 42 plaguicidas analizados en 2010 se detectaron sólo 6 (imazalil, prochloraz, diazinon, malatión y terbutrina) a concentraciones entre 1,84 y 21,61 ng g^{-1} (dw) y de los 50 plaguicidas determinados en 2011, fueron encontrados 7 los cuales son: imazalil, clorpirifos, diazinon, diclofentión, ethion, hexythiazox y terbutryn, a concentraciones entre 0,10 y 36,17 ng g^{-1} (dw)

Los compuestos más frecuentes en 2010 fueron diazinon y clorpirifos, presentes en el 45% de las muestras y en 2011 clorpirifos (82%) y diclofentión (21%) Estos plaguicidas tienen un alto coeficiente de partición octanol/agua (log k_{ow}) (Tabla S-1 apartado VII), por lo tanto, son hidrófobos, poco solubles en agua y tienden a acumularse en los sedimentos

La presencia de más de un residuo de plaguicidas en una misma muestra de sedimento puede verse en la Fig S-4B (apartado VII) En ambas campañas, el 86% de las muestras no presentaron plaguicidas En 2010, el 9% y 2011, el 12% tenía menos 5 plaguicidas Sólo el 5% y el 2% de las muestras, en los años 2010 y 2011 consecutivamente, presentaron hasta 10 plaguicidas

4. **Distribución espacial y temporal de contaminantes emergentes en las cuencas mediterráneas**

Figura 2-A y B (apartado VI) ofrece una visión general de la distribución espacial de plaguicidas en los ríos Turia y Júcar Los puntos de muestreo JUC8 (2010), ALF6

(2013), TUR13 (2012-2013), TUR16 (2013) y TUR19 (2013) (Fig S-3 apartado VI), muestran concentraciones muy altas de plaguicidas superando el umbral de tolerancia (0,5 μg L^{-1}) de la Unión Europea (CE, 1998) Los sitios más contaminados de los ríos Júcar y Turia se localizaron en sus desembocaduras, donde se identificaron gradientes de concentración a lo largo del río (por ejemplo, de JUC6 a JUC8 y de TUR10 a TUR13) (Figura S-3 A y B apartado VI) En esta zona las concentraciones de imazalil fueron altas en ambos ríos, Júcar (68272 ng L^{-1} y 22245 ng L^{-1}) y Turia (75029 ng L^{-1}) El imazalil se utiliza como anti-fúngico en el tratamiento post-cosecha de las naranjas y otras frutas, también se detectaron procloraz (486,21 ng L^{-1}) y clorfenvinfos (148,07 ng L^{-1}) a altas concentraciones El clorfenvinfos es un insecticida utilizado para el control de plagas en cereales, cítricos, vid, árboles frutales y procloraz, al igual que el imazalil, es un fungicida aplicado en la post-cosecha de los cítricos En 2013, se instalaron 6 nuevas estaciones en el curso bajo del río Turia para confirmar los mayores niveles de contaminación en la parte baja del río En estas estaciones se detectó el 66% de los plaguicidas seleccionados La Figura S-3 (apartado VI) muestra la distribución de cada familia de plaguicidas en ambos ríos

La Figura 1A y B (apartado VII) muestra la distribución espacial de los plaguicidas a lo largo del río Ebro y sus afluentes, que podría estar relacionada con el uso del suelo [7-9] Las concentraciones de plaguicidas fueron moderadas a bajas en la mayor parte del curso del río Los sitios más contaminados fueron los afluentes Zadorra (ZAD) y Segre (SEG) en la cabecera de la cuenca, así como el Delta del Ebro en la desembocadura La Estación ZAD, ubicada en Alava, forma parte de la Red Natura 2000 y está rodeado de cultivos de cereales, remolacha azucarera y patatas, e influenciada por una planta de tratamiento de aguas residuales (Crispijana) En este punto, el diurón excede 100 ng L^{-1},

límite establecido para concentraciones individuales en agua potable de acuerdo con la legislación de la UE (EC, 1998) El punto de muestreo del río Segre (SEG) tuvo las mayores concentraciones en relación a los otros afluentes En 2010, este punto supera los 500 ng L^{-1}, límite establecido para los plaguicidas en su conjunto en el agua potable, y el imazalil excede 100 ng L^{-1} En 2011, la concentración total fue de 233,33 ng L^{-1} Las altas concentraciones de fungicida imazalil en ambas campañas podrían estar relacionadas con los tratamientos post-cosecha de manzanas y peras, cultivo frecuente en el área La distribución espacial mostró claramente un gradiente creciente de concentración para ambas campañas en los puntos de muestreo EBR-7, EBR-8 y EBR-9 (Fig 1-A apartado VII)

La distribución espacial de los plaguicidas en los sedimentos se muestra en la Fig 1B (apartado VII) y la distribución de cada familia de plaguicidas se detalla en la Fig S - 4B (apartado VII) En 2010, los plaguicidas más ubicuos fueron los organofosforados (38,99 ng L^{-1}), triazinas (25,57 ng L^{-1}) y azoles (11,94 ng L^{-1}) Sin embargo, en 2011 sólo se encontraron organofosforados (225,62 ng L^{-1}) en todos los puntos muestreados

En cuanto a la distribución temporal de plaguicidas en la Cuenca del Río Jucar, en 2010 se detectaron más plaguicidas con mayor frecuencia y concentraciones que en 2011 Por el contrario, en la cuenca del río Turia se detectaron más plaguicidas a mayor frecuencia y concentraciones en 2013 que en 2012 Este comportamiento puede ser influenciado por el caudal del río (detallado en la Tabla S-8 apartado VI) El caudal del río en cada punto se clasificó como alto, medio o bajo comparando su valor durante el muestreo con las mediciones de caudal en los últimos 50 años en cada punto donde existían datos disponibles y finalmente normalizados en porcentajes El río Júcar presentó caudales altos y medio-bajos y el Turia caudales medio-bajos y altos En el análisis de los caudales y las concentraciones de plaguicidas detectados se observa que los caudales

más bajos se relacionaron con las concentraciones más altas, y los caudales bajos con las altas concentraciones

El río Ebro mostró resultados similares en el análisis de la distribución temporal de plaguicidas (Tabla S-6 apartado VII) Considerando todas las mediciones de caudal en los últimos diez años se pudo observar que a caudales más altos, existe mayor frecuencia y mayor número de plaguicidas y, en consecuencia, en 2010, la frecuencia y numero fue superior a 2011 (Tabla 1 y Fig S-4 apartado VII) En cuanto al bajo caudal, existen informes que señalan que los caudales más bajos están relacionados con mayores concentraciones [10, 11] Sin embargo, en este caso, las concentraciones también fueron bajas La concentración podría variar teniendo en cuenta las propiedades fisicoquímicas de los plaguicidas, pero también otras condiciones ambientales como la precipitación o las temperaturas (Tabla S-1 apartado VII)

5. Cargas de plaguicidas de las cuencas mediterráneas

El ecosistema costero del mediterráneo, por sus características climáticas, geográficas y demográficas se conforma en un ecosistema sensible y vulnerable, por ello es importante conocer el impacto real de los contaminantes emergentes a través de las cargas que llevan los ríos en su trayecto natural hacia sus costas y el propio mar y de tal forma entender su impacto en los ecosistemas costeros y marinos Para ello, se realizó una estimación de las cargas de plaguicidas (toneladas $año^{-1}$) que anualmente descargan los ríos en función a las concentraciones encontradas La concentración de plaguicidas hallados en las muestras de agua en los diferentes sitios de muestreo se multiplicó por el caudal para obtener cargas ambientales expresadas en miligramos de compuestos por segundo (Ver Fig S-5 apartado VI) Las cargas más altas aparecen en la parte baja de dos ríos En el caso del río Turia, la carga máxima es de hasta 2,08 mg S^{-1} y en el Júcar

13,33 mg S^{-1} (Ver Fig S-5 apartado VI) La carga total de plaguicidas liberados por el río Júcar al mar Mediterráneo se estimó en 539 kg $año^{-1}$ en 2010 y 226 kg $año^{-1}$ en 2011 El río Turia la carga fue menor, 156 kg $año^{-1}$ en 2012 y 98 Kg $año^{-1}$ en 2013 Estos son valores mínimos de vertidos de plaguicidas debido a que las variaciones estacionales no se han tenido en cuenta Estudios previos señalaron que las cargas de plaguicidas son más altas en primavera que en cualquier otra estación Estas cargas de plaguicidas, incluso en períodos de baja concentración, podrían tener un impacto en la biota y en los ecosistemas marinos

Las cargas anuales de plaguicidas en el río Ebro se estimaron en 4359 kg en 2010 y 1606 kg en 2011 Estas estimaciones corresponden al período de octubre a noviembre, caracterizado por una menor descarga de plaguicidas en comparación con la primavera Las Tablas 4 y 5 (apartado VII) describen la concentración de plaguicidas en muestras de agua del río Ebro y de otros ríos mediterráneos desde 2001 hasta la actualidad

6. Evaluación ecotoxicológica de los contaminantes emergentes en las cuencas mediterráneas

La evaluación ecotoxicologica se realizó por medio del coeficiente de riesgo (RQ), unidades toxicas (TU) y la suma de las unidades toxicas (TU_{site}) por cada punto de muestreo para tres niveles tróficos, dafnias, algas y peces El Cociente de Riesgo (RQ) se calculó de acuerdo con las directrices europeas (Directiva 93/67/EEC) [12] para cada plaguicida Se consideró que, si el valor del índice RQ> 1, se podrían esperar efectos nocivos debido a la presencia del contaminante en el agua Por el contrario, si el valor del índice RQ< 01, el riesgo ambiental es bajo La situación intermedia en la que el índice RQ está entre 0,1 y 1 implica un riesgo medio

La unidad tóxica (TU) se utilizó para la evaluación del riesgo ecotoxicológico de las concentraciones medidas de plaguicidas La UT de cada compuesto se basó en valores de toxicidad aguda El estrés tóxico específico del sitio (TU_{site}) se calculó sumando todos las TU individuales de cada compuesto detectado en todos los puntos de muestreo estudiados Las UT > 1 indican un riesgo ambiental sobre algas, dafnias o peces

Los resultados del coeficiente de riesgo (RQ) en las cuencas de los ríos Turia y Júcar (Table S-10 apartado VI) indican que plaguicidas como carbendazima, clorfenvinfos, clorpirifos, etión, fenitrotión, hexitiazox, imazalil, metolaclor, piriproxifeno, procloraz y azinfos-metil presentaron RQ > 1 para las dafnias, realizando los cálculos con las concentraciones media y máxima, demostrando un alto potencial de causar efectos negativos en este organismo acuático Para las algas, el RQ de hexythiazox, imazalil, metolachlor y prochloraz fue > 1 Azinfos metilo, clorpirifos, carbofurano, clorfenvinfos, diclofentión, etión, piriproxifen carbendazim, procloraz e imazalil mostraron también un riesgo para peces tanto en la media como en la máxima concentración (>1) Los insecticidas mostraron los valores más altos de RQ para dafnias y peces, mientras que los herbicidas para algas Los fungicidas mostraron un RQ alto para todos los tipos de biota (dafnia, algas y peces) Cabe resaltar que el efecto aditivo de muchos plaguicidas puede causar un peligro mayor en los distintos ecosistemas, siendo de particular importancia los plaguicidas organofosforados que actúan con un mecanismo común

La Tabla 8 (apartado VII) muestra los resultados de RQ para muestras del río Ebro Hexythiazox y prochloraz estuvieron presentes en algunas muestras a niveles que implicaron una concentración de riesgo (valores RQ> 1) para las algas en sus concentraciones medias y máximas Carbendazim, clorfenvinfos, clorpirifos, diazinón, diclofentión, fenitrotión, hexitiazox, imazalil, malatión, metiocarb y piriproxifeno

también mostraron un peligro para las dafnias en sus concentraciones media y maxima Por último, clorpirifos, diclorofenilo, imazalil y piriproxifeno presentaron RQ>1 para los peces en sus concentraciones media y máxima La prueba de toxicidad crónica mostró un alto riesgo causado por los plaguicidas en tres niveles tróficos (algas, dafnia y peces) Esto podría causar cambios en las comunidades de peces e invertebrados y la disminución de las especies más sensibles o aumento de las más resistentes, con la consecuente pérdida de biodiversidad Por otro lado, los 6 plaguicidas encontrados con valores superiores a RQ> 1 para las algas son herbicidas y fungicidas Estos compuestos afectan la fotosíntesis en las microalgas reduciendo presencia en los ecosistemas acuáticos En el caso de las dafnias, 16 contaminantes (RQ> 1), en su mayoría insecticidas y fungicidas, podrían producir serios efectos en este nivel trófico Por último, para los peces, 8 plaguicidas superan RQ> 1 Las mezclas de organofosforados, azoles y carbamatos son comunes en las aguas, y se ha demostrado que pueden producir efectos sinérgicos en Carpas expuestas a estas mezclas [13, 14] Esto implica que las evaluaciones de un solo producto subestiman sistemáticamente los riesgos reales para las especies acuáticas en las cuencas donde se producen mezclas de insecticidas Debido a ello, el "efecto cóctel" producido por la presencia simultánea de diferentes tipos de plaguicidas que inducen a interacciones sinérgicas también fue evaluado a través de las UT Existen estudios que señalan a los inhibidores de la colinesterasa (orgafosfatos y carbamatos) y los fungicidas azoles (imazalil y carbendazim) implicados en el 95% de los casos sinérgicos [15]

La suma de las TU puede ayudar a estimar los efectos tóxicos de la mezcla de contaminantes en los puntos específicos de muestreo Las Tablas 6 y 7 (apartado VII) muestran las unidades toxicas para los tres niveles tróficos estudiados en la cuenca del río Ebro Los resultados muestran valores < 1, evidenciando que no existe un riesgo

agudo asociado con la contaminación en aguas o sedimentos Sin embargo, los puntos muestreados EBR6 (0,26), ARG (0,24), ZAD (0,21), SEG (0,12), HUE (0,21), EBR-5 (0,21) y EBR-2 (0,23) muestran cierto riesgo hacia las dafnias (vea Fig-2 apartado VII), ya que aunque los valores no alcanzan la unidad, son indicativos de la sensibilidad a la mezcla de residuos de plaguicidas en comparación con los otros niveles tróficos

RQ y TU son índices importantes que nos ayudan a estimar el riesgo en los diferentes niveles tróficos y con ello la protección de los ecosistemas acuáticos

7. Análisis dirigido, de amplio espectro y no dirigido de contaminantes emergentes mediante la adquisición independiente de datos (DIA): Evaluación del Río Turia 2016

En este apartado se desarrolla un flujo de trabajo basado en cromatografía líquida y espectrometría de masas con analizador de cuadrupolo tiempo de vuelo (QqTOF) que incluye tres enfoques (análisis dirigido, de amplio espectro y no dirigido) para evaluar el perfil completo de los contaminantes emergentes en muestras de aguas y sedimentos del río Turia La identificación de los contaminantes potenciales se basó en el tiempo de retención, exactitud de la masa (comparando el error de la masa experimental respecto a la teórica) (<5,0 ppm), perfil isotópico (coincidencia > 90%), y comparación de la fragmentación experimental con la teórica utilizando el software UNIFI (o con la de un patrón analítico) (5 o más fragmentos en común, precisión de 2 mDa) En el análisis de amplio espectro se usó una base de datos con 2200 contaminantes y el modo de adquisición independiente de los datos (DIA) Estos métodos implican la obtención simultánea de 2 espectros de masas, uno a baja energía de colisión (molécula protonada/desprotonada) y otro a una rampa de energía de 10 a 45 eV que produce una fragmentación especifica Como resultado, se identificaron 68 compuestos de los cuales 6 se confirmaron inequívocamente y se cuantificaron con el estándar analítico

Para el desarrollo de la búsqueda no dirigida se utilizó una base de datos en línea (ChemSpider) por medio de la plataforma UNIFI, y al igual que el análisis de amplio espectro, se utilizó el modo DIA para el análisis de las muestras El filtro utilizado para la identificación de contaminantes estuvo basado en el ancho de pico cromatográfico (resolución de cromo <1), resolución de masa (> 7000) e intensidad de pico (> 25000) Como resultado, Eprosartán, contaminante recientemente identificado cerca de plantas de tratamiento de aguas residuales (EDAR), fue confirmado y cuantificado La estrategia

y el flujo de trabajo para el análisis de amplio espectro y la búsqueda no dirigida demostraron ser funcionales La búsqueda dirigida se basó en análisis de 171 plaguicidas y 33 productos farmacéuticos (incluidos los componentes identificados en el análisis de amplio espectro y búsqueda no dirigida) en modo “full scan” La cuantificación fue realizada por calibración externa Los resultados confirmaron la distribución espacial de contaminantes emergentes en la cuenca del Turia, resultado como áreas más contaminadas la desembocadura y puntos específicos de la cabecera de cuenca Los resultados que se presentan en este apartado resultan de gran interés internacional, en un contexto de establecer influencias del cambio climático y las concentraciones de los contaminantes De otro lado, la versatilidad de detección de QTOF-MS y el potencial de alta resolución permiten una evaluación completa de los contaminantes emergentes en cuencas, resultado en una herramienta importante de forensia ambiental que permite la evaluación completa de estos contaminantes en las cuencas

7.1. Análisis de amplio espectro

El análisis de amplio espectro se realizó en 10 muestras de agua y 10 muestras de sedimento, de un total de 32 muestras de agua y 29 muestras de sedimento La selección de las muestra se basó en estudios previos sobre la presencia de contaminantes emergentes en la cuenca del Turia [11, 16-19] Todos los estudios concluyeron que la contaminación se encuentra principalmente en la desembocadura de la cuenca, específicamente en algunos puntos de la cabecera y en áreas cercanas a plantas de depuradoras de aguas residuales Fundamentalmente se seleccionaron muestras pertenecientes a la desembocadura de la cuenca y platas de tratamiento de aguas residuales (efluentes)

La figura 3 (Apartado VIII) muestra la interfaz de la plataforma UNIFI al procesar los datos: (5A) plantilla de flujo de trabajo, (5B) lista de identificación de componentes, (5C) cromatograma del ion seleccionado y finalmente 5D) el respectivo espectro de masas y fragmentación (ejemplo de cucurmenol identificado por la librería)

Siguiendo este enfoque, se identificaron 68 compuestos (Tabla 1 apartado VIII) Cuarenta y cinco contaminantes en muestras de agua, y 42 contaminantes en sedimentos Con este paso, todos los compuestos identificados pasaron al nivel 2 (estructura probable) Se detectaron un total de 51 plaguicidas, 15 fármacos y 2 micotoxinas Todos los compuestos identificados tuvieron un error inferior a 5 ppm y el tiempo de retención estuvo entre 3,4 (trimetoprim) y 17,6 (betametasona) minutos

En muestras de agua, los contaminantes identificados fueron plaguicidas y fármacos (Tabla S-2 apartado VIII) Las muestras de agua tenían al menos 5 contaminantes distintos Sin embargo, las muestras más contaminadas fueron WWTP-II (17), WWTP-I (10) y ALF-5 (13) Los efluentes de las estaciones depuradoras (EDARs) son el principal foco de entrada en el medioambiente de contaminantes emergentes (EPs) Este flujo de trabajo podría detectar plaguicidas y fármacos para muestras de agua Estudios previos realizados en la cuenca del Turia describen una amplia gama de concentraciones de contaminantes emergentes (en algunos casos por encima de los niveles máximos de residuos MRL), especialmente cerca de las EDARs

También se identificaron plaguicidas, fármacos y micotoxinas en muestras de sedimentos (Tabla S-2B apartado VIII) Los sitios más contaminados fueron ALF1 (23), ALF3 (10) y ALF5 (11) y TUR4 (12) Las muestras de sedimentos del tributario Alfambra (ALF) en el río Turia alcanzaron niveles críticos de contaminación (Especialmente el sitio ALF5 fue una de las muestras más contaminadas) Esto podría estar vinculado principalmente a las actividades de agricultura y ganadería que se

desarrollan en Albarracín y Gúdar como también la presencia de municipios de Albarracin y Alfambra, dónde se concentra la mayor parte de la población de la zona

Finalmente, la identidad de los contaminantes se confirmó comparando su comportamiento cromatográfico y espectro de masas con estándares analiticos (Información complementaria SI-1 Apartado VIII) Dexametasona, isoprocarb, bupirimato y penoxsulam tentativamente identificados en las muestras no coincidieron en términos de masa exacta (error de masa> 5 ppm) y tiempo de retención Sin embargo, compuestos como el imazalil, tebuconazol, nytempiram, matalxilo, tiabendazol y oxitetraciclina fueron confirmados por medio de su estándar correspondiente y posteriormente cuantificados

Las estructuras asignadas basadas en la masa exacta, el patrón isotópico y la comparación de la fragmentación con una librería son un paso importante para identificar contaminantes, sin embargo, su naturaleza teórica a veces coincide con falsos positivos (por ejemplo, dexametasona, figura 4 cuarta publicación) en términos de precisión de masa, RT o fragmentación

7.2. Búsqueda no dirigida

En base a los resultados del análisis de amplio espectro se seleccionaron las ocho muestras más contaminadas (aguas, sedimentos y efluentes) para realizar la búsqueda no dirigida utilizando el modo de adquisición de datos DIA Las 4 muestras de agua fueron ALF5, GUA2, GUA6, TUR8; los 2 efluentes, WWTP1 y WWTP2; y 2 muestras de sedimentos, ALF5 y TUR4

Como resultado del análisis no dirigido, y gracias a la librería en línea se identificaron de 15000 a 20000 posibles “features” (compuestos) La plataforma UNIFI permite crear filtros para reducir o eliminar candidatos poco probables o no tóxicos, mientras que

selecciona solo compuestos de interés El filtro diseñado para este paso se basó en el ancho de pico cromatográfico (resolución de cromo <1), resolución de masa (> 7000) e intensidad de pico (> 25000 recuentos de detectores); Con este filtro se logró reducir de 500 a 1000 “features” por muestra Posteriormente, se añadió al filtro el parámetro de concordancia de halógeno (Cl, Br, F) dando como resultado una reducción de 50-100 Finalmente, los compuestos se identificaron mediante la elucidación de la fórmula molecular y luego se les asigno la estructura tentativamente mediante la fragmentación *in silico* La elucidación se basó en masa exacta (<5 ppm), i-FIT (> 90%), fragmentos en común (<2 mDa, N> 5) e intensidad relativa de fragmentación Entre las diferentes bibliotecas incluidas en ChemSpider, se seleccionaron PubChem (más de 10000000 de estructuras) y Thomson Pharma (más de 2000000) para la identificación; Los resultados fueron clasificados de acuerdo a la coincidencia de los fragmentos (> 5) y el número de referencias bibliográficas (> 10) (Tabla S-3 apartado VIII)

La Tabla 2 (Apartado IX) resume los compuestos identificados por cada muestra Los candidatos potenciales identificados fueron alprenolol (2501814), ampirona (204113), tapentadol (2221855), safingol (3023054), rolipram (2761602), paroxipropiona (1510751), tinabinol (3752353), cyclopent (3202228), Ibuverina (291,1966), tanacaína (235,181), 5- Carboxamidotriptamina (204,1132), eprosartán (425,1539) y crotetamida (227,1762) (figura S-3 apartado VIII) Estos candidatos son en su mayoría analgésicos, antiinflamatorios, anestésicos, antidepresivos y agentes antihipertensivos

Se identificaron alprenolol, ampirona, tepantadol, tanacaina y 5-Carboxamidotriptamina en la muestra WWTP-I, con más de 5 fragmentos comunes y más de 21 citaciones Esto significa que estos compuestos se encontraron en otros trabajos y se registraron en la literatura científica Estos criterios fueron importantes para la clasificación de los candidatos En la muestra WWTP-II se encontró alprenolol,

tanacaina, eprosartán y 5- Carboxamidotriptamina como posibles candidatos con fragmentos comunes (4-218) y más de 21 citaciones Se logró identificar alprenolol, tanacaína y 5- Carboxamidotriptamina en las muestras de efluentes de las dos depuradoras Los fragmentos y citas comunes fueron los criterios principales para su selección y la posterior compra del estándar Rolipram, paraxypropine, ibuverine y cyclopent fueron identificados en la muestra ALF5

El último paso fue seleccionar a los candidatos (potenciales) para confirmar su presencia en las muestras usando un estándar Tras el análisis de los iones precursores y productos de los compuestos en la literatura científica, se adquirieron los patrones analíticos de 5-carboxamidotriptamina y eposartan La primera se seleccionó debido a su presencia en dos importantes muestras (WWTP-I y WWTP-II) y mostró muchas citas (40) y fragmentos comunes (8) en la base de datos ChemSpider Por otro lado, se seleccionó eprosartán (WWTP-II), porque al igual que 5- Carboxamidotriptamina, mostró muchos fragmentos comunes (18) y su presencia en aguas ha sido ampliamente recogida en las publicaciones científicas (319) Sin embargo, los últimos informes sobre el Eprosartan demostraron su presencia cerca de estaciones depuradoras (EDARs), estas pistas fueron importantes para tomar una decisión y comprar un estándar real Finalmente, se inyectaron ambos estándares Como resultado, el patrón de 5-Carboxamidotriptamina no coincidió el tiempo RT y los iones del producto del pico tentativamente identificado Sin embargo, en el caso del Eprosartan patrón y pico tentativamente identificado en la muestra concordaron exactamente y pudo ser cuantificado en la búsqueda dirigida en todas las muestras de agua y sedimento (Figura 5 apartado VIII)

El Eprosartán es un antagonista del receptor de la angiotensina II, que se utiliza en el tratamiento de la hipertensión Recientemente se ha detectado en aguas residuales a

concentraciones moderadas a altas Shah et al [20] analizó al Eprosartan a través de TOF-MS/MS y logró identificar dieciséis fragmentos, uno de ellos es m/z 207 correspondiente pérdida neutra de $C_8H_6O_2$

7.3. Búsqueda dirigida

La búsqueda dirigida se realizó por medio de un UPLC-QTOF MS en modo exploración completa (full scan) El tiempo total de ejecución fue de 20 min, los plaguicidas eluyeron entre 1,27 - 19,14 min y los fármacos entre 1,84 - 17,34 min 171 plaguicidas y 33 fármacos, incluyendo los compuestos identificados en el análisis de amplio espectro y búsqueda no dirigida La cuantificación se basó en la calibración por estándar externo (mezcla en metanol entre 80 y 200 ng mL^{-1})

Se detectaron 33 plaguicidas y 7 fármacos en muestras de agua, y 34 plaguicidas y 6 fármacos en muestras de sedimentos En las muestras de agua, los plaguicidas más frecuentes fueron 3-hidroxicarbofurano (97%), etaconazol (63%), fenarimol (63%), Neburon (90%), propiconazol (84%) y mefenacet (60%) y los fármacos carbamezapina (27%) y lidocaína (18%) Aquellos que se determinaron a más altas concentraciones incluyen 3-hidroxicarbofurano (778 ng L^{-1}), dimetoato (203 ng L^{-1}), eprosartán (1967 ng L^{-1}) y tiabendazol (533 ng L^{-1}), carbamamacepina (1196 ng L^{-1}), trimethropim (2569 ng L^{-1}) y lidocaína (2407 ng L^{-1})

En las muestras de sedimentos, los plaguicidas más frecuentes fueron el 3-hidroxicarbofurano (97%), etaconazol (69%), etofumesato (69%), fenarimol (62%), fenurón (68%) y neburona (100%) y los fármacos carbamezapina (34%) y oxitetraciclina (13%) Sin embargo, las concentraciones más altas fueron para etofumesato (483 ng g^{-1}), 3-hidroxicarbofurano (83 ng g^{-1}), oxitetraciclina (2033 ng g^{-1}), diazepam (1085 ng g^{-1}) y doxiciclina (845 ng g^{-1})

En la cuantificación se incluyeron los dos compuestos identificados y confirmados en el análisis de amplio espectro y búsqueda no dirigida Los compuestos confirmados en el análisis de amplio espectro incluyen algunos muy frecuentes como imazalil (24%) y el tiabendazol (533 ng L^{-1}) que además presentó la mayor concentración en muestras de agua En las muestras de sedimentos, los más frecuentes fueron tebuconazol (31%) e imazalil (17%) y la mayor concentración fue para el tebuconazol (13 ng L^{-1}) En cuanto a los compuestos farmacéuticos, la oxitetraciclina estuvo presente en el 13% de las muestras de sedimento a altas concentraciones (hasta 2033 ng g^{-1})

Eprosartán, fármaco confirmado a través del análisis no dirigido de la muestra WWTP-II, estuvo presente en el 18% de las muestras y la concentración máxima fue de 1967 ng L^{-1} localizada es la desembocadura de la cuenca

Las muestras pueden contener varios contaminantes emergentes El seguimiento del río mostró que el 81% de las muestras de agua y el 86% de las muestras de sedimentos contenía al menos 5 plaguicidas Los productos farmacéuticos se simultanearon menos que los plaguicidas (al menos el 12% de las muestras contenían más de 2 compuestos) Sin embargo, el 21% de las muestras de agua contenían más de 10 plaguicidas

Respecto a la distribución espacial de los contaminantes, este trabajo confirmó el patrón de contaminación previamente descrito en el Río Turia (Fig 6 apartado VIII) La desembocadura y puntos específicos localizados en la cabeza de la cuenca fueron los sitios más contaminados De TUR12 a TUR15, se detectaron plaguicidas y productos farmacéuticos a alta frecuencia En TUR15, se detectaron 20 plaguicidas en agua y 14 en sedimento (Fig S-5 Apartado VIII) Otra área impactada por los contaminantes emergentes fue la cabeza del río, específicamente el afluente de Alfambra, donde el tramo ALF1 a ALF6 mostró una alta frecuencia de contaminantes en muestras de agua y sedimentos Específicamente fármacos como oxitetraciclina, doxiciclina, diazepam y

carbamezapina mostraron altas concentraciones (> 500 ng L^{-1}) Esta situación podría estar vinculada a las actividades de agricultura y ganadería en esta área como a la presencia de poblaciones en los municipios de Alfambra y Albarracín

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X.

CONCLUSIONES

De acuerdo a los objetivos planteados en la presente investigación se llegaron a las siguientes conclusiones:

Primera: Los métodos analíticos basados en la cromatografía líquida de ultra alta resolución y espectrometría de masas en tándem (triple cuadrupolo) utilizando electrospray en modo de ionización positiva y trabajando en modo de monitorización de reacciones seleccionadas son selectivos, sensibles, fiables y eficaces para la separación, detección y cuantificación de plaguicidas en extractos de aguas y sedimentos.

Segunda: Complementariamente, la búsqueda no dirigida de contaminantes a través de un espectrómetro de masas de alta resolución (cuádruplo tiempo de vuelo) permitió identificar contaminantes emergentes no seleccionados "a priori" configurándose como una herramienta indispensable para análisis integral de los patrones de contaminación que presentan las cuencas hidrográficas.

Tercera: La optimización de la microextracción líquido-líquido dispersiva para la extracción simultánea de piretrinas naturales y sintéticas en aguas proporcionó buenas recuperaciones y altos factores de enriquecimiento. Esta técnica es ecológica debido la baja cantidad del disolvente utilizado (200 µL). Sin embargo, su aplicación se restringe a compuestos apolares (K_{ow}>3) a diferencia de la extracción en fase sólida que es capaz de separar y concentrar más de 50 plaguicidas en aguas abarcando un amplio rango de polaridades.

Cuarta: Los métodos de extracción de plaguicidas para sedimentos implican una extracción sólido-líquido con acetonitrilo incluyendo QuEChERS y otros sistemas. La microextracción líquido-líquido dispersiva puede combinarse como una etapa de purificación a estos métodos de extracción proporcionando un incremento de la sensibilidad debido al elevado factor de concentración para piretrinas y piretroides.

Quinta: En las campañas de muestreo que se desarrollaron en el Turia analizando 50 plaguicidas se detectaron 33 en agua en 2012 y 44 en 2013 a concentraciones superiores a los límites de detección. Comparativamente, en 2010-2011 se detectaron 22 y 18 plaguicidas en el Júcar y 29 y 32 en el Ebro, respectivamente. Las principales familias de plaguicidas fueron organofosforados, triazinas, azoles y carbamatos. Algunos plaguicidas encontrados sobrepasaron los límites (> 100 ng mL^{-1}) permisibles establecidos por la normativa de calidad de aguas potables. También se detectaron plaguicidas prohibidos en la UE, su presencia puede justificarse por su capacidad de persistencia y formar depósitos.

Sexta: De los 50 compuestos analizados en los años 2012 y 2013 en los sedimentos del río Turia se detectaron 10 y 5 plaguicidas, respectivamente. En el río Júcar, en la campaña 2010-2011 se encontraron 8 y 12 plaguicidas y en las muestras del Ebro 6 y 7 respectivamente. Los plaguicidas son más frecuentes en aguas que en sedimentos. Esto se debe probablemente a la polaridad de los contaminantes identificados.

Séptima: En 2016, se amplió el número de compuestos analizados a 171 plaguicidas y 33 fármacos. Se detectaron 33 y 7, respectivamente en aguas y 34 y 6, respectivamente en sedimentos. Las principales familias de plaguicidas detectadas en ambas matrices son carbamatos, ureas, organofosforados y los principales fármacos fueron antibióticos y analgésicos. Los fármacos se presentaron en muestras de aguas y sedimentos con similar frecuencia.

Octava: La caracterización de las diferentes unidades de paisaje en la demarcación del Júcar permitió evaluar las fuentes de los contaminantes. El estudio de distribución espacial a lo largo de las tres cuencas confirmó el vínculo entre las actividades antrópicas y el tipo e intensidad de la contaminación, configurando un patrón de contaminación elevada en puntos específicos en las cabeceras y las desembocaduras de las cuencas y baja polución en los paisajes abruptos centrales, debido a la complicada accesibilidad, baja densidad poblacional y escasa área cultivable.

Novena: Combinando la concentración de cada contaminante emergente con el caudal del río, se obtienen las cargas de plaguicidas que llegan al Mar Mediterráneo. La carga total de plaguicidas liberados al río Turia se estimó en 156 y 98 kg por año en la campaña 2012-2013. Los niveles más altos de contaminación se relacionan con los peores valores de los parámetros de la calidad del agua, que a su vez están vinculados a las zonas de mayor presión antrópica y a la creciente intrusión salina en las cuencas del área.

Décima: La evaluación de riesgo a través de los coeficientes de riesgo (RQ) y unidades toxicas (TU) establece el impacto ecotoxicológico de los contaminantes emergentes en tres niveles tróficos: algas, dafnias y peces. Los insecticidas mostraron alto riesgo RQ (>1) para dafnias y peces, los herbicidas para algas y los fungicidas para los tres niveles tróficos. Las mezclas de plaguicidas hallados en la cuenca del rio Turia suponen un mayor riesgo para peces y dafnias.

Undécima: El estudio comparativo a nivel de tres cuencas mediterráneas (Turia, Júcar y Ebro) da a conocer un patrón similar de comportamiento de los contaminantes emergentes. Organofosforados, azoles, carbamatos y triazinas se detectaron con frecuencia y a altas concentraciones. Las cargas de plaguicidas vertidas al mar Mediterráneo se estima que superan los 100 kg por año. La distribución espacial de los plaguicidas a lo largo de los tres ríos, demostró que las desembocaduras de cuencas son las más impactadas por los contaminantes emergentes.

ANEXOS

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II. Plaguicidas Tradicionales

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Abbreviations and Acronyms

AALLME
Air-Agitated Liquid–Liquid Microextraction

APCI-MS
Atmospheric Pressure Chemical Ionization Mass Spectrometry

ASE
Accelerated Solvent Extraction

ATD
Automatic Thermal Desorption

Au-ME
Gold Microelectrode

BAμE–LD/LVI
Bar Adsorptive Micro-Extraction Combined With Liquid Desorption Followed By Large Volume Injection

CD-MEKC
Cyclodextrin-Modified Micellar Electrokinetic Chromatography

CE
Capillary Electrophoresis

CE-DAD
Capillary Electrophoresis With Diode-Array Detection

CNBF
-Chloro-3,5-Dinitrobenzotrifluoride

CPEMNP
Carbon Paste Electrode Modified With Natural Phosphate

CQ
Chlormequat

CZE-AD
Capillary Zone Electrophoresis With Amperometric Detection

DF
Difenzoquat

DLLME
Dispersive Liquid–Liquid Microextraction

DLLME-SFO
Dispersive Liquid–Liquid Microextraction Based On Solidification Of Floating Organic Drop

DP-AdSV
Differential Pulse Adsorptive Stripping Voltammetry

DPV
Differential Pulse Voltammetry

DQ
Diquat

ED
Electrochemical Detection

EI
Electron Impact

EIC
Extracted Ion Chromatogram

ELISA
Enzyme-Linked Immunosorbent Assay
EMIS
Electrochemical Magneto Immunosensor
EPA
Environmental Protection Agency
ESA
Ethanesulfonic Acid
EU
European Union
FL
Fluorescence
FPIA
Fluorescence Polarization Immunoassays
GABA
ɣ-Aminobutyric Acid
GC
Gas Chromatography
GC-FID
Gas With Flame Ionization Detection
GC-ITMS
Gas Chromatography Iontrap Mass Detector
GPC-SPE
Gel Permeation Chromatography-SPE
HA
Heptaflurobutyric Acid
HF-LPME
Hollow Fibre Liquid Phase Microextraction
HGC
Hydroxypropyl-Gamma-Cyclodextrin
HILC
Hydrophilic Interaction Liquid Chromatography
HILIC-SPE
Hydrophilic Solid-Phase Extraction
HLLME-FA
Homogeneous Liquid-Liquid Microextraction Via Flotation Assistance
HMDE
Hanging Mercury Drop Electrode
LC
High-Performance Liquid Chromatography
HPTLC
High-Performance Thin-Layer Chromatography
HPV
High Production Volume
IARC
International Agency Of Research On Cancer

I-IL Au NPs
Imidazole Ionic Liquid Modified Gold Nanoparticles

IL-VALLME
Vortex-Assisted Ionic Liquid Based Liquid-Liquid Microextraction

IMS
Ion Mobility Spectrometry

LC
Liquid Chromatography

LC-DAD
Liquid Chromatography-Diode Array Detector

LC-UV
Liquid Chromatography-Ultraviolet Detector

LD
Lethal Dose 50

LMS
Liquid Chromatography/Mass Spectrometry

LP-GC
Low-Pressure Gas Chromatography

LTP-MS/MS
Low-Temperature Plasma Tandem Mass Spectrometry

MA
Monoclonal Antibodies

MAE
Microwave-Assisted Extraction

MHLLE
Miniaturized Homogeneous Liquid Liquid Extraction

MIP
Molecularly Imprinted Polymers

MISPE
Molecularly Imprinted Solid Phase Extraction

MM-SPD
Modified Matrix Solid-Phase Dispersion

MQ
Mepiquat

MRL
Maximum Residue Level

MRLs
Maximum Residue Limits

MS
Mass Spectrometry

MSPD
Matrix Solid-Phase Dispersion

MSPE
Magnetic Solid-Phase Extraction

MSWV
Multiple Square-Wave Voltammetry

NADPRF
Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form

NP
Normal Phase

OA
Oxanilic Acid

PAD
Pulse Amperometric Detection

PCSFC
Packed Column Supercritical Fluid Chromatography

PI
Postharvest Interval

PLE
Pressurized-Liquid Extraction

PQ
Paraquat

PSA
Primary Secondary Amine

PSE
Pressurized Solvent Extraction

PSL
Pressurized Liquid Extraction

QLIT
Quadrupole Linear Ion-Trap

QQQ
Triple Quadrupole

QTOF-MS
Quadrupole Time-Of-Flight Mass Spectrometry

QuEChERS
Quick, Easy, Cheap, Effective, Rugged and Safe

RIA
Radioimmunoassay

RSD
Relative Standard Deviation

SBSE
Stir-Bar-Sorptive Extraction

SDME
Single Drop Microextraction

SEC
Size Exclusion Chromatography

SERS
Surface Enhanced Raman Spectroscopy

SFC
Supercritical Fluid Chromatography

SFC-MS/MS
Supercritical Fluid Chromatography Tandem Mass Spectrometry

SFE
Supercritical Fluid Extraction

SFODME
Solidified Floating Organic Drop Microextraction

SIM
Select Ion Monitoring

SLE
Solid–Liquid Extraction

SLE-LTP
Solid-Liquid Extraction With Low Temperature Partitioning

SLSDE - DLLME
Solid–Liquid–Solid Dispersive Extraction Followed By Dispersive Liquid-Liquid Microextraction

SPE
Solid-Phase Extraction

SRM
Selected Reaction Monitoring

SWE
Subcritical Water Extraction

SWV
Square Wave Voltammetry

TF
Time Of Flight

UDSA–DLLME
Up-And-Down-Shaker-Assisted Dispersive Liquid–Liquid Microextraction

UETC - IL-DLLME
Ultrasound-Enhanced Temperature-Controlled - Ionic Liquid Dispersive Liquid–Liquid Microextraction

UPLC
Ultra-Performance Liquid Chromatography

UMAE-SLSDE
Ultrasound/Microwave-Assisted Solid-Liquid-Solid Dispersive Extraction

UPC
Ultraperformance Convergence Chromatography

USA
Ultrasound Assisted

USAEME
Ultrasound-Assisted Emulsification Magnetic Microextraction

USAEME-SFO
Ultrasound-Assisted Emulsification Microextraction With Solidification of Organic Droplet

UV
Ultraviolet

VSLLME-SFO
Vortex-Assisted Surfactant-Enhanced-Emulsification Liquid–Liquid Microextraction with Solidification of Floating Organic Droplet

WBE
Water-Based Extraction

CIDE Centro de Investigaciones sobre Desertificación

CSIC
Consejo Superior de Investigaciones Científicas

Universitat de València

Gobierno de España
Ministerio de Economía, Industria y Competitividad

Generalitat Valenciana