VNIVERSITAT DE VALÈNCIA

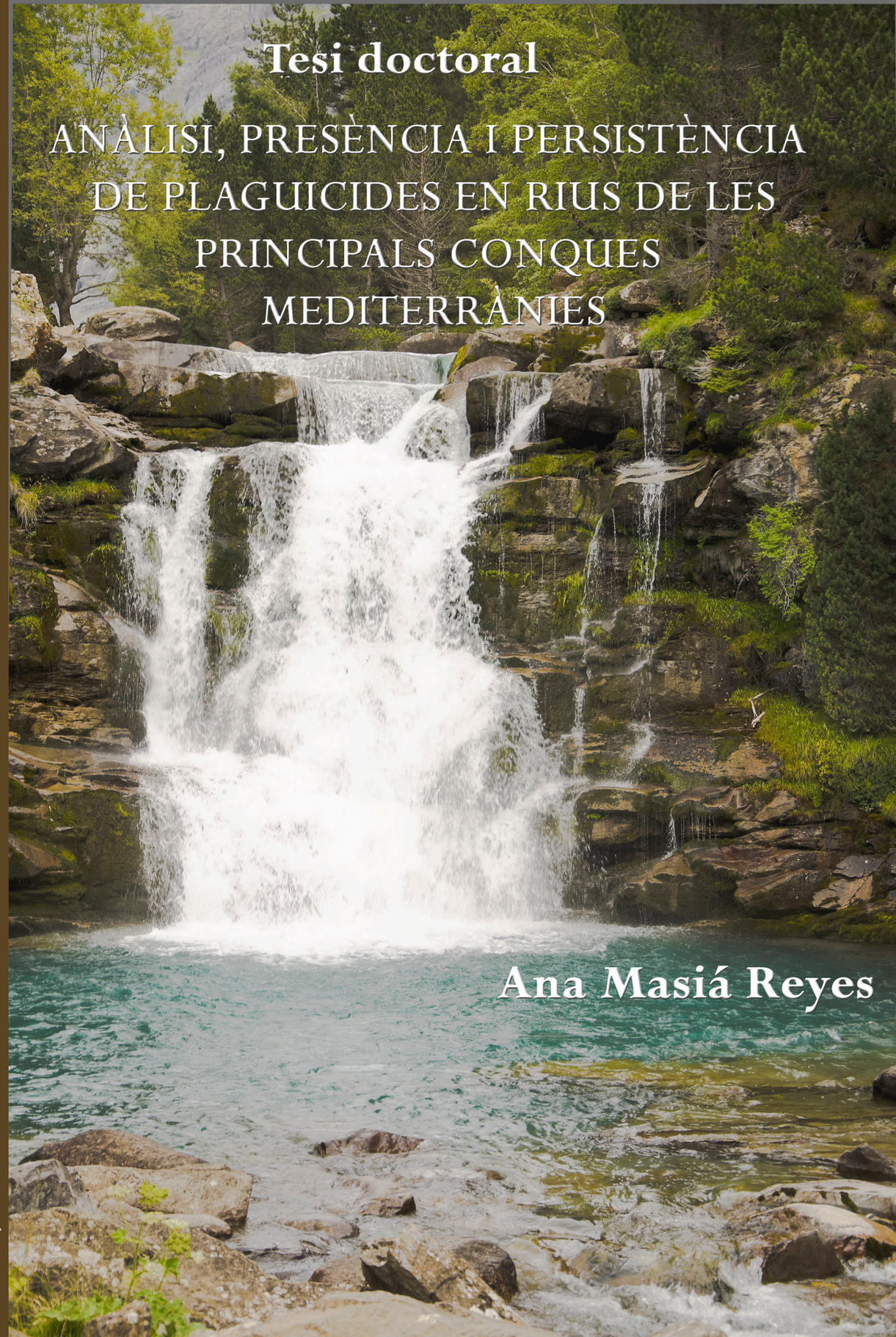
ANA MASIÁ REYES

TESI DOCTORAL

2014

Tesi doctoral

ANÀLISI, PRESÈNCIA I PERSISTÈNCIA DE PLAGUICIDES EN RIUS DE LES PRINCIPALS CONQUES MEDITERRÀNIES



Ana Masiá Reyes

VNIVERSITAT DE VALÈNCIA Facultat de Química

Programa de doctorat 03056
QUIMICA

Anàlisi, presència i persistència de plaguicides en rius de les principals conques mediterrànies

Análisis, presencia y persistencia de plaguicidas en ríos de las principales cuencas mediterráeas

Analysis, presence and persistence of pesticides in the main Mediterranean river basins

Ana Masiá Reyes

Dirigida per:

Dra. Yolanda Picó García
Catedràtica
Facultat de Farmàcia
Universitat de València

Dra. Cristina Blasco Giraud
Professora titular
Facultat de Farmàcia
Universitat de València

Burjassot, 15 d'octubre de 2014

VNIVERSITAT DE VALÈNCIA Facultat de Química
Dpt. Química Analítica
Av. Dr. Moliner s/n
46100 Burjassot, Valencia, Espanya

Yolanda Picó García i Cristina Blasco Giraud, Doctores en Farmàcia i catedràtica i professora titular, respectivament, de l'àrea de Nutrició i Bromatologia

CERTIFIQUEN QUE:

La Llicenciada Ana Masiá Reyes ha estat treballant durant quatre anys en l'elaboració de la tesi "***ANÀLISI, PRESÈNCIA I PERSITÈNCIA DE PLAGUICIDES EN RIUS DE LES PRINCIPALS CONQUES MEDITERRÀNIES***" amb un contracte de investigador pre-doctoral adscrit al projecte Consolider-Ingenio 2010 CSD2009. Aquest treball s'ha plasmat en nou treballs publicats o que es publicaran en les següents revistes:

1. "***Last trends in pesticide residue determination by liquid chromatography-mass spectrometry***", A.Masiá, C. Blasco, Y. Picó, Trends in Environmental Analytical Chemistry, 2 (2014) 11-24 (El primer nombre d'esta revista es publicà l'any 2014 per tant no està encara inclosa en el Journal Citation Reports)

2. "***Combined use of liquid chromatography triple quadrupole mass spectrometry and liquid chromatography quadrupole time-of-flight mass spectrometry in systematic screening of pesticides and other contaminants in water samples***", A. Masiá, M. Ibañez, C. Blasco, J.V. Sancho, Y. Picó, F. Hernández, Analytica Chimica Acta, 761 (2013) 117-127 (factor d'impacte: 4.517)

3. "***Ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry to identify contaminants in water: An insight on environmental forensics***", A. Masiá, J. Campo, C. Blasco, Y. Picó, Journal of Chromatography A, 1345 (2014) 86-97 (factor d'impacte: 4.258)

4. "***Multiresidue analysis of organic pollutants by in-tube solid phase microextraction coupled to ultra-high performance liquid chromatography-electrospray-tandem mass spectrometry***", A. Masiá, Y. Moliner-Martínez, M. Muñoz-Ortuño, Y. Picó, P. Campíns-Falcó, Journal of Chromatography A, 1306 (2013) 1-11 (factor d'impacte: 4.258)

5. "***Assessment of two extraction methods to determine pesticides in soils, sediments and sludges. Application to the Túria River Basin***",A. Masiá, K. Vásquez, J. Campo, Y. Picó, J. Chromatography A (enviat, factor d'impacte: 4.258)

6. "***Occurrence and removal efficiency of pesticides in sewage treatment plants of four Mediterranean River Basins***", J. Campo, A. Masiá, C. Blasco, Y. Picó, Journal of Hazardous Materials, 263P (2013) 146-157 (factor d'impacte: 4.331)

7. "***Screening of currently used pesticides in water, sediments and biota of the Guadalquivir River Basin (Spain)***", A. Masiá, J. Campo, P. Vázquez-Roig, C. Blasco, Y. Picó, Journal of Hazardous Materials, 263P (2013) 95-104 (factor d'impacte: 4.331)

8. "***Pesticide monitoring in the basin of Llobregat River (Catalonia, Spain) and comparison with historical data***", A. Masiá, J. Campo, A. Navarro-Ortega, D. Barceló, Y. Picó, Science of Total Environment-*En premsa* (factor d'impacte: 3.163)

9. "***Integrate different forecasting models for pesticide concentration calculation as well as information derived from environmental monitoring campaigns. The example of glyphosate in Italy and several other herbicides in Spain***", A. Masiá, Y. Picó, M. Calliera, L. Lamastra, F. Ferrari, Revista de L'Alguer.

Vuit dels nou treballs han sigut presentats pel doctorand com a primer autor i en l'altre signa en segon lloc. A més no hi ha cap article que haja sigut o vaja a ser utilitzat implícita o explícitament per a la realització d'una altra tesi, per la qual cosa autoritzem la seva presentació per a optar al grau de doctor.

Burjassot, 15 d'octubre de 2014

Dra. Yolanda Picó García

Dra. Cristina Blasco Giraud

Aquesta tesi doctoral s'ha realitzat mitjançant un contracte pre-doctoral per a personal investigador finançat pels projectes: "Assessing and Predicting Effects on Water Quantity and Quality in Iberian Rivers Caused by Global Change (SCARCE)" (No. CSD2009-00065, http://www.scarceconsolider.es) i "Evaluation of Emerging Contaminants in the Turia River Basins: From Basic Research to the Application of Environmental Forensics (EMERFOR)" (GCL2011-29703-C02-02, http://mefturia.es).

AGRAÏMENTS

En aquestes línies m'agradaria expressar la meua més sincer agraïment a totes les persones que d'alguna manera han contribuït a l'elaboració d'aquest treball:

- A la meua directora, la Dra. Yolanda Picó García, per la seua dedicació permanent en la direcció, orientació i supervisió d'aquest projecte. Voldria reconèixer i admirar la seua motivació, esforç i treball diari, a més d'agrair-li la seua confiança dipositada en mi durant tots aquests anys.
- A la meua co-directora Cristina Blasco Giraud per totes les seues aportacions científiques i principalment per la seua amistat, fortalesa i optimisme.
- A la Dra. Pilar Campíns Falcó per les seues encertades observacions i suggeriments durant la supervisió del meu treball final de màster, així com, per oferir-me l'oportunitat de col·laborar amb ella i el seu grup de recerca en un dels treballs que es presenten en aquesta tesi.
- Al Dr. Damià Barceló per la seua generositat en brindar al nostre grup de recerca l'oportunitat de participar en el projecte SCARCE, el finançament del qual ha permès el desenvolupament d'aquest treball.
- A Eric Carmona i Alberto Perpinyà, pel seu excel·lent treball i l'ajuda que m'han oferit amb el disseny i traducció d'aquesta tesi.
- Als meus companys de grup (María Jesús, Julián, Eric, Jose Manuel i Pablo) amb els quals he compartit incomptables hores de treball i de riures. Gràcies per crear tan bon ambient de treball.
- A tota la gent del laboratori de toxicologia (Jesús, Pepi, Belén,...) pels bons moments compartits.
- Als meus pares i la meua germana pel seu amor i el seu suport incondicional. Per ensenyar-me a aconseguir les meues metes a través de la perseverança i l'esforç. A vosaltres us dec gran part del que sóc ara.
- A Jose per creure en mi i recolzar totes les meues decisions, així com per la seua comprensió, paciència i ànims rebuts durant aquests anys.

A tots ells, gràcies!

Objectiu i Pla de Treball

El continu creixement demogràfic de la població en l'últim segle ha creat la necessitat d'incrementar la producció d'aliments. No obstant això, les plagues i malalties destrueixen prop de la tercera part de les collites durant la seua producció, transport i emmagatzematge. Davant aquestes circumstàncies la utilització de plaguicides que controlen l'acció de les plagues és imprescindible.[1]

La utilització de productes fitosanitaris ha aportat indiscutibles beneficis econòmics ja que augmenten la productivitat agrícola, i disminueixen la mà d'obra així com també el cost dels aliments. Junt amb els avantatges derivats del seu ús agrícola, la utilització de plaguicides també ha beneficiat el camp de la sanitat, on han contribuït al control i, fins i tot, l'eradicació en algunes zones de malalties transmeses per vectors [2].

A tot això, si afegim que la seua síntesi és econòmica, la seua estabilitat química excel·lent i el seu risc toxicològic per a la salut humana i el medi ambient s'ha evidenciat relativament tard, que, a més a més, ha provocat el seu ús massiu i indiscriminat. [3]

Els plaguicides no són selectius a la plaga que pretenen controlar, i afecten no solament als vectors relacionats amb aquesta, sinó que també a altres espècies, provocant desequilibris en els ecosistemes. A partir dels estudis realitzats als Estats Units d'Amèrica (EUA), dels 500 milions de quilos de plaguicides utilitzats anualment només l'1 % dels productes arriba als organismes nocius als quals van destinats. El 99 % restant roman en els ecosistemes, es transfereixen a l'atmosfera per volatilització, al sòl i als aqüífers [4]. Si bé els productes fitosanitaris augmenten la producció d'aliments, els efectes negatius en el medi ambient són indiscutibles. Des de les aigües superficials els plaguicides entren en els nivells més baixos de la cadena alimentària en concentracions baixes, es van bioacumulant en cada nivell tròfic i es biomagnifiquen successivament fins a arribar a la part més alta de la cadena tròfica (aus rapaços, peixos o mamífers depredadors), on aconsegueixen concentracions entre 10 y 100 vegades més altes que les originals.

Aquesta bioacumulació afecta la biodiversitat ocasionant una disminució en les seues poblacions, ja que tots els integrants de la cadena tròfica estan exposats a concentracions subletals que provoquen efectes en els individus que poden anar des d'indetectables fins a seriosos danys que afecten la seua reproducció i supervivència.

La Figura 1 mostra com els contaminants arriben als ecosistemes aquàtics. Els productes químics que es troben en els fitosanitaris penetren en les vies fluvials per efecte de la difusió de la polvorització. El grau i extensió de la difusió depén de factors climàtics externs com la temperatura ambient i el règim de pluges i de vents.

Figura 1. Cicle dels plaguicides en el medi ambient

La pluja mobilitza els plaguicides del lloc on es van aplicar per fenòmens d'escolament superficial o per infiltració a través del sòl cap a les aigües subterrànies. Igual que la pluja, el reg incrementa el grau de lixiviació disminuint la quantitat de plaguicida que es volatilitza des del sòl. Un excés de reg pot percolar els plaguicides directament en l'aqüífer. Part dels plaguicides s'evaporen passant a l'aire o unint-se a les

partícules del sòl, com a vapor o com a pols. Poden ser transportats grans distàncies i novament ser depositats sobre la terra o aigües superficials a través de les pluges.

En les últimes dècades, ha augmentat la preocupació per la conservació dels recursos naturals des d'una perspectiva sostenible i la disponibilitat de l'aigua s'ha convertit en una qüestió important que preocupa tots els governs.

Esta contaminació generalitzada dels ecosistemes aquàtics i del sòl ha obligat a l'adopció de mesures legislatives restrictives per part de la Unió Europea:

- Directiva Marc de l'Aigua (DMA) (Directiva 2000/60/CE), estableix les bases per a regular els recursos hídrics amb l'objectiu de preservar, protegir i millorar la seua qualitat i el seu ús sostenible [5].
- Decisió 2455/2001/CE, estableix una llista de 33 substàncies prioritàries que han de ser controlades, la tercera part de les quals són plaguicides [6].
- Directiva 2008/105/CE defineix les normes de qualitat ambiental (NQA), mitjana anual (MA) i concentracions màximes admissibles (CMA) en les aigües superficials per a la llista de substàncies prioritàries abans mencionades. Encara que diversos plaguicides estan inclosos actualment en esta llista en els reglaments de la Unió Europea (UE), molts altres encara no estan regulats [7].
- Directiva 98/83/CE, estableix límits per als plaguicides en les aigües destinades al consum humà (100 ng/L per als plaguicides individuals i 500 ng/L per a la suma de tots els plaguicides) [8].

Les conques hidrològiques mediterrànies estan sotmeses principalment a pressions que provenen de l'expansió i el desenvolupament humà en les seues diferents expressions (agrícola, industrial, urbà...). Els models de canvi climàtic conclouen que les regions mediterrànies seran les més impactades en els pròxims anys i les conseqüències del canvi

global afectaran tant a la disponibilitat com a la qualitat de l'aigua. Encara que aquests contaminants es troben generalment a nivell de traça en els ambients aquàtics, és important conéixer la seua distribució i evolució en el medi ambient per al seu control i evolució, ja que l'exposició crònica a aquestes dosis baixes pot suposar una amenaça per a la biota aquàtica i la salut humana [9].

Tot el que hem dit fins ara posa de manifest que l'anàlisi de residus de plaguicides representa un instrument fonamental per a la protecció humana i del medi ambient. Aquesta circumstància, crea la necessitat de realitzar un major esforç en el desenvolupament de metodologies ràpides i versàtils que permeten la detecció de contaminants emergents i els seus possibles metabòlits en matrius mediambientals [10].

Els estudis sobre la incidència dels contaminants emergents en la Península Ibèrica són escassos [11-16]. En general, a Europa hi ha pocs estudis que determinen l'aparició dels plaguicides utilitzats en l'actualitat en els distints compartiments ambientals diferents de l'aigua i la majoria d'ells són mostrejos erràtics realitzats per a demostrar la fiabilitat d'un mètode d'anàlisi, però no estudis sistemàtics que avaluen la incidència i els nivells de plaguicides en una conca hidrogràfica [17-20]. Aquests estudis, tot i que no tenim molts antecedents, són més freqüents als EUA [21-28].

L'escàs nombre de publicacions relacionades amb la presència de plaguicides en sediments i biota són a conseqüència, en part, de la falta de NQA per als contaminants orgànics en aquestes matrius, inclosos els plaguicides i a la dificultat de la seua extracció. Els sediments i els peixos són matrius variables i molt complexes, a causa de les fortes interaccions que enllacen els anàlits amb els diferents constituents de la mostra (matèria orgànica i argiles en sediments; greixos i proteïnes en els peixos), convertint a aquestes dues matrius en un dipòsit d'aquests compostos i dificultant la seua extracció [29].

Atenent a les necessitats mediambientals existents es va definir l'objectiu global d'aquesta tesi doctoral, basada en oferir una visió general de la qualitat de les aigües de les conques mediterrànies representatives de la Península Ibèrica, així com l'avaluació del risc toxicològic dels plaguicides sobre la biota (algues i bacteris, macroinvertebrats i peixos). Tot i això, aquesta tesi doctoral pretén contribuir al coneixement de la funcionalitat de les conques mediterrànies, i de la seua capacitat de recuperació enfront de l'impacte de les activitats humanes, a més a més, de ser útil com una base sòlida per al desenvolupament i la planificació d'estratègies i metodologies més adequades per a la recuperació i protecció dels ecosistemes mediterranis distribuïts per tot el planeta, prenent com a cas d'estudi la presència de residus de plaguicides.

Per tant, la metodologia utilitzada en l'elaboració d'aquesta tesi és:

1. Desenvolupament i validació de metodologies analítiques basades en l'extracció en fase sòlida (SPE), la metodologia QuEChERS (acrònim de Quick, Easy, Cheap, Effective, Rugged, Safe) i l'extracció amb líquids pressuritzats (PLE) seguida de cromatografia líquida acoblada a espectrometria de masses en tàndem (LC-MS/MS), utilitzant un analitzador de triple quadrupol així com quadrupol temps de vol, per a la determinació de plaguicides en mostres d'aigua, sediments i peixos.
2. Estudiar les concentracions, distribució i destí d'aquests contaminants en ecosistemes fluvials mediterranis de la Península Ibèrica (Guadalquivir, Llobregat i Túria) tractant de determinar l'origen i el patró de distribució d'aquests compostos.
3. Avaluar l'amenaça que suposen aquests compostos per a la fauna aquàtica, basat en les dades toxicològiques disponibles de plaguicides. Els organismes diana pertanyien als tres nivells de la cadena tròfica, per a

obtindré una imatge completa del potencial d'impacte d'aquestes substàncies per al medi aquàtic.

Per al seu desenvolupament, les publicacions que conformen aquesta tesi s'han distribuït en 9 capítols que es poden agrupar en tres blocs:

- Capítols 1-5, enfocats a la posada a punt dels mètodes analítics que resolguen els reptes que dificulten l'anàlisi de residus de plaguicides.
- Capítols 6-8, basats en l'aplicació de la metodologia anteriorment desenvolupada sobre diversos ecosistemes fluvials mediterranis de la Península Ibèrica, a través d'una enquesta de seguiment realitzada en les campanyes 2010 i 2011, en què es van analitzar més de 40 plaguicides emprats en l'actualitat.

  Per tal d'avaluar els principals factors d'estrés d'aquestes conques es va establir una xarxa de punts de mostreig representatius que cobrien les zones més afectades des del punt de vista de la indústria química, hidrològic, morfològic i ecològic, així com els llocs de referència en els quals s'esperava major qualitat.
- Capítol 9 se centra en l'estudi de models sobre el comportament dels plaguicides en les conques mediterrànies.

A continuació, detallem l'objectiu de cada capítol:

El *capítol 1* és la introducció general en la qual abordarem diferents aspectes. En primer lloc oferirem una visió crítica de l'actual esquema de treball dins de l'anàlisi de residus de plaguicides per LC-MS, perquè moltes de les revisions que tracten aquest tema són més generals i se centren en altres tipus de tècniques, a més de LC-MS. També prestarem especial atenció a proporcionar una cobertura completa de les últimes

innovacions dins d'aquest camp. Finalment, analitzarem breument les possibles tendències futures i desenvolupaments en aquesta àrea.

Dels capítols 2 al 9 recollirem el treball experimental realitzat durant el doctorat, recopilats en forma de publicacions, el qual va ser planificat i dissenyat per tal d'aconseguir els objectius proposats.

En el *capítol 2* analitzarem més de 60 mostres d'aigües superficials i residuals per LC-triple quadrupol (QqQ)-MS/MS i LC-quadrupol temps de vol (QqTOF)-MS/(MS) després d'una SPE convencional, per tal d'investigar si l'ús combinat dels sistemes és útil en la realització rutinària de la determinació sistemàtica de residus de plaguicides.

En el *capítol 3* farem un pas endavant en les estratègies analítiques desenvolupades per a l'anàlisi no dirigit quantitatiu i no quantitatiu de contaminants utilitzant l'última generació LC-QqTOF-MS (ABSciex TripleTOF^{TM}5600) mitjançant l'extracció dels ions amb una exactitud de massa de 20 mDa d'acord a una base de dades de més de 2.000 compostos amb informació de massa exacta i, si estan disponibles, els temps de retenció. Un mètode d'informació d'adquisició depenent (IDA) permet obtenir automàticament espectres MS/MS dels ions precursors més intensos (sense selecció prèvia). D'altra banda, per primera vegada, i amb la finalitat d'identificar contaminants rellevants inesperats per als sistemes d'aigua i definir canvis en les empremtes dactilars d'aigua, anàlisi de dades estadístiques utilitzant l'anàlisi de components principals (PCA) i els components principals agrupació variables (PCVG), es va combinar amb càlcul de la fórmula empírica, cercada en base de dades en línia (chemspider o altres bases de dades d'Internet), i la interpretació de fragments d'ions MS/MS per a detectar amb èxit contaminants desconeguts com una visió dins de la forensia mediambiental. Finalment, les capacitats quantitatives del sistema van ser explorades per a 42 plaguicides utilitzats en l'actualitat, la determinació dels quals va ser validada d'acord amb les directrius

europees [30]. La possibilitat de limitar l'anàlisi a un sol instrument proporcionarà avantatges en termes d'estalviar temps i costos.

En el *capítol 4*, desenvoluparem un mètode analític per microextracción en fase sòlida "en tub" (IT-SPME) acoblat a cromatografia d'ultra alta pressió- electrospray - espectrometria de masses en tàndem (UHPLC-QqQ-MS/MS) per a l'anàlisi multiresidu de nou substàncies prioritàries incloses en la Directiva 2008/105/CE en aigües superficials continentals. Es mostra per primera vegada un procediment que permet aconseguir la selectivitat i la sensibilitat necessària per a determinar contaminants orgànics en mostres d'aigua, mitjançant l'acoblament d'una tècnica que necessita una pressió relativament baixa (IT-SPME), i una altra que opera a una pressió relativament alta (UHPLC). El nou acoblament es compara amb l'establert prèviament IT-SPME i Cromatografia Líquida capil·lar (CapLC).

En el *capítol 5* descriurem, avaluarem i compararem els procediments PLE i QuEChERS per a l'extracció de 50 plaguicides en sòls, sediments i fangs i la seua posterior determinació mitjançant LC-MS /MS. La combinació QuEChERS amb LC-MS/MS, va ser la més avantatjosa i es va aplicar per primera vegada per a determinar els residus de plaguicides en sòls, sediments i fangs de la conca del riu Túria.

En el *capítol 6* analitzarem les concentracions de plaguicides en l'influent, l' efluent i els fangs deshidratats de les principals estacions depuradores d'aigües residuals (EDARs) situades al llarg dels rius Ebre, Guadalquivir, Xúquer i Llobregat a Espanya. Amb aquestes dades, es calculen i relaten les eficiències d'eliminació d'aquestes EDARs. L'objectiu final d'aquest estudi és millorar el coneixement sobre les causes de la contaminació dels ambients aquàtics considerant les EDARs com a fonts puntuals de contaminants com són els plaguicides.

En el *capítol 7* monitoritzem la concentració de plaguicides en aigua, sediments i biota (només 2010) de la Conca Hidrogràfica del Guadalquivir. Aquest és el primer estudi pilot dut a terme en aquesta extensa conca hidrogràfica espanyola i té la intenció de millorar el coneixement de la incidència d'aquests plaguicides en el medi ambient aquàtic. Les concentracions dels plaguicides utilitzats en l'actualitat associats als sediments i la biota també poden determinar quins plaguicides tenen més probabilitat de repartir-se en la fase de sediments en suspensió, o de bioacumular-se en la cadena tròfica aquàtica, i aquesta informació serà útil per a altres conques on aquests compostos són aplicats.

En el *capítol 8* analitzarem les concentracions de plaguicides en mostres d'aigua superficial, efluents d'aigües residuals, sediments i biota (només 2010) al llarg de tot el curs del riu Llobregat i els seus afluents, amb la finalitat d'establir la incidència dels plaguicides i la seua distribució. Els resultats obtinguts en aquest seguiment es van comparar amb les dades històriques recopilades durant altres programes de monitoreig en aquesta conca per a descriure les tendències en l'estat de la qualitat de l'aigua i determinar els riscos potencials per a la salut humana.

Aquesta és la primera vegada que (i) es determina un nombre tan elevat de plaguicides a la conca, (ii) s'estudien tres compartiments ambientals (aigua / sediments / biota) i (iii) les dades relacionades amb la incidència dels plaguicides en aquest riu s'han recopilat i comparat d'una manera detallada. A més a més, els resultats d'aquest estudi són àmpliament aplicables a altres conques que segueixen el patró hidrològic dels rius mediterranis i que pateixen l'efecte del canvi climàtic en augment.

Finalment, en el *capítol 9* identificarem les forces impulsores dels processos involucrats en el moviment de plaguicides en algunes regions italianes i espanyoles. A més a més, es desenvolupen eines per a simular el comportament dels plaguicides a diferents escales amb l'objectiu de definir els permisos d'ús de plaguicides (o

restriccions) a nivell regional, planificant programes de seguiment, optimitzant el pressupost dels estudis al centrar el mostreig en les àrees on és probable que es troben les concentracions de plaguicides més altes.

Els resultats d'aquesta tesi han contribuït al desenvolupament de mètodes analítics més sensibles i fiables, així com, a l'avanç en el coneixement sobre la distribució d'aquests compostos tòxics en el medi ambient.

Referències bibliogràfiques:

[1] La ciència que protegeix als consumidors des del camp fins a la taula. http://www.efsa.europa.eu/en/corporate/doc/efsacorporatebrochure_es.pdf.

[2] J. Cooper, H. Dobson, Crop Prot. 26 (2007) 1337.

[3] Els plaguicides, com a contaminants de l'aigua. http://www.fao.org/docrep/w2598s/w2598s06.htm.

[4] Promoció de l'Agricultura ecològica: la seua producció i consum, de la Xarxa Carrefour d'Informació i Animació Rural de la Comissió Europea. http://www.crie.uji.es/agric/santa.htm.

[5] Comissió Europea, Directiva 2000/60/CE del Parlament Europeu i del Consell de 23 d'octubre de 2000per la qual s'estableix un marc comunitari d'actuació en l'àmbit de la política d'aigües (Directiva Marc de laigua), D. Of. Comun. Eur. L327 (2000).

[6] Decisió No 2455/2001/CE del Parlament Europeu i del Consell de 20 de novembre de 2001 per la qual s'aprova la llista de substàncies prioritàries en l'àmbit de la política d'aigües, i per la qual es modifica la Directiva 2000/60/CE, D. Of. Comun. Eur. L 331 (2001).

[7] Comissió Europea, Directiva 2008/105/CE Parlament Europeu i del Consell de 16 de desembre de 2008relativa a les normes de qualitat ambiental en l'àmbit de la política d'aigües, per la qual es modifiquen i deroguen ulteriorment les Directives 82/176/CEE, 83/513/CEE, 84/156/CEE,84/491/CEE i 86/280/CEE del Consell, i per la qual es modifica la Directiva 2000/60/CE, D. Of. Comun. Eur. L348 (2008).

[8] Directiva 98/83/CE del Consell de 3 de novembre de 1998 relativa a la qualitat de les aigües destinades al consum humà, D. Of. Comun. Eur. L330 (1998).

[9] Scarce 2014. http://www.scarceconsolider.es/publica/P000Main.php.

[10] A. Masiá, C. Blasco, Y. Picó, TrAC 2 (2014) 11.

[11] M.A. Fernández, C. Alonso M.J. Gonzalez, L.M. Hernández, Chemosphere 38 (1999) 33.

[12] P. Palma, M. Kock-Schulmeyer, P. Alvarenga, L. Ledó, I.R. Barbosa, M. Lopez de Alda, D. Barceló, Sci. Total Environ. 488-489 (2014) 208.

[13] C. Flores, F. Ventura, J. Martin-Alonso, J. Caixach, Sci. Total Environ 461 (2013) 618.

[14] V. Matamoros, V.Salvado, J. Environ Manage 117 (2013) 96.

[15] J.C. Lopez-Doval, N. De Castro-Català, I. Andrés-Domenech, J. Blasco, A. Ginebreda, I. Muñoz, Sci. Total Environ 440 (2012) 194.

[16] C. Planas, J. Caixach, F.J. Santos, J. Rivera, Chemosphere 34 (1999) 2393.

[17] A. Navarro-Ortega, V. Acuña, R.J. Batalla, J. Blasco, C. Conde, F.J. Elorza, A. Elosegi,F. Francés, F. La-Roca, I. Mu˜noz, M. Petrovic, Y. Picó, S. Sabater, X. Sanchez-Vila,M. Schuhmacher, D. Barceló, Environ. Sci. Pollut. Res. 19 (2012) 918.

[18] A. Belmonte Vega, A. Garrido Frenich, J.L. Martínez Vidal, Anal. Chim. Acta 538(2005) 117.

[19] A. Lazartigues, C. Fratta, R. Baudot, L. Wiest, C. Feidt, M. Thomas, C. Cren-Olive, Talanta 85 (2011) 1500.

[20] Y.B. Li, F.S. Dong, X.G. Liu, J. Xu, J. Li, Z.Q. Kong, X. Chen, W.C. Song, Y.H. Wang, Y.Q. Zheng, J. Chromatogr. A 1218 (2011) 6667.

[21] M.P. Ensminger, R. Budd, K.C. Kelley, K.S. Goh, Environ. Monit. Assess. 185(2013) 3697.

[22] K.L. Smalling, T.J. Reilly, M.W. Sandstrom, K.M. Kuivila, Sci. Total Environ. 447(2013) 179.

[23] L.H. Nowell, P.W. Moran, R.J. Gilliom, D.L. Calhoun, C.G. Ingersoll, N.E. Kemble,K.M. Kuivila, P.J. Phillips, Arch. Environ. Contam. Toxicol. 64 (2013) 32.

[24] M.L. Hladik, J.L. Domagalski, K.M. Kuivila, Sci. Total Environ. 408 (2009) 356.

[25] L.A. Knight, M.K. Christenson, A.J. Trease, P.H. Davis, A.S. Kolok, Environ. Toxicol. Chem. 32 (2013) 1544.

[26] N.E. Kemble, D.K. Hardesty, C.G. Ingersoll, J.L. Kunz, P.K. Sibley, D.L. Calhoun, R.J. Gilliom, K.M. Kuivila, L.H. Nowell, P.W. Moran, Arch. Environ. Contam. Toxicol.64 (2013) 52.

[27] S.J. Kalkhoff, A.V. Vecchia, P.D. Capel, M.T. Meyer, J. Environ. Qual. 41 (2012)1566.

[28] A.V. Vecchia, R.J. Gilliom, D.J. Sullivan, D.L. Lorenz, J.D. Martin, Environ. Sci.Technol. 43 (2009) 9096.

[29] A. Masiá, K. Vásquez, J. Campo, Y. Picó, J. Chromatogr. A. (2014) enviat.

[30] Mètode de Validació i de control de qualitat dels procediments d'Anàlisi de Residus de plaguicides en aliments i pinsos. Comissió Europea Document No. SANCO/12495/2011, Brussel·les, 1 gener 2012.

Evolució del consum de Fitosanitaris

L'organització per a l'Alimentació i l'Agricultura (FAO) ha definit com a plaguicida qualsevol substància o mescla de substàncies destinades a prevenir, destruir o controlar qualsevol plaga, incloent els vectors de malalties humanes o animals, les espècies no desitjades de plantes o animals, que causen perjudici o interfereixen en la producció, transformació, emmagatzematge, transport o comercialització d'aliments, productes agrícoles, fusta i productes de fusta o aliments per a animals, o substàncies que poden administrar-se als animals per a combatre insectes, aràcnids o altres plagues en els seus cossos o sobre aquests. El terme inclou les substàncies destinades a utilitzar-se com a reguladores del creixement de les plantes, defoliants, dessecants, agents per a reduir la densitat de la fruita o evitar la caiguda prematura de la fruita. També s'utilitzen com a substàncies aplicades als cultius abans o després de la collita per a protegir el producte contra la deterioració durant l'emmagatzematge i el transport [1].

La utilització més comuna dels plaguicides és com a productes fitosanitaris (també coneguts com a productes de protecció de cultius), substàncies actives i preparats que contenen una o més substàncies actives presentats en la forma en què són subministrats a l'usuari, que en general protegeixen a les plantes de les influències perjudicials com són les males herbes, malalties de plantes o insectes. Aquest ús dels plaguicides és tan comú que el terme plaguicida es tracta sovint com a sinònim de producte fitosanitari, encara que en la realitat és un terme més ampli, ja que els plaguicides també s'utilitzen per a finalitats no agrícoles [2].

Els productes fitosanitaris són mitjans imprescindibles per a la producció agrícola, ja que protegeixen els cultius de les plagues i malalties que els amenacen; no obstant això, poden tenir altres efectes no desitjables i perillosos per a la salut humana i el medi ambient.

La necessitat de reduir els riscos i efectes derivats del seu ús en la salut humana i el medi ambient, ha portat a la implementació de la Directiva Sostenible (RD 1311/2012 trasllada part de la Directiva 2009/128/CE) [3,4]. Sobre la base d'aquest objectiu, la indústria fitosanitària s'ha compromés activament a anar adaptant els productes fitosanitaris a les necessitats i demandes de la societat amb la finalitat de minimitzar la quantitat de productes que poden arribar a les aigües superficials i subterrànies com a conseqüència del seu ús irresponsable [5].

D'aquesta manera, el sector agrícola és capaç d'atendre la demanda global d'aliments, i assegurar la sostenibilitat econòmica, social i mediambiental de les zones en les quals es desenvolupa [6].

L'objectiu d'aquest capítol és oferir una idea sobre el consum de productes fitosanitaris a Espanya, considerant allò que hem exposat al capítol anterior; la magnitud del problema mediambiental que pot generar-se a causa de la seua presència.

A Espanya, el consum de productes fitosanitaris per a la protecció dels cultius s'ha incrementat gradualment des de la meitat dels anys noranta. No obstant això, en 2011 es produeix una reducció del 5,4% en el seu consum, expressat en kg d'ingredient actiu, a causa de la situació econòmica i les condicions meteorològiques de la primavera d'eixe any (Figura 2).

Aquesta variació anual trenca la tendència que s'havia iniciat l'any anterior en la qual el consum s'havia incrementat quasi un 12%, per a tornar a valors de consum similars als experimentats en els anys previs a la situació d'inestabilitat econòmica (2005-2007) [6].

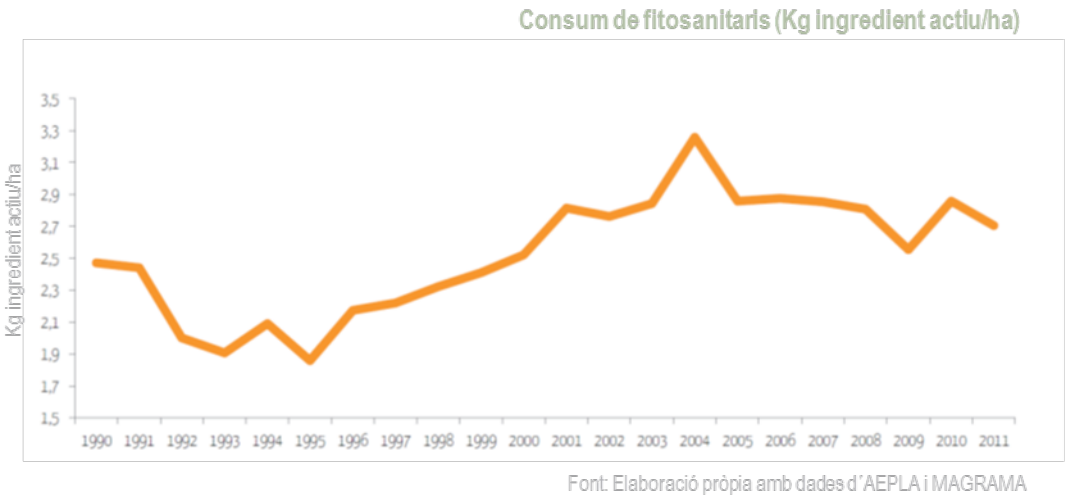


Figura 2. Evolució del consum de fitosanitaris a Espanya

La Figura 3 mostra l'evolució del mercat fitosanitari espanyol, europeu i mundial en els últims anys, segons dades facilitades per l'Associació empresarial per a la protecció de les plantes [5].

Figura 3. Evolució del mercat fitosanitari

Respecte als tipus de productes fitosanitaris més emprats en 2011, segons dades facilitades per AEPLA, podem citar els insecticides, acaricides i nematocides, amb un 31,2%, sent els productes més utilitzats, seguits dels herbicides (30,2%) i els fungicides (22,4%) [6].

En aquest mateix any, les comunitats autònomes amb un major ús de productes fitosanitaris per ha són Canàries, amb 79,2 kg/ha, seguida de la Regió de Múrcia (23,0

kg/ha), Cantàbria (22,1 kg/ha), la Comunitat Valenciana (19,7 kg/ha), i Astúries (14,2 kg/ha), mentre que les comunitats que menor consum han registrat han sigut Castella-La Manxa (2,2 kg/ha), Castella i Lleó (2,3 kg/ha) i Aragó (2,9 kg/ha) (Figura 4) [6].

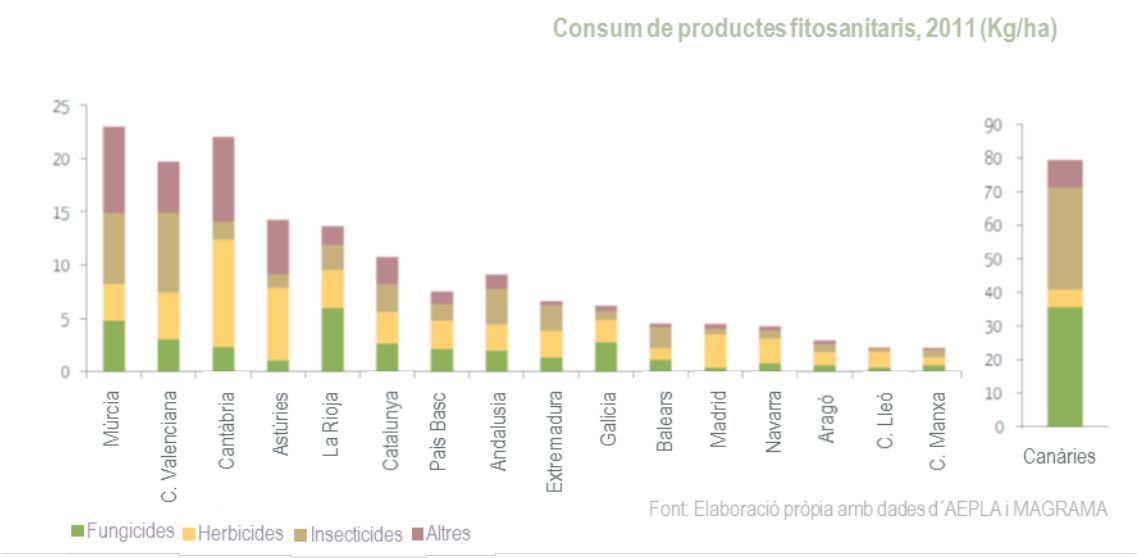


Figura 4. Consum de plaguicides per CCAA l'any 2011

La Taula 1 mostra la tendència general observada en comparar les dades de les substàncies comercialitzades durant l'any 2011 respecte a l'any 2012, d'acord als resultats de l'Enquesta de Comercialització de Productes Fitosanitaris que va dur a terme el Ministeri d'Agricultura, Alimentació i Medi ambient l'any 2012 [7].

COMPARATIVA DELS PRINCIPALS GRUPS DE FITOSANITARIS ENTRE 2011 I 2012 (expressat en Tones)		
	2012	2011
FUNGICIDES I BACTERICIDES	26.540	31.232
HERBICIDES	13.624	13.615
INSECTICIDES I ACARICIDES	7.706	8.030
MOLUSQUICIDES, REGULADORS DEL CREIXEMENT I ALTRES	14.899	19.855

Taula 1. Comparativa de les principals categories de substàncies químiques comercialitzades entre els anys 2011 i 2012

Podem assenyalar un descens en el total de les substàncies comercialitzades durant l'any 2012 així com en els principals grups de famílies, a excepció del grup dels Herbicides on pràcticament el total dels herbicides comercialitzats durant el 2012 és similar a la quantitat arreplegada en el 2011 [7].

El principal descens es produeix en el grup format per molusquicides, reguladors del creixement i altres protectors on el descens respecte a l'any 2011 és del 25%. Els fungicides i bactericides són el segon grup a arreplegar el major descens, sent aquest del 15% respecte a l'any anterior. Finalment, quant al descens de substàncies comercialitzades, comparant 2012 respecte a les dades de 2011, el grup dels Insecticides arreplega un descens del 4% [7].

Els herbicides han crescut en un 4% respecte a l'any 2011 sent els herbicides amides i anilides el subgrup que ha experimentat un major creixement [7].

Aquestes xifres de consum de plaguicides posen de manifest la magnitud del problema mediambiental, ja que reflecteixen la quantitat de substàncies tòxiques

procedents de l'activitat agrícola que aconsegueixen el medi ambient, i conseqüentment l'alt risc que els residus de plaguicides contaminen el sòl i els aqüífers.

Encara que actualment s'observa una tendència a la reducció en l'ús dels fitosanitaris en els països desenvolupats, encara es detecta una tendència a l'alça en el consum d'aquests productes en països tropicals, on se segueixen aplicant intensivament.

Tot això, ha generat que en l'última dècada, les polítiques ambientals es dirigisquen cap a l'aplicació de normes reguladores més exigents a fi de reduir les aportacions d'aquestes substàncies al medi ambient. En l'àmbit científic, es continuen duent a terme nombrosos estudis i recerques que permeten conéixer la destinació dels fitosanitaris en el medi ambient i més concretament el seu impacte sobre la qualitat de les aigües superficials i subterrànies.

Referències bibliogràfiques:

[1] Organització per a l'Agricultura i l'Alimentació de les Nacions Unides (2002), el Codi Internacional de Conducta per a la Distribució i Utilització de Plaguicides. Consultat el 2007-10-25.

[2] Document d'orientació acordat entre els serveis de la Comissió i les autoritats competents dels Estats membres per als productes biocides Directiva 98/8/CE, i per als productes fitosanitaris de la Directiva 91/414/CEE sobre: Línia fronterera entre la Directiva 98/8/CE relativa a la posada en el mercat de productes biocides i la Directiva 91/414/CEE relativa a la posada en el mercat dels productes fitosanitaris. http://ec.europa.eu/food/plant/protection/evaluation/ borderline_en.htm.

[3] Reial decret 1311/2012, de 14 de setembre, pel qual s'estableix el marc d'actuació per a aconseguir un ús sostenible dels productes fitosanitaris. BOE 223 (Setembre) (2012) Sec. I. – 65127-65171.

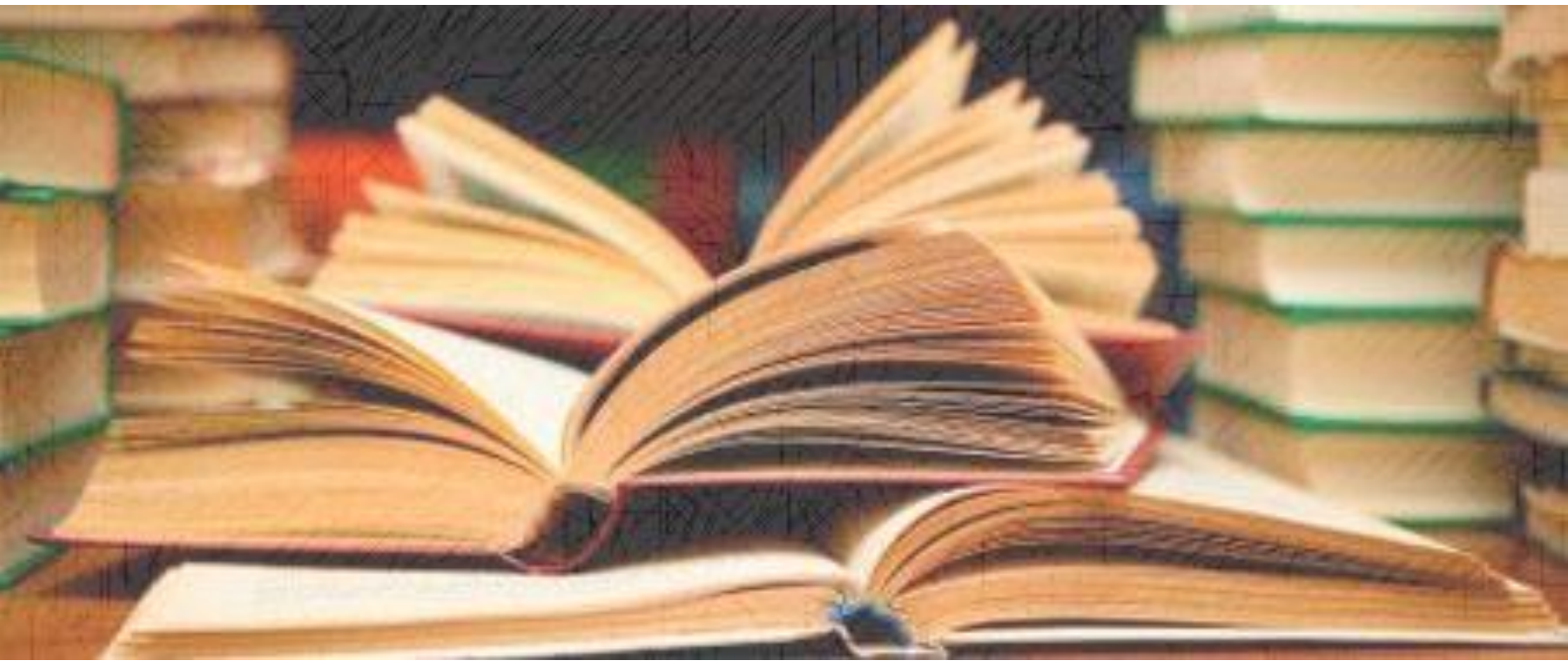
[4] Directiva 2009/128/CE del parlament europeu i del consell de 21 d'octubre de 2009 per la qual s'estableix el marc de l'actuació comunitària per a aconseguir un ús sostenible dels plaguicides. D. Of. Unió Eur.L 309 (2009) 71-86.

[5] AEPLA 2013, Memòria d'activitats de l'Associació Empresarial per a la protecció de les Plantes (2013). http://www.aepla.es/files/Publicaciones/Memoria AEPLA 2013.pdf.

[6] MAGRAMA 2012. Perfil Ambiental d'Espanya (2012), http://www.magrama.gob.es/es/calidad-y-evaluación-ambiental/publicaciones/agricultura tcm7-303856.pdf.

[7] MAGRAMA 2012, Resultats Enquesta de Comercialització (2012). http://www.magrama.gob.es/es/estadistica/temas/estadisticas-agrarias/resultados 2012 para el PDF tcm7-319697.pdf.

CAPÍTOL 1



Antecedents bibliogràfics

Publicació científica 1

Last trends in pesticide residue determination by liquid chromatography-mass spectrometry

A.Masiá, C. Blasco, Y. Picó

Trends in Environmental Analytical Chemistry, 2 (2014) 11-24

Trends in Environmental Analytical Chemistry 2 (2014) 11–24

Contents lists available at ScienceDirect

Trends in Environmental Analytical Chemistry

journal homepage: www.elsevier.com/locate/teac

Review

Last trends in pesticide residue determination by liquid chromatography–mass spectrometry

Ana Masiá *, Cristina Blasco, Yolanda Picó

Food and Environmental Safety Research Group, Department of Medicine Preventive, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés, s/n, 46100, Burjassot, Valencia, Spain

ARTICLE INFO

Article history:
Received 30 January 2014
Received in revised form 1 March 2014
Accepted 3 March 2014

Keywords:
Pesticide residues
Liquid chromatography–mass spectrometry (LC–MS)
Sample preparation
Water
Environmental samples
Food
Biological samples

ABSTRACT

Liquid chromatography–mass spectrometry (LC–MS) is as an excellent analytical tool in the determination of pesticides. Multiresidue analysis of these compounds at trace levels is one of the oldest analytical schemes within environmental and food safety. However, the issue of "pesticide residue determination" is still a hot topic for the analytical community. This review discusses current approaches and recent advances in using LC–MS for pesticide identification and quantification. We outline how MS has influenced the sample preparation process. We critically assess and compare various mass spectrometers, highlighting their strengths and limitations. We, then, review the main applications of LC–MS in pesticide residue determination in the past three years. We also look at the implications for the future of the field.

Contents

1. Introduction

Synthetic pesticides are used in agriculture throughout the world since the middle of the 20th century to avoid pest in plants and animals [1,2]. Any environmental and food sample is susceptible to contain pesticide residues because they are widely dispersed from their application areas, reaching environment and food chain [3]. As a result, people are exposed to pesticide residues at low concentrations through the environment, their diets, etc. Scientists are interested about the health effects because they are not clearly understood yet. Moreover, new alarms regarding the prevalence and effects of these compounds in the environment, and concerns of synergies between them have recently emerged [2,4,5].

Consequently, in the 70s and 80s, the European Union (EU) established strict regulations banning many pesticides [6,7]. Since that moment, new groups of these substances coined as "currently used" or "modern" pesticides have been introduced in the market replacing the oldest ones [2].

In case of environmental samples, EU adopted the Water Framework Directive 2000/60/EU [8]. The adopted Decision No 2455/2001/EC [9], which amends Directive 2000/60/EC [8], has established a list of 33 priority substances in the field of water policy, the third part of which are pesticides. Previously, another

* Corresponding author. Tel.: +34 963543092.
E-mail address: ana.masia@uv.es (A. Masiá).

http://dx.doi.org/10.1016/j.teac.2014.03.002
2214-1588/

directive, 98/83/EC [10], had set limits for pesticides in water intended for human consumption (100 ng/L for individual pesticides and 500 ng/L for the sum of all pesticides) [11].

For food samples, pesticide residues have been regulated by several legislative authorities throughout the world, basically concerned with the quality, efficacy and safety in the use of pesticides, however, there is not a global harmonized legislation [12]. EU has set through maximum residue levels (MRL) (European Commission, 1999) [13]. In general, the MRLs are in the range of 0.01–10 mg/kg, depending on the combination commodity and pesticide. The lowest is characteristic of banned compounds because is considered as the minimum limit of detection (LODs) achievable. A value of zero is considered below the LOD because of the slight inaccuracies in the measurement methods available [12].

The analysis of pesticide residues represents a basic instrument not only for the protection of human health, but also for trade and official control purposes [12]. Despite of multiresidue analysis of these compounds at trace levels has been carried out since the 70s [14], analysis of pesticides still remains a challenge because different chemical classes are present at low concentrations in complex matrices. Therefore, it is necessary to continue developing multi-residue analytical methods with higher recoveries and lower limits of detection [11] as well as incorporating the ultimate innovations to them.

Nowadays, liquid chromatography–mass spectrometry (LC–MS) is preferred over gas chromatography (GC) because currently used pesticides are quite polar, thermally labile or not easily vaporized [14], and consequently, worst detected by GC. Therefore, LC–MS has become one of the most powerful analytical tool for organic compound analysis at sub μg/L level providing the sensitivity, selectivity, and specificity needed to meet EU legisla-tion for the analysis of pesticides in water and food samples [15,16]. As a demonstration of the growing interest of this topic, Fig. 1.1, summarizes the number of articles published from 2009 to 2013 shorted from the ISI Web of Science and the SCOPUS using the keywords "Pesticides" and "liquid chromatography–mass spectrometry". The number of articles increases year after year. Furthermore, the number of recent review articles dealing with this subject [1,12,17–25] can add elements to the evidence already pointed out in Fig. 1.1. This review offers a critical overview of the current workflow within pesticide residue analysis by LC–MS because the many reviews that treat this topic are more general and focus not only on LC–MS but on a sort of techniques. Special attention is paid to provide comprehensive coverage of ultimate innovations in the field. Finally, possible future trends and developments in this area are briefly discussed.

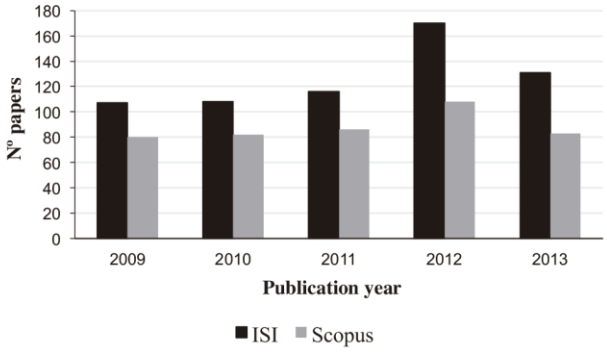


Fig. 1.1 Pesticide residues and liquid chromatography–mass spectrometry.

2. Analysis

Table 1.1 includes a summary of the most significant studies regarding LC–MS for pesticide residue determination. These reports are classified according to the mass analyzer and to the matrix and show the number of pesticides, the matrix selected, the sample preparation procedure, the separation setup and the determination with an insight in sensitivity.

2.1. Extraction procedures

Sample preparation step is necessary to isolate and concentrate pesticide residues. These procedures often take up most of the total analysis time, contributing highly to its total cost, and greatly influencing its reliability and accuracy [65]. Extraction and purification of pesticides has evolved a lot since the first multiresidue extraction scheme proposed by Mills [66]. Many of these analyses are made on food and other perishable products. Therefore, method rapidity, or at least, method feasibility in a reasonable time is important. The first methods developed, even though they were milestones in the analysis of organic contaminants, were long, tedious, consumed large amount of sample and organic solvents and required a sophisticated purification involving solvent partition and column chromatography clean-up that could require several days [67].

Fig. 2.2 provides an outline of the evolution of extraction methods parallel to the increase in the specificity of detection systems. MS, particularly when combined with LC has been architect of a chemist's dream: to reduce the sample preparation. For economic reasons and practicality and thanks to selectivity and specificity of MS, extraction tends to be generic. This means that these procedures are able to extract the widest number of organic contaminants, not only pesticides, to later identify them unequivocally by LC–MS [65].

Nowadays, among the different analytical methods developed, solid-phase extraction (SPE) in combination with LC is the most applied technique for the extraction of pesticide residues in water [68]. On-line SPE is considered the elegant alternative (includes automation and miniaturization) and solid-phase microextraction (SPME) the environmental friendly (requires little quantity of water samples and is almost solvent free) but both are less used. With the aim of offering a clear idea of the current situation, of 9 studies in water presented in Table 1.1, 6 were performed using conventional SPE, 1 using a variant of the SPME (in-tube SPME), 1 applying a liquid–liquid microextraction procedure (LLME), based on supramolecular solvents (SUSME), and finally, other just made direct injection.

Similarly, the determination of pesticide residues in solid samples is mostly afforded by the QuEChERS "Quick, Easy, Cheap, Effective, Rugged, and Safe" method [69]. It provides a versatile platform of many different protocols, depending on the type of pesticide (influence of pH, degradability), the type of matrix (fatty, acidic or especially complex), the purpose of the analysis, etc. Of 40 studies collected in Table 1.1, 40% apply some kind of QuEChERS protocol. The other studies on matrices different to water are based on an array of techniques that can be used alone or combined. These alternative techniques include liquid–liquid (LLE) [31,33,48,55,56,58,59], membrane assisted (MASE) [5], matrix solid phase dispersion (MSPD) [35,41], turbulent flow chromatography [36], microwave-assisted (MAE) [38], pressurized liquid (PLE) [39], SPME [39], stir bar sorptive (SBSE) [51], SPE [54] and high throughput planar SPE (HTpSPE) [47] extractions or just dilution of the sample and direct injection [44].

LC–MS has made possible that sample preparation directs toward simple, miniaturized and environmental friendly methods (according to the principles of Green Chemistry). This

Table 1.1
Selected LC–MS applications to determine pesticide residues published from 2011.

Matrix	Pesticide	Extraction	Separation		Determination		Refs.
			Column	Mobile phase	Detection	Sensitivity	
QqQ							
Environmental matrix							
Surface and waste water	43 pesticides	SPE[a]	LC- Luna C18 (150 mm × 2.1 mm, 3 μm)	Gradient MeOH–H_2O 10 mM $HCOONH_4$[v] Flow rate: 0.4 mL/min	Agilent 6410 ESI in PI mode Dynamic SMR	LOD 0.04–2 ng L^{-1}	[26]
Surface, waste water, Sediments and biota	50 pesticides	SPE QuEChERS[b]	LC- Luna C18 (150 mm × 2.1 mm, 3 μm)	Gradient MeOH–H_2O 10 mM $HCOONH_4$ Flow rate: 0.4 mL/min	Agilent 6410. ESI in PI mode MS/MS in SRM mode	LODs (water) 0.01–2 ng/L LODs (sediment) 0.03–1.67 ng/g LODs (biota) 0.08–3.75 ng/g	[3]
Influent, effluent wastewater and dehydrated sludge	50 pesticides	SPE QuEChERS	LC- Luna C18 (150 mm × 2.1 mm, 3 μm)	Gradient MeOH–H_2O 10 mM $HCOONH_4$ Flow rate: 0.4 mL/min	Agilent 6410. ESI in PI mode. MS/MS in SRM mode	LOQ (influent/effluent) 0.01–5 ng/L LOQ (sludge) 0.1–5.0 ng/g	[27]
River, coastal and wastewater	8 pesticides and DEHP	IT-SPME[c]	LC- Luna C18 (150 mm × 2.1 mm, 3 μm)	Gradient MeOH–H_2O 10 mM $HCOONH_4$ Flow rate: 0.4 mL/min	Agilent 6410. ESI in PI mode. MS/MS in SRM mode	LODs 0.025–2.5 μg/L	[28]
Fish and waters	50 pesticides	SPE QuEChERS	LC- Luna C18 (150 mm × 2.1 mm, 3 μm)	Gradient MeOH–H_2O 10 mM $HCOONH_4$ Flow rate: 0.4 mL/min	Agilent 6410. ESI in PI mode. MS/MS in SRM mode	LOD (fish) 0.01–3.8 ng/g LOQ (fish) 0.03–11.25 ng/g LOD (water) 0.01–2 ng/L LOQ (water) 0.04–6 ng/L	[29]
Fishpond water	Pentachlorophenol, niclosamide and fenpropathrin	LE[d] with DMC[e] and acetone (4:1)	LC-acclaim 120-C18 column (150 × 3 mm, 3 μm)	Gradient MeOH–20 mM NH_4OAc[w] pH 4.5 Flow rate: 0.3 mL/min	Thermo Finnigan LTQ-MS ESI in PI and NI mode SIM for pentachlorophenol SRM for niclosamide and fenpropathrin	LOD 0.02–1.2 ng/ml	[30]
Biological matrix							
Plasma	OPPs-protein bound (BuChE) and albumin	1 ml *n*-hexane	UHPLC column Acquity HSS T3 (100 mm × 2.1 mm, 1.8 μm)	Gradient AcN–H_2O 0.2% HCOOH[x] Flow rate: 0.1 mL/min	Thermo Scientific, TSQ Quantum Ultra triple quad SRM mode	–	[31]
Food (cucumber, lemon, wheat flour, rocket, and black tea)	300 pesticides	10 mL AcN[f]	First dimension: YMC-Pack Diol (100 mm × 2.1 mm; 5 μm; 120 Å). Second dimension: Poroshell 120 EC-C18 (100 mm × 2.1 mm; 2.7 μm; 120 Å). Trapping column: ZORBAX SB-C8 (12.5 mm × 4.6 mm; 5 μm; 80 Å)	First dimension: Gradient H_2O–AcN/H_2O (90:10) 5 mM/L $HCOONH_4$ and 0.1% CH_3COOH Flow rate: 0.2 mL/min RP separation: Gradient MeOH–H_2O5 mM/L $HCOONH_4$ and 0.1% CH_3COOH. Flow rate: 0.2 mL/min	Agilent 6460 ESI in PI and NI mode Dynamic SRM	LOD < 0.01 mg/kg	[32]
Infant formulas	Fungicides (Genistein and dicarboximide)	UAE[g] with AcN/SPE	LC–LUNA C8 (50 mm × 2 mm, 5 μm)	Gradient AcN-MilliQ H_2O 0.05% CH_3COOH Flow rate:200 μL/min	AB-SCIEX, API 3000 ESI SRM mode, 2 transitions	LOD 0.6–16.5 ng/g	[33]

Table 1.1 (*Continued*)

Matrix	Pesticide	Extraction	Separation		Determination		Refs.
			Column	Mobile phase	Detection	Sensitivity	
Red wine	18 pesticides	Comparison MASE[h] and QuEChERS	LC-RP C18 Aqua column (50 mm × 2 mm, 5 μm, 125 Å)	Gradient: (A) 30% MeOH–70% H_2O (B) 90% MeOH–10% H_2O; 2 mM $HCOONH_4$ Flow rate: 0.2 mL/min	AB Sciex, API 2000 ESI in PI mode SRM mode, 2 MS/MS transitions	LOQ 3 ng/L	[5]
Meat products	188 OPPs and others	LE EtAc[i]-cyclohexane, GCP, and PSA/silica-gel SPE	LC-Ascentis C18 (100 mm × 3 mm, 3 μm)	Gradient AcN–H_2O10 mM NH_4OAc. Flow rate: 500 μL/min	Agilent MSD SL ESI in PI and NI mode	LOQ < 0.01 μg/g	[34]
Fruit and vegetables	9 OPPs	MSPD[j]	UHPLC column Zorbax RRHT SB-C18 (100 mm × 2.1 mm, 1.8 μm)	Gradient 0.1% HCOOH – 10 mM/L NH_4OAc,AcN–0.1% HCOOH	Agilent 6410. ESI in PI mode SRM, 2 MS/MS transitions	LOD 0.06–0.15 μg/kg LOQ 0.2–0.5 μg/kg	[35]
Grapes, baby food and wheat flour	48 pesticides	On-line chromatographic cleanup (Turbulent flow chromatography): MCX-2 50 mm × 0.5 mm TurboFlow™ (TX) column	LC-Hypersil BDS C18 (100 mm × 3 mm, 3 μm)	Gradient MeOH–H_2O, 0.1% $HCOONH_4$ Flow rate: 1 mL/min	Thermo Fisher Scientific, TSQ Access Max triple quadrupole ESI in PI mode, SRM	LOD < 0.8–6.0 ng/g (baby food) LOD < 0.8–10.3 ng/g (grapes and wheat flour)	[36]
Vegetable (eggplant and lettuce) and fruit (strawberry)	Thiram (dithiocarbamate fungicide)	Na_2SO_4[k],EDTA and AcN	LC-Discovery C18 (50 mm × 2.1 mm, 5 μm)	MeOH–H_2O 0.1 mM NH_4OAc Flow rate: 300 μL/min	Waters, Quattro Micro ESI in PI mode. Full MS and 4 MS/MS transitions, one for quantification and 3 for confirmation	LOD < 0.0012 mg/kg	[37]
Infant milk formula	Pesticides	MAE[l] and SPE	LC- Zorbax Rx-SIL (250 mm × 4.6 mm, 5 μm)	Gradient (A) 5 mM $HCOONH_4$ and 0.1% HCOOH in H_2O and (B) 5 mM $HCOONH_4$ and 0.1% HCOOH in AcN Flow rate: 1 mL/min	Agilent 6410 ESI in PI mode SRM, 2 transitions	LOD 0.12–2.53 μg/kg LOQ 0.41–8.42 μg/kg	[38]
IT							
Biological matrix							
Honey	12 Insecticides (OPPs and carbamates)	Comparison: Quechers SPE PLE[m] SPME[n]	LC- Luna C18 (250 mm × 4.6 mm, 5 μm)	Gradient MeOH–H_2O Flow rate: 0.7 mL/min	Esquire 3000 (Brucker) APCI in both PI and NI modes. Full MS and SRM for MS^n	CCα[y]-0.01–1.1155 μg g^{-1}	[39]
Baby food	Fungicide residue	QuEChERS	LC- Zorbax Eclipse XDB-C8 (50 mm × 4.6 mm, 5 μm)	H_2O 0.1% HCOOH and AcN Flow rate: 0.6 mL/min	Esquire 6000 (Brucker) ESI in PI mode, Full-MS and MS/MS	LODs 0.5–3.0 μg kg^{-1}	[40]
Fruits	Pesticides	MSPD	LC- Zorbax Eclipse XDB-C18 (75 mm × 4.6 mm, 3.5 μm)	Gradient MeOH–H_2O10% CH_3COOH Flow rate: 0.5 mL/min	Thermo Fisher (LCQ Advantage), ESI in PI mode, SRM mode	LOD < 1.4 ng g^{-1} LOQ < 5 ng g^{-1}	[41]
LIT							
Environmental matrix							
River and underground waters	Chiral pesticides (enantiomers of mecoprop and dichlorprop)	SUSME[*] with THF and re-extraction in acetate buffer (pH 5.0)	LC-Nucleodex α-PM (200 mm × 4 mm, 5 μm)	Isocratic: 65% MeOH and 35% 100 mM HCOOH/$HCOONH_4$ (pH 4.0) Flow rate: 0.5 mL/min	ABSciex 4000 Turbo Ion Spray (TIS) interface in NI mode SRM mode	LOQ 1–4 ng/L	[42]
Groundwaters and surface water	150 pesticide metabolites	Direct injection	LC-Synergy Fusion-RP 100 A (50 mm × 2 mm, 2.5 μm)	Gradient of 90/10 (v/v) H_2O/MeOH-10/90 (v/v) H_2O/MeOH 0.2% CH_3COOH. Flow rate:250 μL/min	ABSciex, API 5500 ESI in PI and NI mode SRM with 2 mass transitions	LOQ 0.025–0.1 μg/L	[43]

Biological matrix							
Fruit juices	53 pesticides (OPPs and others)	Centrifugation and mix 100 μL of juice with 900 μL of AcN	LC- Reverse-phase C8 column (150 mm × 4.6 mm, 5 μm)	Gradient AcN-H_2O 0.1% HCOOH Flow rate: 400 μL/min	ABSciex, 5500 QTRAP ESI in PI and NI mode, SRM transitions with scheduled SRM mode. 2 MS/MS transitions per compound	LOQ 0.1–5 μg/L	[44]
Fruits, cereals, spices and oil seeds	288 pesticides, 38 mycotoxins	Comparison 3 methods: (A) Aqueous AcN extraction followed by partition (QuEChERS) (B) Aqueous AcN extraction (C) AcN extraction.	UHPLC column Acquity UHPLC HSS T3 (100 mm × 2.1 mm, 1.8 μm)	ESI (+): 5 mM $HCOONH_4$ and 0.2% HCOOH in Milli-Q H_2O and MeOH ESI (−): 5 mM NH_4OAc in Milli-Q H_2O–MeOH Gradient of flow rate: 0.3–0.7 mL/min	ABSciex, 5500 QTRAP Turbolon^{TM} electrospray (ESI) in PI and NI mode. SRM mode	LOQ (method A) < 10 μg/kg	[45]
Human whole blood and urine	Disulfoton and its oxidative metabolites	QuEChERS	LC-CAPCELL-PAK MG II column (35 mm × 2 mm, 5 μm)	Gradient of (A) 95% 10 mmol/L $HCOONH_4$ 5% MeOH and (B) 5% 10 mmol/L $HCOONH_4$ 95% MeOH Flow rate: 0.2 mL/min	ABsciex 3200 ESI (SRM-EPI) scan mode	LOD (whole blood) 0.90–1.15 ng/mL LOD (urine) 0.46–1.05 ng/mL LOQ (whole blood & urine) < 5 ng/mL	[46]
Green and BlackTea	Pesticide residues	Comparison 3 methods: QuEChERS–dSPE, AcN–dSPE, AcN–HTpSPE[o]/HTpSPE clean-up	LC- Chromolith Performance RP-18 endcapped (100 mm × 3 mm)	GradientAcN-10 mM $HCOONH_4$ with 2% of MeOH	AB SCIEX, 5500 QTRAP ESI in PI mode SRM mode, two specific precursor-to-product ion transitions	LOQ < 0.002 mg/kg	[47]
Cereals	19 triazine pesticides and degradation products	LLE[p] (petroleum ether saturated with AcN)-SPE	LC-CAPCELL PAK CR 1:20 (100 mm × 2.0 mm, 3 μm).	Gradient (A) $HCOONH_4$ 10 mM and HCOOH, 0.1%, v/v and (B) AcN Flow rate: 0.2 mL/min	Thermo Finnigan ESI in PI mode SRM mode LIT-MS^3	CC_α 0.0020–0.4200 μg kg^{-1} CC_β[z] 0.0024–0.4500 μg kg^{-1}	[48]
Paddy field water	70 pesticides	Direct injection	LC-Zorbax Eclipse XDB-C8 (150 mm × 4.5 mm, 5 μm)	Gradient AcN- H_2O 0.1% HCOOH Flow rate: 0.6 mL/min	AB SCIEX, 5500 QTRAP LC/MS/MS system Turboionspray source in PI and NI mode SRM mode, 2 MS/MS transitions per compound IDA experiment in PI mode: SRM/scan/EPI	LODs 0.4–80 ng L^{-1} LOQs 2–150 ng L^{-1}	[49]
TOF							
Environmental matrix							
Surfacewater and soil samples	Pesticides and other pollutants	Waters: SPE Soils: ultrasonic bath with EtAc	UHPLC column Acquity UHPLC BEH C18 (150 mm × 2.1 mm, 1.7 μm)	MeOH -H_2O, acidified 0.01% HCOOH Flow rate: 300 μL/min	Waters, Q-oaTOF Premier ESI in PI and NI mode Accurate-mass Full MS	–	[50]
Biological matrix							
Wine	15 Fungicides	SBSE[q]	LC-Zorbax Eclipse XDB-C18 (100 mm × 2.1 mm, 3.5 μm)	Gradient: AcN -Ultrapure H_2O NH_4OAc 1 mM	Agilent 6520 Dual-Spray ESI source Operated in the 2-GHz Extended Dynamic Range resolution mode	LOQs- 0.1–2.2 ng mL^{-1}	[51]
Rice	4 herbicides, 9 fungicides, 2 insecticides	QuEChERS	UHPLC column XDB-C18 (4.6 mm × 50 mm, 1.8 μm)	Gradient: (A) AcN (95/5) 0.1% HCOOH–H_2O, (B) AcN–H_2O (95/5) HCOOH Flow rate: 0.6 mL/min	Agilent MSDTOF ESI in PI mode. TIC in Full MS	LOD < 10 μg/kg	[52]
Palm oil	Pesticides	QuEChERS	UHPLC column Zorbax Eclipse SB-C18 (50 mm × 2.1 mm, 1.8 μm)	Gradient AcN–H_2O 0.1% HCOOH Flow rate: 0.25 mL/min	Agilent MSD TOF ESI in PI mode Accurate mass spectra	LOD < 5 ng g^{-1} LOQ < 9 ng g^{-1}	[53]

Table 1.1 (*Continued*)

Matrix	Pesticide	Extraction	Separation		Determination		Refs.
			Column	Mobile phase	Detection	Sensitivity	
Fruits, cereals, spices and oil seeds	288 pesticides, 38 mycotoxins	Comparison 3 methods: (A) Aqueous AcN extraction followed by partition (QuEChERS), (B) Aqueous AcN extraction, (C) AcN extraction.	UHPLC column Acquity UHPLC HSS T3 (100 mm × 2.1 mm, 1.8 μm)	ESI (+): 5 mM $HCOONH_4$ and 0.2% HCOOH in Milli-Q H_2O-MeOH ESI (−): MeOH–H_2O 5 mM NH_4OAc Gradient of flow rate: 0.3–0.7 mL/min	Waters, LCT Premier XE (ESI) in PI and NI mode SRM mode	–	[45]
Human body fluids	Neonicotinoid pesticides	Acidic, neutral and basic SPE	UHPLC column Acquity UHPLC HSS T3 (2.1 mm × 50 mm)	Linear gradient AcN–H_2O 0.1% HCOOH Flow rate: 0.4 mL/min	Waters, LCT Premier XE Mean intensities obtained for both PI and NI modes.	LOQ (acetamiprid) 0.068 ng/mL LOQ (AM-2) 0.55 ng/mL	[54]
QTOF							
Environmental matrix							
Surface and waste water	43 pesticides, 13 pharmaceuticals, 2 drugs	SPE	UHPLC column Acquity UHPLC BEH C18 (100 mm × 2.1 mm, 1.7 μm)	Gradient MeOH–H_2O, 0.01% HCOOH Flow rate: 300 μL/min	Waters, Q-oaTOF Premier ESI interface in PI mode	–	[26]
Biological matrix							
Red pepper	Isobaric pesticides	MeOH[r]/H_2O[s] (80:20)	UHPLC column Zorbax C8 (150 mm × 4.6 mm, 1.8 & 3.5 μm). Zorbax C18 (150 mm × 4.6 mm, 1.8 μm). Phenyl column (150 mm × 4.6 mm, 1.8 μm)	Gradient 10% AcN–90% H_2O 0.1% HCOOH Flow rate: 0.6 mL/min	Agilent 6540 ESI in PI mode. Single MS Full MS and MS/MS to discriminate isobars	–	[55]
Apple and pear		SE[t] EtAc	UHPLC column Waters Acquity C18 (150 mm × 2.1 mm 1.7 μm)	MeOH–H_2O, 10 mM $HCOONH_4$ Flow rate: 200 μL/min	Waters Micromass ESI PI mode. Resolution ~ 10,000 FHWM. Full MS and MS/MS	LOQ: 0.05–1 μg	[56]
Food	240 pesticides	QuEChERS	LC-Restek Ultra Aqueous C18 (100 mm × 2.1 mm, 3.0 μm)	Gradient H_2O 10 mM $HCOONH_4$/0.1% HCOOH and MeOH 10 mM $HCOONH_4$/0.1% HCOOH	ABSciex, 5600 Q-TOF ESI, PI mode SRM mode	–	[57]
Oranges and bananas	Multiclass pesticides	SE MeOH/H_2O (80:20, v/v)	UHPLC column Acquity C18 BEH (150 mm × 2.1 mm, 1.7 μm)	MeOH -H_2O, 0.01% HCOOH Flow rate: 0.3 mL min^{-1}	Waters, Premier ESI in PI and NI mode Resolution > 10,000 FHWM. Full MS and MS/MS	–	[58]
Onion	Imidacloprid metabolites	MeOH/H_2O (80/20%) with 0.1% CH_3COOH[u]	LC-Zorbax C8 (150 mm × 4.6 mm, 3.5 μm)	Gradient 10% AcN–H_2O 0.1% HCOOH Flow rate: 0.6 mL/min	Agilent 6540 ESI in PI mode. Single Full MS and MS/MS	–	[59]
ORBITRAP							
Biological matrix							
Fruits and vegetables	166 pesticides	QuEChERS	UHPLC column Acquity UHPLC BEH C18 (100 mm × 2.1 mm, 1.7 μm)	Gradient AcN–H_2O 10 mM NH_4OAc	ThermoFisher Q-Exactive ESI in PI mode Full MS-SIM and full MS/dd-MS^2 (TopN) 70,000 FWHM resolution	LCL[**] 5–100 μg/kg	[60]
Oranges and hazelnuts	116 pesticides	On-line sample preparation	UHPLC column Hypersil GOLD. (100 mm × 2.1 mm, 1.9 μm)	GradientAcN–H_2O, 0.1% HCOOH Gradient of flow rate	ThermoFisher Exactive™ Benchtop Orbitrap. ESI in PI mode Full MS 50,000 FWHM resolution	LOQs < MRL	[61]

Fruit and vegetable peel (apples, pears, citrics)	Postharvest compounds and other xenobiotics	UAE (MeOH)	UHPLC column Kinetic C18. (100 mm × 2.1 mm, 1.7 μm)	Gradient MeOH-ultrapure H_2O, 10 mM $HCOONH_4$ Flow rate: 0.3 mL/min	Thermo Fisher, LTQ Velos ESI in PI mode Full MS	LOQ 1 ng	[62]
Environmental matrix							
Agricultural soils	Polar pesticides (OPPs and TPs)	10 mL of H_2O with ammonia (5%, v/v)	UHPLC column Hypersil Gold Phenyl (100 mm × 2.1 mm, 1.9 μm)	Gradient MeOH- aqueous solution of HCOOH (0.01%, v/v) Flow rate: 0.3 mL min^{-1}	ThermoFisher Exactive™. ESI in NI mode. Full MS and MS/MS 50,000 FWHM resolution	LOQ from 10 to 50 μg kg^{-1}	[63]
Wastewater	Acidic pesticide and pharmaceutical contaminants	SPE	LC-Eclipse XDBC18 (150 mm × 2.1 mm, 5 μm)	Gradient AcN–H_2O, 1 mM NH_4OAc	Thermo Scientific, LTQ Orbitrap Discovery ESI in NI mode. FullMS 30,000 FWHM resolution	LOQ 2.1–27 ng/L	[64]
Food and environmental samples	54 pesticides Pharmaceuticals Drugs of abuse Mycotoxines and their metabolites	QuEChERS SPE	UHPLC column Kinetic XB-C18 100 Å (5 mm × 2.1 mm, 1.7 μm)	Gradient H_2O/MeOH, 0.1% HCOOH Flow rate: 0.3 mL min^{-1}	Thermo Fisher, LTQ Velos ESI in PI and NI mode. Full MS 30,000 FWHM resolution	LOD < 2 ng/mL	[14]

[a] Solid phase extraction.
[b] Quick, easy, cheap, effective, rugged and safe.
[c] In-tube solid phase micro extraction.
[d] Liquid extraction.
[e] Dichloromethane.
[f] Acetonitrile.
[g] Ultrasonic assisted extraction.
[h] Membrane-assisted solvent extraction.
[i] Ethyl acetate.
[j] Matrix solid phase dispersion.
[k] Sodium sulphate anhydrous.
[l] Microwave assisted extraction.
[m] Pressure liquid extraction.
[n] Solid phase micro extraction.
[*] Supramolecular solvent-based microextraction.
[o] High-throughput planar solid phase extraction.
[p] Liquid–liquid extraction.
[q] Stir bar sorptive extraction.
[r] Methanol.
[s] Water.
[t] Solvent extraction.
[u] Acetic acid.
[v] Ammonium formate.
[w] Ammonium acetate.
[x] Acid formic.
[y] Decision limit.
[z] Detection capability.
[**] Lowest concentration level.

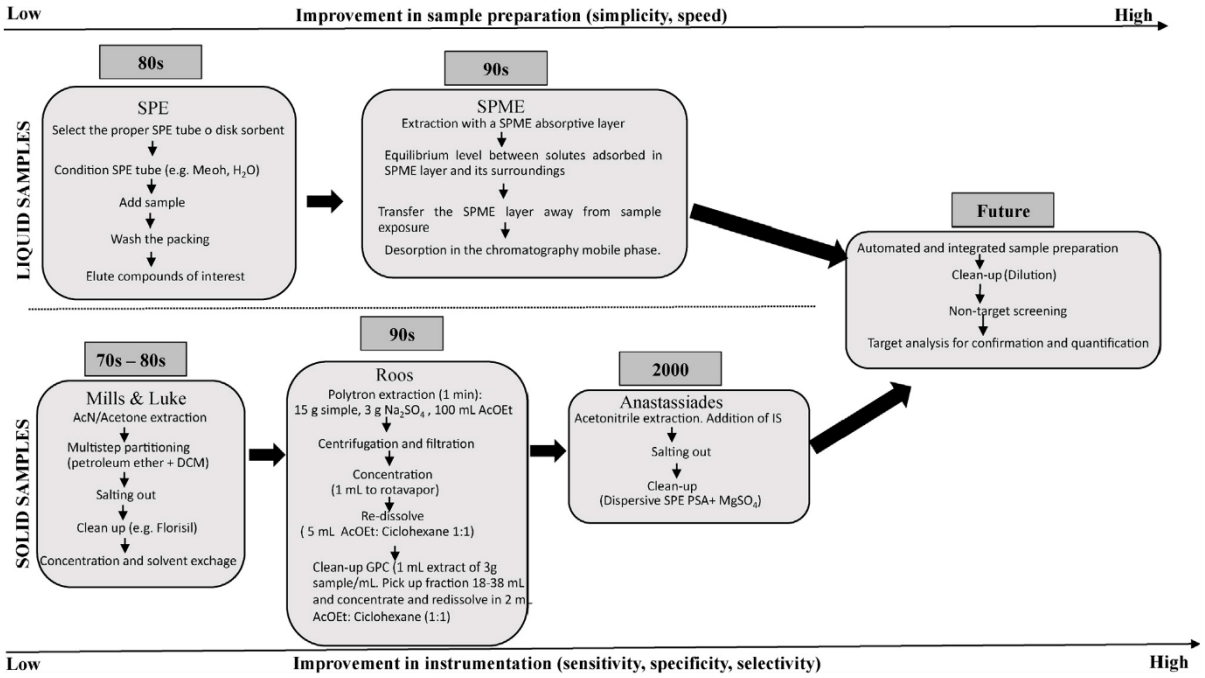


Fig. 1.2 Trends in multi-residue pesticide analysis.

simplification ensures the elimination or, at least, the reduction of organic solvents, highly toxic reagents and energy consumed in the processes as well as the determination in a single run a broad spectrum of target analytes [67]. Future trends are toward automation of analytical techniques in order to reduce labor, time and energy as well as eliminate the human factor [67]. The development of automated procedures that will provide fast, accurate, precise, solventless, inexpensive and amenable to on-line treatment is ongoing [65].

2.2. Liquid chromatography

LC separation is important to ensure the highest quality data. Reversed-phase LC (RP-LC) covers more than 95% of applications. The correct choice of the column and mobile phase is important to achieve a good separation not only between analytes but also between analytes and some polar and apolar interfering compounds [70].

In the last years, miniaturization strategies on LC columns have been developed to obtain increased sensitivity and peak resolution, efficiency, together with shorter analysis times than those achieved with conventional LC [5,26]. These strategies are mostly based on reducing the inner diameter of the LC columns or the particle size [37,71]. On the former strategy, the literature shows a clear shift toward smaller diameters in the use of normal-bore columns [from 4.6 to 2.1 mm that offer a better electrospray (ESI)-MS compatibility and lower mobile phase consumption] [3,5,26–29,32–34,37,43,46,48,51,57,64,72–85]. On the second strategy, the introduction of ultra-high performance liquid chromatography (UHPLC) columns, with smaller particle size (1.7 and 1.8 μm), have allowed improved peak resolution and, therefore, increase sensitivity in chromatographic separations [14,26,31,35,45,50,52–56,58,60–63]. Although UHPLC technology has been launched recently and its separation capabilities when combined with MS are not fully exploited, it is used in more than 50% of the applications reported in Table 1.1.

However, there are still some key challenges of LC in pesticide residue analysis that are difficult to overcome even by UHPLC, such as peak coelution and poorly retained peaks eluting in the solvent front. Multidimensional chromatography is getting interest to solve the separation challenges that cannot be achieved by one-dimension technique [86]. Different coupling techniques with various interface solutions have been developed. Recently, a fully automated system was developed for the determination of more than 300 different pesticides in various food commodities. The separation of analytes and matrix compounds in the first dimension was carried out by a YMC-Pack Diol HILIC column. All analytes eluted within one small fraction at the beginning of the run. With a packed loop interface this fraction was transferred to the second dimension, an analytical reversed phase separation performed on an Agilent Poroshell 120 EC-C18. Some very polar compounds with a stronger retention on the HILIC column were measured directly. The method was validated for over 300 pesticides in cucumber, lemon, wheat flour, rocket, and black tea. Fig. 1.3 shows the principles and the achieved separation [32]. In spite of the injection of a pure sample extract, the method showed robust results even with dirty matrices like hops and tea constituting a promising prospect within the field.

2.3. Mass analyzers

The most important advances within the field have been done in mass analyzers. There are many different mass analyzers successfully applied to pesticide residue determination. Single quadrupole, triple quadrupole (QqQ), tridimensional ion trap (^{3D}IT), linear ion trap (QqLIT), time of flight (TOF), quadrupole-time of flight (Q-TOF), and orbitrap have been used to the moment. All of them are characterized by parameters such as: accuracy, resolution, mass range, tandem analysis capabilities and scan speed, but each one present advantages and disadvantages depending on the requirements of the particular analysis. Those that directly affect to the determination of pesticide residues are outlined in Table 1.2.

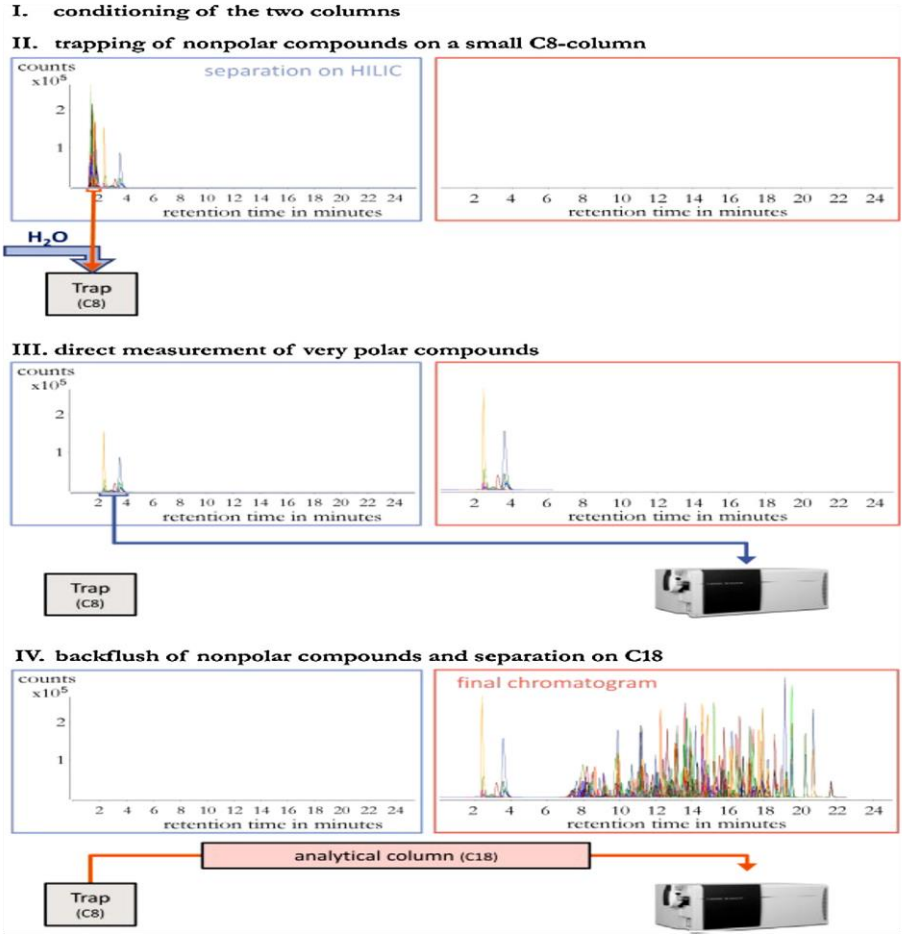


Fig. 1.3 Principle of the two-dimensional method. Reproduced with permission from [32].

Progresses are carried out in each of them in order to improve advantages and diminish disadvantages. Commonly, the price is the top to the capabilities and versatility of each instrument.

First applications of LC–MS in pesticide residue analysis were carried out with a *single quadrupole (Q)*. Nevertheless, single quadrupole does not meet the newest criteria of specificity established by EU because only in few occasions can achieve required fragmentation of the target molecules [12]. For this reason, single quadrupole MS was almost fully replaced by QqQ at the end of 90s. In fact, there are not applications reported in Table 1.1. LC–QqQ–MS/MS using ESI is a powerful analytical tool and has become the most widely used technique for the routine multiresidue screening of pesticides in water and food [12]. Table 1.1 shows selected examples of the multiresidue method developed for a wide variety of matrices including water, fish, red wine, infant formula, plasma, meat and fruits and vegetables. This mass analyzer is able to detect more than 100 pesticides simultaneously (up to 188 in those examples reported in table), with enough sensitivity to determine residues at levels below 0.01 mg kg^{-1}.

Ion-Trap offers the possibility of performing multiple stage MS fragmentation (MS^n) in the ion-trapping process, in which target ions are "trapped" and unwanted ones are eliminated selectively [39–41], resulting in a reduction of background noise in spectra MS^n. The ion trap has been used to determine pesticide residues in two configurations, the conventional tridimensional trap and the linear ion trap. The limitations of the former instrument are (i) poor resolution and mass shit, (ii) narrow dynamic range because the space charge effects diminish performance, (iii) inability to trap products ions below 50 *m/z* and existence of an upper limit on the ratio between the precursor mass and the lowest trapped fragment ion mass (ca. 0.3 depending on the *m/z* value), and (iv) reduced number of ions that can be simultaneously isolated [40,41]. Three applications to honey, baby food and fruits and vegetables are still reported in Table 1.1, even though this instrument is being replaced by the more accurate and sensitive ones.

The hybrid configuration, the QqLIT, combines the capabilities of a QqQ and a ^{3D}IT. Then, it can operate either as any of the two analyzers and perform novel scan modes not available on other instruments. It offers a range of operating modes, such as "Enhanced MS" or EMS scan and "Enhanced product ion scan" (EPI). In EMS scan mode, Q1 operates as an ion guide, Q2 cell is set to a low energy and Q3 as a linear ion trap to trap the ions, which are scanned out of the Q3 ion trap in an axial direction toward the ion detector to yield a highly sensitive MS scan. In order to acquire MS/MS scans, EPI employs the tandem-in-space capabilities of the ion path with the high sensitivity ion trap mass scan. In this mode, the ion selection and fragmentation are performed as in a triple quad instrument, precursor ion is selected in Q1 and fragments generated in Q2 are trapped in Q3 that works as a trap prior detection [87]. Usui et al. [46] exploited the EPI mode to distinguish disulfoton and its oxidized metabolites in blood and urine establishing its major patterns of oxidation. However, the

Table 1.2
Performance of different instruments.

Mass analyzer	Advantages	Limitations	Refs.
QqQ	• High sensitivity in SRM, selectivity and dynamic range • Compound identification is typically performed monitoring 2 SRM transitions and calculating the ratio quantifier/qualifier ions. • LODs in the ppt range • Dynamic range ≥3 orders of magnitude	• Medium sensitivity in full scan • Nominal mass resolution can cause of false positives. • False negatives if one of the SRM is affected by matrix effects. • Only useful for target analysis	[1]
QqLIT	• High sensitivity in full scan and SRM. • Reduction of the potential risk of false positives and negatives by acquiring EPI[a] spectra followed by library searching. • High selectivity. Full MS, MS^2 and MS^3 with high sensitivity • LODs in the ppt range • Dynamic range ≥3 orders of magnitude	• Low accuracy • Nominal mass resolution can cause false positives. • More versatile to perform target than non-target analysis • Complexity of the different working modes that sometimes are not fully exploited	[64]
TOF-MS	• High sensitivity in full scan • High acquisition speed • High mass range and resolution power > 10,000 FWHM • High accuracy • LODs	• Low selectivity • Medium dynamic range of two orders of magnitude that compromise quantification of target pesticides at ultra-trace levels. • Lack of MS/MS capabilities	[135]
QTOF-MS	• Full spectrum acquisition and accurate mass • High sensitivity in full scan, mass resolution and accuracy for both MS and MS/MS • High selectivity, high acquisition speed, resolution power > 10,000 FWHM and MS/MS • LODs	Medium dynamic range of two orders of magnitude due to the ion saturation at the upper part of the concentration range	[147]
Orbitrap	• High sensitivity in full scan, selectivity and accuracy. • High speed scan, accuracy and resolving power (>60,000 FWHM)	• Medium dynamic range • Lack of MS/MS capabilities	[35,61]
LTQ-Orbitrap	• High sensitivity in full scan, selectivity and accuracy • High speed scan, accuracy and resolving power (>60,000 FWHM) and MS^n • Many different working modes	• Medium dynamic range • The software does not allow to fully exploit the identification possibilities of the software • Low scan speed and then, long cycles times.	[35,60]
Q-orbitrap	• High sensitivity in full scan, selectivity and accuracy. • High speed scan, accuracy and resolving power (>60,000 FWHM) and MS^2	• Medium dynamic range • Low scan speed and then, long cycles times	[35,62]

[a] Enhanced product ion.

determination for routine monitoring was carried out by conventional selected reaction monitoring (SRM) because its high sensitivity. In contrast, Pareja et al. [49] programmed an information dependent acquisition (IDA) experiment in the positive mode combining SRM as the survey scan and an EPI scan as the dependent scan, in the same injection. This experiment was developed for the confirmation of quinclorac, due to the absence of a second precursor → product ion transition. The ability to perform both the very selective MS/MS scans of a QqQ MS instrument and the extremely sensitive product ion scans of a ^{3D}IT reduces the limits of identification and detection [87]. QqLITs can achieve MS^3, this feature was also used for the simultaneous determination of 19 triazines and degradation products in cereals [48]. The detection limits of MS^3 in comparison with MS^2 were lower because of less baseline noise. However, most of the applications do not exploit these features and use the QqLIT just as conventional and sensitive QqQ [42–45,47] able to detect up to 288 pesticides and other food contaminants with limits of quantification below 10 $\mu g\ kg^{-1}$ for food samples.

The first application of accurate MS to identify pesticide residues was reported by Thurman et al. [88] using TOF-MS instrument. To this moment, the analysis of pesticides was always carried out using target procedures developed for a number of compounds selected a priori and previously optimized with analytical standards. This application led to an evolution in pesticide residue determinations materialized by the development of schemes for non-target screening and "unknown" identifications that have enlarged the analytical possibilities. Fig. 1.4 shows the basic principles of each type of scheme.

Nowadays the range of mass analyzers able to perform accurate mass spectra has been increased with the QqTOF and orbitrap. *TOF-MS* offers high mass range, high sensitivity in full scan due to the high acquisition speed in the detection of all ions, high resolution power (10,000 or more) and mass accuracy which allows to guess elemental composition of parent and fragment ions and mass interferences with analytes having the same nominal mass and chromatographic retention time. Applications of this mass analyzer are mostly reported for target analysis [51,52] and non-target screening [45,50,54,89] and cover water, wine, rice, oil, fruits, cereals, spices and human body fluids. The main disadvantage of this mass spectrometer is that cannot perform MS/MS. However, some useful information referent to fragment ions can be obtained because some of them are very characteristics of the molecule. If one of these characteristics fragment ions shows up at a different retention time than the protonated molecule, this may indicate the presence of a possible degradation product that either presents the same mass or contains that fragment ion, which could be useful for unknown identification.

Although these additional confirmations are possible without MS/MS, the most successful and widely applied mass analyzers for non-target screening and unknown identification is the hybrid *QqTOF*. It combines high sensitivity, mass resolution and mass accuracy for both precursor (MS) and product ions (MS/MS). The instrument is an attractive analytical tool for rapid detection and reliable identification of a large number of pesticides thanks to the full spectrum acquisition at accurate mass with satisfactory sensitivity. This process is readily boosted when combined with specialized software packages, together with theoretical exact mass databases [26,55,57,58]. Despite of its limited dynamic range (defect that has almost disappeared in the recent instruments), quantitative analysis has been successfully afforded in some applications [52,53]. The higher resolution of QqTOF increases specificity and consequently it provides *S*/*N* benefit in some analytical situations [90]. This mass spectrometer has been mostly used for the identification of non-target, unexpected compounds including transformation products of pesticides or even unknown

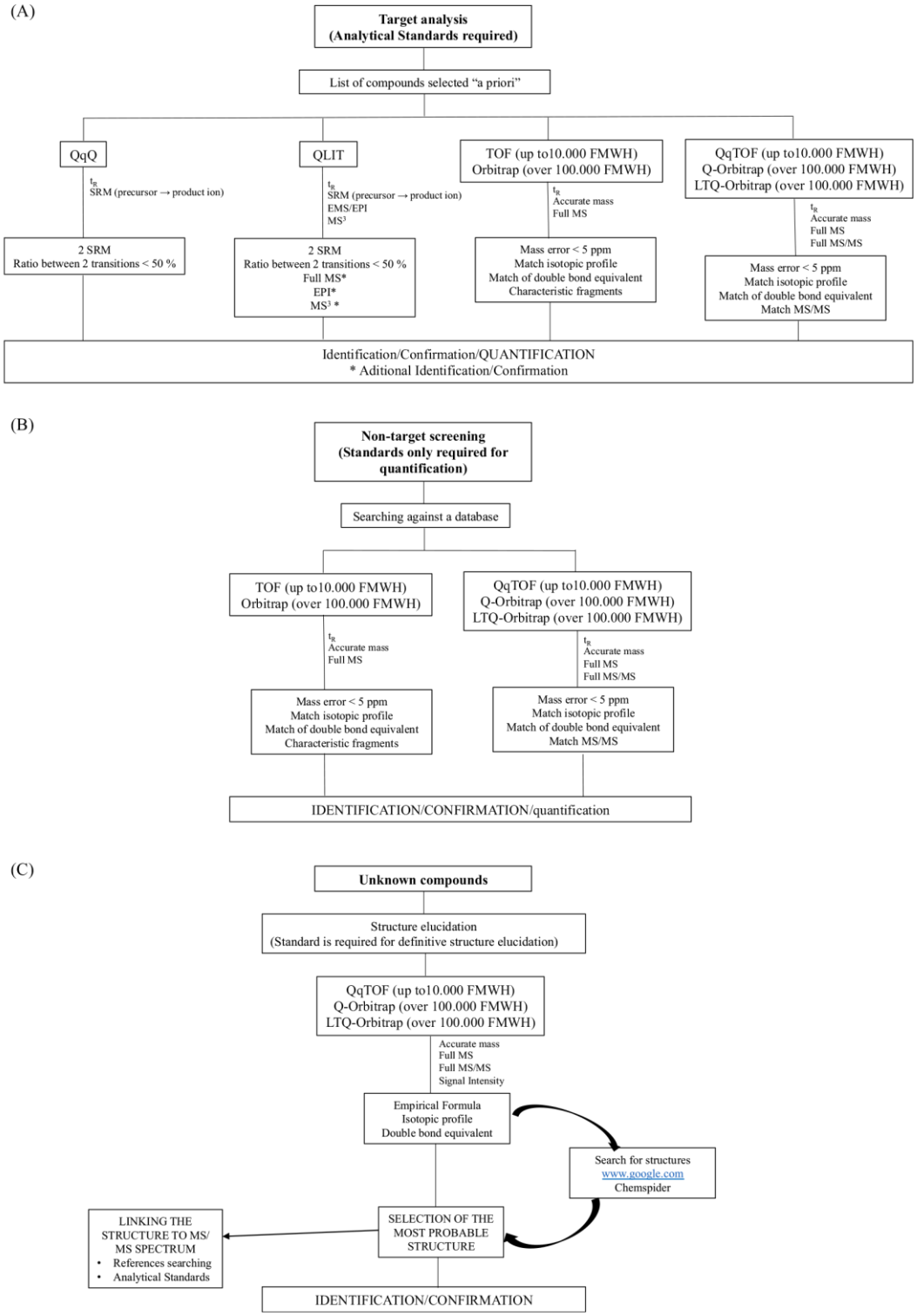


Fig. 1.4 Working flows in pesticide residue determination by LC–MS: (A) targetanalysis; (B) non-target screening; (C) unknown compounds.

compounds [45]. The process of unknown identification is very complicated. In this case, the limit is the patience and expertise of the analyst. However, the software could be an unquestionable beneficial tool to be successful.

The last addition to the extend family of mass analyzers is *Orbitrap*, developed by Makarov ten years ago and commercially introduced in 2005. It implements the principles of Fourier transform (FT) through an electrostatic axially harmonic orbital trapping technique to yield high resolution mass spectra. The standalone instrument, which provides high mass resolution (15.000 FMWH) and high accuracy (<2 ppm) as well as its combinations with a linear ion trap (LTQ-Orbitrap) and more

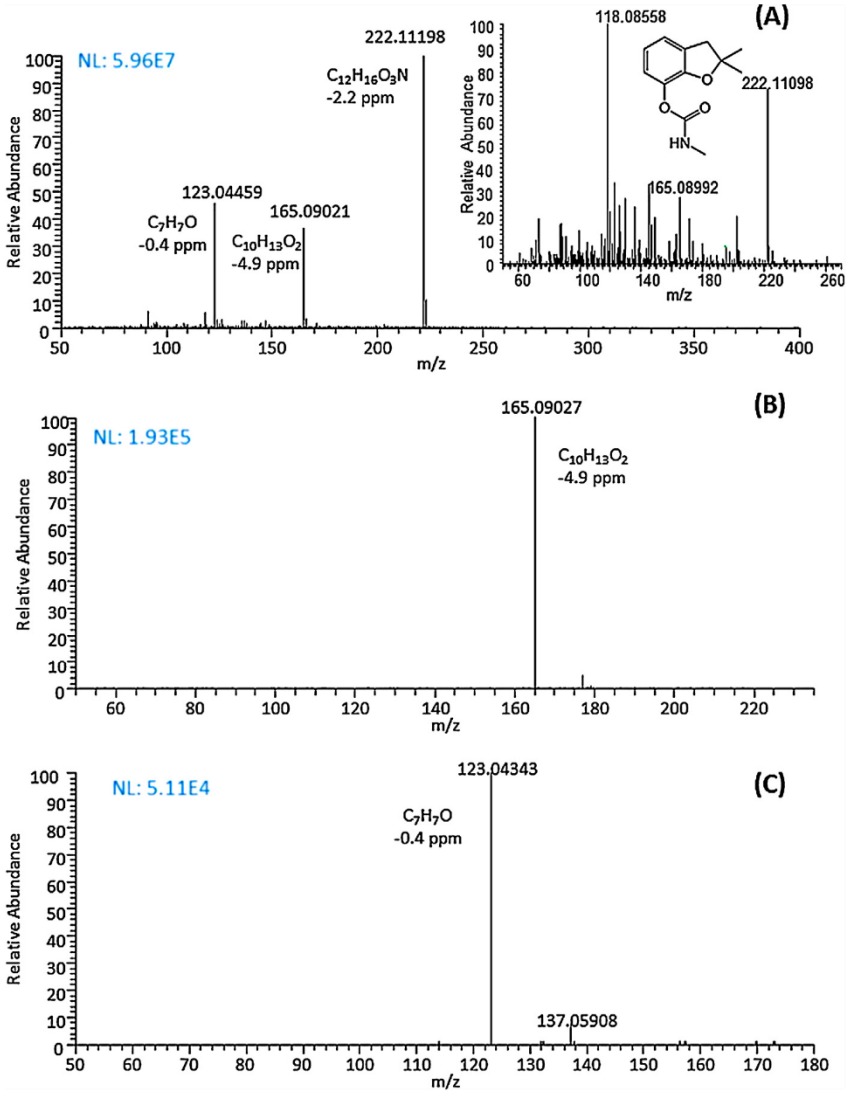


Fig. 1.5 Sequential mass spectra obtained by MS/ddMS3 for carbofuran in honeybees (A) full MS (the insert shows the mass spectrum without background subtraction), (B) full ddMS^2 from the peak at *m/z* 222 (peak was automatically selected by the instrument) and (C) full dd MS^3 from the peak at *m/z* 165 (Reprinted from [14] with permission).

recently with a quadrupole (Q-Orbitrap), have already successfully used in routine for determining pesticide residue [14,60–64,87]. The standalone orbitrap is only able to perform MS whereas Q-orbitrap performs MS/MS and LTQ-Orbitrap preserves many different working modes because both mass analyzers LTQ and Orbitrap remain highly functional. LTQ-Orbitrap offers the possibility to perform data dependent experiments that comprises a full scan experiment followed by a data dependent MS^2 or MS^3. Fig. 1.5 illustrates additional MS^3 data for carbofuran in bees.

The major drawback in quantitative analysis with LC–MS is the matrix effect – unexpected suppression or enhancement of the analyte response due to co-eluting matrix constituents that influence the ionization process. The matrix effect is related to the ionization source of the mass spectrometer, and then all mass analyzers are susceptible to suffer matrix effects. Method performance parameters such as limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy and precision, are affected by the loss of sensitivity and selectivity.

The calibration approaches most frequently used to reduce or compensate matrix effects are calibration using external standards prepared in sample matrix (matrix-matched standards) [91–93] and calibration using internal standard [68,94]. The advantage of using matrix-matched standards is the simplicity of application and economy, however, the need of obtaining a blank matrix for every sample type is mandatory, and turns it into unpractical technique for routine analysis. Otherwise, calibration with internal standards is the best option if the isotopically labeled standards of the target compounds are available. This system corrects signal suppression, as both labeled and native compounds suffer the same suppression effect. An isotopically labeled compound is the best solution for a quantitative compensation but they are sometimes not available and/or highly expensive.

3. Conclusions and future trends

In the last years, there have been an increase in the number of LC–MS applications in the field of pesticide residues. In this text, we have discussed the most recent approaches; addressing different aspects concerning all the steps of the analysis.

The improvements in sample preparation tend to reduce time and solvent consumption. In the recent years, the most used extraction techniques for liquid and solid samples are SPE and QuEChERS, respectively. Although new extraction methods based on microextraction techniques and some conventional procedures are published time-to-time, they are not implemented in routine analysis. Future trends within sample preparation for pesticide residue analysis go through miniaturization and automation and the use of solvent-free techniques, in order to reduce the time required and also decrease the possibility of introducing contaminants.

In relation to LC, miniaturization strategies based on reducing the inner diameter of the LC columns or the particle size have been developed. The introduction of UHPLC columns have improved peak resolution and, therefore, increased sensitivity in chromatographic separations. Multidimensional chromatography coupled to mass spectrometry provides more separation power and, at this moment, is an interesting prospect. However, this approach has been very restrictively applied yet.

With the introduction of QqQ-MS, IT-MS^n, LIT, TOF, QqTOF and orbitrap instruments, all major classes of pesticides can be detected, identified and quantified satisfactorily. Currently, the first option is typically QqQ-MS or LIT-MS in multiple SRM for target pesticide analysis. However, the gradual introduction of the newly developed accurate mass spectrometers, such as the QqTOF or the orbitrap, with the distinctly enhanced selectivity and the possibility to calculate elemental composition has improved the performance of the analysis and has opened the door to the non-target screening and the identification of non-expected pesticides as well as unknown metabolites. These multi-instrumental platforms are already a reality, particularly for water analysis. Future prospects, will be devoted to the fully implementation for more complex samples.

Acknowledgments

This work has been supported by the Spanish Ministry of Economy and Competitiveness through the Program Consolider-Ingenio 2010 (Project No. CSD2009-00065) and Program on Fundamental Research (Projects Nos. CGL2011-29703-C02-00, CGL2011-29703-C02-01 and CGL2011-29703-C02-02).

References

[1] E.G. Primel, S.S. Caldas, A.L.V. Escarrone, Cent. Eur. J. Chem. 10 (2012) 876.
[2] V. Andreu, Y. Pico, Anal. Bioanal. Chem. 404 (2012) 2659.
[3] A. Masia, J. Campo, P. Vazquez-Roig, C. Blasco, Y. Pico, J. Hazard. Mater. 263 (2013) 95.
[4] B. Gilbert-Lopez, J.F. Garcia-Reyes, A.R. Fernandez-Alba, A. Molina-Diaz, J. Chromatogr. A 1217 (2010) 3736.
[5] M. Moeder, C. Bauer, P. Popp, M. van Pinxteren, T. Reemtsma, Anal. Bioanal. Chem. 403 (2012) 1731.
[6] Off. J. Eur. Comm. L33 (1979) 36.
[7] Off. J. Eur. Comm. L230 (1991) 1.
[8] Off. J. Eur. Comm. L37 (2000) 1.
[9] Off. J. Eur. Comm. L331 (2001) 1.
[10] Off. J. Eur. Comm. L330 (1998) 32.
[11] M. Kuster, M. Lopez de Alda, D. Barcelo, Mass Spectrom. Rev. 25 (2006) 900.
[12] A.K. Malik, C. Blasco, Y. Pico, J. Chromatogr. A 1217 (2010) 4018.
[13] Off. J. Eur. Union L70 (2005) 1.
[14] M. Farre, Y. Pico, D. Barcelo, J. Chromatogr A. 1328 (2014) 66.
[15] Document N° SANCO/12495/2011, 2012.
[16] Off. J. Eur. Comm. L221 (2002) 8.
[17] S. Grimalt, J.V. Sancho, O.J. Pozo, F. Hernandez, J. Mass Spectrom.: JMS 45 (2010) 421.
[18] M.M. Gómez-Ramos, C. Ferrer, O. Malato, A. Agüera, A.R. Fernández-Alba, J. Chromatogr. A 1287 (2013) 24.
[19] M. Gomez-Ramos, C. Ferrer, O. Malato, A. Agueera, A. Fernandez-Alba, J. Chromatogr. A 1287 (2013) 24.
[20] B. Kmellar, L. Pareja, C. Ferrer, P. Fodor, A.R. Fernandez-Alba, Talanta 84 (2011) 262.
[21] A. Kaufmann, Anal. Bioanal. Chem. 403 (2012) 1233.
[22] F. Hernandez, J.V. Sancho, M. Ibanez, E. Abad, T. Portoles, L. Mattioli, Anal. Bioanal. Chem. 403 (2012) 1251.
[23] F. Busetti, W.J. Backe, N. Bendixen, U. Maier, B. Place, W. Giger, J.A. Field, Anal. Bioanal. Chem. 402 (2012) 175.
[24] H.V. Botitsi, S.D. Garbis, A. Economou, D.F. Tsipi, Mass Spectrom. Rev. 30 (2011) 907.
[25] M. Petrovic, M. Farre, M.L. de Alda, S. Perez, C. Postigo, M. Kock, J. Radjenovic, M. Gros, D. Barcelo, J. Chromatogr. A 1217 (2010) 4004.
[26] A. Masia, M. Ibanez, C. Blasco, J. Sancho, V.Y. Pico, F. Hernandez, Anal. Chim. Acta 761 (2013) 117.
[27] J. Campo, A. Masia, C. Blasco, Y. Pico, J. Hazard. Mater. 263 (2013) 146.
[28] A. Masia, Y. Moliner-Martinez, M. Munoz-Ortuno, Y. Pico, P. Campins-Falco, J. Chromatogr. A 1306 (2013) 1.
[29] F. Belenguer, A. Martinez-Capel, Masiá, Y. Picó, J. Hazard. Mater. 265 (2014) 271.
[30] H. Jiang, Y. Zhang, X. Chen, J. Lv, J. Zou, Anal. Methods 5 (2013) 111.
[31] M.J. van der Schans, A.G. Hulst, van der Riet-van Oeveren, D. Noort, H.P. Benschop, C. Dishovsky, Chemico-biological Interact. 203 (2013) 96.
[32] S. Kittlaus, J. Schimanke, G. Kempe, K. Speer, J. Chromatogr. A 1283 (2013) 98.
[33] S. Maggioni, R. Bagnati, M. Pandelova, K.W. Schramm, E. Benfenati, Food Chem. 136 (2013) 116.
[34] T. Matsuoka, Y. Akiyama, T. Mitsuhashi, J. Pest. Sci. 36 (2011) 73.
[35] S.x. Guan, Z.g. Yu, H.n. Yu, C.h. Song, Z.q. Song, Z. Qin, Chromatographia 73 (2011) 33.
[36] L. Hollosi, K. Mittendorf, H.Z. Senyuva, Chromatographia 75 (2012) 1377.
[37] A. Peruga, S. Grimalt, F.J. Lopez, J.V. Sancho, F. Hernandez, Food Chem. 135 (2012) 186.
[38] G. Fang, H.F. Lau, W.S. Law, S.F.Y. Li, Food Chem. 134 (2012) 2473.
[39] C. Blasco, P. Vazquez-Roig, M. Onghena, A. Masia, Y. Pico, J. Chromatogr. A 1218 (2011) 4892.
[40] B. Gilbert-Lopez, J.F. Garcia-Reyes, A. Molina-Diaz, Food Chem. 135 (2012) 780.
[41] M.M. Radisic, T.M. Vasiljevic, N.N. Dujakovic, M.D. Lausevic, Food Anal. Met. 6 (2013) 648.
[42] C. Caballo, M. Sicilia, S. Rubio, Anal. Chim. Acta 761 (2013) 102.
[43] T. Reemtsma, L. Alder, U. Banasiak, J. Chromatogr. A 1271 (2013) 95.
[44] C. Ferrer, M. Martinez-Bueno, A. Lozano, A. Fernandez-Alba, Talanta 83 (2011) 1552.
[45] O. Lacina, M. Zachariasova, J. Urbanova, M. Vaclavikova, T. Cajka, J. Hajslova, J. Chromatogr. A 1262 (2012) 8.
[46] K. Usui, Y. Hayashizaki, T. Minagawa, M. Hashiyada, A. Nakano, M. Funayama, Legal Med. 14 (2012) 309.
[47] C. Oellig, W. Schwack, J. Chromatogr. A 1260 (2012) 42.
[48] P. Li, X. Yang, H. Miao, Y. Zhao, W. Liu, Y. Wu, Anal. Chim. Acta 781 (2013) 63.
[49] L.a. Pareja, M.J. Mart+¡nez-Bueno, V.n. Cesio, H. Heinzen, A.R. Fernández-Alba, J. Chromatogr. A 1218 (2011) 4790.
[50] F. Hernandez, T. Portoles, M. Ibanez, M. Bustos-Lopez, R. Diaz, A. Botero-Coy, C. Fuentes, G. Penuela, Sci. Total Environ. 439 (2012) 249.
[51] A. Fontana, I. Rodriguez, M. Ramil, J. Altamirano, R. Cela, Anal. Bioanal. Chem. 401 (2011) 767.
[52] L. Pareja, M. Colazzo, A. Perez-Parada, N. Besil, H. Heinzen, B. Boecking, V. Cesio, A.R. Fernandez-Alba, J. Agric. Food Chem. 60 (2012) 4440.
[53] E. Sobhanzadeh, N.K. Abu Bakar, M.R. Bin Abas, K. Nemati, Environ. Monit. Assess. 184 (2012) 5821.
[54] K. Taira, K. Fujioka, Y. Aoyama, Plos One 8 (2013), http://dx.doi.org/10.1371/journal.pone.0080332, e80332.
[55] E.M. Thurman, I. Ferrer, P. Zavitsanos, J.A. Zweigenbaum, J. Agric. Food Chem. 61 (2013) 2340.
[56] Y. Pico, M. la Farre, R. Segarra, D. Barcelo, Talanta 81 (2010) 281.
[57] K. Zhang, J.W. Wong, P. Yang, D.G. Hayward, T. Sakuma, Y. Zou, A. Schreiber, C. Borton, N. Tung-Vi, B. Kaushik, D. Oulkar, Anal. Chem. 84 (2012) 5677.
[58] R. Diaz, M. Ibanez, J.V. Sancho, F. Hernandez, Anal. Methods 4 (2012) 196.
[59] E.M. Thurman, I. Ferrer, P. Zavitsanos, J.A. Zweigenbaum, Rapid Commun. Mass Spectrom. 27 (2013) 1891.
[60] J. Wang, W. Chow, D. Leung, J. Chang, J. Agric. Food Chem. 60 (2012) 12088.
[61] Y. Shi, J. Chang, C. Esposito, C. Lafontaine, M. Berube, J. Fink, F. Espourteille, Food Addit. Contam. A: Chem. Anal. Control Expo. Risk Assess. 28 (2011) 1383.
[62] M. Farre, Y. Pico, D. Barcelo, Anal. Chem. 85 (2013) 2638.
[63] J. Antonio Padilla-Sanchez, P. Plaza-Bolanos, R. Romero-Gonzalez, A. Grande-Martinez, E.M. Thurman, A. Garrido-Frenich, J. Mass Spectrom. 47 (2012) 1458.
[64] M.G. Cahill, B.A. Dineen, M.A. Stack, K.J. James, J. Chromatogr. A 1270 (2012) 88.
[65] A. Beyer, M. Biziuk, Food Chem. 108 (2008) 669.
[66] P. Mills, J. Assoc. Off. Agric. Chem. 42 (1959) 734.
[67] M. Tankiewicz, J. Fenik, M. Biziuk, Talanta 86 (2011) 8.
[68] M. Kock-Schulmeyer, M. Olmos, M. Lopez de Alda, D. Barcelo, J. Chromatogr. A 1305 (2013) 176.
[69] M. González-Curbelo, A.V. Herrera-Herrera, L.M. Ravelo-Pérez, J. Hernández-Borges, TrAC Trends Anal. Chem. 38 (2012) 32.
[70] G. Martínez-Domínguez, P. Plaza-Bolaños, R. Romero-González, A. Garrido-Frenich, Talanta 118 (2014) 277.
[71] M. Noestheden, D. Noot, R. Hindle, J. Chromatogr. A 1263 (2012) 68.
[72] A. Kruve, M. Haapala, V. Saarela, S. Franssila, R. Kostiainen, T. Kotiaho, R.A. Ketola, Anal. Chim. Acta 696 (2011) 77.
[73] Z.L. Xu, D.P. Zeng, J.Y. Yang, Y.D. Shen, R.C. Beier, H.T. Lei, H. Wang, Y.M. Sun, J. Environ. Monit. 13 (2011) 3040.
[74] K.H. Park, J.H. Choi, A. Abd El-Aty, S.K. Cho, J.H. Park, B.M. Kim, A. Yang, T.W. Na, M.M. Rahman, G.J. Im, J.H. Shim, Food Chem. 134 (2012) 2552.
[75] T. Schwarz, T.A. Snow, C.J. Santee, C.C. Mulligan, T. Class, M.P. Wadsley, S.C. Nanita, J. Agric. Food Chem. 59 (2011) 814.

[76] J. Wong, C. Hao, K. Zhang, P. Yang, K. Banerjee, D. Hayward, I. Iftakhar, A. Schreiber, K. Tech, C. Sack, M. Smoker, X. Chen, S.C. Utture, D.P. Oulkar, J. Agric. Food Chem. 58 (2010) 5897.
[77] C. Ferrer Amate, H. Unterluggauer, R. Fischer, A. Fernandez-Alba, S. Masselter, Anal. Bioanal. Chem. 397 (2010) 93.
[78] K. Tran, D. Eide, S.M. Nickols, M.R. Cromer, A. Sabaa-Srur, R.E. Smith, Food Chem. 134 (2012) 2398.
[79] J. Wang, W. Chow, D. Leung, Anal. Bioanal. Chem. 396 (2010) 1513.
[80] F. Camino-Sanchez, A. Zafra-Gomez, B. Oliver-Rodriguez, O. Ballesteros, A. Navalon, G. Crovetto, J. Vilchez, Food Addit. Contam. 27 (2010) 1532.
[81] A. Yang, J.H. Park, A. Abd El-Aty, J.H. Choi, J.H. Oh, J.A. Do, K. Kwon, K.H. Shim, O.J. Choi, J.H. Shim, Food Control 28 (2012) 99.
[82] S.W. Chung, B.T. Chan, J. Chromatogr. A 1217 (2010) 4815.
[83] A.E.-M. Afify, M.A. Mohamed, H.A. El-Gammal, E.R. Attallah, J. Food Agric. Environ. 8 (2010) 602.
[84] B. Kanrar, S. Mandal, A. Bhattacharyya, J. Chromatogr. A 1217 (2010) 1926.
[85] Y. Li, F. Dong, X. Liu, J. Xu, X. Chen, Y. Han, X. Liang, Y. Zheng, J. Hazard. Mater. 250/251 (2013) 9.
[86] G. Purcaro, S. Moret, L. Conte, TrAC Trends Anal. Chem. 43 (2013) 146.
[87] M. Himmelsbach, J. Chromatogr. B 883/884 (2012) 3.
[88] E.M. Thurman, I. Ferrer, J.A. Zweigenbaum, J.F. García-Reyes, M. Woodman, A.R. Fernández-Alba, J. Chromatogr. A 1082 (2005) 71.
[89] O. Malato, A. Lozano, M. Mezcua, A. Agueera, A.R. Fernandez-Alba, J. Chromatogr. A 1218 (2011) 7615.
[90] C. Ferrer, A. Lozano, A. Agueera, A. Giron, A. Fernandez-Alba, J. Chromatogr. A 1218 (2011) 7634.
[91] A.M. Botero-Coy, J.M. Marin, M. Ibanez, J.V. Sancho, F. Hernandez, Anal. Bioanal. Chem. 402 (2012) 2287.
[92] Y. Pico, M. Farre, C. Soler, D. Barcelo, Anal. Chem. 79 (2007) 9350.
[93] Z. Jia, X. Mao, K. Chen, K. Wang, S. Ji, J. AOAC Int. 93 (2010) 1570.
[94] J. Campo, A. Masiá, Y. Picó, M. Farré, D. Barceló, Sci. Total Environ. 472 (2014) 912.

CAPÍTOL 2

Screening sistemàtic de plaguicides per Cromatografia Líquida-Espectrometria de masses

Publicació científica 2

Combined use of liquid chromatography triple quadrupole mass spectrometry and liquid chromatography quadrupole time-of-flight mass spectrometry in systematic screening of pesticides and other contaminants in water samples

A. Masiá, M. Ibañez, C. Blasco, J.V. Sancho, Y. Picó, F. Hernández

Analytica Chimica Acta, 761 (2013) 117-127

Analytica Chimica Acta 761 (2013) 117–127

Contents lists available at SciVerse ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Combined use of liquid chromatography triple quadrupole mass spectrometry and liquid chromatography quadrupole time-of-flight mass spectrometry in systematic screening of pesticides and other contaminants in water samples

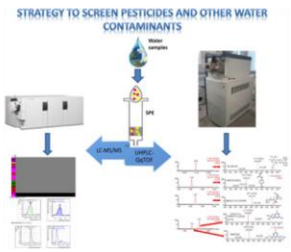
A. Masiá[a], M. Ibáñez[b], C. Blasco[a], J.V. Sancho[b], Y. Picó[a,*], F. Hernández[b]

[a] Environmental and Food Safety Research Group, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n., 46100 Burjassot, València, Spain
[b] Research Institute for Pesticides and Water, Universitat Jaume I, Av. Sos Baynat s/n, 12071 Castelló de la Plana, Castellón, Spain

HIGHLIGHTS

- HPLC–QqQ-MS/MS combined with UPLC–QTOF MS to screen pesticides.
- LC–QqQ-MS/MS was validated to determine 43 pesticide residues.
- UPLC–QTOF MS identify several family compounds by searching in a home-made database containing more than 1100 organic pollutants.
- Waste and surface samples were successfully analyzed using the developed method.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 17 May 2012
Received in revised form 16 October 2012
Accepted 18 November 2012
Available online 26 November 2012

Keywords:
Surface water
Waste water
Pesticides
Organic pollutants
LC–MS/MS
LC–QTOF MS

ABSTRACT

As a suitable way for routine screening of pesticides and control of other organic contaminants in water, the combination of liquid chromatography triple quadrupole tandem mass spectrometry (LC–QqQ-MS/MS) and liquid chromatography–hybrid quadrupole time-of-flight mass spectrometry (LC–QTOF-MS) has been applied to the analysis of 63 surface and waste water samples after conventional solid-phase extraction (SPE). The extracts were screened for 43 pesticides or degradation products by LC–QqQ-MS/MS achieving limits of detection (LOD) ranged from 0.04 to 2 ng L^{-1}. Of the 43 selected pesticides, 33 were detected in water samples. The ESI–QTOF MS instrument was run using two simultaneous acquisition functions with low and high collision energy (MS^E approach) and acquiring the full mass spectra. A home-made database containing more than 1100 organic pollutants was used for substance identification. Around 250 of these compounds were available at the laboratory as reference standards. Five pesticides and 3 of their degradation products, different to those selected in the QqQ method, were detected by QqTOF-MS. Thirteen pharmaceuticals and two drugs of abuse were also identified in the samples. In practice, the sample preparation proved to be suitable for both techniques and for a wide variety of substances with different polarity. Mutual confirmation and evidence of co-occurrence of several other organic contaminants were the main advantages of the combination of both techniques.

1. Introduction

Pesticides are widespread contaminants in agricultural crops as well as in surface and waste waters, where they enter as runoff from the treated sites [1–4]. Systematic pesticide residue determination

* Corresponding author. Tel.: +34 963543092; fax: +34 963544954.
E-mail address: Yolanda.Pico@uv.es (Y. Picó).

0003-2670/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aca.2012.11.032

is an ongoing topic of research in environmental and food safety because of the large number of possible pesticides, which may occur at very low concentrations in samples [5,6]. Most of the analytical methods proposed to determine pesticide residues are aimed at the quantification of target compounds. However, water contamination includes but is not limited to pesticides [7]. Pharmaceuticals, illicit drugs, personal care products and other substances coming from the human activity provide a cocktail of organic contaminants that may result in multiple substances acting in "an additive, synergistic or antagonistic manner" that may render impacts relatively difficult to discern [8]. The simultaneous detection and identification of these co-occurring contaminants [9–11], which is highly dynamic and requires complex analytical platforms [12,13], has become a mandatory issue. In these cases, multiresidue schemes permit the analysis of a wide range of pollutants with varying physico-chemical parameters in a single run. Currently, these schemes apply an extraction procedure as generic as possible, which, in terms of cost and time, is beneficial because the extract can "capture" all the chemicals present in a sample.

These multiresidue methods are typically carried out using liquid chromatography coupled to triple quadrupole mass spectrometry (LC–QqQ-MS) [14–23]. This technique provides high sensitivity and selectivity operating in selective reaction monitoring mode (SRM), but the number of compounds that can be simultaneously analyzed in a single run is limited to those previously selected. Mass analyzers with improved mass resolution and mass accuracy, as detectors in liquid chromatography, have been a big step forward in the use of LC–MS for contaminants screening in water, soil, sediment, biological and food samples [12,24–27]. The accurate mass together with the isotopic pattern provides valuable information for substance identification. In addition, structural information can be obtained from the fragment ion spectra generated. A home-made database of approximately 1100 organic contaminants containing information about their (de)protonated molecules and adducts, as well as fragment ions and retention time when reference standards were available, was described for use in systematic screening and monitoring by LC–QTOF MS and proved to be very useful for substance identification [27].

The present study investigated, taken as example surface and waste water samples, whether the combined use of LC–QqQ-MS/MS and LC–QTOF MS is a suitable way in routine performance of systematic pesticide residue determination. More than 60 surface and waste water samples were analyzed by both systems, after a conventional off-line solid-phase extraction (SPE). LC–QqQ-MS/MS is still one of the most efficient methods for target analysis because of its relatively simple practical performance, high robustness, sensitivity and reproducibility. It is needed for regulatory monitoring and tolerance enforcement performed routinely on agricultural runoff by certain Spanish authorities. The selection of the target pesticides and metabolites was based on extend of use, water solubility and amenability to LC–MS analysis. The list included selected compounds from different families, such as organophosphorus, ureas, phenylureas, azoles, neonicotinoids, carbamates, triazines, chloroacetanilides and acetanilides. LC–QTOF MS in combination with a large compound database is highly specific to obtain information on other contaminants present and to obtain additional confirmation of the target analytes.

2. Experimental

2.1. Reagents

High purity (98–99.9%) standards of selected pesticides, namely, acetochlor, alachlor, atrazine, atrazine-deisopropyl, atrazine-deethyl, azinphos-ethyl, azinphos-methyl, buprofezin, carbofuran, carbofuran-3-hydroxy, chlorfenvinphos, chlorpyriphos, diazinon, dichlofenthion, dimethoate, diuron, ethion, fenitrothion, fenthion, fenthion-sulfoxide, fenthion-sulfone, hexythiazox, imazalil, imidacloprid, isoproturon, malathion, methiocarb, metolachlor, molinate, omethoate, parathion-ethyl, parathion-methyl, propanil, prochloraz, propazine, pyriproxyfen, simazine, tolclophos-methyl, and terbutryn, were purchased from Sigma–Aldrich (Steinheim, Germany). Fenoxon, fenoxon-sulfoxide, fenoxon-sulfone as 1 mL solution at a concentration of 10 μg mL^{-1} in acetonitrile were from Dr. Ehrenstorfer (Augsburg, Germany).

Individual standard solutions were prepared in methanol at a concentration of 1000 mg L^{-1}. The working standard solution was prepared by mixing the appropriate amounts of the individual standard solutions and diluting with methanol to a final concentration of 0.5 mg L^{-1}. Working solutions were prepared daily by diluting this solution with water. All solutions were stored in amber glass bottles at 4 °C in the dark.

Ammonium formate, sodium hydroxide and formic acid were purchased from Sigma–Aldrich (Steinheim, Germany), and dichloromethane and methanol (gradient grade for liquid chromatography), were obtained from Merck (Darmstadt, Germany). High purity water was prepared using a Milli-Q water purification system (Millipore, Milford, MA, USA). Dichloromethane-methanol (50:50) (v/v) was used to elute the pesticides from the OASIS HLB SPE cartridge (200 mg sorbent/6 mL cartridge, Waters). Milli-Q water and methanol, both with ammonium formate 10 mM, were used as mobile phase in LC–MS/MS whereas Milli-Q water and methanol, both with 0.01% formic acid (HCOOH) were used as mobile phase in LC–QTOF-MS.

2.2. Sampling and sample preparation

Surface water samples (35) were taken from Jucar, Ebro, Llobregat and Guadalquivir rivers. They were collected in the middle of the current river with 2.5 L amber glass bottles. Influent (14) and effluent (14) waste water samples were also taken in different WWTPs located along these four rivers using automatic 24-h volume-proportional composite sampling (device takes a constant sample volume at variable time intervals after a certain volume of sewage has passed the sampling point). Samples were obtained from WWTP in 2.5 L polyethylene bottles. Immediately after sampling, they were transported to the laboratory, where they were kept frozen at −20 °C until the analysis.

Before the analysis, both surface and waste waters were vacuum filtered through 1 μm glass fiber filters followed by 0.45 μm nylon membrane filters (VWR, Barcelona, Spain).

The pre-concentration applied to the water samples is based on the off-line SPE procedure described by de Almeida Azevedo et al. [28]. 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 will vary through the extraction by the clogging of the sorbent, consequently, the flow rate is somewhat variable). The cartridges 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) drop by drop (flow rate ca. 1 mL min^{-1}). Extracts were evaporated to dryness at 40 °C under a stream of nitrogen in a Zymark TurboVap LV evaporator (Hopkinton, MA, USA) and reconstituted with 1 mL of methanol. Then, they were filtered through 0.45 μm PTFE filters into the autosampler vials for LC–MS analysis.

2.3. Liquid chromatography triple quadrupole mass spectrometry

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). Data were processed using a MassHunter Workstation Software for qualitative and quantitative analysis (A GL Sciences, Tokyo, Japan).

The chromatographic column was a Luna C18 (15.0 cm × 0.21 cm) with a 3 μm particle size (Phenomenex, Torrance, USA). The column temperature was kept at 30 °C and the volume injected was 5 μL. A binary mobile phase at flow rate of 0.4 mL min^{-1} with a gradient elution was used. Solvent A was Milli-Q water with 10 mM ammonium formate and solvent B was methanol also with 10 mM ammonium formate. The linear gradient was as follows: 0 min (50% B), 10 min (83% B), 12 min (83% B), 12.5 min (98% B), and 15.5 min (98% B). Then, the mobile phase returns to the initial conditions with an equilibration time of 12 min.

Ionization and fragmentation settings were optimized by direct injection of pesticide standard solutions. MS/MS was performed in the SRM mode using ESI in positive mode. For each compound, two characteristic product ions of the protonated molecule $[M+H]^+$ were monitored, the first and most abundant one was used for quantification, while the second one was used as a qualifier. Collision energy and cone voltage were optimized for each pesticide (Table 2.1). Nitrogen was used as collision, nebulizing and desolvation gas. The ESI conditions were: capillary voltage 4000 V, nebulizer 15 psi, source temperature 300 °C and gas flow 10 L min^{-1}. In order to maximize sensitivity, dynamic MRM was used, with MS_1 and MS_2 at unit resolution and cell acceleration voltage of 7 eV for all the compounds.

2.4. Ultra high-pressure liquid chromatography (UHPLC)–QTOF MS

A Waters Acquity UPLC system (Waters, Milford, MA, USA) was interfaced to a hybrid quadrupole-orthogonal acceleration-TOF mass spectrometer (Q-oaTOF Premier, Waters Micromass, Manchester, UK), using an orthogonal Z-spray-ESI interface operating in positive ion mode. Chromatographic separation was performed using an Acquity UPLC BEH C_{18} 1.7 μm particle size analytical column 100 × 2.1 mm (Waters) at a flow rate of 300 μL min^{-1}. The mobile phases used were (A) formic acid 0.01% in water and (B) formic acid 0.01% in methanol. The following gradient profile was used: 0 min (10% B), 14 min (90% B), 16 min (10% B), and 18 min (90% B). Nitrogen was used as drying gas and nebulizing gas. The gas flow was set at 600 L h^{-1}. TOF-MS resolution was approximately 10,000 full width half maximum (FWHM) at m/z 556. MS data were acquired over an m/z range of 50–1000. The micro-channel plate (MCP) detector potential was set to 1850 V. A capillary voltage of 3.5 kV and cone voltage of 25 V were used. Collision gas was argon 99.995% (Praxair, Valencia, Spain). The interface temperature was set to 350 °C and the source temperature to 120 °C. The column temperature was set to 40 °C.

For MS^E experiments, two acquisition functions with different collision energies were created [29]. The first one, the low energy function (LE), selecting a collision energy of 4 eV, and the second one, the high energy (HE) function, with a collision energy ramp ranging from 15 to 40 eV in order to obtain a greater range of fragment ions. The LE and HE functions settings were for both a scan time of 0.2 s and an inter-scan delay of 0.05 s. The automated attenuated function was also selected to correct for possible peak saturations (extended mode).

Calibrations were conducted from m/z 50 to 1000 with a 1:1 mixture of 0.05 M NaOH:5% HCOOH diluted (1:25) with acetonitrile–water (80:20). For automated accurate mass measurement, the lock-spray probe was utilized, using as lockmass a solution of leucine enkephalin (2 μg mL^{-1}) in acetonitrile–water (50:50) at 0.1% HCOOH, pumped at 30 μL min^{-1} through the lock-spray needle. A cone voltage of 65 V was selected to obtain adequate signal intensity for this compound (~500 counts s^{-1}). The protonated molecule of leucine enkephalin at m/z 556.2771 was used for recalibrating the mass axis and ensuring a robust accurate mass measurement along time.

It should be noted that all the exact masses shown in this work have a deviation of 0.55 mDa from the "true" value, as the calculation performed by the MassLynx software uses the mass of hydrogen instead of a proton when calculating $[M+H]^+$ exact mass. However, because this deviation is also applied during mass axis calibration, there is not negative impact on the mass errors presented in this article.

MS data were acquired in centroid mode and were processed by the ChromaLynx XS application manager (within MassLynx v 4.1; Waters Corporation).

The compound database used in this work included around 1100 organic contaminants of different families. Compounds searched included pharmaceuticals, pesticides, drugs of abuse, hormones, UV-filter agents, colorants, preservatives, phenols and surfactants, and a notable number of transformation products/metabolites. Around 250 reference standards were available at our laboratory, and therefore information about retention time, fragmentation and adduct formation was also included in the target list for those compounds to facilitate and enhance reliability in the identification/elucidation process. For the rest of compounds, only the elemental composition (i.e., exact mass) was included. Different strategies were followed depending on the availability or not of the reference standard for a detected compound [27], as described in Section 3.2.

2.5. Data analysis and validation parameters

The linearity of the MS/MS method was established with eight calibration points, using external standards over a concentration range of 3000 to 2×10^{-6} ng L^{-1} (equivalent to 15–10,000 ng L^{-1} in water samples) for all pesticides. Peak area of target analytes was calculated using Mass Hunter software (Agilent). Each point was obtained as the mean of three injections. The data were fit to a linear least-squares regression curve with a $1/x$ weighting, and was not forced through the origin. Linearity was assumed when regression coefficient was >0.99 with residuals lower than 30%. Although the calibration line did not force the line through the origin, concentrations were estimated with good reproducibility compared to those added initially to the samples.

The sensitivity of the method was estimated by establishing the limits of detection (LODs) and quantification (LOQs). LODs were calculated using standard solutions prepared in spiked influent waste water samples (the most complex matrix). If one compound was initially in the waste water samples (e.g. imazalil), another waste water free of the compound was used to establish LODs and LOQs for it. The LODs were determined as the lowest pesticide concentration whose qualified transition (SRM_2) presented a signal-to-noise ratio (S/N) ≥ 3. The LOQs were determined also in pure solvent and in waste water, as the minimum detectable amount of analyte with a S/N ≥ 10 for the SRM_1 transition.

Influent waste water samples were extracted and analyzed according to the described procedure in order to assess interferences from extraction or matrix, or potential ion suppression. For the assessment of matrix effects, a comparison of the slopes of the regression lines, in pure solvent and in matrix-matched

Table 2.1
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)	SMR_2[f]	CE[e] (V)	Frag[d] (V)	SMR_2/SRM_1 (%) (%RSD)[g]
Acetochlor	10.07	2	270	224	120	10	148	120	10	82.3 (21)
Alachlor	10.07	2	270	238	80	15	162	80	10	61.8 (18)
Atrazine	6.52	2.63	216	132	120	15	174	120	20	71.6 (17)
Atrazine-deethyl	2.54	2.5	188	146	120	15	104	121	24	37.1 (13)
Atrazine-deisopropyl	1.75	2.08	174	96	120	15	132	120	15	84.1 (11)
Azinphos-ethyl	10.16	1.71	346	97	80	20	137	80	32	85.2 (8)
Azinphos-methyl	8.17	1.24	318	125	80	8	132	80	12	39.3 (10)
Buprofezin	14.5	1.1	306	201	120	10	116	120	15	45.1 (7)
Carbofuran	4.37	2.91	222	123	120	10	165	70	15	78.7 (13)
Carbofuran-3-hydroxy	1.85	2.48	255	163	70	5	220	70	15	64.2 (12)
Chlorfenvinphos	11.74	1.61	359	155	120	10	127	120	15	66.2 (8)
Chlorpyriphos	15.33	2.23	350	350	92	13	198	97	92	83.3 (12)
Diazinon	11.77	1.89	305	169	128	17	153	128	21	74.7 (11)
Dichlofenthion	14.68	2	315	259	120	10	287	120	5	37.4 (16)
Dimethoate	2.06	2.59	230	199	80	10	171	80	5	50.1 (8)
Diuron	7.5	1.25	233	72	120	20	160	120	20	5.5 (11)
Ethion	14.88	1.23	385	199	80	5	171	80	15	45.3 (12)
Fenitrothion	10.03	1.18	278	125	140	15	109	121	12	95.6 (14)
Fenoxon	8.17	1.73	263	231	128	9	216	128	21	35.4 (18)
Fenthion	11.51	1.83	279	247	114	5	169	114	13	71.6 (11)
Fenoxon sulfoxide	4.95	1.83	279	247	114	5	169	114	13	80.9 (9)
Fenoxon sulfone	5.49	3	295	280	136	33	109	136	13	91.2 (13)
Fenthion sulfoxide	5.85	2.68	295	109	136	33	280	136	13	99.8 (14)
Fenthion sulfone	6.22	2.3	311	125	146	21	109	146	17	62.9 (12)
Hexythiazox	15.11	1.15	353	228	120	20	168	120	10	69.1 (10)
Imazalil	11.4	1.71	297	159	120	20	201	120	15	49.5 (13)
Imidacloprid	1.61	1.96	256	209	80	10	175	80	10	94.9 (10)
Isoproturon	6.83	2.37	207	72	120	20	165	120	10	14.7 (16)
Malathion	9.36	1.96	331	99	80	10	127	80	5	92.4 (9)
Methiocarb	8.64	1.93	226	121	80	5	169	80	10	90.3 (12)
Metholachlor	10.49	2.04	284	252	120	15	176	120	10	17.8 (15)
Molinate	9.41	1.98	188	126	80	20	55	80	10	70.0 (18)
Omethoate	1.06	2.67	214	125	80	5	183	80	20	59.6 (5)
Parathion-ethyl	11.11	1.91	292	236	88	4	264	88	8	36.1 (9)
Parathion-methyl	8.17	1.5	264	125	120	20	232	110	5	37.6 (17)
Prochloraz	12.08	1.91	376	308	80	10	266	80	10	18.7 (15)
Propanil	8.6	2.01	218	162	120	20	127	120	15	72.9 (12)
Propazine	8.74	2	230	146	120	15	188	120	20	94.2 (7)
Pyriproxyfen	14.78	1.33	322	227	120	10	185	120	10	51.8 (13)
Simazine	4.53	1.76	202	124	120	20	132	120	20	77.9 (10)
Terbutryn	10.63	1.2	242	186	120	20	71	120	15	5.9 (15)
Tolclofos-methyl	12.13	1.71	301	125	115	12	269	120	15	73.8 (19)

[a] t_R = retention time.
[b] Δt_R = delta retention time, that is the centered 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.

standards, was performed. Matrix-matched calibration solutions were prepared by spiking influent waste water extracts at equal concentrations in the final extract as the solvent standards. If one compound initially existed in the waste water samples, other waste water free of the compound was used to establish matrix effects for it. Three different waste waters were used in order to assess the matrix effects.

Recovery and precision, expressed as percentage relative standard deviation (%RSD), were determined by analyzing influent waste water samples spiked at two concentrations (15 and 150 ng L^{-1}). Quantification to validate the method was carried out using matrix matched standards to avoid overlap between concepts such as recovery and matrix effects. Intra-day precision data were obtained from five analyses performed on one day; inter-day data were obtained by analyzing a total of 10 extracts of the same concentration over 5 days (2 samples per day for 5 days; $n = 10$). The recovery was determined as the average of five analyses of a spiked influent waste water sample at both concentrations. If one compound initially existed in the waste water samples, background-subtracted peak area was used to calculate the recovery. Three different waste waters were used to perform these assays.

3. Results and discussion

3.1. Liquid chromatography–triple quadrupole tandem mass spectrometry

3.1.1. Optimization of LC–MS/MS conditions

For the SRM method, two transitions per compound were selected in order to comply with EU requirements for confirmatory analysis (Commission Decision 2002/657/EC or No. SANCO/12495/2011) [30,31]. Both documents establish the same criteria to confirm the results. The less intense transition (SRM_2) was used for the confirmation of each analyte, while for quantitative purposes the peak area of the most intense transition (SRM_1) was considered. The ratios between the two transitions (SRM_2/SRM_1) and their RSD were calculated from mean values

A. Masiá et al. / Analytica Chimica Acta 761 (2013) 117–127

obtained from the matrix-matched calibration curves, so as to consider how they varied depending on concentration. In all cases, variations observed were lower than 19% (except for acetochlor). The ratio of deviation was used as the identification criterion along with the retention time, according to Refs. [30,31]. The results are presented in Table 2.1. The low intensity of the second transition of diuron (5.5%), isoproturon (14.7%), metolachlor (17.8%) and terbutryn (5.9%) limited the confirmation for those particu-lar pesticides. This is a drawback when identifying these analytes, especially at low concentrations, at which the signal-to-noise ratio for confirmation must be higher than three; however, in pesti-cide identification, the importance of having a qualifying transition was considered greater than sensitivity because in any case LODs achieved were low.

Other important aspects are peak shape and resolution. Analytes that give poor peak shapes have higher LODs, are more difficult to identify and integrate. They are more prone to interferences than analytes that give narrow peaks. Acetamide herbicides particularly can be challenging, often yielding broad peak shapes or excessive tailing making reliable quantitation at low levels more problematic. Kmellára et al. [32] evaluated the most common modifiers employed in the analysis of pesticides everywhere and concluded that they were formic acid (0.01–0.2%), ammonium formate (2–10 mmol L^{-1}) and ammonium acetate (1–20 mmol L^{-1}). These authors also showed that using the same column the differences in the retention time obtained with formic acid as modifier instead of ammonium salts were in the range of ±1 min in majority of the cases.

Fig.2.1 shows the chromatograms obtained in dynamic SRM mode corresponding to the LC–MS/MS analysis of a waste water spiked at 15 ng L^{-1} and includes the signal to noise (S/N) ratios obtained for the pesticide transitions. There were important differences in the S/N ratios for the different pesticides and transitions, which are directly related to the LODs and LOQs (see Table 2.2). It is interesting

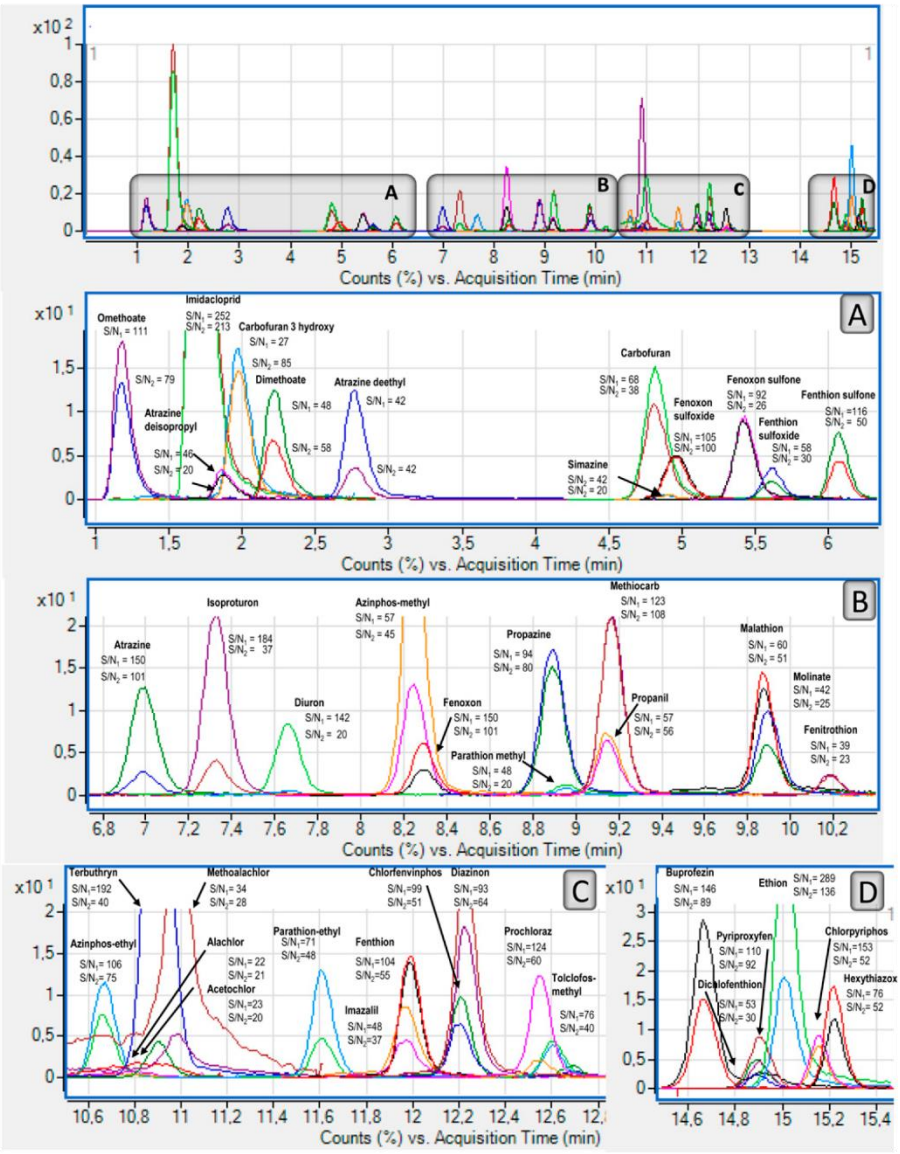


Fig. 2.1 LC–MS/MS chromatogram in the dynamic SRM mode corresponding to a waste water effluent sample spiked at 15 ng L^{-1}. Signal-to-noise (S/N) ratios for each transition have been detailed. S/N_1 = signal-to-noise ratio for the precurson → product ion transition used to quantify and S/N_2 = signal-to-noise ratio for the precurson → product ion transition used to qualify.

Table 2.2
Main validation parameters of the LC–QqQ-MS/MS method: LOD, LOQ, coefficient of determination (R^2), interday and intraday precisions and slope in matrix/slope in solvent ratio obtained for the developed method (the results correspond to spiked influent waste water).

Pesticide	LOD (ng L^{-1})	LOQ (ng L^{-1})	Interday precision (% RSD_{wR})		Intraday precision (% RSD_r)		R^2	Slope in matrix/slope in solvent
			15 ng L^{-1}	150 ng L^{-1}	15 ng L^{-1}	150 ng L^{-1}		
Acetochlor	2	6	17	14	16	14	0.9980	0.73
Alachlor	2	6	18	12	11	10	0.9972	0.82
Atrazine	1.3	4	14	10	6	2	0.9929	0.94
Atrazine-deethyl	2	6	10	12	8	7	0.9947	0.65
Atrazine-deisopropyl	2	6	13	5.0	5	5	0.9938	0.55
Azinphos-ethyl	0.5	1.5	14	7	4	5	0.9916	0.93
Azinphos-methyl	0.5	1.5	10	9	12	6	0.9975	0.99
Buprofezin	0.5	1.5	11	6	6	8	0.9901	0.94
Carbofuran	0.2	0.6	8	2	4	4	0.9968	1.35
Carbofuran-3-hydroxy	0.2	0.6	9	7	4	3	0.9978	1.45
Chlorfenvinphos	0.2	0.6	9	9	13	7	0.9951	0.81
Chlorpyriphos	0.2	0.6	14	12	7	7	0.9973	0.86
Diazinon	0.04	0.2	12	9	2	5	0.9959	1.10
Dichlofenthion	0.5	1.5	14	11	4	8	0.9958	0.95
Dimethoate	1	3	14	10	6	4	0.9912	0.98
Diuron	1	5	8	9	4	7	0.9973	0.88
Ethion	0.5	1.5	10	8	5	1	0.9945	0.99
Fenitrothion	2	6	11	10	4	3	0.9941	1.00
Fenoxon	0.2	1	10	5	3	1	0.9954	0.98
Fenthion	0.2	1	12	10	5	5	0.9951	1.00
Fenoxon sulfoxide	0.2	1	11	5	2	2	0.9979	0.96
Fenoxon sulfone	0.2	1	7	9	7	8	0.9941	1.00
Fenthion sulfoxide	0.2	1	10	10	4	7	0.9935	0.98
Fenthion sulfone	0.2	1	12	9	12	10	0.9990	0.92
Hexythiazox	0.2	1	10	8	7	7	0.9980	0.98
Imazalil	0.3	1	12	10	6	1	0.9978	0.95
Imidacloprid	0.04	0.2	11	10	6	3	0.9983	0.91
Isoproturon	0.3	1.0	15	11	5	6	0.9973	0.82
Malathion	0.3	1	12	10	4	1	0.9986	0.87
Methiocarb	0.3	1	15	8	6	9	0.9994	0.85
Metolachlor	0.3	1	19	15	16	12	0.9974	0.75
Molinate	0.5	1.5	12	9	9	6	0.9985	1.03
Omethoate	0.3	1	15	10	2	2	0.9914	1.39
Parathion-ethyl	2	6	17	8	4	3	0.9994	0.92
Parathion-methyl	2	6	11	7	3	3	0.9982	0.97
Prochloraz	0.8	6	14	9	8	4	0.9977	0.87
Propanil	0.3	1	14	13	8	9	0.9971	0.88
Propazine	0.3	1	11	13	3	4	0.9915	0.76
Pyriproxyfen	0.5	1.5	12	8	3	6	0.9978	1.03
Simazine	2	6	16	14	3	2	0.9934	0.93
Terbutryn	0.5	1.5	10	8	4	2	0.9964	0.98
Tolclofos-methyl	0.5	1.5	15	13	6	5	0.9968	0.80

to note that S/N and peak shapes for the acetamide herbicides (mostly acetochlor and alachlor) are the worst. However, the chromatogram showed in the figure can be improved by smoothing (using the layout of the integration software). Smoothing improves results with noisy data and is also used to avoid split peaks.

Using this instrument, it would be possible to target a larger number of pesticides than those selected in this study.

3.1.2. Validation of the LC–MS/MS method

Validation studies were carried out using a real water sample. As no certified pesticide-free water sample could be obtained to be used as blank, influent waste water (the most difficult case) was used, which was previously analyzed and the presence of the target compounds considered.

All the compounds presented LODs (in influent samples) far below 100 ng L^{-1}, which is the tolerance for individual pesti-cides in drinking water (Table 2.2). The LODs were in the range of 0.04–2 ng L^{-1} (see Table 2.2). The lowest LODs corresponded to imidacloprid and diazinon (0.04 ng L^{-1}) and the highest to acetochlor, alachlor, atrazine-deethyl, atrazine-deisopropyl, fenitrothion, parathion-ethyl, parathion-methyl and simazine (2 ng L^{-1}). These LODs are comparable to those reported in previous studies, and appropriated to determine pesticide residues in surface and waste water samples [8,33–35].

The linearity along the studied range was good, with correlation coefficients higher than 0.99 for all target compounds, as shown in Table 2.2. Matrix effects, either as signal suppression or enhancement, are a major drawback for quantitative trace analysis by LC–ESI-MS. Matrix co-extractives can compromise the quantitative analysis of the compounds at trace levels, as well as greatly affect the method accuracy and reproducibility. The analysis of water samples based on SPE implies a pre-concentration step, thus matrix effects are generally observed. In order to evaluate their extent, the ratios between the slopes of matrix-matched (from influent waste waters extracts) and solvent-based calibrations curves were calculated for each pesticide (see Table 2.2). Thirty-five of the pesticides under study did not present relevant matrix effect (slopes ratio between 0.8 and 1.2, which means signal suppression or enhancement < 20%), whereas eight of the analytes showed moderate matrix effects (signal suppression or enhancement in the range of 20–50%). Acetochlor, atrazine-deethyl, atrazine-deisopropyl, metolachlor and propazine showed moderate signal suppression (slope ratio of 0.5–0.8), and carbofuran, carbofuran- 3-hydroxy and omethoate showed moderate signal enhancement (from 1.2 to 1.5). No strong signal enhancement or suppression was observed.

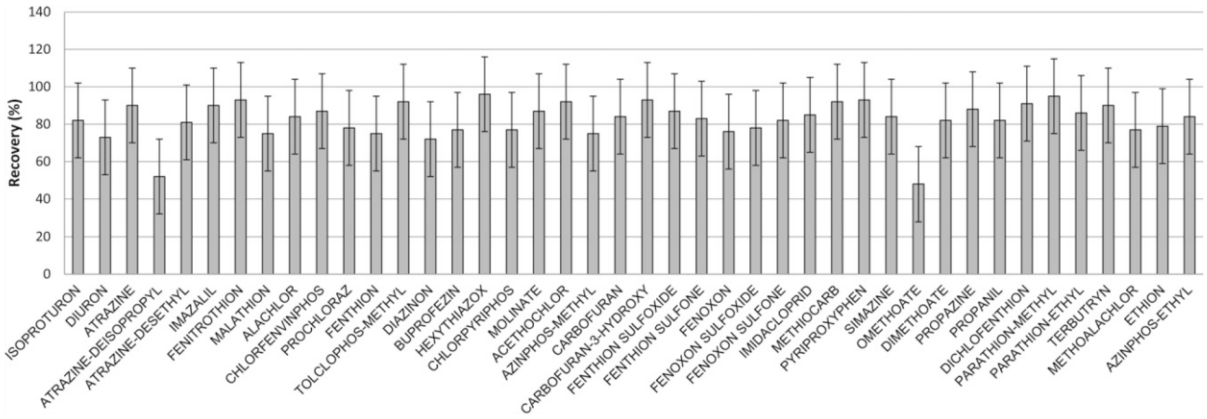


Fig. 2.2 Recoveries of the selected pesticides and RSD (%) at a concentration of 15 ng L^{-1} for each pesticide.

However, the chemical composition of water samples could be very different and they could contain different concen-trations of ionization enhancing or suppressing compounds (e.g. humic acids). The use of an internal standard (I.S.) is recommended in the European Guidelines on quality control [30,31]. However, as no relevant matrix effects seemed to occur, even in the worst sample types tested and due to economical restrictions to acquire a large number of isotope-labeled reference standards (matrix effect are compound dependent), ISs were not used. The validation of the method was carried quantifying with matrix-matched standard in order to avoid overlapping of recovery with matrix effects.

The precision of the method was estimated by determining the intra- and interday, % RSD. As it is shown in Table 2.2, the RSD ranged between 2–19% and below 16% for the interday and intraday precisions, respectively.

The target compounds have been extracted using SPE, the performance of which is summarized in Fig. 2.2. As can be observed, the recoveries were higher than 70% for all analytes, except for omethoate and atrazine-deisopropyl, which yielded recoveries of 48% and 52%, respectively. The low recovery is no drawback for a reliable determination of these compounds in the real samples because the other performance data, especially repeatability (RSD < 16%) and sensitivity (LOQs < 5 ng L^{-1}), were acceptable.

3.1.3. Application to real samples

Table 2.3 shows the occurrence and the maximum and minimum concentrations found in the samples. All the 63 analyzed samples present more than one pesticide. In Fig. 2.3, LC–MS/MS chro-matograms of the two monitored transitions of several pesticides in surface waters are represented along with the three criteria used for the identification of the pesticide. In the Qualifier Chro-matogram panel, two chromatograms, quantifier and qualifier, are shown overlapped. The qualifier is normalized with the expected signal and not the actual one. If the amount of the qualifier sig-nal is exactly what was expected, the qualifier and quantifier peaks overlap. If the amount of the qualifier signal is lower than what was expected, the qualifier peak is shown below that of quantifier and vice versa.

The most frequently pesticide in waste water was the insecticide diazinon, whereas the herbicide diuron was present at the highest concentration. Other highly frequent pesticides were chlorfenvinphos, chlorpyriphos, dimethoate, hexythiazox, prochloraz and pyriproxyfen and less often the post-harvest fungicide imazalil and the herbicide atrazine together with the TPs atrazine-deethyl and atrazine-deisopropyl. Similar pattern of pesticides were found in the rivers but their concentrations were much lower than in waste waters.

The compounds found in the samples are pesticides widely used in Mediterranean agriculture [36]. Considering the European Union (EU) Directive on the quality of drinking water [37], which has set the maximum admissible concentrations of each pesticide at 100 ng L^{-1}, and the total concentration of all pesticides at 500 ng L^{-1}, the levels of the individual or multiple pesticides in the analyzed samples were lower than this tolerance, with the exception of the waste water samples.

3.2. Ultra high-pressure liquid chromatography quadrupole time of flight mass spectrometry

Different water extracts, previously analyzed by LC–MS/MS, were also analyzed by UHPLC–QTOF MS in MS^E mode, and processed in a "post-target" mode, trying to find other potential organic contaminants not investigated by triple quadrupole. To this aim, a large list of more than 1000 contaminants was used, as explained later in this section. A mobile phase with formic acid instead of ammonium formate was used as this was the mobile phase employed when building the database. Otherwise, retention times, and/or adduct formation could not be comparable. MS^E experiments involve the simultaneous acquisition of accurate mass data at low and high collision energy. With MS^E experiments, both (de)protonated molecule and fragment ion data are enabled in a single acquisition, without the need of selecting the precursor ion. Using this approach, the QTOF instrument is used in TOF mode, but promoting fragmentation in the collision cell during the HE function. A possible concern of the MS^E is that co-eluting compounds would lead to overlapping product ion spectra, i.e., the observed product ion spectra might contain ions unrelated to the parent compound. Here, the use of UHPLC will likely to substantially ameliorate this problem, given the excellent resolving power of this technique [24,33].

The "post-target" processing method was based on monitoring exact masses of the selected analytes using narrow mass extraction windows (e.g. 10–20 mDa). When a compound is detected, its mass error is displayed, and it is feasible to filter the findings according to the Rt deviation tolerance established, when this information is available. In addition, the accurate mass full spectrum of the positive findings is also obtained.

To enhance reliability in the identification/elucidation process, improvements in the post-target approach can be made, consisting on including additional entries in the target list for those contaminants with high degree of fragmentation and abundant adducts formation (e.g. sodium adducts). Therefore, exact masses of relevant fragments and/or adduct ions can be also included in the target list.

Table 2.3
Target analytes detected and quantified in surface and waste water samples.

Pesticide	Influent waste waters ($n=14$)				Effluent waste waters ($n=14$)				Surface waters ($n=35$)			
	Conc. (ng L^{-1})		Freq. QqQ	Freq. QTOF	Conc. (ng L^{-1})		Freq. QqQ	Freq. QTOF	Conc. (ng L^{-1})		Freq QqQ	Freq QTOF
	Min	Max			Min	Max			Min	Max		
Atrazine	6.0	22.9	6	–	5.1	23.4	6	–	4.6	18.6	10	1
Atrazine-deethyl	16.0	158.4	10	2	20.4	153.6	9	5	<LOQ	97.2	10	2
Atrazine-deisopropyl	17.4	28.1	2	–	17.4	41.8	4	–	<LOQ	30.2	13	–
Azinphos-ethyl	–	–	–	–	6.4	65.5	2	–	–	–	–	–
Buprofezin	1.6	9.0	10	–	2.9	9.3	11	–	1.9	7.9	7	–
Carbofuran	4.3	6.4	4	–	2.8	42.1	5	–	1.0	4.1	2	–
Carbofuran-3-hydroxy	10.4	10.4	1	–	10.4	140.4	3	–	2.9	8.5	2	–
Chlorfenvinphos	3.1	268.1	12	1	11.2	258.6	13	–	1.0	87.3	25	–
Chlorpyriphos	1.1	163.7	12	–	1.1	123.2	13	–	1.0	21.2	26	–
Diazinon	5.9	316.0	14	7	3.6	135.4	14	11	0.1	23.7	32	5
Dichlofenthion	8.2	34.9	7	–	8.3	30.8	7	–	13.6	22.7	13	–
Dimethoate	2.8	620.6	13	–	3.1	432.6	12	–	2.3	69.3	14	–
Diuron	28.4	2526.1	11	4	29.1	2393.1	11	3	16.0	67.7	5	1
Fenitrothion	23.8	23.8	1	–	–	–	–	–	–	–	–	–
Fenoxon sulfone	13.1	50.4	3	–	5.2	15.7	3	–	1.6	20.8	8	–
Fenthion sulfone	13.0	35.3	6	–	11.0	20.9	6	–	13.7	13.7	1	–
Hexythiazox	<LOQ	13.8	12	–	0.9	15.7	14	–	1.3	10.6	7	–
Imazalil	7.3	2120.8	10	2	5.2	1170.7	10	4	1.3	409.8	19	4
Imidacloprid	2.0	5.2	8	–	2.0	6.7	9	–	1.1	6.1	18	–
Isoproturon	1.0	90.0	9	–	2.5	101.8	10	1	2.6	9.1	5	–
Malathion	848.0	848.0	1	–	–	–	–	–	2.6	116.1	2	–
Methiocarb	3.9	5.7	3	–	5.7	5.7	1	–	–	–	–	–
Metolachlor	70.0	313.5	6	2	70.0	126.9	5	6	17.5	124.4	2	1
Molinate	19.2	19.2	1	–	5.8	19.2	2	–	–	–	–	–
Omethoate	2.4	4.0	2	–	4.1	4.6	2	–	8.6	11.7	2	–
Parathion-ethyl	–	–	–	–	–	–	–	–	14.5	14.5	2	–
Prochloraz	<LOQ	63.2	11	–	2.9	59.1	11	–	<LOQ	34.5	12	–
Propanil	49.8	49.8	2	–	4.6	4.6	1	–	–	–	–	–
Propazine	9.0	277.4	5	–	9.0	14.2	5	–	1.2	13.3	3	–
Pyriproxyfen	0.5	75.5	12	–	1.9	72.1	14	–	<LOQ	37.7	16	–
Simazine	<LOQ	15.0	6	3	5.0	15.0	5	5	<LOQ	48.0	9	4
Terbutryn	5.0	182.9	9	2	8.8	45.8	9	1	4.2	14.9	5	1
Tolchlofos-methyl	–	–	–	–	–-	–	–	–	1.5	16.8	6	–

The presence of the protonated molecule measured at its accurate mass, at the expected retention time, was evaluated in surface water and in both, influent and effluent, waste water samples. Additionally, collision induced dissociated fragments (in any of the two functions acquired, at low or high collision energy) or characteristic isotopic ions (when available) were also evaluated. These were calculated theoretically and then compared to the found isotopic ratios (according to the ion ratio approach

Table 2.4
Contaminants non-investigated by LC–QqQ-MS but detected in surface and waste water samples by UHPLC–QTOF MS.

Type of contaminant	Compound	Frequency in influent waste water ($n=14$)	Frequency in effluent waste water ($n=14$)	Frequency in surface water ($n=35$)
Pesticides	Thiabendazole	1	1	2
	Carbendazim	2	–	1
	Terbumeton	1	1	–
	Tebuconazole	–	–	2
	Terbuthylazine	7	7	14
Transformation products of pesticides	Terbumeton-deethyl	1	1	–
	Terbuthylazine-2-hydroxy	1	1	–
	Terbuthylazine-deethyl	3	2	6
Pharmaceuticals	Atorvastatin	–	2	–
	Ketoprofen	3	4	–
	Valsartan	14	12	5
	Irbesartan	14	14	12
	Noscapine	–	–	2
	Paracetamol	5	–	1
	Venlafaxine	1	9	2
	Carbamazepine	1	–	1
	Gabapentin	3	–	–
	Lincomycin	–	–	1
	Naproxen	2	–	–
	Bezafibrate	3	–	–
	Diclofenac	2	1	–
Drugs of abuse	Benzoylecgonine	9	1	–
	Caffeine	7	–	–

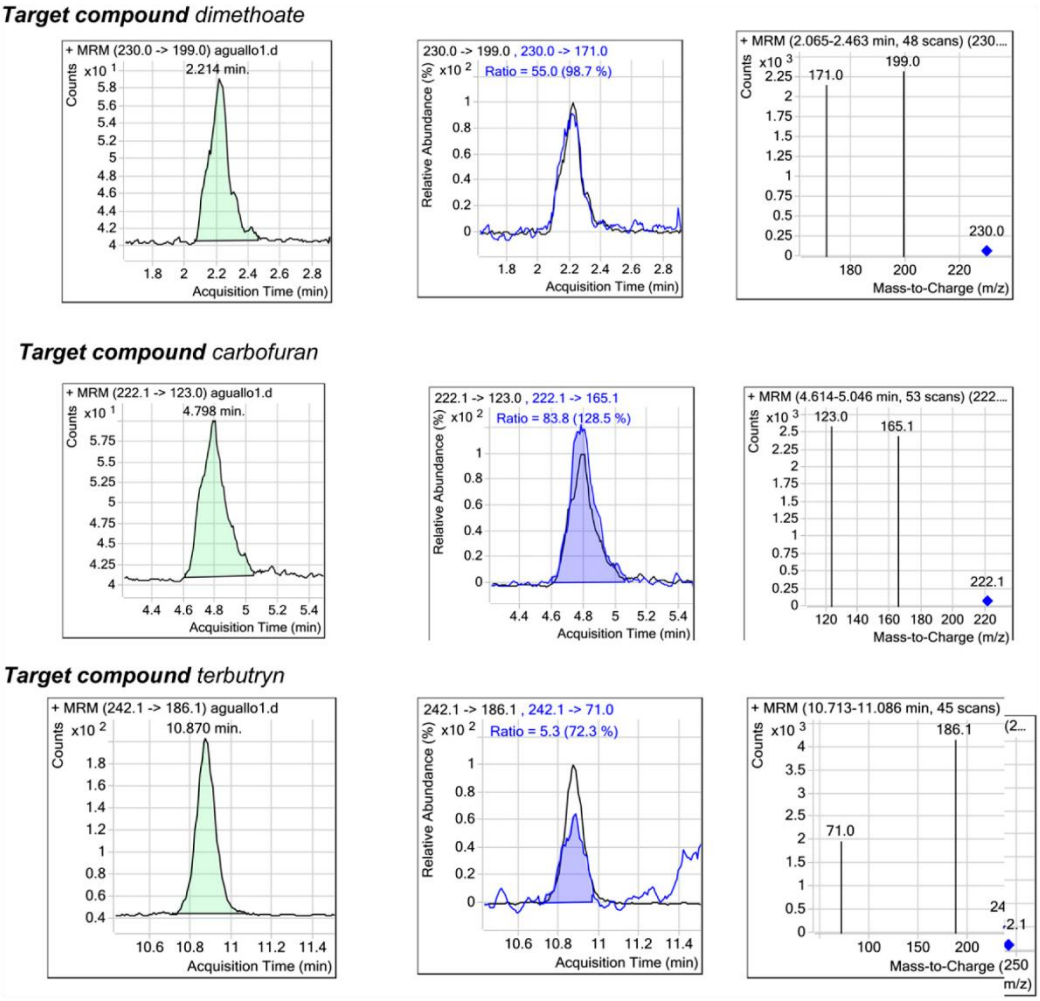


Fig. 2.3 LC–MS/MS chromatograms of several identified pesticides in a sample from the Llobregat River. Calculated pesticide concentrations: dimethoate = 6.62 ng L^{-1}, carbofuran = 6.80 ng L^{-1}; terbutryn = 8.60 ng L^{-1}.

included in 2002/657 [30]. Following this approach, a notable number of compounds were detected and identified in the samples, some of them not investigated by LC–QqQ-MS/MS (Table 2.4). Triazine herbicides and transformation products (terbuthylazine, simazine, terbumeton, terbutryn, terbuthylazine-deethyl, terbuthylazine-2-hydroxy, atrazine-deethyl), and fungicides such as thiabendazole, carbendazim, tebuconazole and imazalil, were identified. Herbicides (diuron and metolachlor), or insecticides (chlorfenvinphos), were also detected. Among pharmaceuticals, angiotensin II receptor antagonists valsartan and irbesartan (used for treatment of high blood pressure), anti-inflammatory drugs naproxen, diclofenac and ketoprofen, analgesic drug paracetamol, antidepressant venlafaxine and lipid regulator bezafibrate were identified. Gabapentin, used primarily for the treatment of seizures and neuropathic pain, was also detected in several samples. Regarding drugs of abuse, the main metabolite of cocaine (benzoylecgonine) was the most frequently detected compound. Codeine and caffeine were also identified. Table 2.4 shows the list of identified compounds together with the frequency of identification. Since the purpose of the QqTOF screening is mostly qualitative, the efficiency of the extraction method for pharmaceuticals and drugs of abuse was not evaluated. However, a number of methods in the literature used a similar extraction method as that used in this study reporting recoveries from 70 to 101% for pharmaceuticals and from 40 to 95% for drugs of abuse [18,14,32].

As an example, Fig. 2.4 shows LE UHPLC–TOF MS chromatograms and accurate mass spectra for several contaminants detected in an influent waste water sample collected between September and October 2010: pesticide simazine and the transformation product terbuthylazine-deethyl (which share the same elemental composition and, therefore, the same exact mass), pharmaceuticals (paracetamol and valsartan), and benzoylecgonine.

In almost all cases of positive findings, reference standards were available and the presence of the detected compounds was unequivocally confirmed. When the reference standard of a detected compound was not available in our laboratory, as in the case of noscapine, the chemical structure of all fragment ions was justified using specialized software (MassFragment) based on a systematic bond-disconnection approach and accurate mass measurements. Noscapine is a benzylisoquinoline alkaloid from plants of the Papaveraceae family, without significant painkilling properties. This agent is primarily used for its antitussive (cough-suppressing) effects. However, noscapine has a history of over-the-counter drug

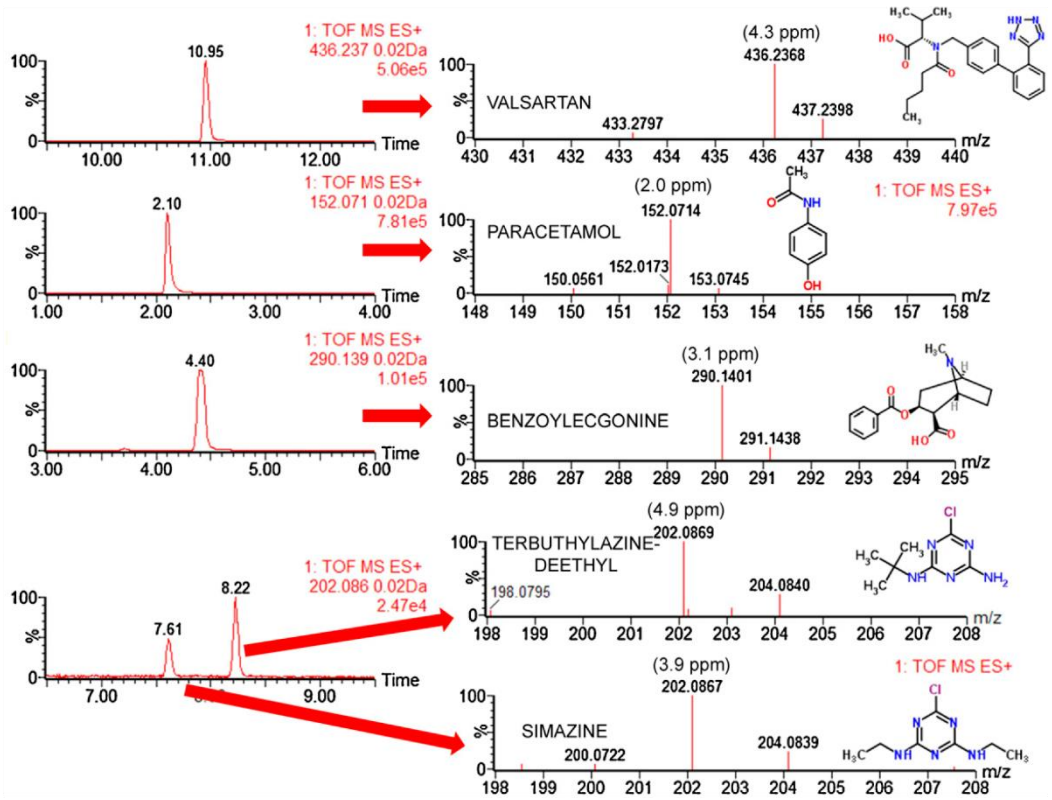


Fig. 2.4 UHPLC–QTOF MS extracted-ion chromatograms at different *m/z* (mass window 20 mDa) for pharmaceuticals valsartan and paracetamol, the main metabolite of cocaine (benzoylecgonine), pesticide simazine and a transformation product (terbuthylazine-deethyl).

abuse in several countries. Fig. 2.5 illustrates the detection and identification of this pharmaceutical in river surface water. The protonated molecule of noscapine was detected in the LE function, with a mass error of 0.5 ppm. In the HE function, three fragments were assigned to this compound as were detected at the same retention time, obtaining errors for experimental accurate masses below 2 ppm in relation to the theoretical exact masses predicted. In order to avoid spectrum interferences that would complicate the identification process, recognizing which ions are fragments, and which are not, becomes mandatory. In this sense, UHPLC turned

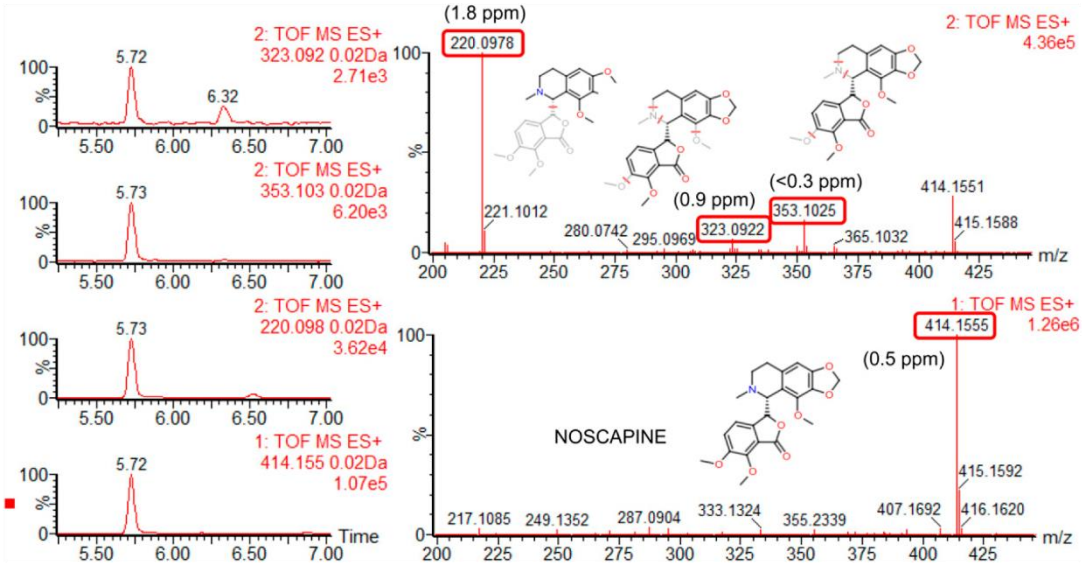


Fig. 2.5 Detection and identification of pharmaceutical noscapine in a water sample by UHPLC–QTOF MS (MSE approach). LE and HE spectra for sample (with justified fragments using MassFragment software) and XICs at 20 mDa mass window for different ions observed in HE function.

valuable for choosing perfectly co-eluting ions. The strategy followed to improve the confidence in the identification process was based on the comparison of the main TOF fragments with MS/MS product ions reported in the literature for the suspect compound. In this way, noscapine was tentatively identified by the presence of three fragments at *m*/*z* 353.1025, 323.0922 and 220.0978 in the HE spectrum. Two of these fragment ions had been previously reported (in nominal mass) for the determination of this compound by QqQ [38]. A reliable elemental composition for these two fragments was calculated obtaining errors of <0.3 and 0.4 ppm, respectively. After this careful evaluation process, a medicament containing this compound was purchased and injected to definitively confirm the identity of the compound detected. This information was added to the target list in order to facilitate future screenings.

Moreover, since detection and identification is performed by using full MS acquisition data, data can be reprocessed and re-evaluated using new or modified databases to search for other interesting compounds, simply by including their theoretical mass and empirical formulae into the database [39].

4. Conclusions

The combination of LC–QqQ-MS/MS and UHPLC–QTOF MS proved to be a feasible and efficient way for systematic pesticide residue determination. The simple and fast sample preparation is suitable for both techniques and covers a large variety of substances with different polarity. LC–QqQ-MS/MS is able to detect more target compounds because of its higher sensitivity.

The method usefulness was established through the confirmation of 6 different pesticides during the analysis of real samples. Furthermore other co-occurrent contaminants, such as pharmaceuticals valsartan and irbesartan were identified in the samples using QTOF MS. All the analyzed samples presented pesticides, being buprofezin, chlorfenvinphos, chlorpyriphos, atrazine-deethyl, diazinon, dimethoate, diuron, hexythiazox, imazalil, prochloraz, and pyriproxyfen the compounds most often found. Some of these samples presented a concentration of pesticides higher than 0.5 $\mu g\,L^{-1}$ which is the maximum concentration allowed by the EU legislation in drinking water. These results highlight the consistent and good sensitivity and the high identification power that can be achieved using the developed method. The increased efficiency and ease of sample processivity make this method a key tool for the routine quality control of priority pesticides in water.

Acknowledgements

This work has been supported by the Spanish Ministry of Economy and Competitiviness through the project Consolider-Ingenio 2010 CSD2009, as well as by the European Regional Development Funds (ERDF) (project CGL2011-29703-C02-02). We also would like to thank the persons of the research groups from IDEA and ICMAN for taking the samples of the Ebro, Llobregat and Guadalquivir Rivers. Finally, we wish to thank the Serveis Centrals d'Instrumentació Científica (SCIC) of University Jaume I for using the UHPLC–QTOF MS instrument.

References

[1] M.D. Hernando, A. Rodriguez, J.J. Vaquero, A.R. Fernández-Alba, E. Garcia, Crit. Rev. Environ. Sci. Technol. 41 (2011) 699.
[2] Z.G. Yu, Z. Qin, H.R. Ji, X. Du, Y.H. Chen, P. Pan, H. Wang, Y.Y. Liu, Chromatographia 72 (2010) 1073.
[3] D.P. Oliver, R. Kookana, J.S. Anderson, J.W. Cox, N. Waller, L.H. Smith, Agric. Water Manage. 106 (2012) 70.
[4] C. Blasco, Y. Picó, Trends Anal. Chem. 28 (2009) 745.
[5] D.J. Beale, S.L. Kaserzon, N.A. Porter, F.A. Roddick, P.D. Carpenter, Talanta 82 (2010) 668.
[6] K. Tani, Y. Matsui, K. Iwao, M. Kamata, T. Matsushita, Water Res. 46 (2012) 741.
[7] P. Vazquez-Roig, V. Andreu, M. Onghena, C. Blasco, Y. Picó, Anal. Bioanal. Chem. 400 (2011) 1287.
[8] A.C. Navarro-Ortega, R. Tauler, S. Lacorte, D. Barceló, J. Hydrol. 383 (2010) 5.
[9] F. Hernández, O.J. Pozo, J.V. Sancho, F.J. López, J.M. Marín, M. Ibáñez, Trends Anal. Chem. 24 (2007) 596.
[10] K. Wille, H.F. De Brabander, L. Vanhaecke, E. De Wulf, P. Van Caeter, C.R. Janssen, Trends Anal. Chem. 35 (2012) 87.
[11] K. Fischer, E. Fries, W. Körner, C. Schmalz, C. Zwiener, Appl. Microbiol. Biotechnol. 94 (2012) 11.
[12] M. Kock, M. Farré, E. Martínez, K. Gajda-Schrantz, A. Ginebreda, A. Navarro, M. López de Alda, D. Barceló, J. Hydrol. 383 (2010) 73.
[13] L. Pareja, M.J. Martinez-Bueno, V. Cesio, H. Heinzen, A.R. Fernández-Alba, J. Chromatogr. A 1218 (2011) 4790.
[14] C. Postigo, M.J. López de Alda, D. Barceló, A. Ginebreda, T. Garrido, J. Fraile, J. Hydrol. 383 (2010) 83.
[15] J.A. Shoemaker, Anal. Methods 3 (2011) 1628.
[16] H. Singer, S. Jaus, I. Hanke, A. Luck, J. Hollender, A.C. Alder, Environ. Pollut. 158 (2010) 3054.
[17] J.V. Sancho, O.J. Pozo, F. Hernández, Analyst 129 (2004) 38.
[18] S. Kern, H. Singer, J. Hollender, R.P. Schwarzenbach, K. Fenner, Environ. Sci. Technol. 45 (2011) 2833.
[19] J.M. Marín, E. Gracia-Lor, J.V. Sancho, F.J. López, F. Hernández, J. Chromatogr. A 1216 (2009) 1410.
[20] M. Ibáñez, J.V. Sancho, F. Hernández, D. McMillan, R. Rao, Trends Anal. Chem. 27 (2008) 481.
[21] N. Dujaković, S. Grujić, M. Radišić, T. Vasiljević, M. Laušević, Anal. Chim. Acta 678 (2010) 63.
[22] J. Fenoll, P. Hellín, C.M. Martínez, P. Flores, S. Navarro, Talanta 85 (2011) 975.
[23] L. Diaz, J. Llorca-Pórcel, I. Valor, Anal. Chim. Acta 624 (2008) 90.
[24] F. Hernández, J.V. Sancho, M. Ibáñez, S. Grimalt, Trends Anal. Chem. 27 (2008) 862.
[25] Y. Picó, D. Barceló, Trends Anal. Chem. 27 (2008) 821.
[26] Y. Picó, M. Farré, C. Soler, D. Barceló, Anal. Chem. 79 (2007) 9350.
[27] R. Díaz, M. Ibáñez, J.V. Sancho, F. Hernández, Anal. Methods 4 (2012) 196.
[28] D. Almeida Azevedo, S. Lacorte, T. Vinhas, P. Viana, D. Barceló, J. Chromatogr. A 879 (2000) 13–26.
[29] F. Hernández, L. Bijlsma, J.V. Sancho, R. Díaz, M. Ibáñez, Anal. Chim. Acta 684 (2011) 96.
[30] Commission Decision 2002/657/EC of 12 August 2002, implementing Council Directive 96/23/EC Brussels, Off. J. Eur. Commun. L221 (August) (2002) 8.
[31] Method validation and quality control procedures for pesticide residue analysis in food and feed, Document No. SANCO/12495/2011 implemented by 01/01/2012.
[32] B. Kmellára, L. Parejaa, C. Ferrera, P. Fodorb, A.R. Fernández-Alba, J. Chromatogr. A 84 (2011) 262.
[33] O.J. Pozo, J.V. Sancho, M. Ibañez, F. Hernandez, W.M.A. Niessen, Trends Anal. Chem. 25 (2006) 1030.
[34] A. Giordano, M. Fernández-Franzon, M.J. Ruiz, G. Font, Y. Picó, Anal. Bioanal. Chem. 393 (2009) 1733.
[35] Spanish Ministry of Agriculture, Food and Environment (Eds.), Avance Anuario de Estadística 2011, Spanish Ministry of Agriculture, Food and Environment, Madrid, Spain, 2012.
[36] F. Hernández, J. Beltrán, J.V. Sancho, Sci. Total Environ. 132 (1993) 297.
[37] Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption, Off. J. Eur. Commun. L 330 (December) (1998) 32.
[38] J.R. Wickens, R. Sleeman, B.J. Keely, Rapid Commun. Mass Spectrom. 20 (2006) 473.
[39] F. Hernández, M. Ibáñez, E. Gracia-Lor, J.V. Sancho, J. Sep. Sci. 34 (2011) 3517.

CAPÍTOL 3

Cromatografia Líquida d'Ultra Alta Pressió acoblada a un quadrupol temps de vol per a identificar contaminants en aigües. Una introducció a la forensia mediambiental

Publicació científica 3

Ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry to identify contaminants in water: An insight on environmental forensics

A. Masiá, J. Campo, C. Blasco, Y. Picó

Journal of Chromatography A, 1345 (2014) 86-97

Journal of Chromatography A, 1345 (2014) 86–97

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Ultra-high performance liquid chromatography–quadrupole time-of-flight mass spectrometry to identify contaminants in water: An insight on environmental forensics☆

Ana Masiá, Julian Campo, Cristina Blasco, Yolanda Picó*

Food and Environmental Safety Research Group, Facultat de Farmàcia, Universitat de València, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, València, Spain

ARTICLE INFO

Article history:
Received 9 December 2013
Received in revised form 14 March 2014
Accepted 7 April 2014
Available online 18 April 2014

Keywords:
UHPL-QqTOF-MS
Screening
Non-target identification
Target quantification
Water
Principal component analysis

ABSTRACT

Ultra-high pressure liquid chromatography–quadrupole time-of-flight mass spectrometry (UHPLC–QqTOF-MS) acquiring full scan MS data for quantification, and automatic data dependent information product ion spectra (IDA-MS/MS) without any predefinition of the ions by the user was checked for identifying organic contaminants in water samples. The use of a database with more than 2000 compounds achieved high confidence results for a wide number of contaminants based upon retention time, accurate mass, isotopic pattern and MS/MS library searching. More than 20 contaminants, mostly pharmaceuticals, but also mycotoxins and polyphenols were unambiguously identified. Furthermore, the combination of statistical data analysis using principal component analysis (PCA) followed by empirical formula calculation, on-line database searching and MS/MS fragment ion interpretation achieves not only the successful detection of unknown contaminants but also the selection of those relevant to different types of waters. Unknown compounds, such as $C_{20}H_{34}O_3$, were identified in waste water showing the prospects of this technique. A group of 42 currently used pesticides were selected as target compounds to evaluate the quantitative possibilities. Mean recoveries and percentage relative standard deviation (RSD) were 48–79% (4–20% RSD). The limit of detections ranged from 0.02 to 2 ng L^{-1}, with a validated limit of quantification of 2 ng L^{-1} for water after solid-phase (SPE) isolation and concentration. The quantitative data obtained using UHPLC–QqTOF-MS were compared with those obtained using conventional LC–MS/MS with a triple quadrupole (QqQ).

1. Introduction

In the last years, a large number of compounds, known as "emerging", have been pointed out as possible environmental contaminants [1–3]. Pesticide residues are one of the most frequently detected compounds in water analysis [4–6]. However, water contamination is not limited to them but also includes pharmaceuticals, illicit drugs, personal care products and other substances from the human activity [1,4,7–9]. Not only constant and comprehensive monitoring of organic trace substances is essential to protect the quality of water [10] but also determine the possible sources of them, estimating the approximate timing of its release and distribution into the environment and appropriation of the liability for the damages among the sources (the so called environmental forensics) [11]. The standard technique in modern screening for medium-polar and polar substances is liquid chromatography–mass spectrometry (LC–MS) [10] with several mass analyzers, such as ion trap, triple quadrupole (QqQ), quadrupole linear ion trap (QTRAP), time-of-flight (TOF) or orbitrap [12]. The traditional target screening, in which the analytes are previously selected and any other contaminant present in the sample becomes undetectable [10], is currently the most common and well-established approach able to quantify hundreds of contaminants in a single run [13–19].

However, in recent years, there is a growing movement to analyze water beyond target compound list, and a shift towards non-targeted or general unknown screening that detects all the

☆ Presented at the XIII Scientific Meeting of the Spanish Society of Chromatography and Related Techniques (SECyTA2013), 8–11 October 2013, Puerto de la Cruz, Tenerife, Canary Islands, Spain.

* Corresponding author. Tel.: +34 963543092; fax: +34 963544954.
E-mail address: Yolanda.Pico@uv.es (Y. Picó).

http://dx.doi.org/10.1016/j.chroma.2014.04.017
0021-9673/

substances accessible for a particular technique [16,20–22]. Both, TOF and QqTOF have already been used for screening of non-target and/or unknown compounds in different matrices [23–26]. Although the gain in popularity and the increasing demand for retrospective and non-targeted analyses, there are still few methods in the literature that use full-scan mode to screen for non-target compounds in waste and surface waters [4,7,10,27,28]. In this sense, the QqTOF technology combines the best attributes of a QqQ and accurate mass TOF analyzers in a single instrument allowing high confidence identification based on MS and MS/MS information [29]. Therefore, acquired chromatograms are very rich in information and can easily contain thousands of ions from any compound present in the sample. In return, powerful software tools are needed to explore such data to identify unexpected compounds [30]. These tools are within the "omics" and "fingerprint" terms, and include amenable software, calculations, databases searching and statistical analysis that help in understanding the differences and the significance of the quantified pollutants that give rise to these differences. Recently, Martinez Bueno et al. [31] using the same instrument test in our study already assessed its performance for the simultaneous quantitative screening of 10 target pharmaceuticals and the qualitative identification of non-targets after direct water injection. These approaches are at the forefront of a movement from traditional monitoring to environmental forensics applications. Recent examples are the identification of biomarkers as a potential way to disentangle sewage and manure sources [32], to analyze anthropogenic sources [33] or to investigate compositional changes of marine oil spills [34].

Our previous study [4] investigated whether the combined use of LC–QqQ-MS/MS and LC–QTOF- MS is a suitable way in routine performance of systematic pesticide residue determination. Forty-three target pesticides or degradation products were screened by LC–QqQ-MS/MS with limits of detection ranged from 0.04 to 2 ng L^{-1}. The further application of ESI–QTOF-MS using two simultaneous acquisition functions with low and high collision energy (MS^E approach) and acquiring the full mass spectra allowed to identify 14 additional compounds against a home-made database (≈1100 organic pollutants) but with no idea on their concentration.

This study goes an step forward into the analytical strategies developed for quantitative and non-quantitative non-target screening of contaminants using a last generation LC–QTOF-MS (ABSciex TripleTOF™ 5600) by ion extraction in front of a database of more than 2000 compounds with accurate mass information and, if available, retention times. An information dependent acquisition (IDA) methods allow to obtain automatically MS/MS spectra of the most intense precursor ions (without previous selection) to additionally confirm the identity of the detected compounds by MS/MS library searching. That means the isolation and further fragmentation of the precursor ion, providing higher confidence in the identification. Furthermore, for the first time and in order to identify relevant unexpected contaminants for the water systems and define changes in the water fingerprints, statistical data analysis using principal component analysis (PCA) and principal components variable grouping (PCVG) was combined with empirical formula calculation, online database (chemspider or other internet databases) searching, and MS/MS fragment ion interpretation to successfully detect unknown contaminants as an insight within environmental forensics. Last but not least, the quantitative capabilities of the system were explored for 42 currently used pesticides, the determination of which was validated according to the European guidelines [35]. The possibility to limit the analysis to one instrument will provide advantages in terms of saving time and cost of the determination.

2. Experimental

2.1. Reagents

Acetochlor, alachlor, atrazine, deisopropylatrazine, deethylatrazine, azinphos-methyl, buprofezin, carbofuran, 3-hydroxycarbofuran, chlorfenvinphos, chlorpyriphos, diazinon, diuron, fenitrothion, fenthion, fenthion-sulfoxide, fenthion-sulfone, hexythiazox, imazalil, imidacloprid, isoproturon, malathion, methiocarb, prochloraz, pyriproxyfen, simazine, tolclophos-methyl, molinate, omethoate, dimethoate, propazine, propanil, diclofenthion, parathion-methyl, parathion-ethyl, terbutryne, metolachlor, ethion and azinphos-ethyl were purchased from Sigma–Aldrich (Steinheim, Germany). Fenoxon, fenoxon-sulfoxide, fenoxon-sulfone were obtained from Dr. Ehrenstorfer (Augsburg, Germany).

Individual standard solutions were prepared in methanol at the concentration of 1000 mg L^{-1} and the working standard solution was prepared by mixing the appropriate amounts of each standard solutions and diluting them with methanol to a final concentration of 0.5 mg L^{-1}. Solutions were stored at 4 °C in the dark.

Ammonium formate was purchased from Sigma–Aldrich (Steinheim, Germany), and dichloromethane and methanol (gradient grade for liquid chromatography), were obtained from Merck (Darmstadt, Germany). High purity water was prepared using a Milli-Q water purification system (Millipore, Milford, MA, USA). Dichloromethane–methanol (50:50) (v/v) was used to elute the pesticides from the OASIS HLB SPE cartridge (200 mg sorbent/6 ml cartridge, Waters). MilliQ water and methanol, both with ammonium formate 10 mM were used as mobile phase in LC–MS/MS.

2.2. Sampling and sample preparation

Eight water samples were taken from Turia River. They were collected in the middle of the current river with 1 L polypropylene bottles. Immediately, samples were transported to the laboratory, where they were kept refrigerated at 4.5 °C and extracted within 48 h.

Four influent and their respective effluent waste water samples were provided by four waste water treatment plants of the area. Water samples were 24 h integrated, collected also in polypropylene bottles by the operators.

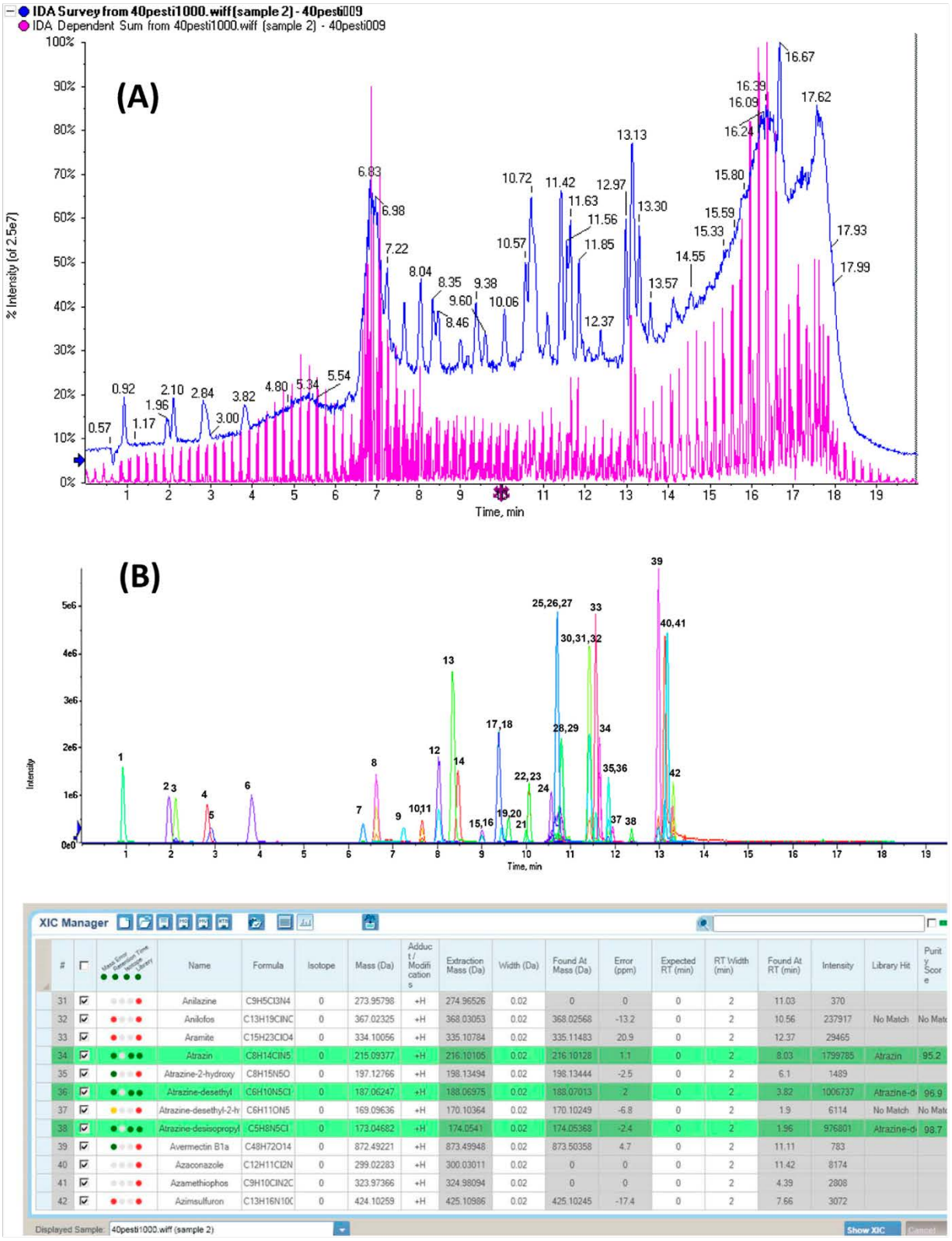
An off-line SPE already reported elsewhere, was used for the pre-concentration of the water samples [4]. Oasis HLB cartridges were preconditioned with 5 ml dichloromethane–methanol (50:50) (v/v) followed by 10 ml of deionized water. Water samples (200 ml) were loaded at a flow rate of 1 ml min^{-1}. The cartridges were then dried under vacuum for 10 min and analytes were eluted with 10 ml of the mixture dichloromethane–methanol (50:50, v/v). Extracts were evaporated to dryness and reconstituted with 1 ml of methanol. Then, they were filtered through 0.45 μm PTFE filters into the autosampler vials for LC–MS/MS analysis.

2.3. Liquid chromatography–quadrupole time-of-flight mass spectrometry

The chromatography was performed on an Agilent 1260 Infinity (Agilent, Waldbronn, Germany) using a Poroshell 12 D EC-C18 column 50 mm × 30 mm internal diameter, 2.7 μm (Agilent). Flow rate was 0.4 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 20 min under the following conditions: 0 min, 30% B; 10 min, 85% B; 15 min, 98% B. The column was equilibrated for 10 min prior to each analysis. The UHPLC system was coupled to a hybrid

Table 3.1
Average retention times (Rt), mass accuracies (ppm) of the protonated molecules $[M+H]_+$, % of difference of the isotopical pattern and average purity scores of the MS/MS identification against a library for each target pesticide spiked at 10 ng L_{-1}. Values reported are mean of eight experiments ($n = 8$) plus the standard deviation (SD).

Peak number	Pesticide	Empirical formula	Mass (Da)	Ion	Extraction mass (Da)	Average mass found (Da)	Mass error		t_r		Isotope ratio			Purity score	
							ppm	SD	min	SD	% of difference	SD		%	SD
1	Omethoate	$C_5H_{12}NO_4PS$	213.0225	$[M+H]^+$	214.0297	214.0300	1.3	0.8	0.92	0.06	1	3		85.7	−3
2	Atrazine-deisopropyl	$C_5H_8N_5Cl$	173.0468	$[M+H]^+$	174.0541	174.0540	−0.9	0.2	1.96	0.09	5	3		91.3	1
3	Imidacloprid	$C_9H_{10}ClN_5O_2$	255.0523	$[M+H]^+$	256.0596	256.0596	0.2	0.5	2.11	0.01	2	3		95.6	4
4	Dimethoate	$C_5H_{12}NO_3PS_2$	228.9996	$[M+H]^+$	230.0069	230.0067	−0.7	0.1	2.82	0.01	2	3		87.8	0
5	Fenoxon sulfoxide	$C_{10}H_{15}O_5PS$	278.0378	$[M+H]^+$	279.0451	279.0443	−2.6	0.7	2.97	0.05	2	2		90.1	−3
6	Atrazine-deethyl	$C_6H_{10}N_5Cl$	187.0625	$[M+H]^+$	188.0698	188.0701	2	0.7	3.81	0.08	4	2		85.3	2
7	Simazine	$C_7H_{12}ClN_5$	201.0781	$[M+H]^+$	202.0854	202.0855	0.3	0.9	6.32	0.07	4	1		85.2	−4
8	Carbofuran	$C_{12}H_{15}NO_3$	221.1052	$[M+H]^+$	222.1125	222.1124	−0.5	0.3	6.62	0.04	5	3		88.4	−4
9	Fenoxon sulfone	$C_{10}H_{15}O_6PS$	294.0327	$[M+H]^+$	295.0400	295.0398	−0.6	0.4	7.22	0.06	1	2		93.6	5
10	Fenthion sulfoxide	$C_{10}H_{15}O_4PS_2$	294.0149	$[M+H]^+$	295.0222	295.0217	−1.7	0.5	7.63	0.01	3	1		92.4	3
11	Fenthion sulfone	$C_{10}H_{15}O_5PS_2$	310.0099	$[M+H]^+$	311.0171	311.0170	−0.5	0.3	7.65	0.09	2	1		89.3	−3
12	Atrazine	$C_8H_{14}ClN_5$	215.0938	$[M+H]^+$	216.1011	216.1012	0.7	0.6	8.03	0.07	5	1		87.5	−5
13	Isoproturon	$C_{12}H_{18}N_2O$	206.1419	$[M+H]^+$	207.1492	207.1494	0.8	0.9	8.35	0.04	5	2		87.7	1
14	Diuron	$C_9H_{10}Cl_2N_2O$	232.0170	$[M+H]^+$	233.0243	233.0242	−0.3	0.6	8.46	0.05	5	3		85.9	−2
15	Azinphos-methyl	$C_{10}H_{12}N_3O_3PS_2$	317.0058	$[M+H]^+$	318.0131	318.0129	−0.5	0.4	9	0.05	5	2		91.8	−4
16	Fenoxon	$C_{10}H_{15}O_4PS$	262.0429	$[M+H]^+$	263.0502	263.0484	−6.7	0.8	9.07	0.07	1	3		92.1	1
17	Propazine	$C_9H_{16}N_5Cl$	229.1094	$[M+H]^+$	230.1167	230.1169	0.6	0.8	9.38	0.03	2	3		95.4	−1
18	Propanil	$C_9H_9Cl_2NO$	217.0061	$[M+H]^+$	218.0134	218.0127	−3.2	0.8	9.45	0.03	3	1		86.3	0
19	Fenitrothion	$C_9H_{12}NO_5PS$	277.0174	$[M+H]^+$	278.0247	278.0244	−0.9	0.3	9.52	0.06	3	1		93.6	0
20	Parathion-methyl	$C_8H_{10}NO_5PS$	263.0017	$[M+H]^+$	264.0090	264.0094	1.4	0.5	9.59	0.04	3	2		92.7	2
21	Methiocarb	$C_{11}H_{15}NO_2S$	225.0824	$[M+H]^+$	226.0896	226.0894	−1.2	0.7	9.96	0.02	3	1		88.5	3
22	Molinate	$C_9H_{17}NOS$	187.1031	$[M+H]^+$	188.1104	188.1100	−2	0.4	10	0.04	4	3		93.9	1
23	Malathion	$C_{10}H_{19}O_6PS_2$	330.0361	$[M+H]^+$	331.0434	331.0432	−0.5	0.1	10.06	0.09	1	1		93.6	4
24	Azinphos-ethyl	$C_{12}H_{16}N_3O_3PS_2$	345.0371	$[M+H]^+$	346.0444	346.0445	0.5	0.3	10.56	0.01	1	2		99.1	−4
25	Alachlor	$C_{14}H_{20}ClNO_2$	269.1183	$[M+H]^+$	270.1255	270.1255	0.0	0.4	10.64	0.08	1	1		97.2	−3
26	Acetochlor	$C_{14}H_{20}ClNO_2$	269.1183	$[M+H]^+$	270.1255	270.1255	0	0.7	10.65	0.07	2	1		93.4	2
27	Terbutryn	$C_{10}H_{19}N_5S$	241.1361	$[M+H]^+$	242.1434	242.1438	1.6	0.9	10.69	0.06	5	3		87.1	3
28	Carbofuran-3-hydroxy	$C_{12}H_{16}NO_4$	238.1079	$[M+H]^+$	239.1152	239.1154	0.8	0.8	10.73	0.09	4	3		85.9	1
29	Metolachlor	$C_{15}H_{22}ClNO_2$	283.1339	$[M+H]^+$	284.1412	284.1407	−1.7	0.1	10.79	0.05	4	3		88.6	−5
30	Parathion-ethyl	$C_{10}H_{14}NO_5PS$	291.0330	$[M+H]^+$	292.0403	292.0398	−1.8	0.6	11.41	0.04	3	1		91.8	4
31	Fenthion	$C_{10}H_{15}O_3PS_2$	278.0200	$[M+H]^+$	279.0273	279.0273	0.1	0.4	11.42	0.03	3	1		93.1	−4
32	Imazalil	$C_{14}H_{14}Cl_2N_2O$	296.0483	$[M+H]^+$	297.0556	297.0561	1.5	0.7	11.42	0.03	3	2		91.3	−2
33	Diazinon	$C_{12}H_{21}N_2O_3PS$	304.1011	$[M+H]^+$	305.1083	305.1082	−0.5	0.8	11.57	0.07	3	2		86.8	1
34	Chlorfenvinphos	$C_{12}H_{14}Cl_3O_4P$	357.9695	$[M+H]^+$	358.9768	358.9771	0.7	0.3	11.64	0.05	3	2		87.2	3
35	Tolclophos-methyl	$C_9H_{11}Cl_2O_3PS$	299.9544	$[M+H]^+$	300.9616	300.9615	−0.4	0.6	11.84	0.06	1	1		89.5	−5
36	Prochloraz	$C_{15}H_{16}Cl_3N_3O_2$	375.0308	$[M+H]^+$	376.0381	376.0379	−0.5	0.7	11.85	0.01	2	1		90.4	−4
37	Buprofezin	$C_{16}H_{23}N_3OS$	305.1562	$[M+H]^+$	306.1635	306.1637	0.9	0.6	11.97	0.02	1	1		90.9	3
38	Ethion	$C_9H_{22}O_4P_2S_4$	383.9876	$[M+H]^+$	384.9949	384.9951	0.4	0.9	12.47	0.01	1	1		91.8	1
39	Piryproxyfen	$C_{20}H_{19}NO_3$	321.1365	$[M+H]^+$	322.1438	322.1439	0.5	0.8	13.17	0.03	1	1		93.1	5
40	Chlorpyriphos	$C_9H_{11}Cl_3NO_3PS$	350.9234	$[M+H]^+$	351.9307	351.9308	0.4	0.1	13.29	0.02	3	2		94.7	−2
41	Dichlofenthion	$C_{10}H_{13}Cl_2O_3PS$	313.9700	$[M+H]^+$	314.9773	314.9774	0.3	0.5	13.3	0.02	5	3		86.8	−3
42	Hexythiazox	$C_{17}H_{21}ClN_2O_2S$	352.1012	$[M+H]^+$	353.1085	353.1086	0.3	0.1	13.31	0.08	4	3		90.9	1



#	Name	Formula	Isotope	Mass (Da)	Adduct / Modification	Extraction Mass (Da)	Width (Da)	Found At Mass (Da)	Error (ppm)	Expected RT (min)	RT Width (min)	Found At RT (min)	Intensity	Library Hit	Purity Score
31	Anilazine	C9H5Cl3N4	0	273.95798	+H	274.96526	0.02	0	0	0	2	11.03	370		
32	Anilofos	C13H19ClNC	0	367.02325	+H	368.03053	0.02	368.02568	-13.2	0	2	10.56	237917	No Match	No Mat
33	Aramite	C15H23ClO4	0	334.10056	+H	335.10784	0.02	335.11483	20.9	0	2	12.37	29465		
34	Atrazin	C8H14ClN5	0	215.09377	+H	216.10105	0.02	216.10128	1.1	0	2	8.03	1799785	Atrazin	95.2
35	Atrazine-2-hydroxy	C8H15N5O	0	197.12766	+H	198.13494	0.02	198.13444	-2.5	0	2	6.1	1489		
36	Atrazine-desethyl	C6H10N5Cl	0	187.06247	+H	188.06975	0.02	188.07013	2	0	2	3.82	1006737	Atrazine-d	96.9
37	Atrazine-desethyl-2-hy	C6H11ON5	0	169.09636	+H	170.10364	0.02	170.10249	-6.8	0	2	1.9	6114	No Match	No Mat
38	Atrazine-desisopropyl	C5H8N5Cl	0	173.04682	+H	174.0541	0.02	174.05368	-2.4	0	2	1.96	976801	Atrazine-d	98.7
39	Avermectin B1a	C48H72O14	0	872.49221	+H	873.49948	0.02	873.50358	4.7	0	2	11.11	783		
40	Azaconazole	C12H11Cl2N	0	299.02283	+H	300.03011	0.02	0	0	0	2	11.42	8174		
41	Azamethiphos	C9H10ClN2C	0	323.97366	+H	324.98094	0.02	0	0	0	2	4.39	2808		
42	Azimsulfuron	C13H16N10	0	424.10259	+H	425.10986	0.02	425.10245	-17.4	0	2	7.66	3072		

Fig. 3.1 UHPLC–QqTOF-MS/idaMS chromatograms obtained from an spiked distilled water sample extract (A) total ion chromatogram (TIC) showing the survey MS scan (blue) and the IDA product ion scan (pink) and (B) extracted ion chromatogram (XIC) of the 43 target pesticides against a XIC manager Table with data on 1212 pharmaceuticals, 546 pesticides, 378 polyphenols and 233 mycotoxins. For peak identification see Table 3.1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 3.2
Non-target compounds identified in the river water samples analyzed against a XIC Table manager including precursor ion mass error, isotope ration and MS/MS identification against a library (peak area was >40,000).

Family/compound	Formula	Mass (Da)	Error (ppm)	Isotope ratio (% difference)	Purity score	Freq.	NI conf.
Pesticides							
Pirimicarb	$C_{11}H_{18}N_4O_2$	238.14298	−2	5	75.3	8	No
Dodemorph	$C_{18}H_{35}NO$	281.27187	−1	9	76.8	12	No
Thiabendazole	$C_{10}H_7N_3S$	201.03607	−2.8	6	81.2	8	No
Sethoxydim	$C_{17}H_{29}NO_3S$	327.18682	0.3	7	74.5	8	No
Fenpropimorph	$C_{20}H_{33}NO$	303.25621	1.6	4	77.9	8	No
Pyrethrin I	$C_{21}H_{28}O_3$	328.20385	−2	5	76.3	8	No
Atrazine-2-hydroxy	$C_8H_{15}N_5O$	197.12766	−2.7	3	80.6	8	No
Spiroxamine	$C_{18}H_{35}NO_2$	297.26678	−1	5	79.2	12	No
Pharmaceuticals							
Irbesartan	$C_{25}H_{28}N_6O$	428.23246	−3.7	5	86.6	16	Yes
Hymecromone	$C_{10}H_8O_3$	176.04734	0.9	4	75.4	16	No
Nabumetone	$C_{15}H_{16}O_2$	228.11503	0.7	7	81.2	12	No
Caffeine	$C_8H_{10}N_4O_2$	194.08038	−0.2	9	79.6	8	Yes
Salbutamol	$C_{13}H_{21}NO_3$	239.15214	−0.5	7	90.1	8	No
Telmisartan	$C_{33}H_{30}N_4O_2$	514.23688	−0.6	8	77.9	16	Yes
Paracetamol	$C_8H_9NO_2$	151.06333	0.7	5	78.6	8	Yes
Atenolol	$C_{14}H_{22}N_2O_3$	266.16304	4.3	6	76.9	16	No
Valsartan	$C_{24}H_{29}N_5O_3$	435.22704	−0.8	5	75.1	8	Yes
Eprosartan	$C_{23}H_{24}N_2O_4S$	424.14568	−1.1	5	83.9	8	No
Climbazole	$C_{15}H_{17}ClN_2O_2$	292.09786	−1.3	8	82.3	12	Yes
Theobromine/Teofilina	$C_7H_8N_4O_2$	180.06473	0.5	7	70.1	4	Yes
Desoximethasone	$C_{22}H_{29}FO_4$	376.20499	0.6	7	72.6	4	No
Fluocortolone	$C_{22}H_{29}FO_4$	376.20499	0.6	9	71.7	4	No
Testosterone benzoate	$C_{26}H_{32}O_3$	392.23515	−4.1	4	76.1	8	No
Carbamazepine	$C_{15}H_{12}N_2O$	236.09496	−2.1	3	83.4	8	No
Exemestane	$C_{20}H_{24}O_2$	296.17763	−0.7	5	74.6	12	No
Cocaine	$C_{17}H_{21}NO_4$	303.14706	−2.5	3	86.5	8	No
Scopolamine	$C_{17}H_{21}NO_4$	303.14706	−2.5	3	91.6	8	No
Crotetamide	$C_{12}H_{22}N_2O_2$	226.16813	−0.8	2	75.2	4	No
Morphine	$C_{17}H_{19}NO_3$	285.13649	−5.2	6	73.6	8	No
Ecgoninemethylester	$C_{10}H_{17}NO_3$	199.12084	−3.8	7	74.1	8	No
Omeprazole	$C_{17}H_{19}N_3O_3S$	345.11471	−5.4	9	71.7	8	No
Benzoylecgonine	$C_{16}H_{19}NO_4$	289.13141	0	5	70.1	16	No
Alprostadil	$C_{20}H_{34}O_5$	354.24062	−0.6	4	76.3	8	No
Dinoprost	$C_{20}H_{34}O_5$	354.24062	−0.6	4	79.1	8	No
Tranexamic acid	$C_8H_{15}NO_2$	157.11028	−0.2	5	75.8	8	No
Clarithromycin	$C_{38}H_{69}NO_{13}$	747.47689	−1.2	8	74.3	4	No
Simvastatin	$C_{25}H_{38}O_5$	418.27192	−1.1	7	71.5	12	Yes
Enalapril	$C_{20}H_{28}N_2O_5$	376.19982	2.6	7	80.6	4	No
Polyphenols							
5-(3′,4′,-Dihydroxyphenyl)-valerolactone	$C_{12}H_{14}O_4$	222.08921	−1.3	4	73.5	12	Yes
Mycotoxins							
Mevinolin	$C_{24}H_{36}O_5$	404.25627	−3.8	3	72.4	4	Yes
Asterric acid	$C_{17}H_{16}O_8$	348.08452	7.3	5	77.9	4	Yes

quadrupole time-of-flight ABSciex Triple TOF™ 5600. The QqTOF was calibrated as recommended by the manufacturer in MS and MS/MS in high sensitivity mode. The MS acquisition was performed in positive ionization using information-dependent acquisition (IDA) that consist of two experiments: the survey scan type that was a full scan mass spectrum between m/z 100–950 and the dependent scan type was a product ion scan in which the system selects ions automatically without any ion predefined by the user. MS parameters were: ion spray voltage, 5500 V; declustering potential (DP) 80 V; collision energy (CE) 10 V; temperature 450 °C with curtain gas (CUR) 30 (arbitrary units); ion source gas 1 (GS1) 35 and ion source gas 2 (GS2) 35. IDA MS/MS was performed using the following criteria: ions that exceeded 100 cps, ion tolerance 50 mDa, collision energy fixed at 45 V and dynamic background subtract activated. Similarly, the QqTOF-MS was also used in negative ionization mode for confirmation purposes. The conditions were the same but some voltages can change their polarity to negative.

Data acquisition and processing was carried out using software Analyst, Peak View 1.0 with the application XIC manager and Formula Finder; and MultiQuant 2.0 in order to perform targeted and non-targeted screening in a single LC–MS/MS run.

The Peak View was used for targeted and non-targeted data processing launching the XIC Manager tool. The XIC Manager automatically calculates XICs, performs compound identification and displays results against a XIC Table containing 1212 pharmaceuticals, 546 pesticides, phenols and mycotoxins, to generate automatically XICs, which are displayed in the chromatogram panel and in a table. These tables include name, formula, adduct/modification, retention time, width and are editable. The results displayed include found mass, mass error (ppm), found at retention time and library (MS and MS/MS) search results.

For unknown compounds not included in the XIC Table, high resolution and accurate mass information was used to empirically calculate the formula of molecular ions and detected fragments ions using Formula Finder of PeakView software. Then, possible structure identities were searched by internet (chemspider and other databases).

Statistical analysis of the samples to find unexpected compounds was performed with the MarkerView™ software (minimum spectral peak with 0.02 Da, noise threshold 100 cps). The statistical analysis was performed using principal component analysis (PCA) and principal components variable grouping (PCPG).

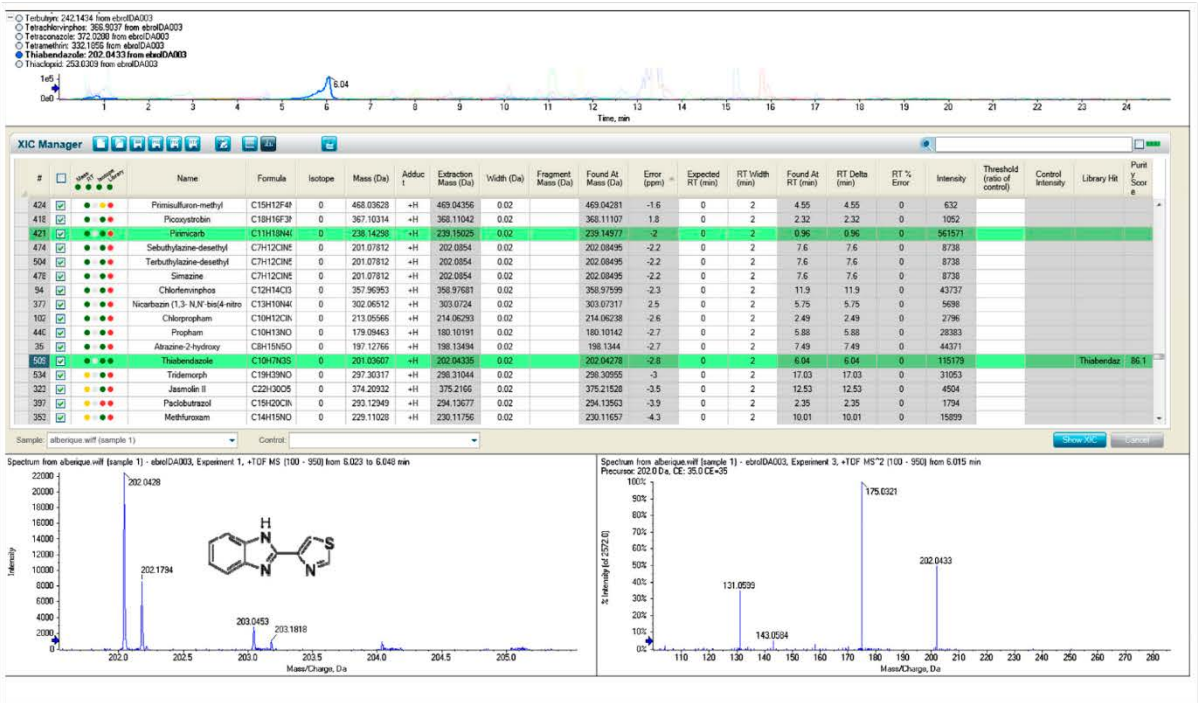


Fig. 3.2 Identification of the non-target pesticide thiabendazole in a river water sample against the XIC manager Table with data on 1212 pharmaceuticals, 546 pesticides, 378 polyphenols and 233 mycotoxins.

2.4. Validation parameters

The method was validated according to the Guidelines on Method Validation and Quality Control procedures for Pesticide Residues [35] and using a within laboratory protocol. Specificity/selectivity, linearity, matrix effect, lowest calibration level (LCL), precision as repeatability and within-lab reproducibility, and recovery were studied. Furthermore, the limit of detection (LOD)

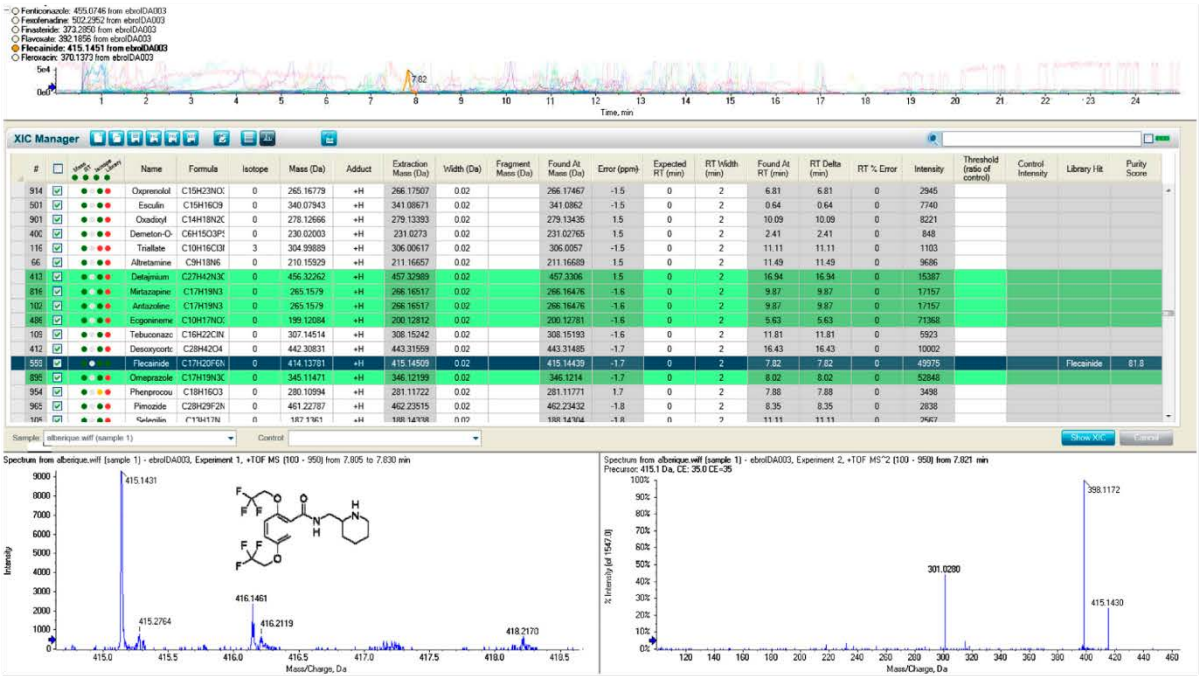


Fig. 3.3 Identification of non-target flecainide in a river water sample against the XIC manager Table with data on 1212 pharmaceuticals, 546 pesticides, 378 polyphenols and 233 mycotoxins.

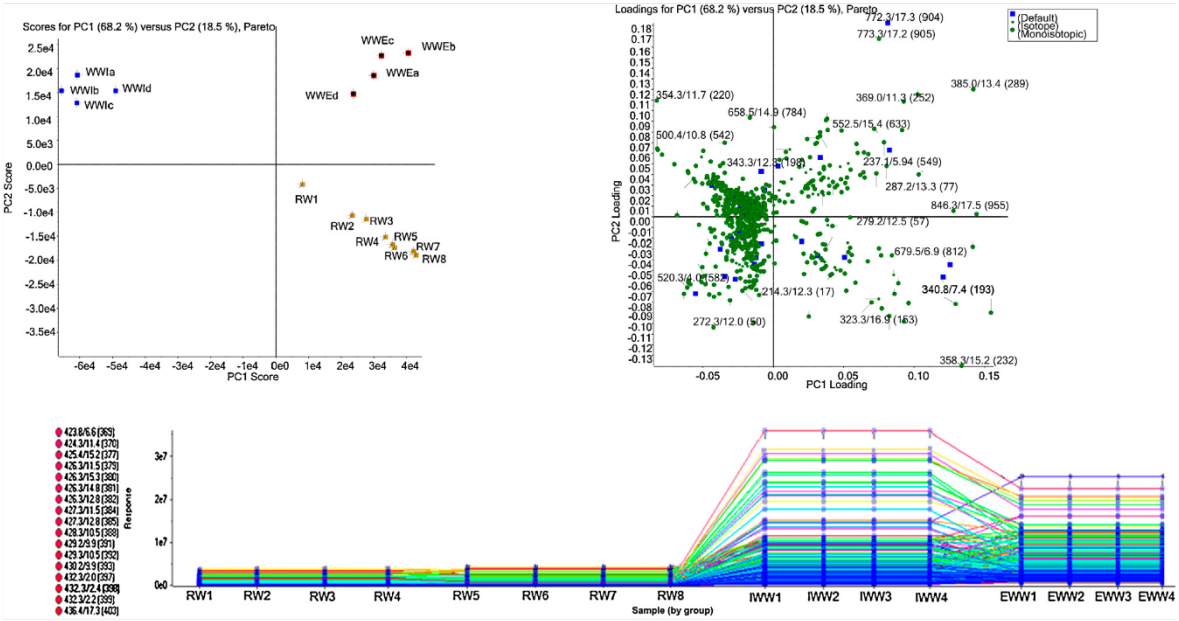


Fig. 3.4 PCA and PCVG of water samples (A) score plot that was used to visualize the difference between samples, (B) the loadings plot with PCVG was used to identify characteristic *m/z*-retention time pairs which were verified in (C) the profile plot.

was estimated by injecting decreasing concentrations of standards and measuring the response to find the concentrations that would give UHPLC peak value around 1.0×10^4 as recommended elsewhere [17,23,27].

Linearity was evaluated by preparing calibration curves in methanol within the range of LCL–1000 ng L^{-1} for each compound. Unspiked raw and treated wastewater as well as surface water were extracted by the Oasis HLB SPE cartridges according to the method described in Section 2.2. The pesticide responses in the presence of matrix ions were compared with their responses dissolved in methanol. For this, the dry residues were reconstituted in 1 ml of a mix of all pesticides at a concentration of 5 ng ml^{-1} of each (equivalent to 20 ng L^{-1} in water) or at 50 ng ml^{-1} of each (equivalent to 200 ng L^{-1}) dissolved in methanol. The samples were filtered through the PVDF syringe filters and injected into the LC–MS/MS system. Each sample was injected three times. As references, filtered standard solutions of at the same concentrations in mobile phase, were injected ($n = 3$) into the LC–MS/MS system. The matrix factors (MF) were calculated by dividing the peak area of the compound in the presence of matrix ions minus the area of the compound in the matrix without spiking by the peak area achieved from the compound dissolved in methanol. No blank matrices were found in this study, all of them contained diazinon or chlorpyriphos at different concentrations.

Recoveries and relative standard deviations (RSDs) of pesticides were calculated by spiking samples in which the target pesticides were not found at three different concentrations – low level, LCL; medium level, 50 ng L^{-1}, and high level, 100 ng L^{-1} – and, then, analyzing them in quintuplicate. The spiking was carried out by adding 100 μl of the appropriate working mixture to 250 ml of water. The precision of the method was determined by the repeatability and reproducibility studies, and expressed as the RSD (%). The intra-day precision was measured by comparing the standard deviation of the recovery percentages of the spiked samples carried out during the same day. The inter-day precision was determined by analyzing the spiked samples in five distinct days.

3. Results and discussion

3.1. Searching of water pollutants against libraries

In relation to the chromatographic separation, generic eluents (water–methanol both 10 mM ammonium formate) were used considering that the selected pesticides belong to several families with different chemical properties. All of the compounds were properly eluted using the gradient profile indicated in Section 2.3. The optimized conditions (see Section 2.3) provided reproducible retention times (RTs), which ranged from 0.92 min (omethoate) to 13.17 min (pyriproxyfen) (Table 3.1). Variations were lower than±0.2 min within and between batches for most of the pesticides. The standard deviation (SD) of the RT of any target pesticides detected in the samples ranged from 0.01 to 0.09. The total run-ning time was 20 min to let the elution of the apolar non-target compounds that could be longer retained in the analytical column.

Fig. 3.1 shows the total ion chromatogram (TIC) of both, the survey scan and the data dependent acquisition, obtained for a river blank water sample spiked with the target pesticides as well as the extracted chromatogram against a XIC manager that contains more than 2000 possible contaminants (1212 pharmaceuticals, 546 pesticides, 378 polyphenols and 233 mycotoxins). As could be observed in the chromatogram, most of the visible identified peaks correspond to the target analytes, which demonstrated appropriate selectivity of the method as well as the lack of false positives and negatives. The quality identification of three target compounds is also noticeable in the figure. Atrazine, deethylatrazine and deisopropylatrazine were identified with mass error < 3 ppm, % of difference in the isotope match < 10% and purity score > 95%.

Table 3.1 also reports mean values for mass accuracies, mass spectral errors of the $[M+H]^+$ ions of target pesticides and the purity score obtained for the identification of the compound by the MS/MS spectrum obtained against a database. Average mass accuracy for all target pesticides was below 0.5 ppm (and never higher than 3.2 ppm for an individual pesticide) and the SD of these error

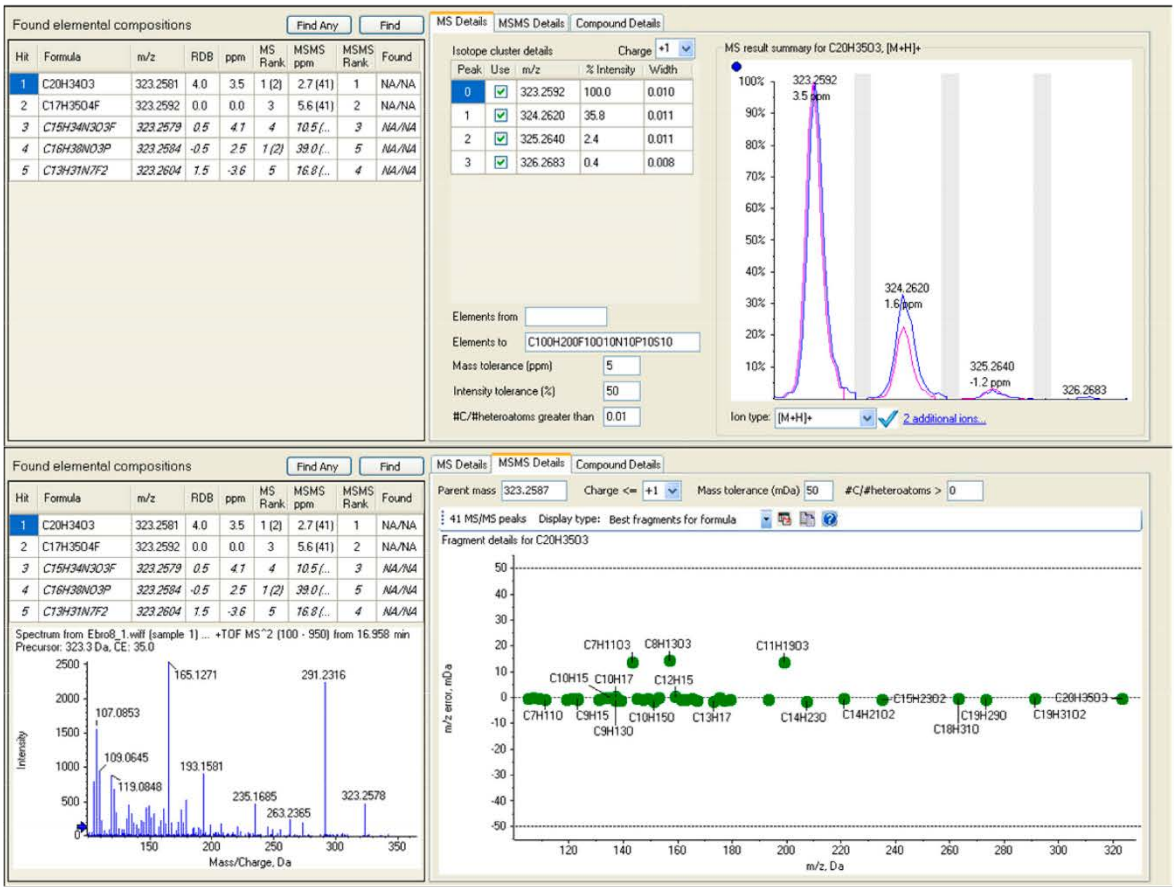


Fig. 3.5 Identification of an unknown compound at *m/z* 323.2582 combining accurate mass, isotopical pattern and MS/MS fragmentation.

were from 0.1 to 0.9, which indicates a very low deviation of the instrument response from the monoisotopic mass. For the parent compound the % of difference in the experimental and theoretical isotope ratio was always lower than 8%. Purity score values higher than 75% were always obtained in MS/MS identification against the library provided by the supplier, even for samples spiked at low concentrations.

In the water samples processed against the XIC Table, 27 target pesticides, in addition to several non-target compounds, mostly pharmaceuticals and illicit drugs were identified with mass error < 5 ppm, % of difference in the isotopic pattern ≤ 10% and MS/MS search library with purity score ≥ 75%. Table 3.2 shows non-target compounds identified and some of the quality parameters obtained in the identification. Peak intensity is an important parameter in this case since is the discriminating factor for the system to perform MS/MS. The MS/MS spectra were obtained for all peaks with an area higher of 40,000. Many peaks with lower area were tentatively identified by only MS with proper mass accuracy and isotope pattern match but were confirmed by MS/MS only in some cases. These data are reported in the supplementary material (Tables S3.1 and S3.2). As can be observed in Table S3.1, peaks with an area >20,000 and <40,000 half of the peaks with appropriate mass error and isotopical pattern also showed MS/MS spectra to con-firm the identity against the mass library. However, in the case of peaks with area ≤ 20,000 the MS/MS spectra were only obtained for the 10% of peaks tentatively identified only by MS. For some of the detected compounds (Table 3.2, last column), an additional confirma-tion was obtained in negative ionization mode because they could ionize in both polarities. Mass spectra obtained for paracetamol in both positive and negative mode are shown in the supplementary material (Fig. S3.1). Negative ESI allowed also sensitive analyses because of the acidic hydroxyl group of paracetamol (pK_a 9.4). How-ever, the sensitivity was lower than in positive mode and, in any water sample, the negative molecule was fragmented. The majority of the analytes identified in this work gave only response in positive ESI (Table 3.2).

It should be noted that the chromatographic separation is strongly related also to the identification because peaks with an improper shape could be undetected. The mobile phase used in this study is appropriate for pesticides but not for pharmaceuticals, mycotoxins and pesticides, which commonly requires an acidic mobile phase. That could be the reason why different compounds remain undetected.

In the supplementary material, two examples in Figs. S3.2 and S3.3 show the identification of target compounds (ethion and meto-lachlor) in one of the river water sample analyzed. Mass errors as low as −0.8 and −0.9, respectively, and purity scores up to 85% were obtained. Fig. 3.2 shows the identification of the non-target thiaben-dazole, the mass error was slightly higher −2.8 but purity score of the MS/MS against a library database was of 86.1. Thiabendazole is extensively used as fungicide to control a variety of fruit and vegetable diseases and as antiparasitic to control roundworm and other helminthics. Furthermore, it is also used as a preservative in paints, carpets, adhesives and textiles. Fig. 3.3 illustrates the positive identification flecainide in a wastewater effluent, which is a classic antiarrhythmic agent used to prevent and treat tachyarrhythmias.

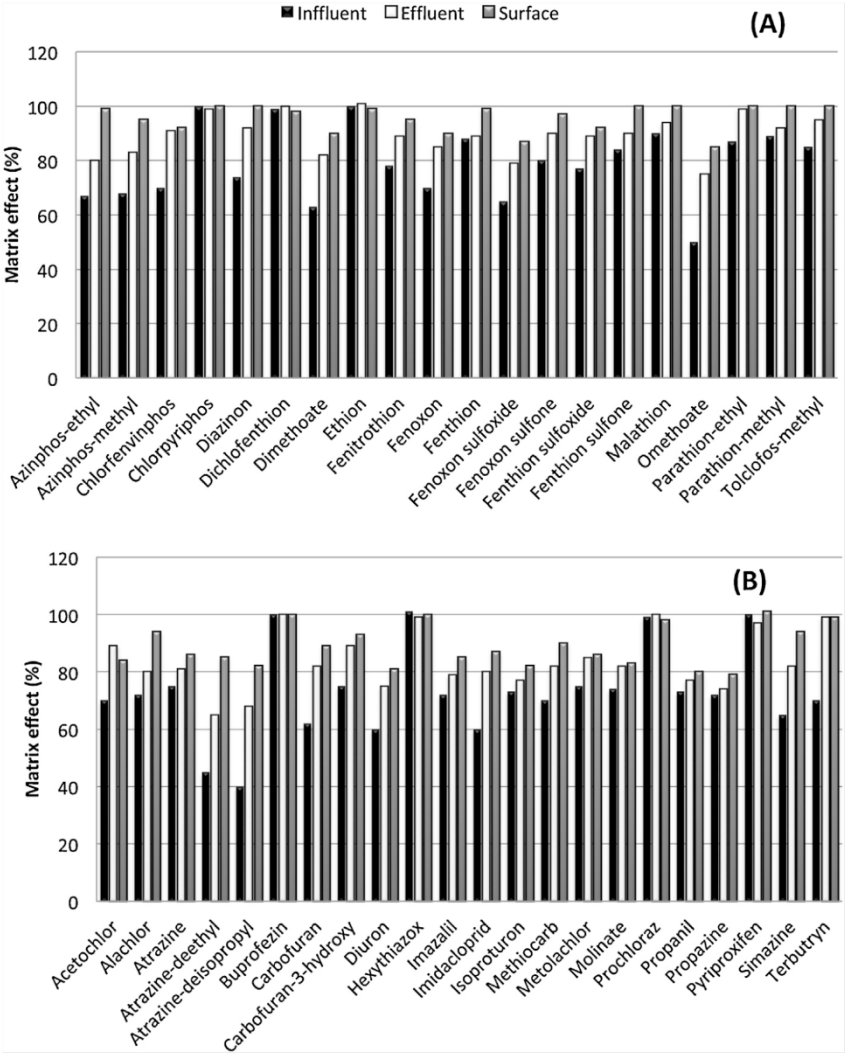


Fig. 3.6 Matrix effects in different water types, influent, effluent and surface waters (A) organophosphorus and (B) other pesticides.

Both, thiabendazole and flecaimide were further confirmed by injection of analytical standards. Figs. S3.4–S3.6 illustrates the identification of some non-target compounds.

3.2. Study with PCA

In addition to the compounds previously identified by the XIC manager, water samples contain thousands of non-target peaks that need to be identified. Every non-targeted peak finding algorithm is capable to doing so. However, the peak list generated by such algorithms can easily contain thousands of chromatographic and mass spectrometric signals. In depth investigation of these signals could be time consuming and inefficient since most of these signals are derived from matrix components (they also can come from the chemical background, but, in this case, most were efficiently removed in a permanent exclusion list). Thus the peak list generated by a non-targeted peak algorithm needs to be reduced to a list containing peaks of interest only.

Statistical data processing is the most effective procedure to find peaks of interest in complex samples. In order to identify other non-target compounds LC–QqTOF-MS/MS data were processed using PCA and PCPG Marker View Software. PCA establishes combinations of variables, in this case based on retention times, mass signals and intensities, that explain most of the variance present in a data set. For each principal component every sample has a score and every variable has a loading that represents its contribution to the combination. It is common to plot the scores and loading of two principal components to visualize the analytical results [36–40]. Fig. 3.4A shows the score plot of all 16 water samples. The scores plot clearly differentiates the samples originating from different water types: influent, effluent and river water. One particular observation is that river water samples are arranged depending on the distance to the mouth of the river. Waste water samples are arranged in the top part of the score plot. Influents to the left and effluent to the right. The corresponding loading plot (Fig. 3.4B) of PCVG shows many peaks responsible for the variation (m/z-retention time pairs in this case). The list of peak is fairly long. Fig. 3.4C shows the profile plot that verifies that all variables for the selected group were charac-teristics of the type of samples. Clearly some signals are different from one to other samples.

One of the higher signals responsible of the differences between wastewaters and natural waters correspond to m/z 323.24 at 16.0 min. The Formula Finder software was used to empirically calculate the molecular formula with information available on

Table 3.3
Linearity equation (concentration range 5–5000 ng L^{-1}), LODs and recovery as well as precision (intra-day RSD, %) at the LCL (5 ng L^{-1}).

Target pesticide	Linearity		LOD (ng L^{-1})	Recovery (%)	RSD (%)
	$y = a + bx$ (ng L^{-1})	r^2			
Acetochlor	$y = 6781x - 1674$	0.99525	0.3	88	5
Alachlor	$y = 6677x - 3802$	0.99305	0.3	74	13
Atrazine	$y = 10{,}844x + 4023$	0.99933	0.06	81	18
Atrazine-deethyl	$y = 8247x + 112$	0.99001	0.1	79	4
Atrazine-deisopropyl	$y = 7224x + 2604$	0.99868	0.1	72	5
Azinphos-ethyl	$y = 5594x + 948$	0.99826	0.3	77	18
Azinphos-methyl	$y = 1587x - 1211$	0.99529	0.4	73	9
Buprofezin	$y = 26{,}418x - 9498$	0.99548	0.02	71	16
Carbofuran	$y = 1757x + 9930$	0.99182	0.4	74	14
Carbofuran-3-hydroxy	$y = 731x + 2662$	0.99881	0.8	88	4
Chlorfenvinphos	$y = 10{,}033x + 12{,}384$	0.99322	0.08	78	19
Chlorpyriphos	$y = 2056x - 3319$	0.99838	0.4	76	3
Diazinon	$y = 18{,}722x + 3e4$	0.99544	0.05	67	8
Dichlofenthion	$y = 30x + 416$	0.99668	2	86	12
Dimethoate	$y = 5415x - 2870$	0.99125	0.3	79	5
Diuron	$y = 9659x - 986$	0.99941	0.1	71	12
Ethion	$y = 11{,}847x + 2e7$	0.99735	0.08	70	6
Fenitrothion	$y = 200x - 328$	0.99542	2	86	11
TableBreak Fenoxon	$y = 9x + 2411$	0.99531	2	99	9
Fenoxon-Sulfone	$y = 2182x + 1329$	0.99078	0.4	74	15
Fenoxon-Sulfoxide	$y = 192x - 47$	0.99846	0.5	72	3
Fenthion	$y = 2307x - 1406$	0.99722	0.4	68	9
Fenthion-Sulfone	$y = 9892x + 5104$	0.99133	0.1	80	4
Fenthion-Sulfoxide	$y = 13{,}494x + 19{,}122$	0.99867	0.08	84	12
Hexythiazox	$y = 4796x - 6235$	0.99168	0.3	89	7
Imazalil	$y = 26{,}388x + 14{,}970$	0.9972	0.02	83	19
Imidacloprid	$y = 6247x + 1812$	0.99873	0.2	82	10
Isoproturon	$y = 24{,}320x + 19{,}246$	0.99815	0.02	79	9
Malathion	$y = 21x + 452$	0.99803	2	69	8
Methiocarb	$y = 2453x + 3769$	0.99837	0.4	83	7
Metolachlor	$y = 9072x + 3e6$	0.99416	0.1	71	9
Molinate	$y = 677x + 35$	0.99602	0.8	82	20
Omethoate	$y = 2221x - 1146$	0.99719	0.4	72	7
Parathion-ethyl	$y = 68x - 22$	0.99939	1.8	82	5
Parathion-methyl	$y = 14x + 85$	0.99622	2	107	18
Prochloraz	$y = 6658x - 2938$	0.99488	0.3	71	14
Propanil	$y = 1466x - 2288$	0.99659	0.4	79	4
Propazine	$y = 14{,}611x + 6648$	0.99892	0.05	80	16
Pyriproxifen	$y = 22{,}891x - 3e4$	0.99479	0.02	89	12
Simazine	$y = 1670x - 171$	0.99049	0.4	75	7
Terbutryn	$y = 28{,}755x + 21{,}308$	0.99855	0.02	84	10
Tolclophos-methyl	$y = 302x - 346$	0.99894	0.5	87	16

the accurate molecular mass quasi-molecular ion, isotopic pattern, cluster ions and MS/MS fragment ions resulting in $C_{20}H_{34}O_3$ (Fig. 3.5). This formula was subsequently searched against on-line databases as chemspider to find possible structures. Thirty-two different structures can fit the empirical formula. All of them are in this moment in use for different applications (for detailed results see Table S3.3 of the supplementary material). Among these structures it is spected according to RDB that the molecule have 4 double bonds. That reduces the possible structures to 7. The suspected structures were compared to the MS/MS spec-trum using the fragment prediction tool in order to establish the most probable one. However, the results were not fully con-clusive, since many of these compounds are structural isomers with very similar structure and fragmentation. Although the most realistic ones were 4-[3-methoxy-4-(nonyloxy)phenyl]butan-2-ol and 1-[4-(hexyloxy)-3-methoxyphenyl]-4,4-dimethylpentan-3-ol, a confirmation using the analytical standard, which was not available is mandatory since the fragmentation of these compounds could be similar.

3.3. Quantification capabilities: pesticides as study case

Table 3 lists the validation parameters for each target pesticide obtained for spiked river water samples.

3.3.1. LODs and LCL

The instrumental LOD was determined empirically, by injecting a series of spiked extract, as the concentration for which the peak height was >1.0 × 10^4, and it is reported in Table 3.3. The LODs ranged from 0.02 ng L^{-1} (buprofezin, imazalil, isoproturon, pyriproxyfen, terbutryn) to 2 ng L^{-1} (dichlofenthion, fenitrothion, fenoxon, malathion, parathion-methyl). Of the 43 studied pesticides, 34 showed LODs lower than 0.5 ng L^{-1}, only 8 had an LOD between 0.6 and 2 ng L^{-1}. Based in these LODs, a LCL of 5 ng L^{-1} equal for all pesticides was established.

At this LCL selected, it was possible to obtain always MS/MS spectra for all target compounds, spiked in any type of matrices even the waste water influents. Thus, this combination of conventional SPE and UHPLC–QqTOF-MS (IDA-MS/MS) attains enough sensitivity to determine pesticide residues at low ng L^{-1} level.

Compared with our previous study in which a UHPLC–QqQ-MS/MS method was developed for the same pesticides, the LOQ ranged from 0.2 to 6 ng L^{-1} [4]. It could be observed that for most pesticides the results are of the same order.

3.3.2. Linearity and matrix effect

The calibration curves were observed to be linear with coefficients of determination (R^2) ≥ 0.99 (Table 2). One of the drawbacks commonly attributed to the QqTOF is the low dynamic range and consequently poor linearity, even though, some studies have

Table 3.4
Examples of the concentrations levels detected of the target pesticides in 4 water samples using this UHPLC–QqTOF-MS(ida-MS/MS) and previously reported UHPLC–QqQ-MS/MS method [15].

Target pesticide	WWI-2 (ng L^{-1})		WWE-1 (ng L^{-1})		RW-3 (ng L^{-1})		RW-10 (ng L^{-1})	
	QqQ	QTOF	QqQ	QTOF	QqQ	QTOF	QqQ	QTOF
Acetochlor								
Alachlor								
Atrazine			20.72	18.52	8.95	9.24	8.13	6.46
Atrazine-deethyl	25.12	21.25	29.76	25.99	19.95	21.25	24.35	21.55
Atrazine-deisopropyl	39	37.21	28.11	27.36	4.35	4.21	5.83	6.46
Azinphos-ethyl								
Azinphos-methyl								
Buprofezin			10.68	8.78	8.04	7.85	7.86	14.3
Carbofuran			4.54	3.52				
Carbofuran-3-hydroxy								
Chlorfenvinphos	53.41	50	268.1	253.45	31.17	29.86	16.15	15.55
Chlorpyriphos	125	145.7	163.72	159.6	120.3	118.1	115.6	112.65
Diazinon	105.00	105.95	40.9	41.23	9.73	8.55	14.21	13.05
Dichlofenthion			34.86	33.27	14.84	14.76	21.02	20.84
Dimethoate	18.00	17.43	16.7	15.28	2.33	2.01		
Diuron	31.03	27.05	163.25	64.4	1.02	0.85	0.92	0.85
Ethion								
Fenitrothion								
Fenoxon								
Fenoxon-Sulfone								
Fenoxon-Sulfoxide			50.36	49.21	3.45	3.12	20.84	20.03
Fenthion								
Fenthion-Sulfone			5.6	4.2				
Fenthion-Sulfoxide								
Hexythiazox	57	55.3	13.76	12.45	8.74	7.69	10.57	10.49
Imazalil	162.65	160.1	675.56	652.23	189.25	182.8	102.62	100.98
Imidacloprid	90	89.78	2.00	1.98	2.77	2.52	2.46	2.42
Isoproturon	90.00	90.03	20.32	19.95				
Malathion	848.00	756.24	8.99	8.65				
Methiocarb								
Metolachlor	894.26	865	239	225	939.4	930	1299	1295
Molinate								
Omethoate	18	18.03						
Parathion-ethyl	1503.65	1498.85					14.45	14.02
Parathion-methyl								
Prochloraz	0.75	0.4	63.15	62.48	34.47	33.21	29.61	29.12
Propanil								
Propazine	25.68	24.45	10.05	9.6	34.98	34.65	22.19	21.8
Pyriproxifen	64.26	63.3	75.46	58.85	33.28	40.95	37.74	41.1
Simazine			5.00	4.78				
Terbutryn	39.00	38.59	56.94	55.21	12.08	10.95	18.23	17.75
Tolclophos-methyl							12.51	11.69

demonstrated that taking the appropriate precautions, it is possible to quantify. However, the design of these new instruments, commonly goes forward and one of the promising features of this new system is an extended and more robust linear dynamic range. As can be observed in Table 3.3, $r > 0.99$ were obtained in a concentration range from 5 ng L^{-1} to 5 μg L^{-1}. These results correspond to calibration curves of the spiked matrix (river waters) extracts (matrix-matches). The peak area vs. concentration showed a linear signal over more than three orders of magnitude. This linearity range is fully comparable to that obtained using QqQ for the same pesticides.

The signal suppression observed for analyzed pesticides dissolved in SPE extract of three different types of water is presented in the Fig. 3.6. Signal suppression was generally observed for all water types analyzed, except for prochloraz, buprofezin, ethion, pyriprox-yfen, chlorphyriphos, dichlofenthion and hexythiazox, which were the last eluting compounds. As expected, signal suppression in influent was notably greater than in the other type of samples (see grey, black and white bars in Fig. 3.6). This fact is in accordance to other studies using UHPLC–MS/MS, where several pesticides were determined in water [4,16,29]. The matrix effect calcu-lated was acceptable (ca. 70%) with the exception of the earlier eluted analytes (peaks from 1. Omethoate to 8. Carbofuran, see Table 3.1). Matrix effects are dependent on the ESI interface design, compound and sample. The effect of the sample matrix is evident as depicted in several figures of this paper, especially for influent waste water samples. In addition, the signal suppression observed for a given sample was generally compound dependent, even within the same pesticide family. Although it could not be the best option and because the high price and low availability of isotopically labelled internal standards, the matrix effect was corrected by the use of matrix matched calibration. After this correction, the matrix effect observed compared to influent, effluents and surface water coming for several sources was lower than 20%.

3.3.3. *Recovery and precision*

Recovery obtained in river water ranged from 67% (diazinon) to 97% (for parathion methyl). The overall repeatability ($n = 5$) of the extraction procedure was satisfactory with RSD values ranging from 3% to 20% for river water. Thus, satisfactory recovery and precision were obtained for all compounds except for diazinon, ethion, fenthion and malathion (recoveries ≤ 70%) as well as for molinate (RSDs ≥ 20%).

3.3.4. Quantification of real samples

The validated method was applied to several types of samples that were analyzed in triplicate. Pesticide residues were detected in all types of samples including water and sediments. Table 3.4 outlines the quantification of target pesticides. The results were compared with those obtained using UHPLC–QqQ-MS/MS according to the instrumental conditions previously reported [4]. The good agreement between both techniques is remarkable. Pesticides were detected in each type of samples. Water samples presented the high number of pesticides (15). In the identification process, the ability to confirm compounds using MS/MS library searching ensured that no false positives were reported.

4. Conclusions

The applicability and efficiency of the LC–QqTOF-MS technique in automated IDA-MS/MS for the qualitative and quantitative analysis has been demonstrated by the development of one of the first applications reported of this technique for the simultaneous determination of 43 pesticides and identification of a large number of non-target pesticides and pharmaceutically active compounds in wastewater and river water samples. The method has been demonstrated to be a very simple, fast, and viable alternative for routine monitoring of organic contaminants in waters.

As well as the obvious advantage of using a TOF analyzer – allowing it to perform full-scan acquisition with sensitivity (detection limits in the ng L^{-1} range) and high mass accuracy (mass errors lower than 2 ppm) – it also makes the qualitative analysis easier, quicker and more accurate, because the monitoring of a specific mass of an analyte is not predefined before data acquisition. This fact is very useful in detecting the presence of an unlimited number of chemical constituents in a sample without re-analysis. Consequently, the method could be readily extended to include additional analytes.

Acknowledgements

This work has been supported by the Spanish Ministry of Economy and Competitiveness through the projects "Assessing and Predicting Effects on Water Quantity and Quality in Iberian Rivers Caused by Global Change (SCARCE)" (No. CSD2009-00065, http://www.scarceconsolider.es) and "Evaluation of Emerging Contaminants in the Turia River Basins: From Basic Research to the Application of Environmental Forensics (EMERFOR)" (GCL2011-29703-C02-02, http://mefturia.es). We also thank to the mass spectrometry section of the Central Services of Support to the Experimental Research (SCSIE) of the Universitat de València for providing us access to the Linear QTOF (Applied Biosciences) and especially to Dr. Sales Galletero to help us in this study beyond the call of duty.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2014.04.017.

References

[1] P. Vazquez-Roig, C. Blasco, Y. Picó, Trends Anal. Chem. 50 (2013) 65.
[2] P. Vazquez-Roig, V. Andreu, M. Onghena, C. Blasco, Y. Pico, Anal. Bioanal. Chem. 400 (2011) 1287.
[3] A. Masia, Y. Moliner-Martinez, M. Munoz-Ortuno, Y. Pico, P. Campins-Falco, J. Chromatogr. A 1306 (2013) 1.
[4] A. Masia, M. Ibanez, C. Blasco, J.V. Sancho, Y. Pico, F. Hernandez, Anal. Chim. Acta 761 (2013) 117.
[5] J. Campo, A. Masiá, C. Blasco, Y. Picó, J. Hazard. Mater. 263 (2013) 146.
[6] C. Vialle, C. Sablayrolles, J. Silvestre, L. Monier, S. Jacob, M. Huau, M. Montrejaud-Vignoles, J. Environ. Manag. 120 (2013) 48.
[7] M. del Mar Gomez-Ramos, A. Perez-Parada, J.F. Garcia-Reyes, A.R. Fernandez-Alba, A. Agueera, J. Chromatogr. A 1218 (2011) 8002.
[8] I. Gonzalez-Marino, J. Benito Quintana, I. Rodriguez, R. Cela, Water Res. 45 (2011) 6770.
[9] J. Magner, M. Filipovic, T. Alsberg, Chemosphere 80 (2010) 1255.
[10] A. Mueller, W. Schulz, W.K. Ruck, W.H. Weber, Chemosphere 85 (2011) 1211.
[11] R.D. Morrison, in: J. Siegel, P. Saukko (Eds.), Encyclopedia of Forensic Sciences, second ed., Elsevier, Oxford, UK, 2013, p. 45.
[12] E.M. Thurman, I. Ferrer, in: I. Ferrer, E.M. Thurman (Eds.), Liquid Chromatography/Mass Spectrometry, MS/MS and Time of Flight MS, ACS Symposium Series, vol. 850, American Chemical Society, Washington, DC, 2003, p. 14.
[13] A. Masiá, J. Campo, P. Vázquez-Roig, C. Blasco, Y. Picó, J. Hazard. Mater. 263 (Part 1) (2013) 95.
[14] R. Lopez-Roldan, M. Lopez de Alda, M. Gros, M. Petrovic, J. Martin-Alonso, D. Barcelo, Chemosphere 80 (2010) 1337.
[15] M. Hurtado-Sanchez, R. Romero-Gonzalez, M. Rodriguez-Caceres, I.I. Duran-Meras, A. Garrido Frenich, J. Chromatogr. A 1305 (2013) 193.
[16] S. Grimalt, J.V. Sancho, O.J. Pozo, F. Hernandez, J. Mass Spectrom. 45 (2010) 421.
[17] R. Loos, R. Carvalho, D.C. Antonio, S. Comero, G. Locoro, S. Tavazzi, B. Paracchini, M. Ghiani, T. Lettieri, L. Blaha, B. Jarosova, S. Voorspoels, K. Servaes, P. Haglund, J. Fick, R.H. Lindberg, D. Schwesig, B.M. Gawlik, Water Res. 47 (2013) 6475.
[18] R. Loos, S. Tavazzi, B. Paracchini, E. Canuti, C. Weissteiner, Anal. Bioanal. Chem. 405 (2013) 5875.
[19] Y. Fillatre, D. Rondeau, A. Jadas-Hecart, P.Y. Communal, Rapid Commun. Mass Spectrom. 24 (2010) 2453.
[20] R. Diaz, M. Ibanez, J.V. Sancho, F. Hernandez, Anal. Meth. 4 (2012) 196.
[21] J.A. Padilla-Sanchez, E.M. Thurman, P. Plaza-Bolanos, I. Ferrer, Rapid Commun. Mass Spectrom. 26 (2012) 1091.
[22] M.M. Aguilera-Luiz, R. Romero-Gonzalez, P. Plaza-Bolanos, Anal. Bional. Chem. 405 (2013) 6543.
[23] M. Ibanez, T. Portoles, A. Rubies, E. Munoz, G. Munoz, L. Pineda, E. Serrahima, J.V. Sancho, F. Centrich, F. Hernandez, J. Agric. Food Chem. 60 (2012) 5311.
[24] Y. Pico, M. Farre, R. Segarra, D. Barcelo, Talanta 81 (2010) 281.
[25] J. Wang, W. Chow, D. Leung, Anal. Bioanal. Chem. 396 (2010) 1513.
[26] J. Nacher-Mestre, M. Ibanez, R. Serrano, J. Perez-Sanchez, F. Hernandez, J. Agric. Food Chem. 61 (2013) 2077.
[27] M. Gomez, M. Gomez-Ramos, O. Malato, M. Mezcua, A. Fernandez-Alba, J. Chromatogr. A 1217 (2010) 7038.
[28] E. Pitarch, T. Portoles, J. Marin, M. Ibanez, F. Albarran, F. Hernandez, Anal. Bioanal. Chem. 397 (2010) 2763.
[29] F. Hernandez, T. Portoles, M. Ibanez, M. Bustos-Lopez, R. Diaz, A. Botero-Coy, C. Fuentes, G. Penuela, Sci. Total Environ. 439 (2012) 249.
[30] R. Diaz, M. Ibanez, J.V. Sancho, F. Hernandez, Rapid Commun. Mass Spectrom. 25 (2011) 355.
[31] M. Martinez Bueno, M.M. Ulaszewska, M. Gomez, M. Hernando, A. Fernandez-Alba, J. Chromatogr. A 1256 (2012) 80.
[32] C. Fenech, K. Nolan, L. Rock, A. Morrissey, Environ. Pollut. 181 (2013) 250.
[33] J. Pascual-Aguilar, V. Andreu, Y. Picó, J. Hazard. Mater. 263 (P1) (2013) 214.
[34] J.R. Radović, C. Aeppli, R.K. Nelson, N. Jimenez, C.M. Reddy, J.M. Bayona, J. Albaigés, Mar. Pollut. Bull. 79 (2014) 268.
[35] Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed. European Commission Document No. SANCO/12495/2011, Brussels, January 1, 2012.
[36] S. Deffontis, A. Breton, C. Vialle, M. Montrejaud-Vignoles, C. Vignoles, C. Sablayrolles, Sci. Total Environ. 452 (2013) 394.
[37] C.J. Houtman, R. ten Broek, K. de Jong, B. Pieterse, J. Kroesbergen, Environ. Toxicol. Chem. 32 (2013) 2449.
[38] Y. Wu, Y. Jia, X. Lu, Ecotoxicol. Environ. Saf. 94 (2013) 138.
[39] A. El Nemr, A.A. Moneer, A. Khaled, A. El-Sikaily, Environ. Monit. Assess. 185 (2013) 4835.
[40] D. Han, X.X. Tong, M. Jin, E. Hepburn, C. Tong, X. Song, Environ. Monit. Assess. 185 (2013) 3413.

Suplementary material

Ultra-high performance liquid chromatography quadrupole time-of-flight mass spectrometry to determine contaminants in water: An insight on environmental forensics

Ana Masiá, Julian Campo, Cristina Blasco, Yolanda Picó

Food and Environmental Safety Research Group, Facultat de Farmàcia, Universitat de València, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, València, Spain

* Corresponding author. Tel: +34-963543092; fax: +34-963544954
E-mail address: Yolanda.Pico@uv.es

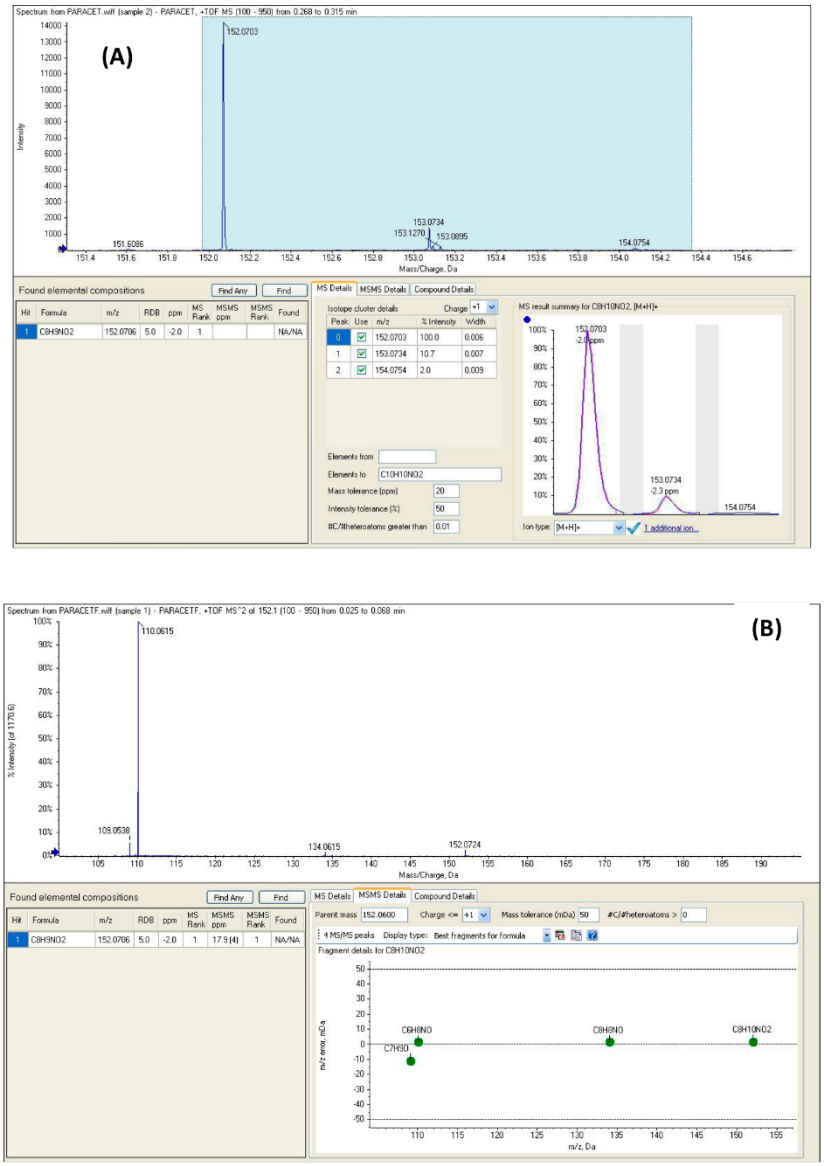


Fig S3.1 Mass spectra of paracetamol in different ionization and MS modes (A) precursor ion mass spectrum by ESI in positive ionization (PI) mode, (B) product ion mass spectrum by ESI in PI mode and (C) precursor ion mass spectrum by ESI in negative ionization (NI) mode.

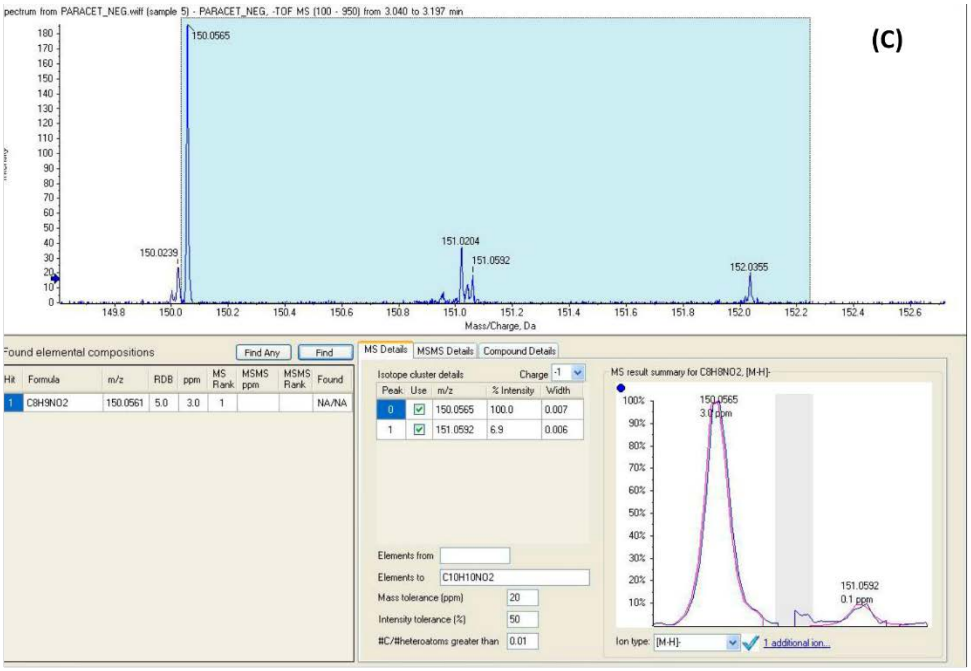


Fig S3.1 Continued

Fig. S3.2 Identification against a XIC manager Table containing 1212 pharmaceuticals, 546 pesticides, 378 polyphenols and 233 mycotoxins of ethion in a wastewater treatment plants (WWE2)

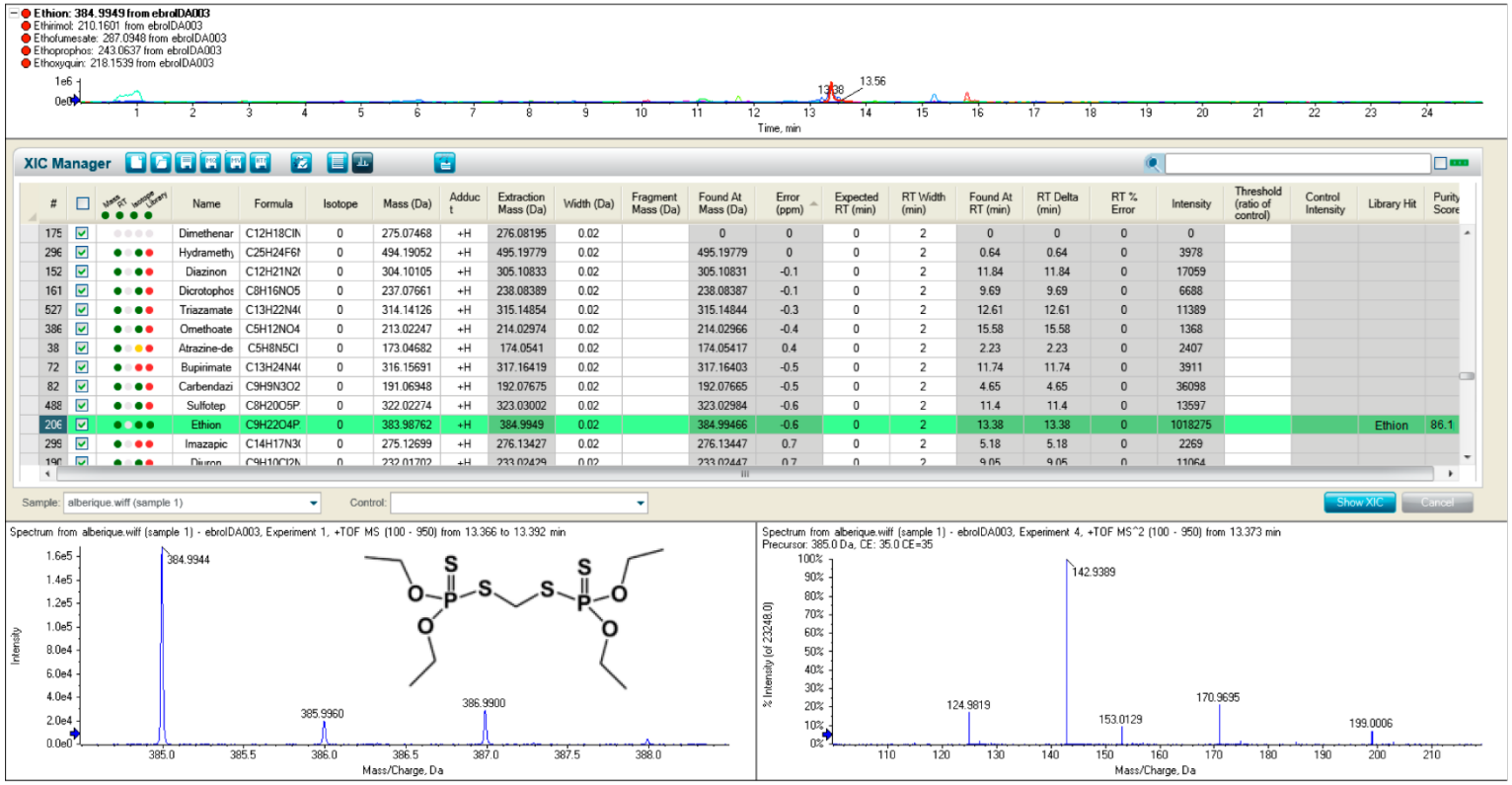


Fig. S3.3 Identification against a XIC manager Table containing 1212 pharmaceuticals, 546 pesticides, 378 polyphenols and 233 mycotoxins of metholachlor in a wastewater treatment plant (WWE2)

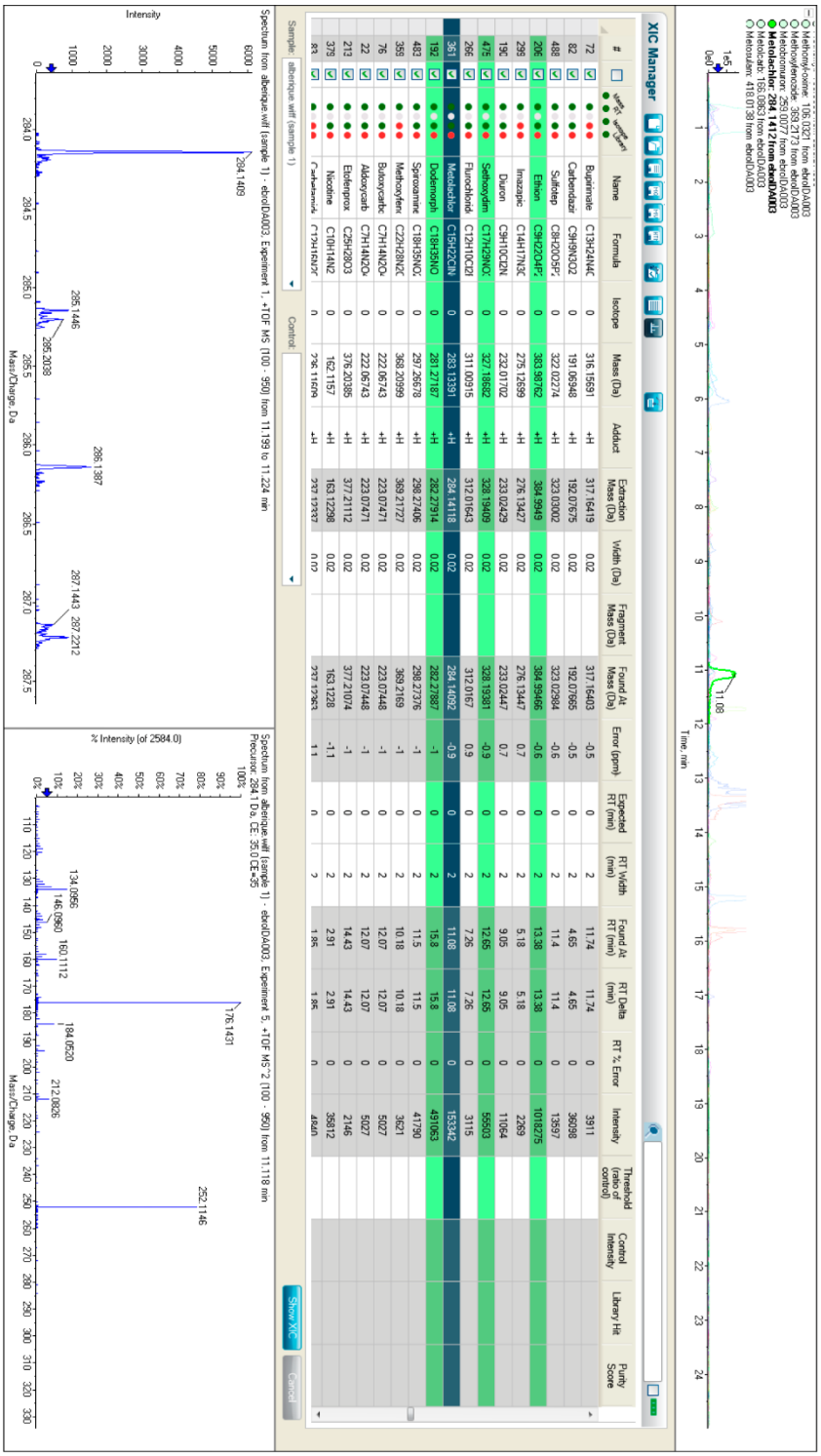
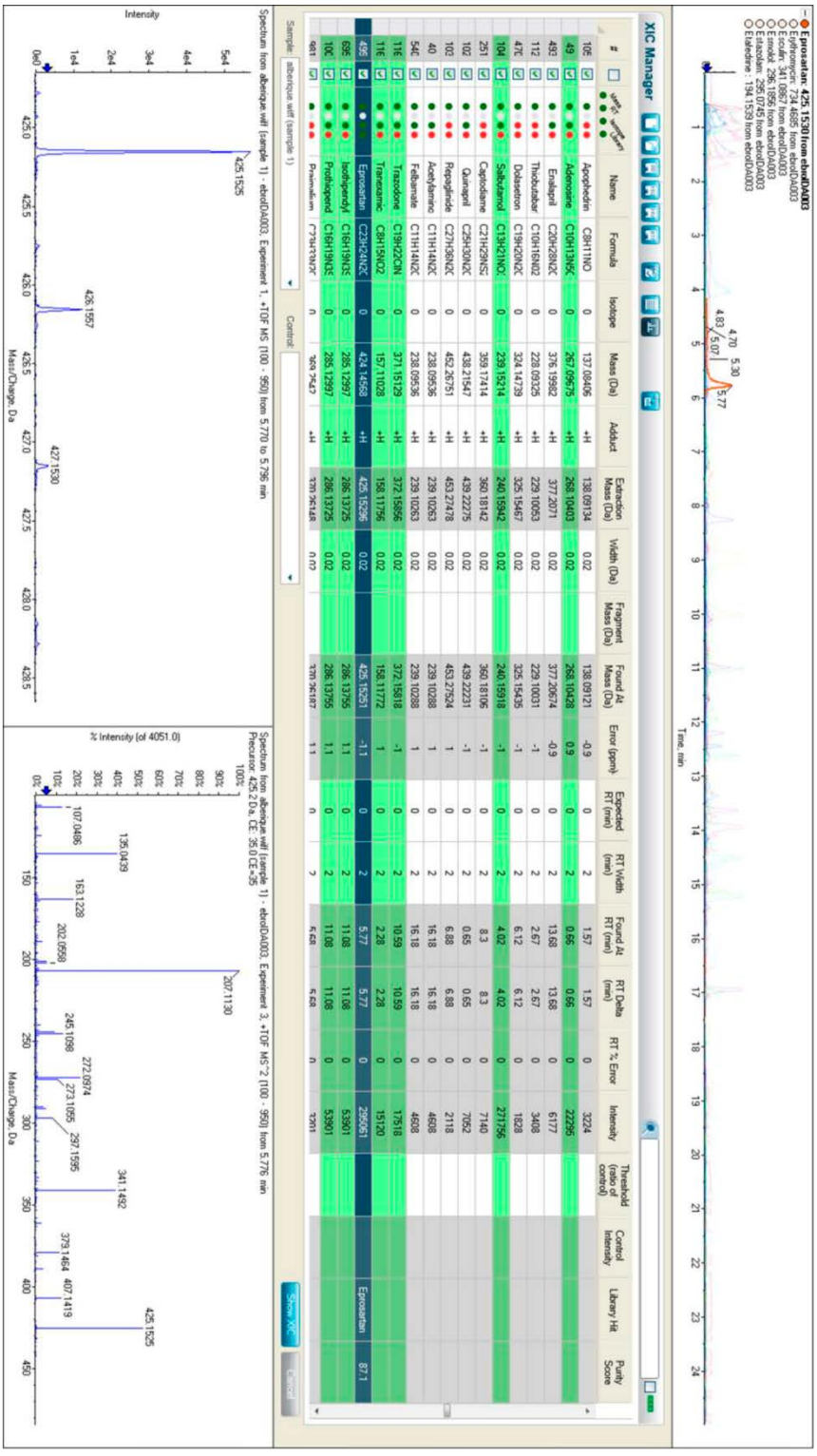


Fig. S3.4 Identification against a XIC manager Table containing 1212 pharmaceuticals, 546 pesticides, 378 polyphenols and 233 mycotoxins of eposartan in a influent waste water (WW12)



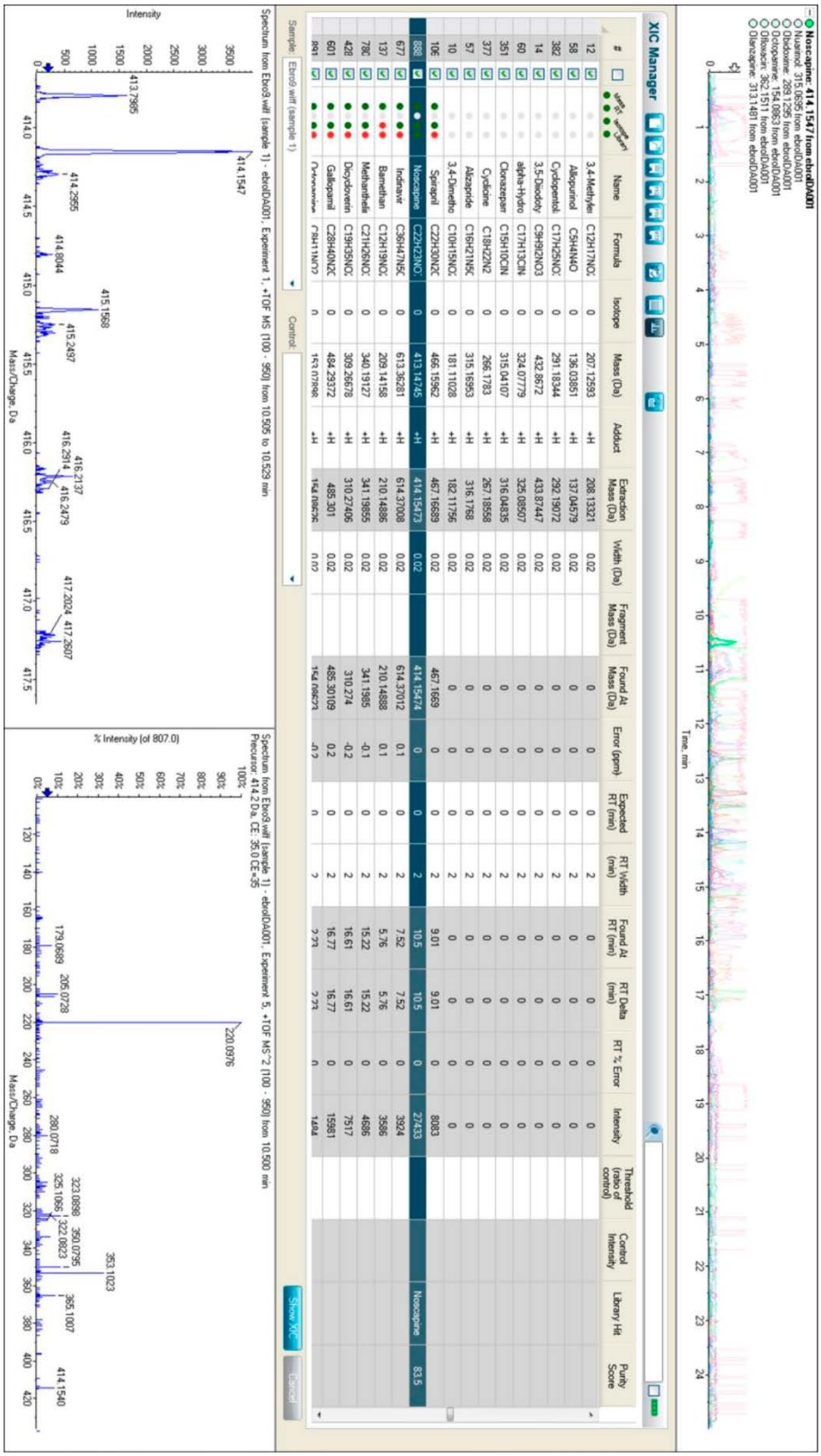


Fig. S3.5 Identification against a XIC manager Table containing 1212 pharmaceuticals, 546 pesticides, 378 polyphenols and 233 mycotoxins of noscapine in a river water (RW7)

Fig. S3.6 Identification against a XIC manager Table containing 1212 pharmaceuticals, 546 pesticides, 378 polyphenols and 233 mycotoxins of omeoprazole in a river water (RW 7)

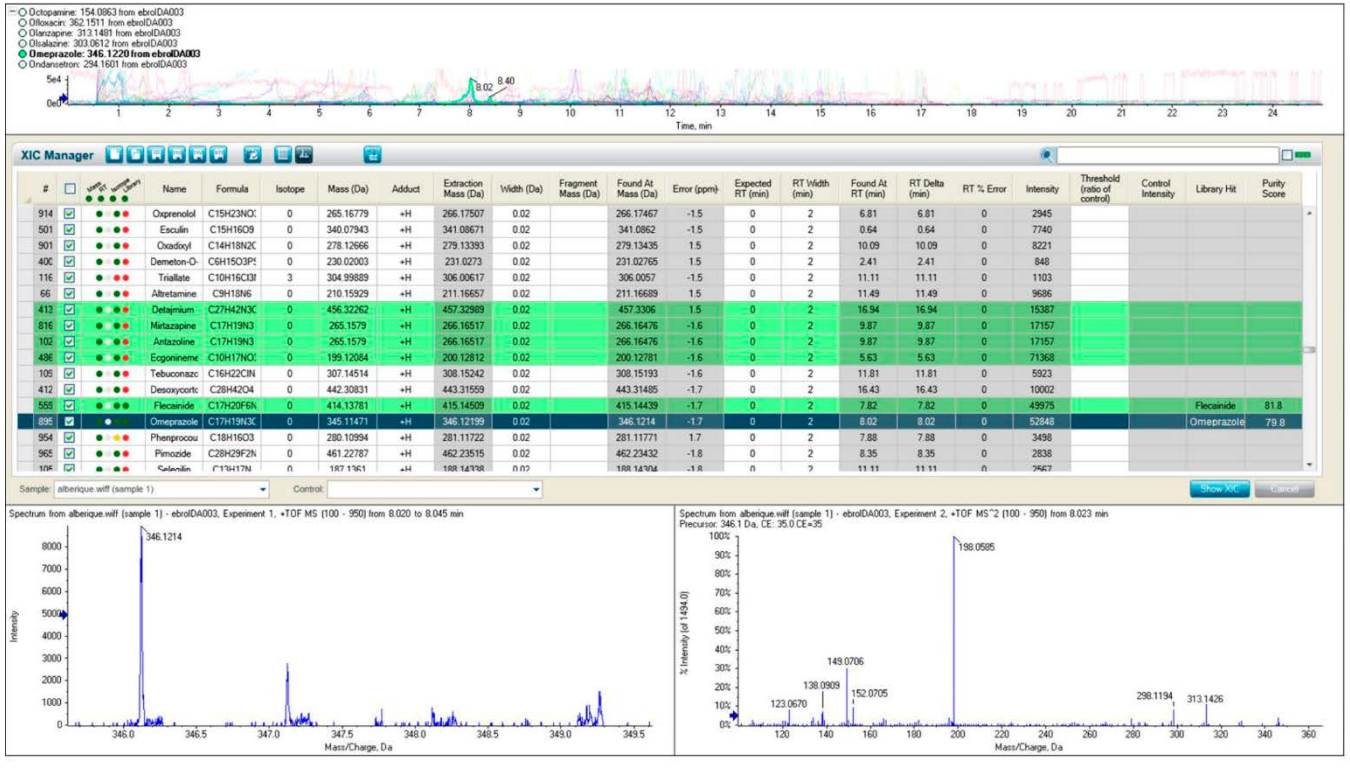


Table S3.1 Non-target compounds tentatively identified in river water samples analyzed against a XIC manager table including MS/MS library search for those peaks with an area > 20,000 and < 40,000

Family/Compound	Formula	Mass (Da)	Error (ppm)	Isotope ratio % difference	Purity Score	Frequency
Pesticides						
Imazamethabenz-methyl	$C_{16}H_{20}N_2O_3$	288.14739	-0.8	6		4
Carbendazim	$C_9H_9N_3O_2$	191.06948	-0.5	7	75.9	8
Tridemorph	$C_{19}H_{39}NO$	297.30317	-0.3	9		8
Tebuconazol	$C_{16}H_{22}ClN_3O$	307.14514	0.2	5	80.2	4
Prometryne	$C_{10}H_{19}N_5S$	241.13612	-1.1	5	73.6	8
Propham	$C_{10}H_{13}NO_2$	179.09463	-2.7	4		4
Methfuroxam	$C_{14}H_{15}NO_2$	229.11028	-3.8	5		4
Pirimiphos-methyl	$C_{11}H_{20}N_3O_3PS$	305.0963	-7.5	6	83.4	4
Pharmaceuticals						
Cyclopentobarbital	$C_{12}H_{14}N_2O_3$	234.10044	-1.2	3		4
Alprenolol	$C_{15}H_{23}NO_2$	249.17288	-0.9	8	76.1	4
Oxycodone	$C_{18}H_{21}NO_4$	315.14706	-4.5	4		4
Amisulpride	$C_{17}H_{27}N_3O_4S$	369.17223	-1.1	4		4
Butoxycaine	$C_{17}H_{27}NO_3$	293.19909	-0.6	5		4
Embutramide	$C_{17}H_{27}NO_3$	293.19909	-0.6	7		4
Lincomycin	$C_{18}H_{34}N_2O_6S$	406.21376	-0.3	5		4
Nicotine	$C_{10}H_{14}N_2$	162.1157	-1.1	10	89.5	4
Phenethylamin	$C_8H_{11}N$	121.08915	-0.3	5		4
Naproxen	$C_{14}H_{14}O_3$	230.09429	-0.4	5	90.5	4
Etofenamate	$C_{18}H_{18}F_3NO_4$	369.11879	-0.9	9		4
Brucine	$C_{23}H_{26}N_2O_4$	394.18926	1.3	7		4
Etafenone	$C_{21}H_{27}NO_2$	325.20418	0.4	3		4
Norpropoxyphene	$C_{21}H_{27}NO_2$	325.20418	0.4	5		4
3,4-Methylenedioxyamphetamine	$C_{10}H_{13}NO_2$	179.09463	-2.7	7	82	4
N-Isopropylsalicylamide	$C_{10}H_{13}NO_2$	179.09463	-2.7	5		4
Phenacetin	$C_{10}H_{13}NO_2$	179.09463	-2.7	6	84.3	4
Phenprobamate	$C_{10}H_{13}NO_2$	179.09463	-2.7	5		4

Family/Compound	Formula	Mass (Da)	Error (ppm)	Isotope ratio % difference	Purity Score	Frequency
Propham	$C_{10}H_{13}NO_2$	179.09463	-2.7	5		4
Fluvoxamine	$C_{15}H_{21}F_3N_2O_2$	318.15551	1.2	5		8
Phencyclidine	$C_{17}H_{25}N$	243.1987	-0.3	7		4
Tolmetin	$C_{15}H_{15}NO_3$	257.10519	-5.3	6		4
Thymopentin	$C_{30}H_{49}N_9O_9$	679.36532	-1.7	8		8
Methfuroxam	$C_{14}H_{15}NO_2$	229.11028	-3.8	2		4
Noscapine	$C_{22}H_{23}NO_7$	413.14745	0	6	77.7	4
Testosterone	$C_{19}H_{28}O_2$	288.20893	-1.6	5	86.2	8
Hydroxymethylpyridine	C_6H_7NO	109.05276	-2.7	5		8
Phenoxymethylpenicillin	$C_{16}H_{18}N_2O_5S$	350.09364	-4.2	4		4
Adenosine	$C_{10}H_{13}N_5O_4$	267.09675	0.9	5		4
Dicycloverine	$C_{19}H_{35}NO_2$	309.26678	0	5	86.3	12
Tetrazepam	$C_{16}H_{17}ClN_2O$	288.10294	0.4	5	74.6	4
Barbital	$C_8H_{12}N_2O_3$	184.08479	3	6		4
Oxedrine	$C_9H_{13}NO_2$	167.09463	-3.4	2		4
Phenylephrine	$C_9H_{13}NO_2$	167.09463	-3.4	9	80.9	4
Poliphenols						
3',4',7-Trihydroxyisoflavan	$C_{17}H_{20}O_6$	320.12599	-5.3	8	72.6	4
4''-O-Methylepigallocatechin 3-O-gallate	$C_{11}H_{12}O_4$	208.07356	-4.8	7	70.8	4
4-O-Methylgallic acid	$C_{11}H_{12}O_4$	208.07356	-4.8	5	75.9	4
3'-Hydroxy-O-desmethylangolensin	$C_9H_{10}O_3$	166.06299	-0.8	5		4
4-Hydroxyhippuric acid	$C_9H_{10}O_3$	166.06299	-0.8	3		4
O-Desmethylangolensin	$C_9H_{10}O_3$	166.06299	-0.8	4		4
Mycotoxines						
Ergosine	$C_{30}H_{37}N_5O_5$	547.27947	4.2	6	76.6	4
hydrolyzed Fumonisin B1	$C_{22}H_{47}NO_5$	405.34542	-2.7	6		4
Penicillic acid	$C_{16}H_{18}N_2O_5S$	350.09364	-4.2	5		4

Table S3.2 Non-target compounds tentatively identified in river water samples analyzed against a XIC manager table including MS/MS library search for those peaks with an area ≤20,000

Family/Compound	Formula	Mass (Da)	Error (ppm)	Isotope ratio % difference	Purity Score	Frequency
Pesticides						
Propoxur	$C_{11}H_{15}NO_3$	209.10519	-1.3	5		4
Tebufenozide	$C_{22}H_{28}N_2O_2$	352.21508	0.4	3		4
Pharmaceuticals						
Diltiazem	$C_{22}H_{26}N_2O_4S$	414.16133	-0.7	5		4
Betamethasone 21-phosphate	$C_{22}H_{30}FO_8P$	472.16623	-2.2	5	75.9	4
Trazodone	$C_{19}H_{22}ClN_5O$	371.15129	-1	4		4
Bezafibrate	$C_{19}H_{20}ClNO_4$	361.10809	-5.2	7	75.4	4
Buflomedil	$C_{17}H_{25}NO_4$	307.17836	-1.1	8		4
Primidone	$C_{12}H_{14}N_2O_2$	218.10553	-2.7	5		4
Bisoprolol	$C_{18}H_{31}NO_4$	325.22531	-0.2	6		4
Captodiame	$C_{21}H_{29}NS_2$	359.17414	-0.6	3		4
Niflumic acid	$C_{13}H_9F_3N_2O_2$	282.06161	-0.9	5		4
Bunitrolol	$C_{14}H_{20}N_2O_2$	248.15248	-5.3	5		4
Pindolol	$C_{14}H_{20}N_2O_2$	248.15248	-5.3	3		4
Dipyridamole	$C_{24}H_{40}N_8O_4$	504.31725	4	7		4
Verapamil	$C_{27}H_{38}N_2O_4$	454.28316	-2.4	5		4
Spirapril	$C_{22}H_{30}N_2O_5S_2$	466.15962	-0.3	4		4
Phenazone	$C_{11}H_{12}N_2O$	188.09496	-1.2	9		4
Butinoline	$C_{20}H_{21}NO$	291.16231	-1.1	5		4
Ranitidine	$C_{13}H_{22}N_4O_3S$	314.14126	-0.3	8		8
Metformin	$C_4H_{11}N_5$	129.10145	-0.5	5	74.6	4
Amezinium	$C_{11}H_{12}N_3O$	202.09804	1.8	4		4
Thioridazine	$C_{21}H_{26}N_2S_2$	370.15374	0.6	5		4
Trimethoprim	$C_{14}H_{18}N_4O_3$	290.13789	0.5	5		4
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	253.05211	0	2		4
Poliphenols						
Dihydrocaffeic acid 3-O-glucuronide	$C_{10}H_{12}O_4$	196.07356	-0.9	1		8

Family/Compound	Formula	Mass (Da)	Error (ppm)	Isotope ratio % difference	Purity Score	Frequency
trans-Resveratrol 4'-O-glucuronide	$C_{13}H_8O_4$	228.04226	-4.6	5		4
Malvidin 3-O-arabinoside	$C_9H_8O_3$	164.04734	-0.9	3		4
Oleuropein	$C_9H_8O_3$	164.04734	-0.9	5	74.9	4
Quercetin 3-O-rutinoside	$C_{14}H_{12}O_3$	228.07864	-4.5	6		4
Tectorigenin 4'-sulfate	$C_{14}H_{12}O_3$	228.07864	-4.5	7		4
2,4-Dihydroxybenzoic acid	$C_{15}H_{12}O_4$	256.07356	1	8		4
Dihydrobiochanin A	$C_{15}H_{12}O_4$	256.07356	1	2		4
Isoferulic acid	$C_{15}H_{12}O_4$	256.07356	1	5		4
Mycotoxines						
Decarestrictine	$C_{10}H_{16}O_5$	216.09977	0.5	5		8
Valinomycin	$C_{27}H_{34}O_9$	502.22028	2.7	6	71.5	4
HT-2-Toxin	$C_{22}H_{32}O_8$	424.20972	-3.1	5	70.6	4
T2-Triol	$C_{10}H_{15}O_3N$	197.10519	-0.9	3		4
alpha-Zearalenol-4-O-glucoside	$C_{24}H_{34}O_{10}$	482.2152	0.4	7		4
beta-Zearalenol-4-O-glucoside	$C_{24}H_{34}O_{10}$	482.2152	0.4	2		4
Fumitremorgin C	$C_{22}H_{25}N_3O_3$	379.18959	0.8	8		4
Fusaproliferin	$C_{27}H_{40}O_5$	444.28757	-1.6	5		4

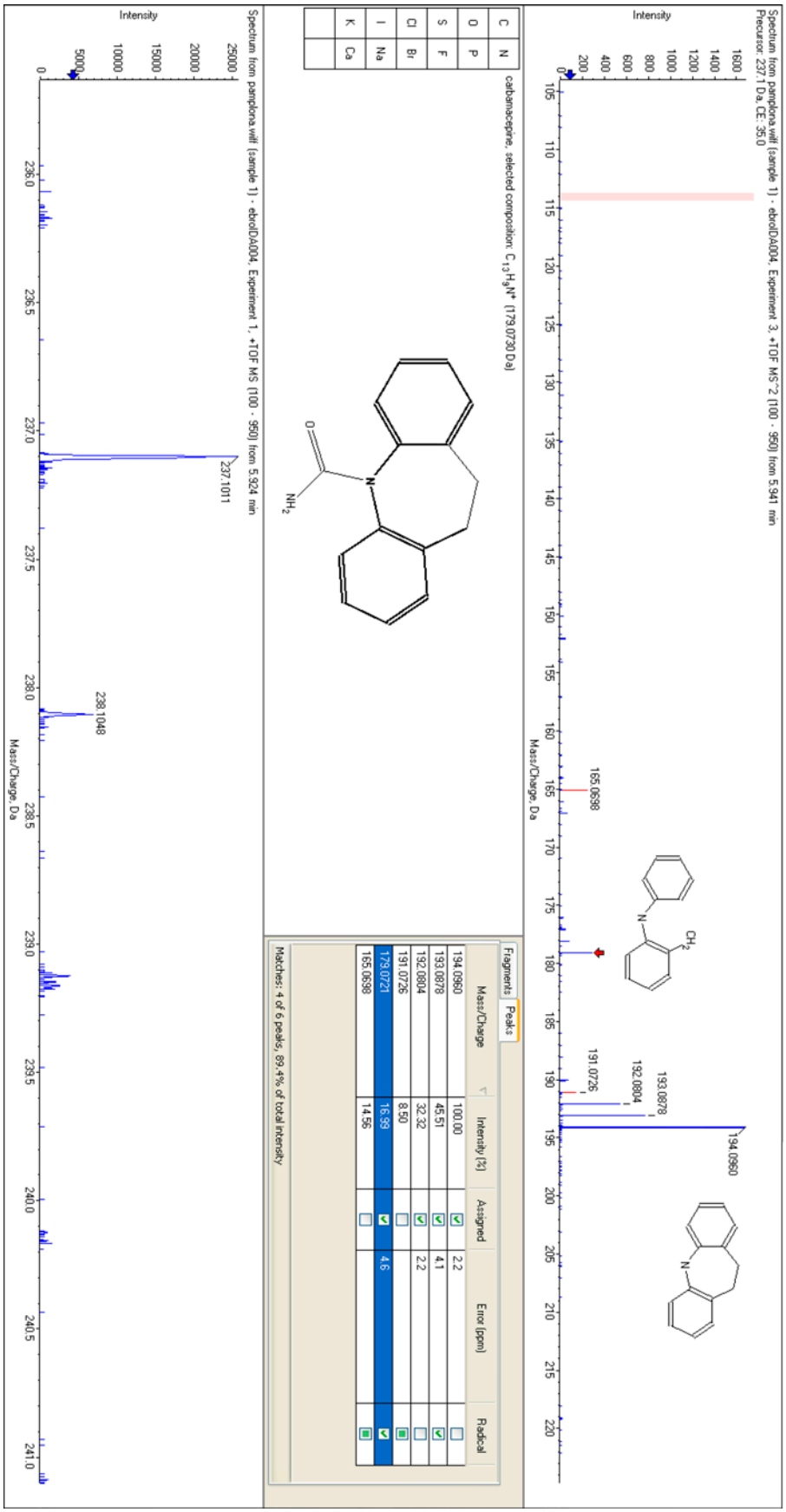
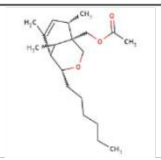
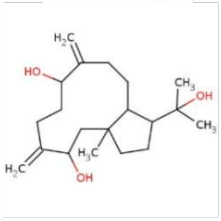
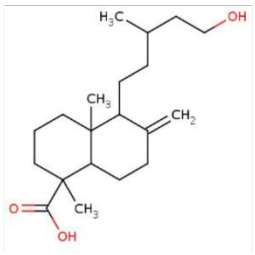
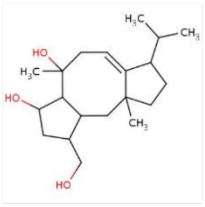
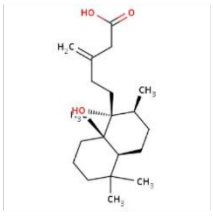
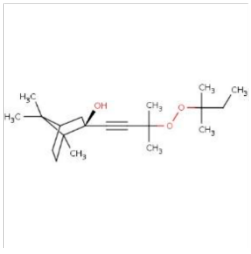
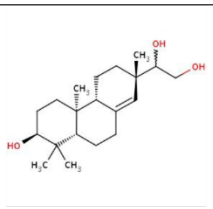
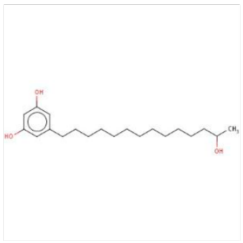
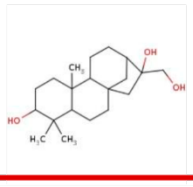
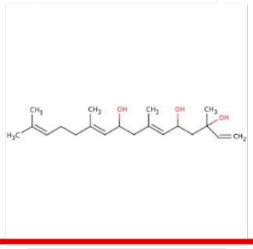
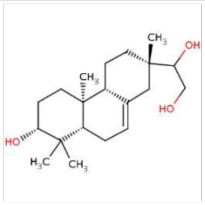
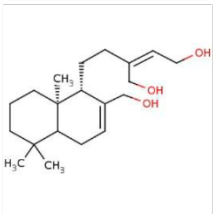
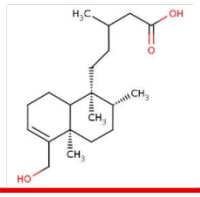
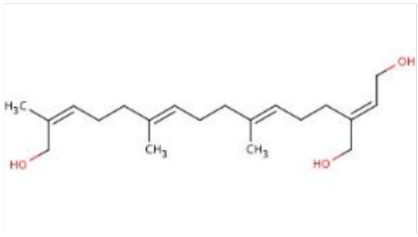
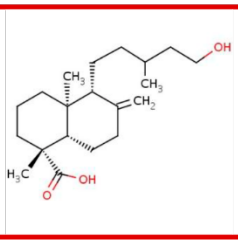
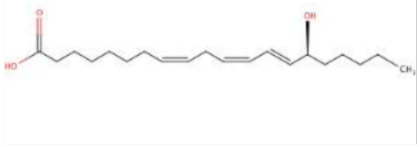


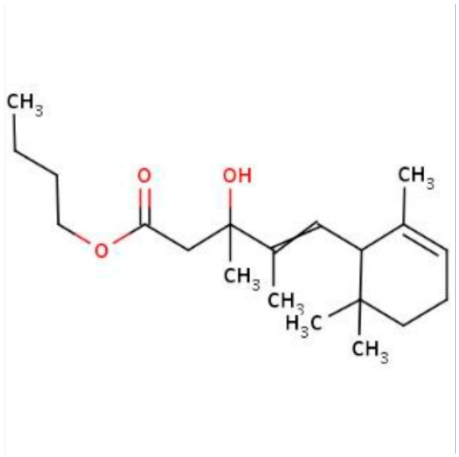
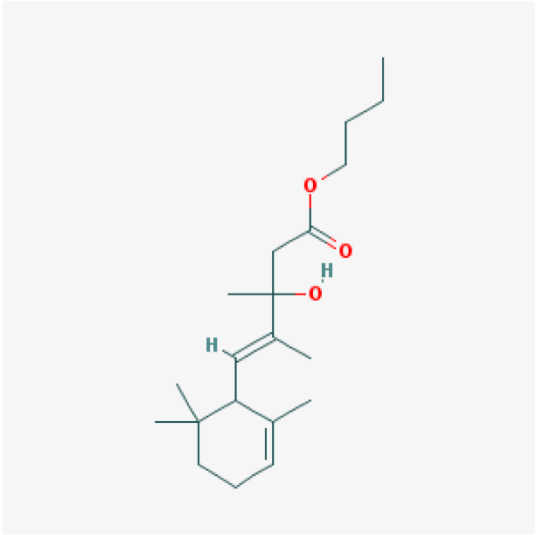
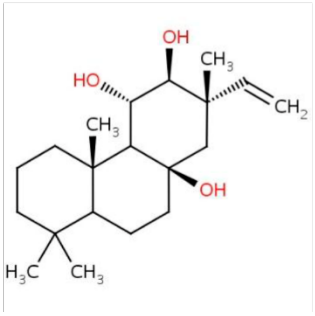
Fig. S3.7 Confirmation of carbamazepine identity by the fomula finder algorithm

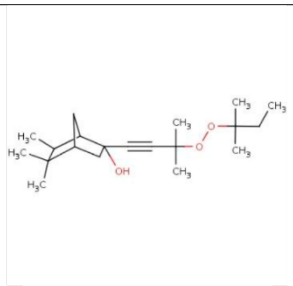
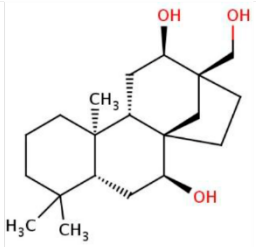
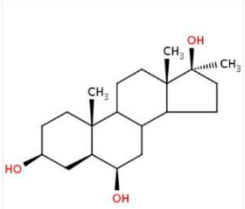
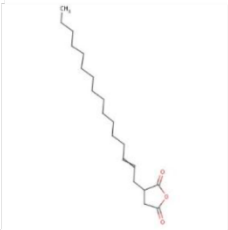
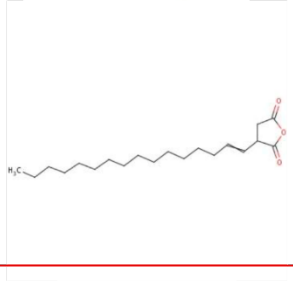
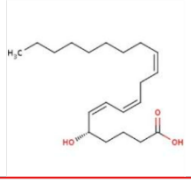
Table S3.3 Possible structures corresponding to the empirical formula $C_{20}H_{34}O_3$

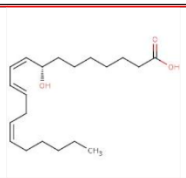
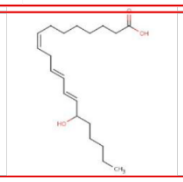
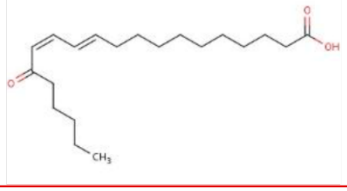
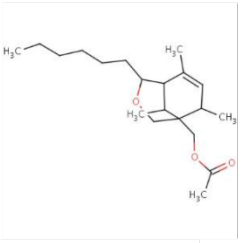
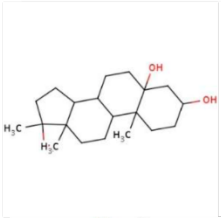
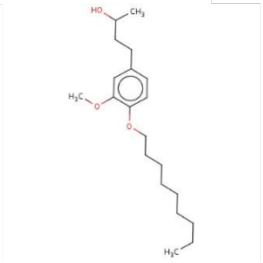
Chemical structure	Compound Name
	{4-hexyl-6,8,9-trimethyl-3-oxabicyclo[3.3.1]non-6-en-1-yl}methyl acetate
	1-(2-hydroxypropan-2-yl)-3a-methyl-6,10-dimethylidene-tetradecahydrocyclopenta[11]annulene-5,9-diol
	5-(5-hydroxy-3-methylpentyl)-1,4a-dimethyl-6-methylidene-decahydronaphthalene-1-carboxylic acid
	(10Z)-4-(hydroxymethyl)-12-isopropyl-1,8-dimethyltricyclo[9.3.0.0^{3,7}]tetradec-10-ene-6,8-diol
	9-Hydroxy-13E-labden-15-oic acid
	(2S)-1,7,7-trimethyl-2-{3-methyl-3-[(2-methylbutan-2-yl)peroxy]but-1-yn-1-yl}bicyclo[2.2.1]heptan-2-ol

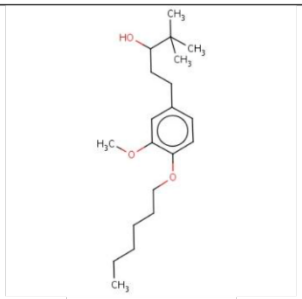
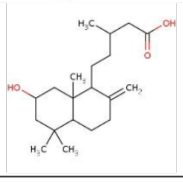
Chemical structure	Compound Name
	1-[(2S,4aR,4bS,7S,8aS)-7-hydroxy-2,4b,8,8-tetramethyl-2,3,4,4a,4b,5,6,7,8,8a,9,10-dodecahydrophenanthren-2-yl]ethane-1,2-diol
	5-(13-hydroxytetradecyl)benzene-1,3-diol
	14-(hydroxymethyl)-5,5,9-trimethyltetracyclo[11.2.1.0^{1,10}.0^{4,9}]hexadecane-6,14-diol
	(6E,10E)-3,7,11,15-tetramethylhexadeca-1,6,10,14-tetraene-3,5,9-triol
	1-[(2S,4aR,4bS,7R,8aS)-7-hydroxy-2,4b,8,8-tetramethyl-1,2,3,4,4a,4b,5,6,7,8,8a,9-dodecahydrophenanthren-2-yl]ethane-1,2-diol
	(2Z)-2-{2-[(1S,8aR)-2-(hydroxymethyl)-5,5,8a-trimethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalen-1-yl]ethyl}but-2-ene-1,4-diol

Chemical structure	Compound Name
	5-[(1S,2R,4aR)-5-(hydroxymethyl)-1,2,4a-trimethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-1-yl]-3-methylpentanoic acid
	(2Z,6E,10E,14E)-14-(hydroxymethyl)-2,6,10-trimethylhexadeca-2,6,10,14-tetraene-1,16-diol
	(1R,4aS,5R,8aS)-5-(5-hydroxy-3-methylpentyl)-1,4a-dimethyl-6-methylidene-hexahydro-2H-naphthalene-1-carboxylic acid
	[(1S,5R)-4-hexyl-6,8,9-trimethyl-3-oxabicyclo[3.3.1]non-6-en-1-yl]methyl acetate

Chemical structure	Compound Name
	(8Z,11Z,13E,15S)-15-hydroxyicosa-8,11,13-trienoic acid
	butyl 3-hydroxy-3,4-dimethyl-5-(2,6,6-trimethylcyclohex-2-en-1-yl)pent-4-enoate
	(2S,3S,4S,4bS,10aR)-2-ethenyl-2,4b,8,8-tetramethyl-decahydrophenanthrene-3,4,10a-triol

Chemical structure	Compound Name
	5,5,6-trimethyl-2-{3-methyl-3-[(2-methylbutan-2-yl)peroxy]but-1-yn-1-yl}bicyclo[2.2.1]heptan-2-ol
	(1R,2S,4R,9R,10S,12R,13R)-13-(hydroxymethyl)-5,5,9-trimethyltetracyclo[11.2.1.0^{1},^{10}.0^{4},^{9}]hexadecane-2,12-diol
	(2R,5S,7S,8R,14S,15S)-2,14,15-trimethyltetracyclo[8.7.0.0^{2},^{7}.0^{11},^{15}]heptadecane-5,8,14-triol
	3-(hexadec-2-en-1-yl)oxolane-2,5-dione
	3-(hexadec-1-en-1-yl)oxolane-2,5-dione
	(5S,6Z,8Z,11Z)-5-hydroxyicosa-6,8,11-trienoic acid

Chemical structure	Compound Name
	(8S,9Z,11E,14Z)-8-hydroxyicosa-9,11,14-trienoic acid
	(8Z,11E,13E)-15-hydroxyicosa-8,11,13-trienoic acid
	(11E,13Z)-15-oxoicosa-11,13-dienoic acid
	{4-hexyl-6,8,9-trimethyl-3-oxabicyclo[3.3.1]non-6-en-1-yl}methyl acetate
	2,14,15-trimethyltetracyclo[8.7.0.0^{2,7}.0^{11,15}]heptadecane-5,7,14-triol
	4-[3-methoxy-4-(nonyloxy)phenyl]butan-2-ol

Chemical structure	Compound Name
	1-[4-(hexyloxy)-3-methoxyphenyl]-4,4-dimethylpentan-3-ol
	5-(7-hydroxy-5,5,8a-trimethyl-2-methylidene-hexahydro-1H-naphthalen-1-yl)-3-methylpentanoic acid

CAPÍTOL 4

Microextracció en Fase Sòlida-en tub acoblada a Cromatografia Líquida d' Ultra Alta Pressió-Espectrometria de Masses

Publicació científica 4

Multiresidue analysis of organic pollutants by in-tube solid phase microextraction coupled to ultra-high performance liquid chromatography-electrospray-tandem mass spectrometry

A. Masiá, Y. Moliner-Martínez, M. Muñoz-Ortuño, Y. Picó, P. Campíns-Falcó

Journal of Chromatography A, 1306 (2013) 1-11

Journal of Chromatography A, 1306 (2013) 1–11

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Multiresidue analysis of organic pollutants by in-tube solid phase microextraction coupled to ultra-high performance liquid chromatography–electrospray-tandem mass spectrometry

A. Masiá[a,*], Y. Moliner-Martinez[b], M. Muñoz-Ortuño[b], Y. Pico[a], P. Campíns-Falcó[b]

[a] Food and Environmental Safety Research Group, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés, s/n, 46100 Burjassot, Valencia, Spain
[b] Department of Analytical Chemistry, Faculty of Chemistry, University of Valencia, C/Dr Moliner, 50, 46100 Burjassot, Valencia, Spain

ARTICLE INFO

Article history:
Received 11 March 2013
Received in revised form 3 July 2013
Accepted 4 July 2013
Available online 9 July 2013

Keywords:
In-tube SPME
UHPLC–MS/MS
Water
Pesticides
Phthalates

ABSTRACT

In this work, in-tube solid phase microextraction (IT-SPME) coupling with ultra-high-pressure liquid chromatography tandem mass spectrometry (UHPLC–MS/MS) multiresidue analytical method has been proposed for the first time for on-line enrichment of 9 analytes included in Water Frame Directive 2000/60/EC (WFD). The device was equipped with a GC TRB-5 capillary column, used as pre-concentration loop, and two conventional six-port injection valves. Water sample and desorption solvent volumes were tested. The optimum conditions were 4 mL of processed sample followed by elution with 40 μL of methanol. The analytes were detected with a mass spectrometer after being ionized positively using an electrospray ionization (ESI) source. The method presents good linearity over the range assayed, 0.025–2.5 μg/L for chlorpyriphos and 0.25–25 μg/L for the other tested compounds and LODs between 0.025 μg/L and 2.5 μg/L. Enrichment factors ranged from 2.5 to 10. Intra and inter-day variation coefficients were <26 and 31.6% respectively. Once validated, the method was applied to several water samples from different sources demonstrating that it achieves the on-line enrichment of the analytes with the advantage of minimum sample manipulation, and the identification and quantification of some organic pollutants in water samples in the range of low parts-per-billion. The method provided similar analytical characteristics as those obtained in the established couple IT-SPME–Capillary Liquid Chromatography (CapLC).

1. Introduction

During the last decade and because of the use of many chemical substances without any control, the study of contaminants course in the environment has become of interest for the European Union (EU) [1]. Many of these contaminants are characterized by a strong persistence which explains their wide presence in the soil–water environment and their accumulation in fatty tissues [2]. In this context, strict regulations have been established. The European Union adopted the Water Framework Directive 2000/60/EC in the field of water policy. The purpose of this directive is to create a protective framework for all inland surface waters, transitional waters, coastal waters, and groundwater in order to prevent deterioration and promote their sustainable use [3]. Directive 2008/105/EC lays down environmental quality standards (EQS) for priority substances and certain other pollutants as provided for in Article 16 of Directive 2000/60/EC, with the aim of achieving good surface water chemical status and in accordance with the provisions and objectives of Article 4 of that Directive [4].

Table 4.1 summarizes the most representative chromatographic procedures described in the literature during the last five years for the analysis of pollutants in water samples. Multiresidue analysis based on gas chromatography (GC) and liquid chromatography (LC) are increasingly common, generally, coupling to mass spectrometry (MS) or tandem MS (MS/MS).

Nowadays, most modern pesticides are characterized by medium to high polarity and thermal stability that make LC the favored analytical separation technique. Indeed, coupling LC to MS, or tandem mass spectrometry (MS/MS) has become a powerful tool for pesticide multiresidue analysis of food and vegetables or in water at sub-μg/L level. Traditionally, liquid–liquid extraction (LLE) has been used to detect organic micropollutants in liquid samples but this procedure requires a large volume of solvents and was, subsequently, largely, replaced by solid-phase extraction (SPE), which uses less solvent- and is less time consuming than the LLE [12]. Nowadays, the general trend is to

* Corresponding author. Tel.: +34 963544118; fax: +34 963544954.
E-mail address: ana.masia@uv.es (A. Masiá).

0021-9673/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2013.07.019

Table 4.1
Summary of different chromatographic procedures described in the literature between 2008 and 2012, for the analysis of organic pollutants in water samples.

Family compounds	Sample	Sample treatment	Sample volume (mL)	Final volume (mL)	Technique	Ext. time	N° comp.	LOD	% Recoveries	Reference
Multiresidue pesticides (atrazine, isoproturon, simazine, terbuthylazine)	Tap water, waste water	Acetonitrile, Sonication/Salting out with NaCl	10 mL	1 mL	LC–MS/MS	15 min	48	ng/L	74.6–111.2	[5]
Multiresidue several compounds (isoproturon, terbuthylazine)	Waste water, river water	On-line SPE	10 mL		LC–ESI-MS/MS (8)	On-line	24	ng/L	70	[6]
Multiresidue pesticides (Simazine, Atrazine, Diuron, Isoproturon)	Ground water	MASE (1)/SPE	13 mL	100 μL	LC–MS/MS	60 min	10	ng/L	71–100	[7]
Multiresidue several compounds (Simazine, Atrazine, Terbuthylazine, Diuron, Isoproturon)	River water	SPE	400 mL	500 μL	LC–MS/MS		34	ng/L	46–89	[8]
OPPs (Chlorpyriphos)	Tap, rain, river and well water	DLLME (2)	5 mL	5 μL	LC–DAD	5 min	4	0.1–5.0 ng/L	>70	[9]
Multiresidue several compounds (atrazine, simazine, trifluralin, di(2-ethylhexyl)phthalate, diuron, isoproturon, chlorfenvinphos, chlorpyriphos)	Waste water	in-tube SPME (3)	2000 μL				28	0.008–0.2 μg/L	70–116	[10]
phthalates (DEHP)	Waste water	in-tube SPME	100 μL		LC–DAD (9)	20 min	2	1–2.5 μg/L	85–115	[11]
Multiresidue pesticides (isoproturon, atrazine)	Flow-weighted composite water	On-line SPME	18 mL		GC–MS	55 min	16	0.016–0.16 μg/L	75–140	[12]
Multiresidue several compounds (trifluralin, terbuthylazine, simazine, atrazine, chlorpyriphos, chlorfenvinphos)	Superficial, underground and waste water	SPE	250 mL	0.5 mL	GC–TOF-MS (10)		150	0.1 μg/L		[13]
Multiresidue several (DEHP)	Plastic-bottled, tap, river, municipal swimming pool, sea harbor and sewage waters	USAEME (4)	10 mL	100 μL	GC–MS	10 min	16	pg/mL; sub ng/mL (phthalates)	78–114	[14]
Multiresidue several (Trifluralin)	River, underground and drinking water	In-tube/on-line SPME	60 mL		GC–MS	38 min	18	6.1–21.8 ng/L	27–78	[15]
Multiresidue several (chlorpyriphos, chlorfenvinphos)	River water	Sequential SBSE-TD (5)	5 mL		TD-GC–MS (11)	2 h	80	<10 ng/L	82–113	[16]
Multiresidue pesticides (chlorpyriphos, chlorfenvinphos, diuron, isoproturon, atrazine, simazine)	Superficial water	SBSE (6)	2 x 50 mL aliquots of surface water	1 mL	LC–MS/MS (QqQ) (12)	1 h	16	0.01–0.4 μg/L	<62	[17]
Multiresidue several (isoproturon, diuron, trifluralin, simazine, atrazine, terbuthylazine, chlorpyriphos, chlorfenvinphos)	River water	SBSE-TD	200 mL		GC-MS/MS (QqQ)	24 h	78		74–116	[18]

Multiresidue several (chlorpyriphos, trifluralin, simazine, chlorfenvinphos, atrazine, DEHP)	Waste water	SPE	500 mL	1 mL	LVI-GC–MS [13]	1 h	75	<ng/L	80–125	[19]
Multiresidue several (DEHP)	Superficial, underground water	SPE	300 mL	0.2 mL	GC–MS and LS–MS/MS	50 min aprox.	33	0.2–88.9 ppt	84–118	[1]
Multiresidue pesticides (atrazine, chlorfenvinphos, chlorpyriphos, diuron, isoproturon, simazine, terbuthylazine)	Mineral water	On-line	0.1 mL		LC–MS/MS		300	0.1 ppb	20–87	[20]
Multiresidue several (Trifluralin, Atrazine, Chlorpyriphos, chlorfenvinphos, simazine, isoproturon, DEHP)	Superficial water	SBSE	100 mL	500 μL GC 1 mL LC	GC–MS and LC–FLD–MS/MS (14)		36	0.2–67 ng/L	59–105	[2]
Multiresidue (Trifluralin, Atrazine, Chlorpiriphos, chlorfenvinphos, simazine, isoproturon, DEHP)	Superficial water	SPE	500 mL	500 μL GC 1 mL LC	GC–MS and LC–FLD–MS/MS		36	0.2–67 ng/L	59–105	[2]
Multiresidue several (simazine, isoproturon, atrazine, diuron, terbuthylazine, chlorfenvinphos, DEHP, chlorpyriphos, trifluralin)	Waste water	in-tube-SPME	4 mL		Capillary-LC–UV–DAD		9	5–50 ng/L	84–103	[21]
Multiresidue pesticides (atrazine)	Water from a deep channel and from an underground well	Direct water injection in GC	5 mL		TOTAD-GC–MS (15)		9	0.02–0.77 ng/L		[22]
Multiresidue pesticides (chlorpyriphos)	River water	RDSE [7]y SBSE	25 mL		GC–MS		7	<3.1 μg/L	76–101	[23]
Multiresidue pesticides (diuron, isoproturon, atrazine, simazine, terbuthylazine)	River water	SPE	200 mL	1 mL	UHPLC–MS/MS (16)		31	<8 ng/L	82–109	[24]
Multiresidue several (trifluralin, simazine, atrazine, chlorpyriphos)	Underground water	dual SBSE	2 x 20 mL aliquots of water		TD-GC–MS		45	0.5–431 ng/L	2.5–89.2	[25]
Multiresidue phthalates sters(DEHP)	Drinking, ultrapure water	SPE (Tenax TA)	1 L		TD-GC–MS		5	36–95 ng/L	15–101	[26]

Table 4.1 (Continued)

Family compounds	Sample	Sample treatment	Sample volume (mL)	Final volume (mL)	Technique	Ext. time	N° comp.	LOD	% Recoveries	Reference
Multiresidue several (atrazine, chlorpyriphos, chlorfenvinphos, simazine, terbuthylazine, diuron, isoproturon)	Surface and waste water	SPE	250 mL	1 mL	LC–MS/MS (QqQ) LC–MS/MS (QTOF)		43	0.04–2 ng/L	>70	[27]

Sample treatment:
(1) MASE: Membrane-assisted Solvent Extraction.
(2) DLLME: Dispersive liquid–liquid microextraction.
(3) SPME: Solid Phase Microextraction.
(4) USAEME: Ultrasound-assisted emulsification–microextraction.
(5) SBSE-TD: Stir bar sorptive extraction- thermal desorption.
(6) SBSE: Stir bar sorptive extraction.
(7) RSDE: Rotating disk sorptive extraction.
Technique:
(8) ESI: Electrospray.
(9) DAD: Photodiode array detector.
(10) TOF: Time of flight.
(11) TD: Thermal desorption.
(12) QqQ: Triple Quadrupole.
(13) LVI: Large volume injection.
(14) FLD: Fluorescence.
(15) TOTAD: Through Oven Transfer Adsorption Desorption.
(16) UHPLC: Ultra high performance liquid chromatography.

simplify the sample preparation, to diminish the sample volume needed, the number of off-line steps and the amount of solvents employed. In this sense, in-tube solid-phase microextraction (IT-SPME) envisages as one of the most useful approaches for sample preparation [10]. IT-SPME is a mode of SPME which typically uses a GC capillary column with a proper coating to extract the analytes.

The aim of this work is to show how to couple IT-SPME, a technique that needs relatively low pressure, and UHPLC that operates to relatively high pressure. A multiresidue analysis of nine priority substances included in Directive 2008/105/EC (Supplementary material, Table 4.1S), by in-tube solid phase microextraction coupled to ultra-high pressure liquid chromatography–electrospray-tandem mass spectrometry (UHPLC–QqQ-MS/MS) in inland surface waters was developed. This procedure has been chosen to reach selectivity and sensitivity necessary to determine organic pollutants in water samples. The new coupling is compared with the previously established IT-SPME and Capillary Liquid Chromatography (CapLC) [21]. In this context, the combination of in-valve IT-SPME and CapLC is a good option for the rapid and cost effective analysis of organic pollutants in waters, because the required sensitivity can be reached by processing relatively large sample volumes. This paper demonstrates that UHPLC coupled to IT-SPME is also a viable alternative.

2. Materials and methods

2.1. Reagents and standard solutions

Atrazine, Chlorfenvinphos, Chlorpyriphos, Di (2-ethylhexyl) phthalate (DEHP), Diuron, Isoproturon, Simazine, Terbuthylazine and Trifluralin were purchased from Sigma–Aldrich (Steinheim, Germany), all of them with the highest available purity (>99.7%).

Individual standard solutions were prepared in methanol at the concentration of 100 mg L^{-1} and the working standard solution was prepared by mixing 100 μL of each individual standard solutions and diluting with methanol to a final volume of 10 mL. Daily working solutions were prepared diluting mixed standard solution containing all analytes at different concentrations. All solutions were stored in amber glass bottles at 4 °C.

Methanol (gradient grade for liquid chromatography) from Merck (Darmstadt, Germany) was used to elute analytes from the capillary column.

MilliQ water and methanol, both with ammonium formate 10 mM, were prepared as mobile phase in LC–MS/MS. High purity water was prepared using a Milli-Q water purification system (Millipore, Milford, MA, USA).

2.2. Glassware

Because of phthalates are present in the analytical laboratory, special care was taken to avoid the contact of reagents and solutions with plastic materials. Scrupulous cleaning and design of quality control procedures ensure analytical results free of systematic errors and false positives.

The glassware used for the analysis was washed with detergent, rinsed with tap water, ultrapure water (Millipore, Milford, MA, USA), acetone from Merck (Darmstadt, Germany) and dried in an oven at 150 °C for 2 h. This material was stored in aluminum foil to avoid adsorption of phthalates from the air.

2.3. Analysis of real water samples

Water samples from different sources were analyzed: 4 waste water samples, 2 influents (IWW) and 2 effluents (EWW), 4 River

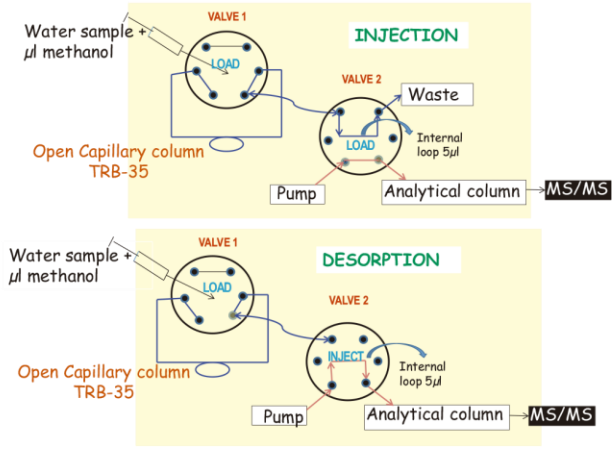


Fig. 4.1 Configuration of IT-SPME–UHPLC–QqQ-MS/MS.

Waters (SW) and 2 coastal waters (CW). In the laboratory, water samples were kept frozen at −20 °C in glass bottles until the analysis. Before the analysis, both surface and waste waters were vacuum filtered through 1 μm paper fiber filter followed by 90 mm glass filter (0.7 μm) (VWR, Barcelona, Spain) and coastal samples were only centrifuged to eliminate particles or any material suspended on them. Surface and wastewater were filtered because of the presence of higher concentrations of suspended solids than in coastal waters.

2.4. In-tube solid phase microextraction (IT-SPME)

The process IT-SPME used in this work was based on that reported by Campins-Falcó et al. [10]. However, substantial modifications of the configuration were carried out for coupling IT-SPME–UHPLC–QqQ-MS/MS (see Fig. 4.1). Two six port valves were used for sample preparation to prevent the high backpressures of the UHPLC column, which could negatively affect the IT-SPME system.

For IT-SPME, a GC TRB-5 capillary (40 cm × 0.32 mm i.d.), coated with 5% diphenyl-95% polydimethylsiloxane (PDMS) (3 μm film thickness) was used (Teknokroma, Barcelona, Spain). This capillary was employed as "injection loop" connected to a conventional six-port injection valve, which at the same time was connected to other injection valve containing a 5 μm internal loop to cancel injector pressure for UHPLC. Capillary connections were facilitated by the use of a 2.5 cm sleeve of 1/16 in. polyether ether ketone (PEEK) tubing at each end of the capillary.

Water samples (4.0 mL) were passed through the capillary in valve 1 by means of a 1.0 mL precision Hamilton syringe. Then, 40 μL of methanol were injected in valve 1 to desorb the analytes from the extractive phase of the GC capillary, and the second valve was manually rotated to INJECT position, so the analytes were transferred to the analytical column by the mobile-phase for separation and detection (see Fig. 4.1).

2.5. CapLC–DAD–MS

The capillary chromatographic system consisted of a capillary pump (Agilent 100 Series, Waldbronn, Germany) equipped with a Rheodyne model 7725 injection valve and coupled to an Agilent Zorbax SB C_{18}-column (150 mm × 0.5 mm, 3.5 μm). A photodiode array detector (DAD, Hewlett-Packard, 1040 M Series II) was coupled to a data system (Agilent, HPLC ChemStation) for data acquisition and calculation. The signal was registered in the DAD detector and it was monitored at 230 nm. The corresponding spectra were saved. A spectra library of the pure compounds was performed. The mobile phase was a mixture of acetonitrile (A)–water (B) in gradient elution mode: 40% B between 0 and 3 min, after up to 60% in 11 min. Afterwards an increase of B up to 100% was applied in 2 min. Once at 100% B this was hold for 10 min to decrease again to 40% in 3 min. A column flow at 10 μL min^{-1} was used throughout the run and the injected sample volume was the internal volume of the IT-SPME capillary loaded with the cleaning solvent (4 mL of standard or sample and 100 μL of nanopure water as cleaning solvent was processed in IT-SPME step). The mass spectrometer was an Agilent G6140A quadrupole system working a drying gas temperature of 350 °C, drying gas flow of 4 L min^{-1}, nebulizer pressure of 35 psi and a capillary voltage of 4000 V. Data were obtained using the same *m/z* precursor ions selected for the UHPLC (see Table 4.2S).

2.6. Ultra high-pressure liquid chromatography–triple quadrupole-mass spectrometry

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).

Specifically for this work and to carry out IT-SPME, autosampler was annulated to perform manual injections through IT-SPME device coupled to the chromatographic system. This was achieved by connecting the pump and the column to the proper positions of the six valve gates injection.

Data were processed using a MassHunter Workstation Software for qualitative and quantitative analysis (A GL Sciences, Tokio, Japan).

The chromatographic column used for UHPLC was a Phenomenex Kinetex XB C18 100A (50 mm × 2.10 mm) with a 1.7 μm particle size (Phenomenex, Torrance, USA). The column temperature was kept at 30 °C and the volume injected was 5 μL, corresponding to the volume of internal loop of the injection valve. A binary mobile phase at flow rate of 0.2 mL min^{-1} with a gradient elution was used. Milli-Q water and methanol both with 10 mM ammonium formate were used as mobile phases. The linear gradient was as follows: 0 min (75% methanol), 5 min (75% methanol), 7 min (98% methanol), 20 min (98% methanol). Then, the mobile phase returns to the initial conditions with an equilibrium time of 12 min.

The ESI conditions were: capillary voltage 4000 V, nebulizer 15 psi, source temperature 300 °C and gas flow 10 L min^{-1}. The ionization and fragmentation of the compounds were optimized injecting individual solutions of analytes directly and using the optimizer program. The empirical formula and the molecular weight of each compound were introduced and a fragmentor range between 10–150 and a collision energy range between 10–100 V were tested. MS/MS was performed in the SRM mode using ESI in positive mode. For the selected transitions and other parameters see Table 4.2S supplementary material. For each compound, two characteristic fragmentations of the protonated molecule $[M+H]^+$ were monitored, with the exception of trifluralin, the first and most abundant one was used for quantification, while the second one was used as a qualifier, according to the Commission Decision 2002/657/EC [28].

3. Results and discussion

3.1. Optimization of IT-SPME

For coupling IT-SPME, a technique that needs relatively low pressure, and UHPLC that operates to relatively high pressure, several valve configurations were tested. To our knowledge, previous studies used LC systems that generated low pressure than UHPLC, such as conventional LC (up to 300 bars) or Cap LC (up to 100 bars). Most of the configurations reported only use one valve and the pass of the mobile phase through the capillary loop is mandatory, independently that desorption were performed in dynamic or static mode [11,12,15,21]. However, in our case, this configuration gave problems because the capillary loop was always broken near to the PEEK sleeve due to the high pressure of the UHPLC (up to 600 bar). To solve this problem, a second valve was coupled to prevent the exposition of the capillary loop to high pressures as shown in Fig. 4.1. This second six-port valve has an internal loop of 5 μL of steel, which is able to support these high pressures. It receives the analytes eluted from the capillary loop engaged to the first valve and isolates it from the system pressure because in the LOAD position it is connected to the capillary and to the waste at atmospheric pressure. Then, to transfer the analytes from this loop to the analytical column, the valve is rotated to the inject position connecting the loop with the UHPLC system and leaving the capillary out of the UHPLC system backpressure.

Sample and eluent volumes were tested to optimize the in-tube procedure and to obtain the maximum enrichment factor: ratio between the analyte concentration in 5 μL injected volume and the initial concentration in the standard or water samples. For this purpose, samples were spiked with 10 ng of each compound studied. The resulting concentration was variable depending on the sample volume (in the range 1–10 mL). Fig. 4.2 shows the chromatograms obtained with the different volumes of water (2, 4, and 6 mL) and methanol (30, 35, 40 and 45 μL) tested. For a certain volume of water, the signal obtained is different depending on the volume of methanol used to elute analytes. However, the optimum methanol volume for desorption can also vary in function of the water volume passed through the capillary because depending on it and by the breakthrough, inherent to the chromatographic process, the analytes could be adsorbed in different parts of the capillary. However, for most of the water volumes, the strongest signal corresponded to an elution volume of 40 μL of methanol. The elution volume must be enough to desorb the analytes from the capillary loop and carry them to the second valve 5 μL internal loop. To select the methanol volumes tested, the volume of the capillary plus the connection between both valves and the second loop (33 μL) was taken into account.

Only desorption using methanol and acetonitrile was tested for optimization because the DEHP is very apolar and the use of the mobile phase as elution solvent or other methanol–water or acetonitrile–water combinations did not give proper results because the desorption of DEHP was not completed. Methanol and acetonitrile provided similar results. Thus, methanol was chosen as was also used for the liquid chromatographic separation.

Fig. 4.3 shows the chromatogram corresponding to 1 ng of chlorpyriphos and 10 ng of the other compounds concentrated from different water volumes, the chromatographic signal is attained more or less constant until 4 mL water, whereas for higher water volume, the chromatographic signal clearly dismishes.

For most analytes, similar signal intensity is obtained with water volume ranged between 1 and 4 mL while the signal decreases gradually for higher volumes because some breakthrough of the analyte occurs. The volume of water processed against the area obtained for each pesticide, using 35, 40 and 45 μL of methanol to elute the compounds are outlined in Figs. 4.1S, 4.2S and 4.3S.

Table 4.2
Recoveries (%) obtained using 40 μL methanol to elute analytes from the loop.

Compounds	Water volume (mL)							
	1	2	3	4	5	6	7	10
Dehp	33	33	31	30	17	13	7	4
Chlorfenvinphos	67	65	64	65	53	49	39	31
Chlorpyriphos	20	18	18	18	21	14	13	5
Trifluralin	14	14	13	12	6	8	7	5
Diuron	3	3	3	3	2	1	1	0
Terbuthylazine	13	13	12	12	9	7	5	3
Atrazine	9	9	9	9	8	3	3	1
Isoproturon	5	5	4	4	4	3	2	2
Simazine	4	4	3	3	2	7	6	3

Table 4.2 shows the recoveries obtained for all tested volumes of water processed and 40 μL methanol. The absolute recoveries of the in-tube SPME were calculated by comparing the amount of analyte in 5 μL extract, which is the amount of the analyte transferred to the analytical column, with the total amount of analyte passed though the GC extraction capillary. The amount of analyte extracted was established from the peak areas in the resulting chromatograms and from the calibration equations constructed through the direct injection of 4 mL of water fortified with increasing concentrations of the analytes of interest, ranging from 0.1 to 10 ng for chlorpyriphos and from 1 to 100 ng for the other compounds.

Absolute recoveries results varied from 0.34 to 67.00%. The highest recoveries were achieved for chlorfenvinphos, chlorpyriphos, terbuthylazine, trifluralin and DEHP. The values obtained are in agreement with the literature for SPME [10]. Although for some compounds the recoveries are low, the technique provides lower LODs due to high volume processed (4 mL).

Fig. 4.4S represents graphically the recoveries obtained in Table 4.2 for each analyte studied. It can be observed that for most compounds, the highest recoveries are reached for volumes ranging from 1 to 4 mL, with exception of chlorpyriphos and simazine, the highest recoveries of which correspond to 5 and 6 mL water respectively.

3.2. Validation parameters

Linear or dynamic range, sensitivity, precision and accuracy of the method were evaluated.

The linear or dynamic range was established using eight calibration points between 0.1 and 10 ng for chlorpyriphos (equivalent to a concentration of 0.025 and 2.5 μg/L) and between 1 and 100 ng for the other analytes (equivalent to 0.25 and 25 μg/L).

Calibration equations obtained are listed in Table 4.3. The linearity through the studied concentration range was good, with correlation coefficients higher than 0.99 for all target compounds except for DEHP, because in most of cases contaminated blanks were detected. Although a scrupulous cleaning of the material was made, DEHP is widely used as additive in the production of plastic, cellulose and many other goodness. DEHP is a ubiquitous contaminant – found at low concentrations in methanol, triazine standards or the plastic conductions of the HPLC. Since DEHP is not bound in plastic or other material, it slowly leaches out causing contamination. Sensitivity was estimated by the limits of detection (LODs) that were determined as the lowest pesticide concentration providing a qualified transition (SRM2) with a signal-to-noise ratio (S/N) ≥ 3.

LODs were estimated by two different ways which were compared. Firstly, the limits of detection were determined experimentally by processing automatically standard solutions containing decreasing concentrations of the analytes in UHPLC–MS/MS system. Next, LODs were determined experimentally by means of direct injection of 4 mL distilled water fortified with increasing concentration of standard solutions in the IT-SPME–UHPLC–MS/MS

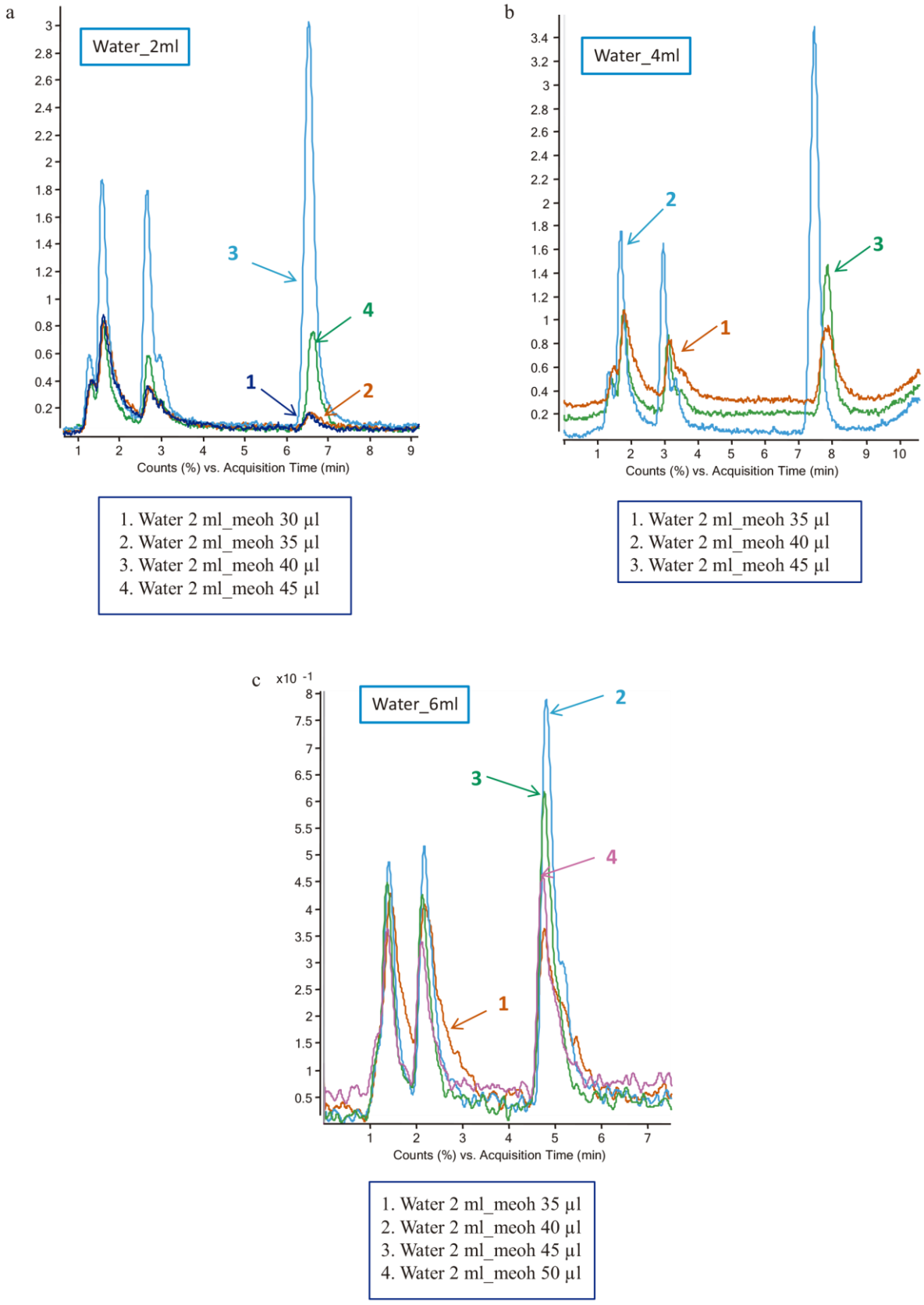


Fig. 4.2 Chromatograms obtained for the different volumes of water and methanol tested.

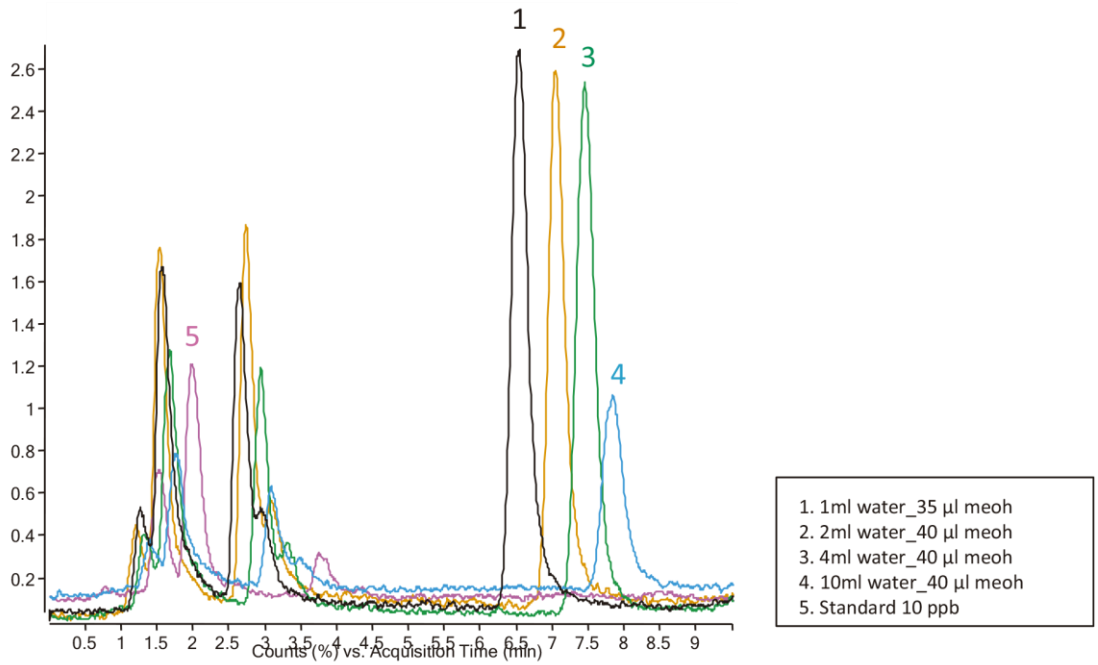


Fig 4.3 Analytical signals obtained passing 1 ng of chlorpyriphos and 10 ng of the other compounds in different water volumes.

system. It can be concluded that for a majority of the analytes the LODs were markedly reduced by the in-tube-SPME approaches with respect to direct injection method (Tables 4.3 and 4.4). The achieved detection limits were compared with those obtained by IT-SPME–CapLC–DAD–MS (see Table 4.4). This table also shows the required LODs in accordance with European Directive [4]. Both couples, UHPLC and CapLC, provide similar results.

Only for an analyte, simazine, LOD according to the requirements of this Directive [4] was not achieved. Therefore, future work will go on the optimization of the method to get lower LODs for all target compounds. A proper solution to improve the LODs would be to use a larger loop on the second valve since only a fraction of 5 μL of the 40 μL used to desorb the analytes was finally injected in the UHPLC. An alternative strategy, also possible, would be to enlarge the in-tube capillary to increase the amount of the analytes adsorbed onto the capillary. LODs obtained by UHPLC–MS/MS system are also summarized in Table 4.3. As it can be seen IT-SPME improved LODs achieved by UHPLC.

Precision was evaluated by determining intra-day and inter-day coefficient of variation. Intra-day values were obtained processing a standard solution of pesticides containing 10 ng/mL of each analyte from three analyses performed on one day, whereas the inter-day results were achieved by analyzing three standard solutions of the same concentration (50 ng/mL) injected in different days.

The intraday and interday relative standard deviations (RSDs) were <26% and 31% respectively (see Table 4.3). They can be considered acceptable at the concentration levels tested and in accordance with published SPME papers [10].

The accuracy of the described in-tube SPME procedure was guaranteed through a recovery study to assess the presence of proportional systematic error attributable to parameters of measure system, procedure or the method.

Recoveries were tested processing real water samples (deionized water, influent-waste water, effluent-waste water and superficial water) spiked with 1 ng chlorpyriphos and 10 ng other compounds. The recovery was determined against the calibration curve obtained as the average of two analyses of the spiked water samples (4 mL) at different concentrations of each analyte. If one compound initially existed in water samples, background-subtracted peak area was used to calculate the recovery. As observed in Table 4.5, the method provided suitable recovery of all the analytes with the exception of DEHP in the different types of water tested.

Finally, the specificity and selectivity of the method was established by the analysis of blank samples. It is well known that the most important problem concerning phthalate analysis is the risk of contamination, resulting in false positive results and overestimated concentrations. The sources of contamination can be present in any step of the analytical procedure. To check the presence of phthalates in the chromatographic system, between two consecutive injections of the analytes a blank of methanol was also processed as a preventive action in order to ensure for possible carryover or other contaminant effects. The presence of phthalate esters was detected in the chromatograms, which means that it was also extracted in the in-tube SPME device; therefore, it was necessary to subtract the peak area of this blank to the area values of the water samples in order to quantify this analyte in the samples. Fig. 4.5S shows analytical signals corresponding to elution time of DEHP in a methanol blank, a standard 0.5 ppb and a water sample. The peaks are not overlapped because the HPLC filters were blocked up, so the retention times are lightly moved.

In comparison with more conventional extraction procedures such as SPE or other reported in Table 4.1, it should be outlined that, even though this method presents very low recoveries (see Table 4.2), it allows to reach high enrichment factors. This fact turns it into a useful and suitable analytical method because it allows to achieve lower limits of detection, increasing sensitivity 100 times approximately compared with preconcentration off-line.

Table 4.3
Analytical parameters calculated for IT-SPME-UHPLC–MS/MS.

Compounds	Linearity $y = a + bx$ (μg/L)					Precision (RSDs, %)		LODs IT-SPME-UHPLC-MS/MS (μg/L)	LODs UHPLC-MS/MS (μg/L)	ENRICHMENT FACTOR
	a	s_a	b	s_b	R^2	Intra-day	Inter-day			
Dehp	340,250	1,239,087	628,961	23,574	0.992	26	31.6	0.025	0.1	4
Chlorfenvinphos	7531	10,591	8662	201	0.997	5.03	15.07	0.025	0.25	10
Chlorpyriphos	422,919	307,255	1,235,846	58,325	0.991	4.4	12.4	0.025	0.1	4
Trifluralin	−470,577	642,733	281525	12228	0.990	16	20.1	0.025	0.1	4
Diuron	35615	20085	9800	382	0.991	4.3	16	0.1	0.25	2.5
Terbuthylazine	37179	201690	129075	3837	0.995	3.5	19.6	0.025	0.1	4
Atrazine	15761	7887	4950	150	0.995	3.7	14.3	0.1	0.25	2.5
Isoproturon	−13238	65914	42820	1254	0.995	3.3	17.2	0.1	0.25	2.5
Simazine	348	406	1143	7	0.999	4.9	16.8	2.5	5	2

Table 4.4
Environmental Quality Standards for the studied analytes and LODs according to the Directive 2008/105/EC and comparison with LODs obtained by a similar method [10].

Compound	Environmental Quality Standards				
	AA-EQS[a] (μg/L)	MAC-EQS[b] (μg/L)	LODdirective[d]	LODCapLC	LODs IT-SPME–UPLC–MS/MS (μg/L)
Atrazine	0.6	2.0	0.2	0.1	0.025
Chlorfenvinphos	0.1	0.3			0.025
Chlorpyriphos-Ethyl	0.03	0.1	0.01	0.01	0.025
Di(2-diethylhexyl)phthalate(dehp)	1.3	Not applicable	0.25	0.25[c]	0.025
Diuron	0.2	1.8	0.1	0.1	0.1
Isoproturon	0.3	1.0	0.2	0.2	0.025
Simazine	1	4	0.1	0.05	0.1
Terbuthylazine	1	1	0.1	0.1	0.1
Trifluralin	0.03	Not applicable	0.01	0.01	2.5

[a] This parameter is the EQS expressed as an annual average value (AA-EQS). Unless otherwise specified, it applies to the total concentration of all isomers.
[b] This parameter is the EQS expressed as a maximum allowable concentration (MAC-EQS). Where the MAC-EQS are marked as 'not applicable', the AA-EQS values are considered protective against short-term pollution peaks in continuous discharges since they are significantly lower than the values derived on the basis of acute toxicity.
[c] Diode array detection in line with mass spectrometer.
[d] LOD directive = (3/10) × 0, 3NCA (Directive 2008/105/CE; Directive 2009/90/CE).

Table 4.5
Recoveries (%) obtained in spiked water samples.

Compounds	Recoveries in spiked water samples			
	Deionized water	Effluent wastewater_2	Influent wastewater_2	Surface water
Dehp	105			
Chlorfenvinphos	134	92	45	94
Chlorpyriphos	80	55	78	79
Trifluralin	88			85
Diuron	121	78	25	110
Terbuthylazine	89	61	45	79
Atrazine	166	52	12	95
Isoproturon	106	75	22	100
Simazine	110	70	8	104

Table 4.6
Concentrations calculated for the water samples by IT-SPME–UHPLC–MS/MS (μg/L).

Compounds	Concentration (g/L)												
	Effluent wastewater			Influent wastewater			Superficial water					Coastal water	
	4 mL EWW1	4 mL EWW2	10 mL EWW1	4 mL IWW1	4 mL IWW2	10 mL IWW1	4 mL SW1	4 mL SW2	4 mL SW3	4 mL SW4	10 mL SW5	4 mL CW1	4 mL CW2
Dehp													
Chlorfenvinphos				0.17	0.20	0.17					0.21	1.98	0.19
Chlorpyriphos	0.03	0.23	0.03	1.2	1.5	2.25	0.14		3.07		1.39	0.24	0.23
Trifluralin		0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12		0.12
Diuron				0.90			1.29	2.02			4.37	0.63	
Terbuthylazine	0.07	0.07	0.07	0.07	0.07	0.07			7.58	8.05		0.07	0.07
Atrazine								0.71				0.01	0.76
Isoproturon	0.08			0.07		0.08	0.07	1.70			2.32	0.21	
Simazine							0.37	2.04	0.11		2.84		

EWW: effluent wastewater, IWW: influent wastewater, SW: surface water, CW: coastal water.

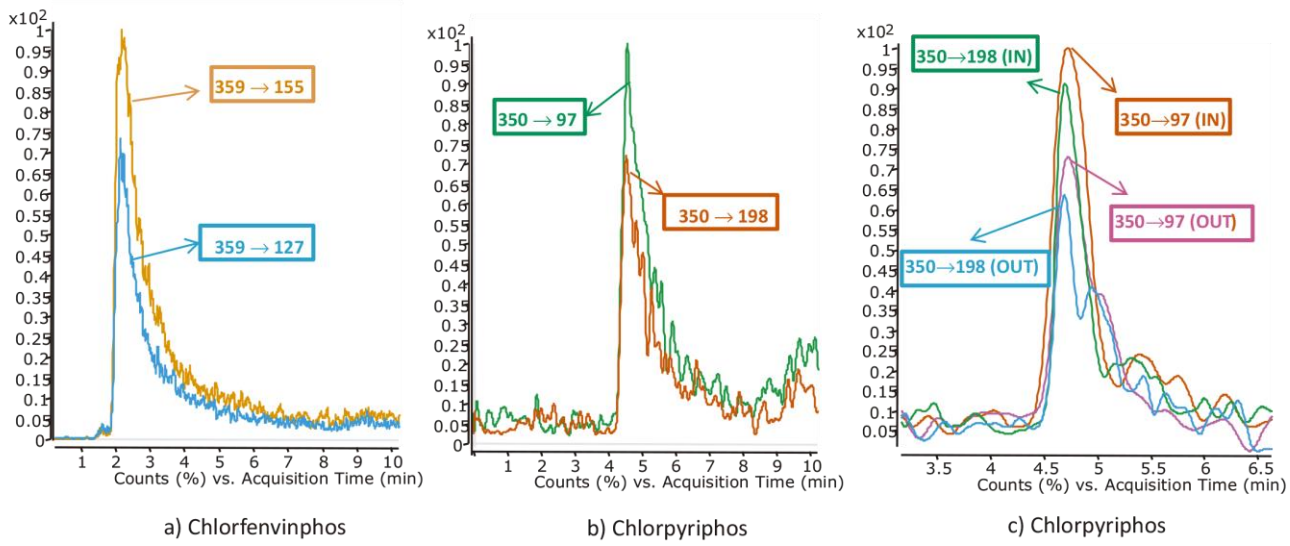


Fig. 4.4 IT-SPME–LC–MS/MS chromatograms of several identified pesticides in the water samples analyzed.

IT-SPME also makes easier the sample preparation favouring the green analytical chemistry in comparison with classical extraction procedures.

3.3. Application to real samples

Table 4.6 shows the results in g/L. In all samples, at least two of the studied compounds were detected. DEHP, chlorpyriphos, trifluralin and terbuthylazine appeared most frequently in samples. DEHP and trifluralin were found in all samples at concentrations over the legislation. Moreover trifluralin is detected in all samples with constant concentration. Meanwhile, chlorpyriphos was detected in 11 samples, 9 of which exceed the maximum allowable limits. Sample SW5 detects the presence of diuron and isoproturon, while SW3 and SW4 are contaminated by terbuthylazine. In all cases, the residue of pesticide found is over the maximum allowed concentration established in the EQS [4].

Fig. 4.4 shows some chromatograms of analytes detected in the sample: in chromatogram A and B are shown both transitions selected for chlorfenvinphos and chlorpyriphos respectively for sample CW1. The less intense transition (SRM2) was

used for the confirmation of each analyte, while for quantitative purposes the peak area of the most intense transition (SRM1) was consid-ered. The chromatogram C presents both selected transitions for chlorpyriphos in waste water samples IWW1 and EWW1. In out sample, peak areas from quantitative and confirmatory transitions are decreased because of depuration treatment.

4. Conclusions

In this study, a suitable method based on in-tube SPME in combination with UHPLC–MS/MS that provides a useful way for identification and quantification of some organic pollutants in water samples has been developed. The proposed configuration is based on two coupled six port valves using static desorption to eliminate the over-pressure problems of UHPLC system.

The in-tube SPME assembly used, permits the on-line enrichment of the analytes in the range of low parts per billion as it increases sensitivity 100 times approximately compared with direct injection 5 μL water sample in chromatograph.

This technique offers the advantages of minimum sample manipulation avoiding efforts for off-line sample preparation because samples only need to be centrifuged if necessary and injected directly in the system. Moreover, in-tube SPME is a free solvent extraction technique. It only uses 40 μL methanol to elute analytes so it assists the development of green analytical chemistry.

On the other hand, the replacement of traditional and less specific detectors like DAD by MS/MS detector increases the selectivity and specificity of the technique.

The described method offers good accuracy and reproducibility and a high enrichment factor (ca. 15), lineal answer ($r > 0.99$) and precision (RSD < 20%) are reached, suitable to control the surface water quality for different pollutants according to the maximum concentration levels established in the legislation. Finally, high sensitivity is achieved (lower detection limits can be reached with the processing of large sample volumes). Some alternative strategies such as increasing the in-tube capillary length or the size of the loop of the second valve can be applied particularly if lower detection limits want to be reached. It joins the advantages of both systems allowing high speed time analysis since separation is achieved in less than 18 min.

Acknowledgements

This work has been supported by the Spanish Ministry of Economy and Competitiveness through the projects SCARCE (CSD2009-665), CGL2011-29703-C02-02 and CTQ 2011-26760. YM-M expresses her gratitude for the JdC (Ministerio de Ciencia e Innovación) research contract.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2013.07.019.

References

[1] J.B. Baugros, B. Giroud, G. Dessalces, M.F. Grenier-Loustalot, C. Cren-Olivé, Anal. Chim. Acta 607 (2008) 191.
[2] S. Barrek, C. Cren-Olivé, L. Wiest, R. Baudot, C. Arnaudguilhem, F. Grenier-Loustalot, Talanta 79 (2009) 712.
[3] European Commission, Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy (Water Framework Directive), Off. J. Eur. Commun. C L327 (2000).
[4] European Commission, Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/156/EEC, 84/491/EEC, 86/280/EEC and amending directive 2000/60/EC of the European Parliament and of the Council, 2008.
[5] J. Fenoll, P. Hellín, C.M. Martínez, P. Flores, S.N. Navarro, Talanta 85 (2011) 975.
[6] H. Singer, S. Jaus, I. Hanke, A. Lück, J. Hollender, A.C. Alder, Environ. Pollut. 158 (2010) 3054.
[7] N.E.S. van Pinxteren, C. Bauer, P. Popp, J. Chromatogr. A 1216 (2009) 5800.
[8] R. Loos, G. Locoro, S. Contini, Water Res. 44 (2010) 2325.
[9] L. He, X. Luo, H. Xie, C. Wang, X. Jiang, K. Lu, Anal. Chim. Acta 655 (2009) 52.
[10] P. Campíns-Falcó, J. Verdú-Andrés, A. Sevillano-Cabeza, R. Herráez-Hernández, C. Molins-Legua, Y. Moliner-Martinez, J. Chromatogr. A 1217 (2010) 2695.
[11] C. Cháfer-Pericás, P. Campíns-Falcó, M.C. Prieto-Blanco, Anal. Chim. Acta 610 (2008) 268.
[12] E. Passeport, A. Guenne, T. Culhaoglu, S. Moreau, J.M. Bouyé, J. Tournebize, J. Chromatogr. A 1217 (2010) 5317.
[13] T. Portolés, E. Pitarch, F.J. López, F. Hernández, J. Chromatogr. A 1218 (2011) 303.
[14] J. Regueiro, M. Llompart, C. Garcia-Jares, J.C. Garcia-Monteagudo, R. Cela, J. Chromatogr. A 1190 (2008) 27.
[15] M. Gupta, A.K.K.V. Pillai, A. Jain, K.K. Verma, Anal. Chim. Acta 618 (2008) 61.
[16] N. Ochiai, K. Sasamoto, H. Kanda, E. Pfannkoch, J. Chromatogr. A 1200 (2008) 72.
[17] A. Giordano, M. Fernandez-Franzon, M. Ruiz, G. Font, Y. Pico, Anal. Bioanal. Chem. 393 (2009) 1733.
[18] F.J. Camino-Sánchez, A. Zafra-Gómez, S. Cantarero-Malagón, J.L. Vílchez, Talanta 89 (2012) 322.
[19] E. Bizkarguenaga, O. Ros, A. Iparraguirre, P. Navarro, A. Vallejo, A. Usobiaga, O. Zuloaga, J. Chromatogr. A. 1247 (2012) 104.
[20] K. Greulich, L. Alder, Anal. Bioanal. Chem. 391 (2008) 183.
[21] Y. Moliner-Martínez, C. Molins-Legua, J. Verdú-Andrés, R. Herráez-Hernández, P. Campíns-Falcó, J. Chromatogr. A 1218 (2011) 6256.
[22] R.M. Toledano, J.M. Cortés, J.C. Andini, J.S. Villén, A. Vázquez, J. Chromatogr. A 1217 (2010) 4738.
[23] A. Giordano, P. Richter, I.S. Ahumada, Talanta 85 (2011) 2425.
[24] G. Gervais, S. Brosillon, A. Laplanche, C. Helen, J. Chromatogr. A 1202 (2008) 163.
[25] P. Tölgyessy, B. Vrana, Z. Krascsenits, Talanta 87 (2011) 152.
[26] H.C. Liu, W. Den, S.F. Chan, K.T. Kin, J. Chromatogr. A 1188 (2008) 286.
[27] A. Masiá, M. Ibáñez, C. Blasco, J.V. Sancho, Y. Picó, F. Hernández, Anal. Chim. Acta 761 (2013) 117.
[28] Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC Brussels, Off. J. Eur. Commun. L221 (2002) 8.

Suplementary material

Multiresidue analysis of organic pollutants by in-tube solid phase microextraction coupled to ultra-high pressure liquid chromatography – electrospray - tandem mass spectrometry

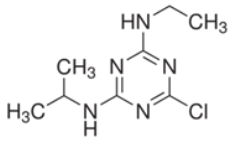
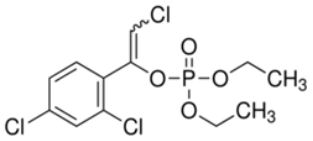
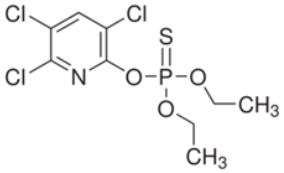
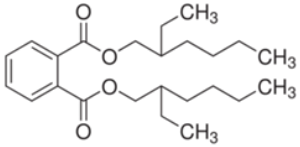
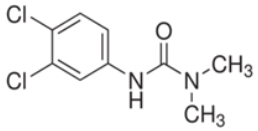
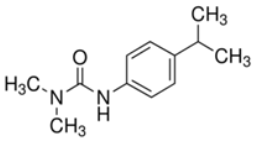
A. Masiá[1], Y. Moliner-Martinez[2], M. Muñoz-Ortuño[2], Y. Pico[1], P. Campíns-Falcó[2]

[1]Food and Environmental Safety Research Group, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés, s/n, 46100 Burjassot, Valencia, Spain.

[2]Department of Analytical Chemistry, Faculty of Chemistry, University of Valencia, C/Dr Moliner, 50, 46100 Burjassot, Valencia, Spain.

Table S4.1

Chemical structure and characteristics of analytes studied.

Compound	CAS Number	Chemical Structure	Molecular Formula	PM (g/mol)	Biocide Action	Chemical Fami
Atrazine	1912-24-9		$C_8H_{14}ClN_5$	215,7	Herbicide	Triazines
Chlorfenvinphos	470-90-6		$C_{12}H_{14}Cl_3O_4P$	359	Insecticide	Organophospho
Chlorpyriphos	2921-88-2		$C_9H_{11}Cl_3NO_3PS$	350,89	Insecticide	Organophospho
Dehp	117-81-7		$C_{24}H_{38}O_4$	390,56	Additive plastic	Phtalates
Diuron	330-54-1		$C_9H_{10}Cl_2N_2O$	233,09	Herbicide	Phenyl urea
Isoproturon	34123-59-6		$C_{12}H_{18}N_2O$	206,28	Herbicide	Urea

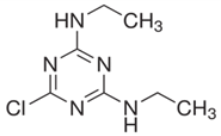
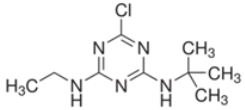
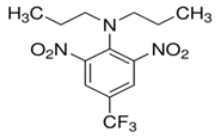
Compound	CAS Number	Chemical Structure	Molecular Formula	PM (g/mol)	Biocide Action	Chemical Fami
Simazine	122-34-9		$C_7H_{12}ClN_5$	201,66	Herbicide	Triazines
Terbuthylazine	5915-41-3		$C_9H_{16}ClN_5$	229,71	Herbicide	Triazines
Trifluralin	1582-09-8		$C_{13}H_{16}F_3N_3O_4$	335,28	Herbicide	Dinitroaniline

Table S4.2

MRM parameters used in LC-MS/MS for organic pollutants determination.

MRM Parameters LC-MS/MS								
Compounds	t_R(min) [a]	Precursor ion	SMR_1[c]	F [d](v)	EC [e](v)	SMR_2[f]	F [d](v)	EC [e](v)
Atrazine	1.193	216	174	122	14	68	122	38
Chlorfenvinphos	2.249	359	155	104	10	99	104	30
Chlorpyriphos	5.036	350	97	104	34	198	104	10
Dehp	13.826	391	149	104	18	65	104	86
Diuron	1.221	233	72	102	18	46	102	14
Isoproturon	1.186	207	72	122	18	46	122	14
Simazine	1.06	202	43	112	42	68	112	34
Terbuthylazine	1.446	230	174	102	14	68	102	42
Trifluralin	14.067	336	336	120	0			

t_R(min) [a]: retention time
SMR_1[c:] selected product ion for quantification
F [d](v): fragmentor
EC [e](v): colision energy
SMR_2[f:] selected product ion for qualification

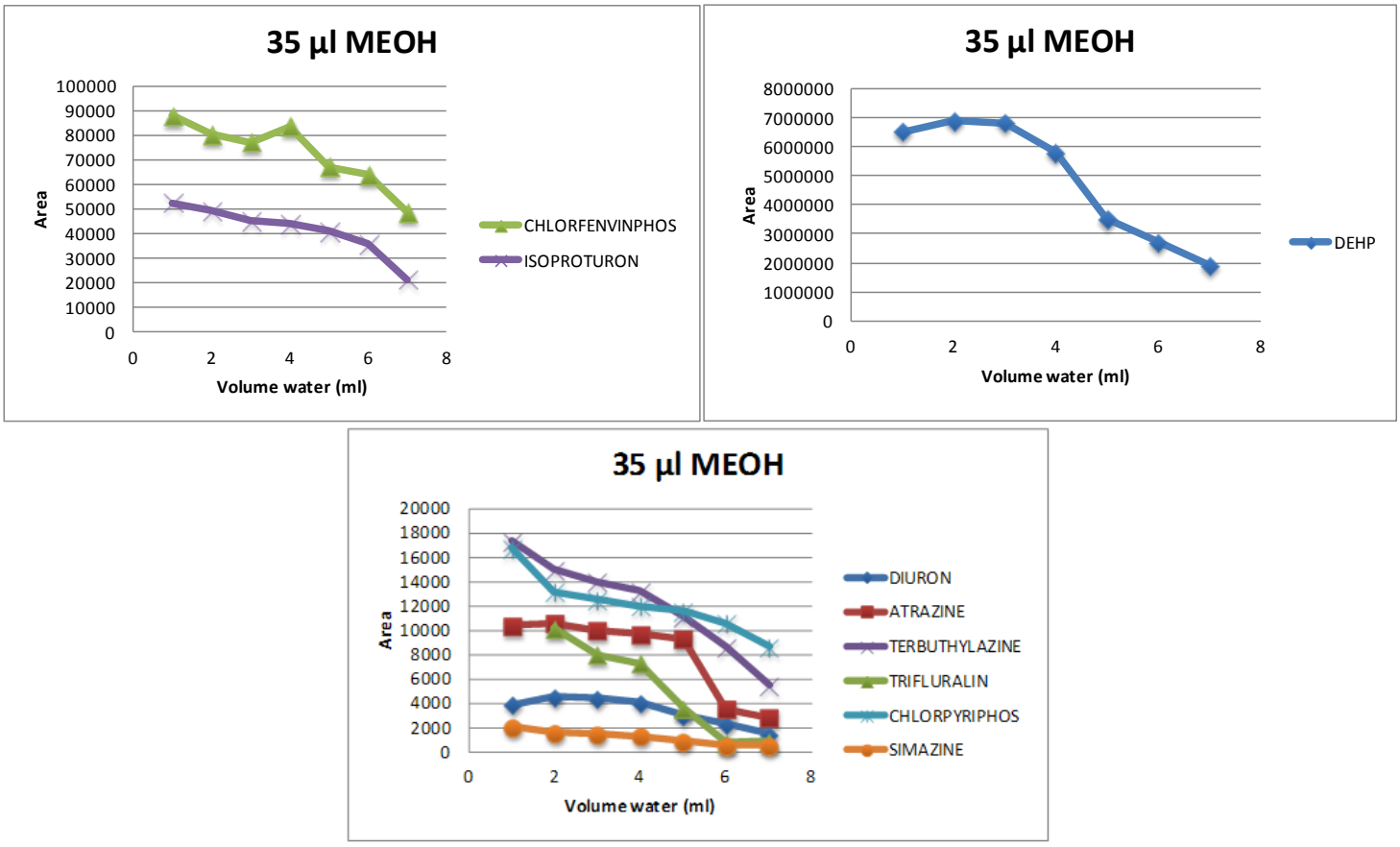


Figure S4.1 Graphic representation of water volumes tested against signal obtained using 35 µl methanol to elute analytes.

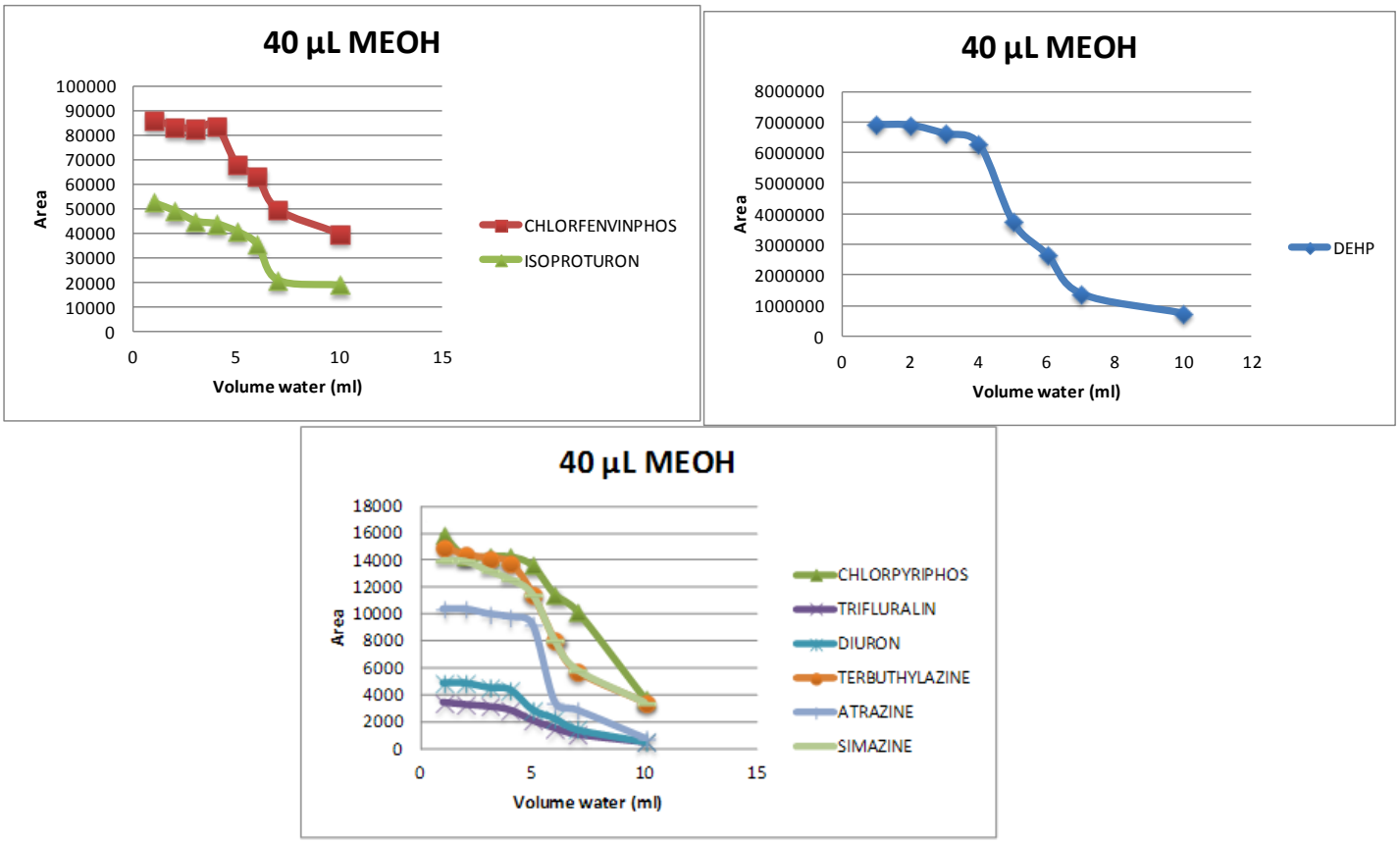


Figure S4.2 Graphic representation of water volumes tested against signal obtained using 40 µl methanol to elute analytes.

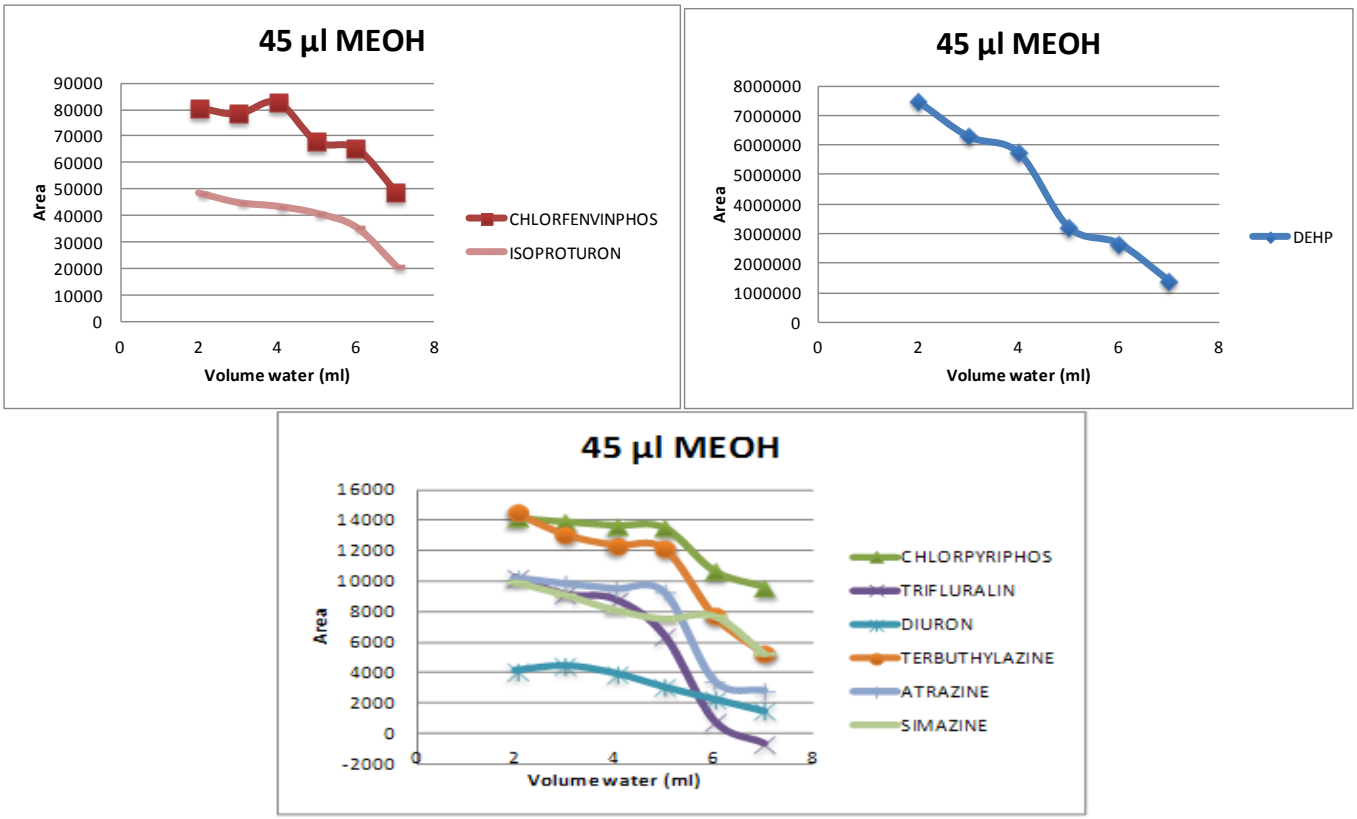


Figure S4.3 Graphic representation of water volumes tested against signal obtained using 45 µl methanol to elute analytes.

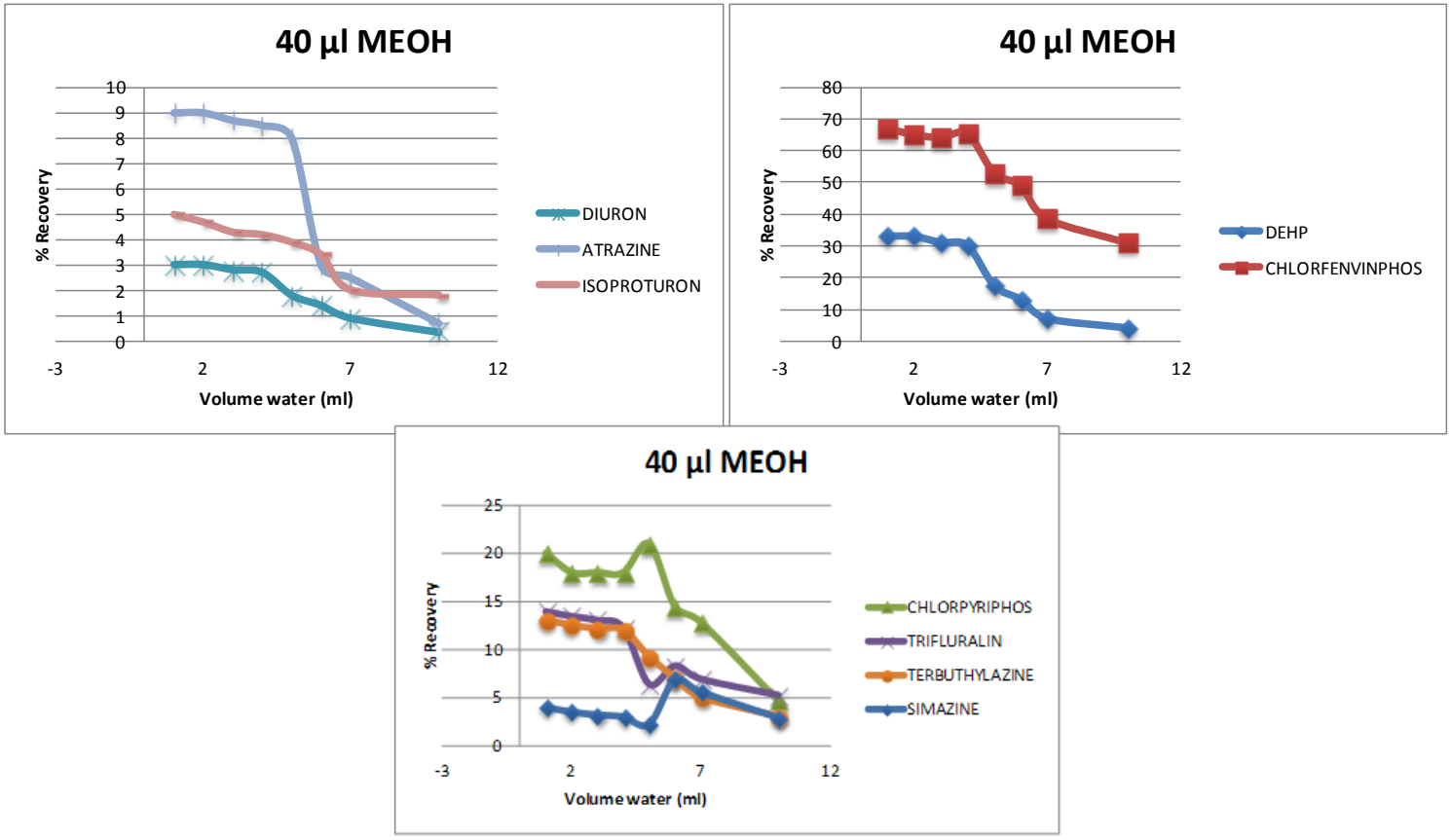


Figure S4.4 Graphic representation of recoveries obtained using 40 µl methanol.

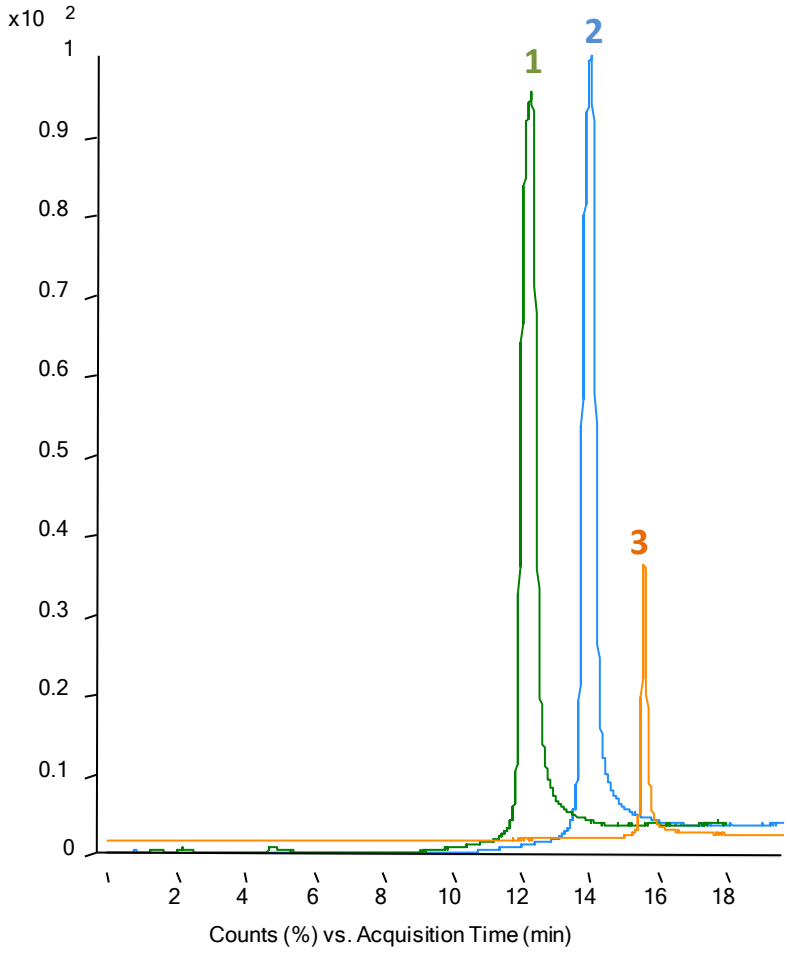


Figure S4.5 Chromatogram showing the presence of DEHP in a blank of methanol. This peak can be due to the existence of DEHP in methanol solvent, plastic conductions in LC-MS/MS or in triazines standards purchased. Peak identification: 1= standard 0.5 ppb; 2 = water 6ml_40 µl meoh; 3 = Blank_meoh.

CAPÍTOL 5

Avaluació de dos mètodes d'extracció per a la determinació de plaguicides en sòls, sediments i fangs

Publicació científica 5

Assessment of two extraction methods to determine pesticides in soils, sediments and sludges. Application to the Túria River Basin

A. Masiá, K. Vásquez, J. Campo, Y. Picó

J. Chromatography A (enviat)

Assessment of two extraction methods to determine pesticides in soils, sediments and sludges. Application to the Túria River Basin.

Ana Masiá, Karina Vásquez, Julián Campo, Yolanda Picó

Highlights

- PLE and QuEChERScompared to extract 50 pesticides in soil, sediment and sludge
- The two methods are able to extract the selected pesticides
- QuEChERS with dSPE using PSA and C_{18} was the best method
- Pesticide residues occurred in the three types samples along the Turia River Basin

Pressurized liquid extraction (PLE) and Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction methodswereoptimized for the simultaneous determination of 50 pesticides in sediment, soils and sewage sludge. While QuEChERS was being developed, several buffers and dispersive solid-phase extraction clean-up (dSPE) sorbents were tested. In the PLE method, various parameters affecting the extraction efficiency such as organic solvent, amount of sample, cell size, temperature, pressure, static time, number of cycles and % of flush as well as sorbent used for the on-line clean up were evaluated.PLE and QuEChERS were assessed and compared in obtained recoveries (33-89 % versus 25-120 %), number of pesticides for which recoveries are in the range of 80-100 % (up to 13 versus up to 35) and cost of the approach. QuEChERS procedure was faster, cheaper and easier to perform. Recoveries were around 80% (at 50 ng g^{-1}d.w.) and the matrix effect was less than -20% for most of the analytes. The limits of quantification were between 0.1 and 10 ng/g (d.w.) except for alachlor and acetochlor. Repeatability and reproducibility were lower than 28 % (%RSD, *n=5*). Soil, sediment and sludge samples taken from the Turia River Basin were analyzed by QuEChERS to determine pesticides. Chlorpyriphos (up to 65,308 ng g^{-1}d.w.) was the most frequent and at higher concentrations. Thiabendazole, imazalil, diazinon, pyriproxyfen, hexythiazox, carbofuran, isoproturon, terbuthylazine andterbumetonwere also found in some samples.

Keywords: Pesticides; Soil; Sediments; Sludges; Liquid-chromatography; Mass spectrometry

5.1 Introduction

Pesticides include a large group of organic compounds belonging to different chemical families, which play an important role in increasing agricultural productivity [1]. However, they are environmental hazards due to their stability, persistence and toxicity and they pose a tremendous danger to wildlife [2, 3].Monitoring programs have focused mainly on their analysis in the aqueous compartment, and scarce information is available on their determination and occurrence in sediments, soil or sludges –these last coming from the waste water treatment plants (WWTPs)-, which are strongly related[4, 5]. The few studies dealing with this issue are focused, predominantly, on organochlorine pesticides [1, 3, 6-11]that were banned more than 30 years ago. These matrices are reservoirs of pollution and should be included in the environmental studies in order to have a more comprehensive picture about the environmental quality status [5]. The low number of publications reporting pesticides in sediments, soil or sludges is influenced by the historical lack of Environmental Quality Standards (EQS) for organic pollutants in these matrices [5].

Only the Directive 2008/105/EC [12]established that Member states should monitor sediment and introduce EQS of those priority substances that tend to accumulate in them. However, the last proposals are still pending of approval and currently EQS for pesticides in sediments, soil or sludges are not included in any directive [5], even though the need to do it is recognized by the EU [12].

Sample preparation still remains a critical step, due to the strong interactions between the analytes and the different constituents of these matrices particularly the organic matter [13, 14].Traditionally, time and solvent consuming techniques, such as Soxhlet extraction were used to the analysis of pesticides in sediments. With current trends towards miniaturization of sample preparation, Soxhlet was replaced by more environmental

friendly procedures that are in agreement with modern green chemistry and analytical principles. Table 5.1 reviews the most representative extraction procedures used in the last 5 years (2010-2014) for the analysis of pesticides (except organochlorine) in sediments, soil and sludge.

Table 5.1 Extraction procedures used in the last five years (2010-2014) for the analysis of pesticides (except organochlorines) in sediment, soil and sludge

Extraction procedure	**Description**	**Determination**	**Recovery (%)**	**Sensitivity (µg kg^{-1} dw)**	**Ref**
MAE	3 g dry sediment + 8 g Na_2SO_4 + 25 mL of ACE-hexane (1:1) at 100 psi, 1,600 W and 60 ºC for 10 min	LC-MS/MS	67 - 123	LOD 0.003-0.024 LOQ 0.009-0.072	[15]
QuEChERS	1 g sediment + 10 ml AcN + 6 g $MgSO_4$, 1.5 g NaCl, 1.5 g (Na_3Cit) + 0.75 g (Na_2HCit sequ). Then, d-SPE of 1 mL with 50 mg PSA, 150 mg $MgSO_4$ and 50 mg C_{18}	LC-MS/MS	40-105	LOD 0.03-1.67 LOQ 0.10-5.00	[16]
QuEChERS	2 g sediment + 10 ml AcN + Acetate buffer buffer (1.5 g NaOAc, 6 g $MgSO_4$ (pH=4.8). Then, d-SPE of 1 mL with PSA/GCB (900mg $MgSO_4$, 150 mg PSA, 15 mg GCB)	LC-MS/MS	40 -98	LOQ 0.5 - 20	[17]
UAE/SBSE (clean-up)	10 g sediments + 10 ml UAE for 30 min at 35 kHz, 60% of intensity and 80 ºC. Then, addition of 85 mL H_2O *SBSE* with a PDMSstir bar for 16 hours at 300 rpm and desorption with 2 mL n-hexane:ACE (9:1) for 30 min at 200 rpm	GC-MS	70 - 111	LOD 0.3 to 4.4* LOQ 0.8 to 14 *	[18]
PLE/SPE (purification)	5 g sediment + 6 g alumina/sand, extracted with ACE:DCM, (1:1) with HCOOH 1% at 100 ºC and 100 bar in 2 cycles of 5 min. Then, the extract are solvent exchanged to MeOH:H_2O, and cleaned up through SPE with Oasis HLB. The analytes are eluted again with MeOH:DCM (1:1)	LC-MS/MS	92-118	LOD 0.02 - 16.98 Ldet 0.13 - 76.60 LOD 2.3 -17	[19]

Extraction procedure	**Description**	**Determination**	**Recovery (%)**	**Sensitivity ($\mu g\ kg^{-1}$ dw)**	**Ref**
MAE	3 g dry sediment + 8 g Na_2SO_4 + 25 mL of ACE-hexane (1:1) at 100 psi, 1,600 W and 60 ºC for 10 min	LC-MS/MS	67 - 123	LOD 0.003-0.024 LOQ 0.009-0.072	[15]
QuEChERS	1 g sediment + 10 ml AcN + 6 g $MgSO_4$, 1.5 g NaCl, 1.5 g (Na_3Cit) + 0.75 g (Na_2HCit sequ). Then, d-SPE of 1 mL with 50 mg PSA, 150 mg $MgSO_4$ and 50 mg C_{18}	LC-MS/MS	40-105	LOD 0.03-1.67 LOQ 0.10-5.00	[16]
QuEChERS	2 g sediment + 10 ml AcN + Acetate buffer buffer (1.5 g NaOAc, 6 g $MgSO_4$ (pH=4.8). Then, d-SPE of 1 mL with PSA/GCB (900mg $MgSO_4$, 150 mg PSA, 15 mg GCB)	LC-MS/MS	40 -98	LOQ 0.5 - 20	[17]
UAE/SBSE (clean-up)	10 g sediments + 10 ml UAE for 30 min at 35 kHz, 60% of intensity and 80 ºC. Then, addition of 85 mL H_2O *SBSE* with a PDMSstir bar for 16 hours at 300 rpm and desorption with 2 mL n-hexane:ACE (9:1) for 30 min at 200 rpm	GC-MS	70 - 111	LOD 0.3 to 4.4* LOQ 0.8 to 14 *	[18]
PLE/SPE (purification)	5 g sediment + 6 g alumina/sand, extracted with ACE:DCM, (1:1) with HCOOH 1% at 100 ºC and 100 bar in 2 cycles of 5 min. Then, the extract are solvent exchanged to MeOH:H_2O, and cleaned up through SPE with Oasis HLB. The analytes are eluted again with MeOH:DCM (1:1)	LC-MS/MS	92-118	LOD 0.02 - 16.98 Ldet 0.13 - 76.60 LOD 2.3 -17	[19]

Extraction procedure	Description	Determination	Recovery (%)	Sensitivity (µg kg^{-1} dw)	Ref
PLE	5 g sediment + sand, extracted with: (a) 3% ACE in hexane, (b) 0.02% TFA in ACE, (c) MeOH at 100 ºC and 100 bar in 3 cycles of 5 min	GC-MS	-	-	[20]
PLE/QuEChERS (clean-up)	4-6 g sediments+ 250 mg diatomaceous earth, extracted with EtAc: ACE (70:30) at 80 ºC. The extract is evaporated to dryness and dissolved in H_2O + 5 ml AcN + 1.6 g $MgSO_4$ + 0.4 g NH_4Cl	LC-ESI-HRMS/MS LC-ESI-APCI/MS	57-139	LOD 0.010 - 4 LOQ 0.030 - 14	[21]
Triple ultrasonication	2 g sediments + 3 x 8 ml ACE sonication for 20 min	GC-MS	70-114	LOD 50 - 100 LOQ 300 -500	[22]
QuEChERS	4 g sediments + 10 ml acetonitrile + 2 g NaCl + 2 g $MgSO_4$ for 10 min	LC-MS/MS	< 1-159	LOD 0.1 -2 LOQ 1 - 6	[5]
Luke	4 g sediments + 10 ml ACE:Hexane: DCM (1:1:1) + 15 g Na_2SO_4 for 1 h	LC-MS/MS	<1-116	LOD 0.1 -2 LOQ 1 - 6	[5]
Method using basic conditions	4 g sediment + 10 ml AcN:H_2O:25% NH_4+ (80:20:1) for 1 h	LC-MS/MS	<1-102	LOD 0.1 -2 LOQ 1 - 6	[5]
Single solid–liquid extraction	4 g sediments + 10 ml AcN/H_2O (50:50) + 4 g $MgSO_4$ for 10 min. Acetonitrile extract was cleaned up with Et.Acet/AcN and then with Et. Act./cyclohexane	LC-MS/MS	35 - 125	LOQ 0.1- 49.0 ng g^{-1}	[23]

Extraction procedure	**Description**	**Determination**	**Recovery (%)**	**Sensitivity (µg kg^{-1} dw)**	**Ref**
UAE/ heated-copper (clean-up)	5 g sediments+ 20 mL ACE/methylene chloride (1:1, v/v) + 5 g Na_2SO_4 at 50–60 Hz for 15 min. *Then, 12 g c*ooper incubation at 60 ºC, evaporation and reconstitution in 6 ml MTBE and pass through Florisil cartridge (pre-washed with 6 ml MTBE)	GC-ECD	94 - 120	LOD 0.22 - 3.72 µg kg^{-1}	[24]
PLE/SBSE	10.0 g sediment at 80 ºC, 1000 psi for 3 cycles of 10 min with 15 mL of methanol. *SBSE with* 10 mL of the methanolic extract +200 mL H_2O+ 60 g NaCl for 12 hours	TD-GC-MS/MS	63-119	LOD 0.001 - 0.3 LOQ 0.002 -0,99	[25]
SFE/DLLME	1.2 g sediment extracted with CO_2 flow-rate of 0.5mLmin^{-1} for 10 min (static) and 30 min (dynamic) at 150 bar and 60 ºC, pesticides collected 1 mL ACN *DLLME:* 7.0 µL Cl_4C	GC-FID	44.4 - 95.4	LOD 1–9	[26]
QuEChERS	10 g sediment + 10 ml ACN + 4 g $MgSO_4$ + 1g NaCl. Then. d-SPE: 330 mg PSA + 330 mg C18 + 1 cm layer $MgSO_4$	GC-MS	48 - 115	LOD 3-20 LOQ 10-50	[27]
LDMHLLE	Amount of sample:5.0 g of homogenized sed. Extraction solvent: 0.5 ml n-hexane (solvent of lower density than water Time: 30 min	GC-MS	-	LOD 0.13-0.26	[28]

Extraction procedure	Description	Determination	Recovery (%)	Sensitivity ($\mu g\ kg^{-1}$ dw)	Ref
UAME/SPE	20 g sediment + 100 ml hexane/ACE (1:1) at 100 W of microvawe and 50 W ultrasounds for 3.6 and 9 min, respectively. Extract passed through a *PSA/GCB SPE* and pesticide eluted with 7m L DCM:hexane (3:7)	GC-MS	65.2–141	LOD 0.31 - 0.70	[29]
PLE/SPE (clean-up)	5 g sed+10g Na_2SO_4 ,+ 10 g sand extracted with DCM/ACE (1:1) at 100 ºC and 2000 psi for 2 cycles of 5 min. Extract passed through a *PSA/GCB SPE* and pesticide eluted with 7 mL DCM:hexane (3:7)	GC-MS	65.7–118.8	LOD 0.68 - 1.43	[30]
UAE/C18 (clean-up)	10 g sediment extracted with MeOH/ACE (3:1) for 20 min. Extract passed through Strata C_{18} SPE Cartridge Conditioned: 2 ml MeOH and 2 ml AcN/H_2O (7:3, v/v) Elution solvent: 3 ml AcN/H_2O (7:3, v/v)	LDTD-MS/MS	38-112	LOD 0.7 - 9.4	[31]
PLE/SPE (clean-up)	5 g sediment + 10g Na_2SO_4 ,+ 10 g sand extracted with DCM/ACE (1:1) at 100 ºC and 2000 psi for 2 cycles of 5 min. Extract passed through a *PSA/GCB SPE* and pesticide eluted with 7 mL DCM:hexane (3:7)	GC-MS	59.7–128	LOD 0.10 - 0.80	[32]

MAE: Microwave-assisted extraction

ACE: Acetone

ACN: Acetonitrile

[Na3C6H5O7·2H2O]: tri-sodium citrate dehydrate

[HOC(COOH)(CH2COONa)2·1.5H2O]: disodium hydrogen citrate sesquihydrate

PSA: Primary secondary amine

GCB: graphitized carbon black

UAE: Ultrasonic assisted extraction

PDMS: polydimethylsiloxane

HCOOH: Acid formic

MeOH: methanol

LDMHLLE: low density miniaturized homogenous liquid–liquid extraction

UAME: Ultrasound assisted microwave extraction

LDTD: Laser diode thermal desorption

MTBE: methyl tertiary butyl ether

PLE: Pressurized Liquid Extraction

SPE: Solid Phase Extraction

DLLM: dispersive liquid–liquid microextraction

SFE: Supercritical fluid extraction

SBSE: stir-bar sorptive extraction

Currently-used technologies are based on new sources of energy, being PLE [5, 20, 21, 25, 27, 30, 33] and UAE [2, 22, 24, 28, 29, 34] the most used procedures. PLE is advantageous for saving time and solvents, but it requires sophisticated and expensive equipment and higher consumption of energy. UAE, which applies ultrasonic radiation in a waterbath or with probes, sonoreactors, microplate horns, is relatively inexpensive[35]. It has been widely employed due to its shorter extraction time, equipment simplicity, and procedural practicability[24]. Moreover, some alternative procedures in minority use have been also described, such as MAE [15], SFE [36], LDMHLLE. The disadvantage of these procedures is that, the obtained extracts require a purification step prior chromatographic analysis to eliminate the interfering co-extractives due to the matrix [35].

Although QuEChERS method (Quick, Easy, Cheap Effective Rugged and Safe) [16, 19, 23] is a reference for foodstuff, it has been scarcely used for the extraction of pesticides from sediment, soil and sludges [23, 27]. However, several studies apply QuEChERS to determine other contaminants, such as pharmaceuticals [17, 37] in these matrices. The major advantages of QuEChERS are low solvent consumption (low costs), speed, high sample throughput and possibility to obtain high recoveries for a wide spectrum of compounds. However, the QuEChERS approach has not been tested yet for the determination of a wide range of pesticides in soil, sediments and sludges.

This study describes, evaluates and comparestwo extraction methods −PLE and QuEChERS− to establish the most suitable technique for the extraction of 50 pesticides (of different classes: organophosphorus, carbamates, triazines, ureas, etc.) in soils, sediments and sludges. The determination was carried out using liquid chromatography-triple quadrupole-mass spectrometry (LC-MS/MS).The QuEChERS and LC–MS/MS were advantageous and provided appropriate recoveries and sensitivity. The methodology

was further applied for the first time to determine the pesticide residues in soil, sediment and sludges collected during a monitoring campaign in Turia River Basin.

5.2 Material and methods

5.2.1 Reagents

Standards of acetochlor, alachlor, atrazine, azinphos-ethyl, azinphos-methyl, buprofezin, carbofuran, chlorfenvinphos, chlorpyriphos, deisopropylatrazine, deethylatrazine, diazinon, dichlofenthion, dimethoate, diuron, ethion, fenitrothion, fenthion, fenthion-sulfoxide, fenthion-sulfone, hexythiazox, 3-hydroxycarbofuran, imazalil, imidacloprid, isoproturon, malathion, methiocarb, molinate, metolachlor, omethoate, parathion-ethyl, parathion-methyl, prochloraz, propanil, propazine, pyriproxyfen, simazine, terbutryn, tolclophos-methyl were purchased from Sigma-Aldrich (Steinheim, Germany) with a purity of at least 99%. Fenoxon, fenoxon-sulfoxide, fenoxon-sulfone were obtained from Dr. Ehrenstorfer (Augsburg, Germany) as 1-mL acetonitrile solutionsat 10 μg/mL. (For more detailed information, see Table S5.1 in the supplementary material).

Individual standard solutions were prepared in methanol at the concentration of 1000 mg L^{-1} and the working standard solution was prepared by mixing the appropriate amounts of each standard solutions and diluting them with methanol to a final concentration of 0.5 mg L^{-1}. Solutions were stored at 4 °C in the dark. Working mixtures, at appropriate concentrations, were daily made by dilution of aliquots of the stock solutions in methanol or in matrix extract.

For PLE procedure, Florisil® from Sigma-Aldrich, Silica gel from Scharlau (Barcelona, Spain) and acidic, neutral and basic alumina (Al_2O_3) from Merk (Darmstadt, Germany) were tested as sorbents. Acetone, ethyl acetate, dichloromethane and acetonitrile were also obtained from Merck.

For QuEChERS, $MgSO_4$, sodium acetate and sodium citrate dibasic sesquihydrate [HOC(COOH) $(CH_2COONa)_2 \cdot 1.5\ H_2O$] were from Alfa Aesar GmbH & Co KG (Karlsruhe, Germany), NaCl, trisodium citrate dehydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) and acetic acid from Prolabovwr (Leuven, Belgium) and primary-secondary amine (PSA), graphitized black carbon (GBC) and C_{18} from AnálisisVínicos (Tomelloso, Spain)

Ammonium formate was purchased from Sigma-Aldrich (Steinheim, Germany) and methanol (gradient grade for liquid chromatography) from Merck (Darmstadt, Germany).High purity water was prepared using a Milli-Q water purification system (Millipore, Milford, MA, USA). Milli-Q water and methanol, both with ammonium formate 10 mM were used as mobile phase in LC-MS/MS.

5.2.2 Sampling

Sampling campaign was performed during 2013 along the Túria River (East of Spain). Sampling locations were homogenously distributed through the course of the river from its source to its mouth [37] (sampling points are georeferenced in Table S5.2, supplementary material).

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 (previously washed with methanol and dried in oven at 100 °C) that was put inside an aluminum box in a portable freezer at 4 °C. Once at the laboratory, sediment samples were frozen (− 20 °C) and freeze-dried with a Virtis SP Scientific lyophilizer(Gardiner, NY, USA) at − 65 °C and with a vacuum of 1–4mT for 48 h. Then, lyophilized sediment was sieved through a series of sieves to collect the fraction < 125 μm.Soil samples of the upper 0–15 layer were collected from sampling points of 16 m^2. In them, 5 sub-samples distributed randomly were taken. Once in the laboratory, samples were dried and passed through a 2 mm Ø sieve, and then, the sub-samples of each

sampling point were homogenized to create a composite one. Dehydrated sludges were provided by the plant operator of the three main wastewater treatment plants (WWTP) of the area in aluminum foils and were treated as the sediments.

Sediment and soil samples that did not show pesticides after a preliminary analysis were used as control blank and for the optimization and validation of the method [38]. Sludge samples always contained one or other pesticides, thus in this case the peak area of the non-spiked sludge were subtracted of the spiked.

5.2.3 Extraction procedures/Sample preparation

5.2.3.1 Pressurized Liquid Extraction

PLE was performed with an ASE 200 system (Dionex, Sunnyvale, CA, USA). A sample of 1 g of lyophilized sample was weighed and homogenized into a mortar using a pestle and then, was introduced into a stainless steel 11 mL extraction cell. The cell was prepared placing a Whatman glass fiber filters at the bottom of the extraction cell to avoid the obstruction of the end caps by particles and 5 g of acidic alumina to clean-up the extract. Then, a glass filter was also place on the top, the cell was closed and positioned in the PLE system connected to a four-bottle solvent controller. Nitrogen, at a pressure of 10 bar, was supplied to assist the pneumatic system and to purge the extraction cells. The extraction cells were preheated for 2 min. Then, the analytes were heat for 5 min and extracted using acetonitrile at 100 ºC and 1.500 psi for 7 minutes of static time, in one cycle, at 100 % flush. Then, extraction cells were purged for 60 s with nitrogen to eliminate any trace of extraction solvent. The total volume extract obtained under those conditions was ca 10 mL. Approximately 1 mL of the extract was filtered through a 0.45 µm PTFE filter into the autosampler vials for LC–MS/MS analysis.

5.2.3.2 QuEChERS

The QuEchERS method, also previously reported in a variety of formats [39], was applied to 1 g of lyophilized sample, which was weighed in a 50 mL falcon tube, and homogenized with 7.5 mL water and 10 mL acetonitrile. Then, $MgSO_4$ (6 g), NaCl (1.5 g), tri-sodium citrate dehydrate [$Na_3C_6H_5O_7 + 2H_2O$ (1.5 g)] and disodium hydrogen citrate sesquihydrate [$HOC(COOH)(CH_2COONa)_2 \cdot 1.5H_2O$ (0.75 g)] were addedfor phase-separation adjustment. The mixture was agitated intensively in a vortex for 1 min and centrifuged at 3000 rpm for 5 min.

An aliquot (1 mL) of the upper organic phase was cleaned up by dispersive solid phase extraction (d-SPE) using PSA (50 mg), $MgSO_4$ (150 mg) and C_{18} (50 mg). This mixture was shaken in a vortex for 1 min and centrifuged at 3000 rpm for 5 min. The supernatant was filtered through a 0.45 µm PTFE filter and introduced in a vial for LC-MS/MS analysis.

5.2.4Liquid chromatography-tandem mass spectrometry

Pesticides were determined by LC-MS/MS, using an HP1200 series LC system, equipped with automatic injector, degasser, quaternary pump and column oven, which was interfaced to an Agilent 6410 triple Quad (QQQ) mass spectrometer, with an electrospray ionization (ESI) interface (Agilent Technologies, Waldbronn, Germany) operating in positive ionization mode. Data were processed using a MassHunter Workstation Software for qualitative and quantitative analysis (A GL Sciences, Tokio, Japan). Detail instrumental and Dynamic MRM conditions are provided in Table S5.3 and S5.4 of Supplementary material.

5.3 Results and discussion

5.3.1. Optimization of the extraction procedures

Some parameters of the PLE and QuEChERS were evaluated to improve the extraction of the target pesticides from sediment samples. The efficiency was evaluated as recoveries obtained.

PLE is influenced by the organic solvent, amount of sample, cell size, temperature, pressure, static time, number of cycles and % of flush as well as sorbent used for the on-line clean-up[26]. The influence of solid-to-solvent ratio was studied by varying the amount of sediment (0.5, 1, 2, 5 and 10 g) used per extraction with a cell of 11 mL. The extraction efficiency of target pesticides per unit mass of sediment decreased with the increase for particulate matter in the extract. The higher the sample amount, the higher the frequency of conductions systems clogging. The best results were obtained using 1 g of sample. Then, preliminary experiments were carried out to determine the appropriate cell size in order to ensure easy and reproducible packing of the sample and co-sorbents as well as proper analyte detectability. After several assays with 5, 11, 22 and 33 mL, the cell of 11 mL was selected because it allowed to pack the sample (1 g) and some sorbent for an effective removal of the interferents. Those that more influenced recovery were solvent, temperature and the addition of an on-line clean-up and the results are summarized in Fig. 5.1 for most representative analytes. Extraction solvent is one of the most important parameters to optimize.

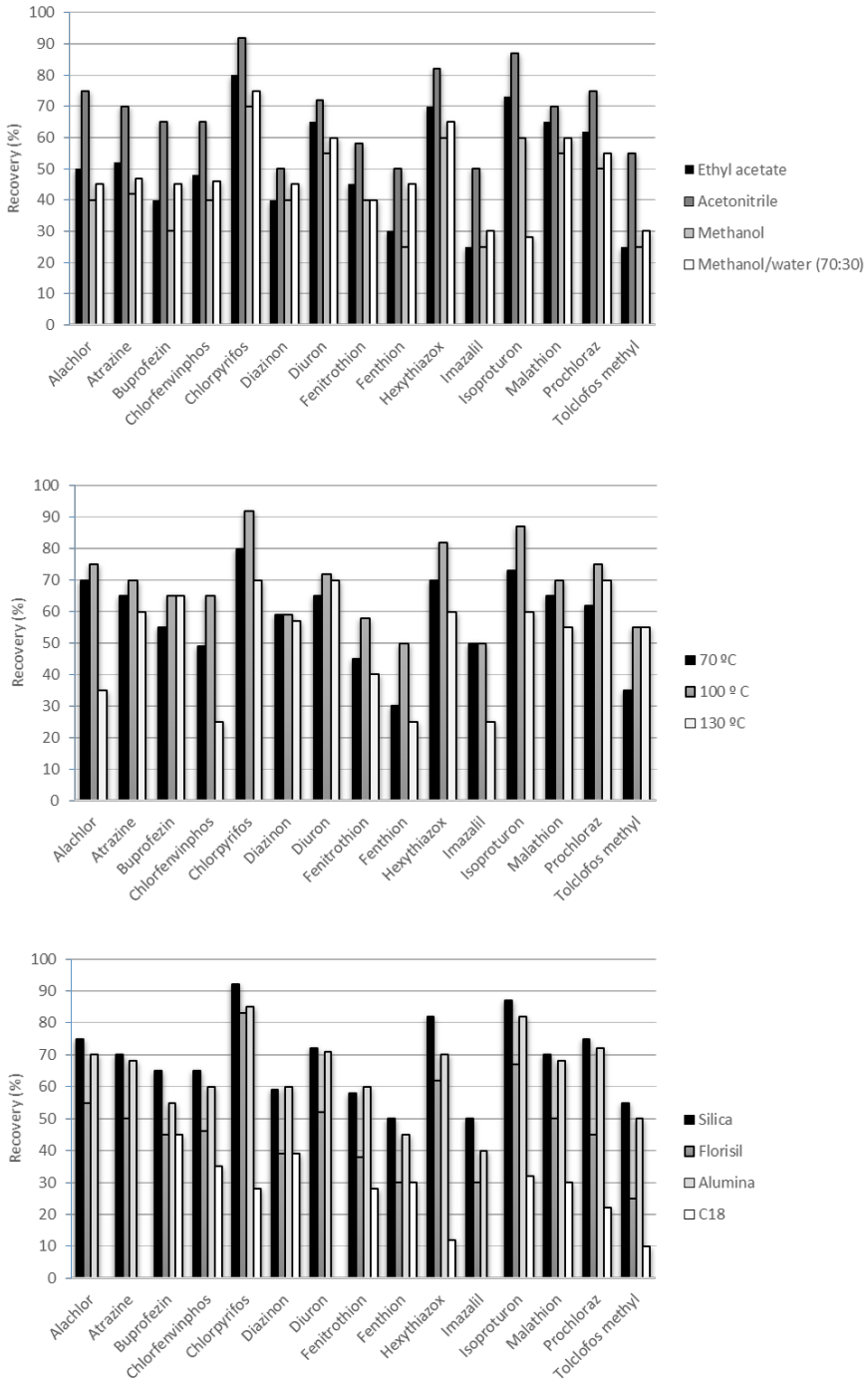


Fig. 5.1 Influence of pressurized liquid extraction parameters: (a) solvent, (b) temperature and (c) different sorbents for the on-line clean-up in the extraction of pesticides using UHPLC-MS/MS. Other conditions: temperature 100 ºC at 1000 psi for 5 min static time.

Ethyl acetate, acetonitrile, methanol and a mixture of methanol-water were tested. Acetonitrile was selected because it yielded acceptable extraction efficiency and allows to better compare both procedures since is also used in the QuEChERS. Temperature impacts the equilibrium (solubility), mass transfer rate (diffusion coefficient), and the stability of pesticides. Three different temperatures (70, 100 and 130 °C), one below and two above the boiling point of acetonitrile (b.p. 81-82 ºC) were selected to evaluate the influence of temperature on the total extraction efficiency and stability of pesticides from sediments. The on-line clean-up was studied comparing between silica, Florisil, alumina, and octadecyl-silica (C_{18}) that were place at the bottom of the cell. The best results were obtained with silica that provides the most transparent extracts and the best recoveries. The amount 1, 5 and 10 g was also tested for the different solvents. Best clean-up keeping the efficiency was obtained with 5 g.

The percentage of flush (from 50% to 150%) gave the best recoveries at 100 % and the number of extraction cycles (from 1 to 5) provides constant recoveries independently of the number of cycles. Then, 1 cycle was used. Three different pressure settings (1000, 1250, and 1500 psi) that are commonly applied with PLE to evaluate whether pressure influences the extractability of pesticides by increasing diffusivity of extraction solvent within the sample matrix were tested. The best and most reproducible results were obtained at 1500 psi. Sediments were extracted with four different static time settings (5, 7, 10 and 15 min). An increase in static time from 5 to 7 min resulted in an increase in the pesticide yield extracted from sediment samples. However, further increase of the static time does not improve the recoveries.

The original QuEChERS method was designed for extracting pesticide residues in fruits and vegetables with high percentage of water. It was based on solvent extraction carried out with acetonitrile and subsequent cleanup based on dispersive solid-phase extraction

(d-SPE) using a PSA sorbent and anhydrous $MgSO_4$ to remove water. Two remarkable modifications of the original unbuffered method have been reported. These modifications have been adopted as the Association of Analytical Communities (AOAC) Official Method 2007.01, which uses strong acetate buffering (pH 4.8), and the European Committee for Standardization (CEN) Standard Method EN 15662, which uses a weaker citrate buffering (pH 5–5.5).An assessment of the three QuEChERS approaches was performed using 1 g of blank lyophilized sediment: (i) 7.5 mL of water + 15 mL of acetonitrile with 1 % acetic acid + 6 g anh. MgSO4 + 1.5 g NaOAc (acetate-buffered version); (ii) 7.5 mL of water + 10 mL of acetonitrile + 6 g anh. MgSO4 + 1 g NaCl + 1 g $Na_3C_6H_5O_7 \cdot 2H_2O$ + 0.5 g HOC(COOH) (CH2COONa)2 • 1.5 H_2O (citrate-buffered version; and (iii)10 mL of acetonitrile + 7.5 mL of water + 6 g anh. MgSO4 + 1 g NaCl (unbuffered version)

Average recoveries obtained from the extraction of sediments with the different QuEChERS approaches are shown in Fig. 5.2a. The important differences in the various salts under the QuEChERS procedure are the resulting pH of the medium and the presence or absence of buffering. The results of the application of sodium citrate increase the recoveries remarkably leading to the adoption of this method as the best.

Three of the most widely used solid-sorbents, PSA, C18 and GCB were examined for their efficiency on the clean-up of the matrix. The sorbents were added in the following composition: (i) 50 mg PSA + 150 mg anh. $MgSO_4$; (ii) 50 mg PSA + 50 mg C_{18} + 150 mg anh. $MgSO_4$; (iii)50 mg PSA + 7.5 mg GBC + 150 mg anh. $MgSO_4$ and (iv) 50 mg C_{18} + 7.5 mg GBC+150 mg anh. $MgSO_4$. The selected extraction did not require a clean-up step GBC due to the low lipid content of the matrix, as well as by the presence of imazalil, which have an acidic nature, increasing the risk of their binding on the sorbent. Furthermore, the GBC retains some pesticides as demonstrated in Fig 5.2b. Particularly,

atrazine, chlorfenvinphos, diuron and imazalil are retained in GBC. Then, this sorbent was not used in the further clean-up.

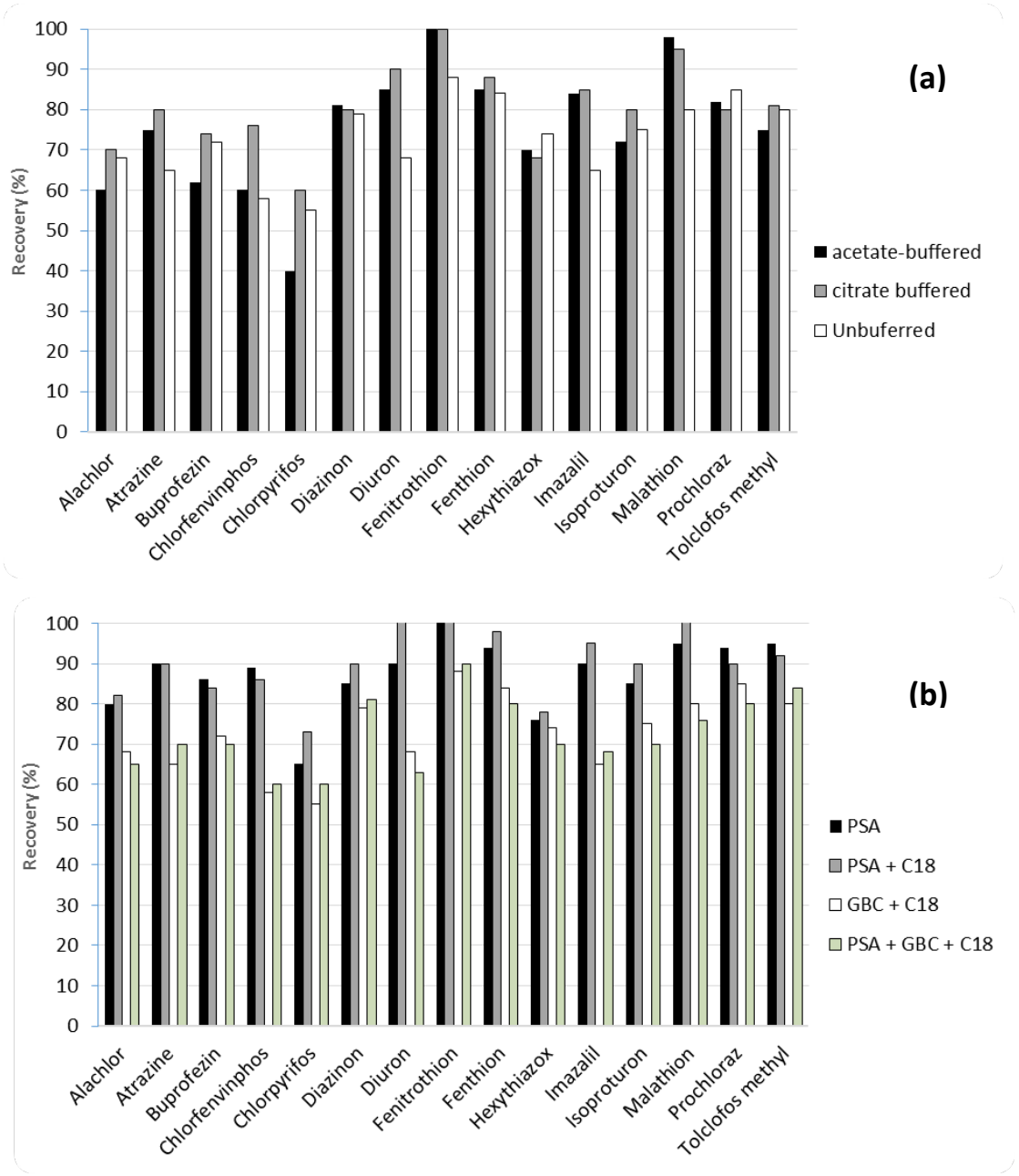


Fig. 5.2 Influence of different QuEChERS approach: (a) extraction and (b) clean-up

5.3.2 Comparison of PLE and QuEChERS procedures

To determine the recoveries (%) obtained by each studied extraction, samples were spiked at 100 ng g^{-1} concentration before the extraction. The samples were left to stand 3 h at

room temperature before the extraction to allow the evaporation of the solvent and to establish equilibration between the pesticides and the matrix. Consecutively, spiked samples were extracted and treated by the previously described protocols. To calculate the recoveries, external matrix-matched calibration was used to minimize the variations between samples. Calibration solutions for external matrix-assisted curve (eight-point calibration) were prepared in blank sediment, soil and sludge extracts obtained following QuEChERS and PLE (no pesticide was present in sediment and soil and the case of the sludge background-subtracted peak area was used to calculate the recovery).

The following step was to evaluate deeply the recoveries obtained by both techniques. Both, PLE and QuEChERS were able to extract all selected pesticides in the three matrices. Recoveries obtained by PLE ranged from 38-85 % for soil, 35-89 % for sediment and 31-120 % for sludges with mean recoveries of 68, 72 and 71 %, respectively. Recoveries obtained by QuEChERS ranged from 25-92 % for soil, 39-120 % for sediment and 31-120 for sludges with mean recoveries of 76, 73 and 82 %, respectively. Thereby, the recoveries obtained were acceptable –considering the wide variety and polarity of the studied pesticides- (Table 5.2) and RSDs were lower than 20%.

Table 5.2 Recovery data for PLE and QuEChERS in soil, sediment and sludge at 100 μg kg^{-1} (mean values, *n = 5*)

	Recovery (%)					
	Soil		**Sediment**		**Sludge**	
	PLE	**QuEChERS**	**PLE**	**QuEChERS**	**PLE**	**QuEChERS**
Acetochlor	64	47	75	50	72	79
Alachlor	62	87	75	42	70	89
Atrazine	66	84	74	78	70	93
Atrazine-desethyl	72	77	76	70	78	85
Atrazine-desisopropyl	83	75	88	93	80	55
Azinphos-ethyl	75	84	81	76	82	93
Azinphos-methyl	72	76	79	76	82	89
Buprofezin	73	80	75	75	75	78
Carbendazim	69	73	77	67	77	82
Carbofuran	67	82	75	63	72	93
Carbofuran-3-hydroxy	80	80	78	120	85	87
Chlorfenvinphos	38	87	35	76	33	91
Chlorpyrifos	70	92	76	72	79	51
Diazinon	65	72	67	72	69	79
Dichlofenthion	70	75	76	70	75	55
Dimethoate	72	83	77	80	78	90
Diuron	63	80	67	83	66	91
Ethion	68	83	79	76	74	81
Fenitrothion	45	81	46	84	44	82
Fenoxon-Sulfone	74	78	83	80	78	82
Fenoxon-Sulfoxide	80	78	70	70	85	82
Fenthion	38	77	45	76	40	87
Fenthion Oxon	75	60	77	41	76	60
Fenthion-Sulfone	70	84	75	45	70	91
Fenthion-Sulfoxide	75	78	80	62	73	82
Hexythiazox	50	79	53	70	49	41
Imazalil	40	54	45	71	43	92
Imidacloprid	85	79	87	78	88	88

	Recovery (%)					
	Soil		Sediment		Sludge	
	PLE	QuEChERS	PLE	QuEChERS	PLE	QuEChERS
Isoproturon	80	84	89	76	87	91
Malathion	50	82	53	89	55	101
Methiocarb	75	79	77	75	77	91
Methoalachlor	72	83	82	107	78	91
Molinate	68	81	75	78	77	85
Omethoate	80	67	70	60	60	67
Parathion-ethyl	73	79	76	94	74	83
Parathion-methyl	76	40	76	47	75	40
Prochloraz	75	79	81	72	80	84
Propanil	77	82	79	84	77	90
Propazine	72	83	81	78	75	93
Pyriproxifen	72	79	86	79	81	83
Simazine	74	74	75	91	76	120
Tebuconazole	79	77	84	69	81	66
Terbumeton	41	82	45	72	44	88
Terbumeton-desethyl	75	81	80	75	78	87
Terbuthylazine	50	83	55	72	53	90
Terbuthylazine-2-hidroxy	75	25	76	39	75	31
Terbuthylazine-deethyl	72	80	82	80	76	89
Terbutryn	61	82	55	73	58	90
Thiabendazole	72	60	76	71	75	102
Tolclofos-methyl	72	83	76	75	73	91

Overview of recoveries data for QuEChERS and PLE is summarized in Fig. 5.3. PLE recoveries were lower than 50 % for 8 pesticides in soil, 5 in sediments and 6 in sludges. Chlorfenvinphos, fenitrothion, fenthion, imazalil and terbumeton were recovered < 50 % in the three matrices. Some of these compounds e.g. chlorfenvinphos is thermolabile and the others can be degraded to some metabolites. Recoveries were never > 90 % and for most of the compounds were between 70 and 80 %. QuEChERS provided better

recoveries because only acetochlor, parathion methyl and terbuthylazine presented recoveries < 50 %. Recoveries for most compounds were between 80-100 %, except in sediment where recoveries were similar by both methods.

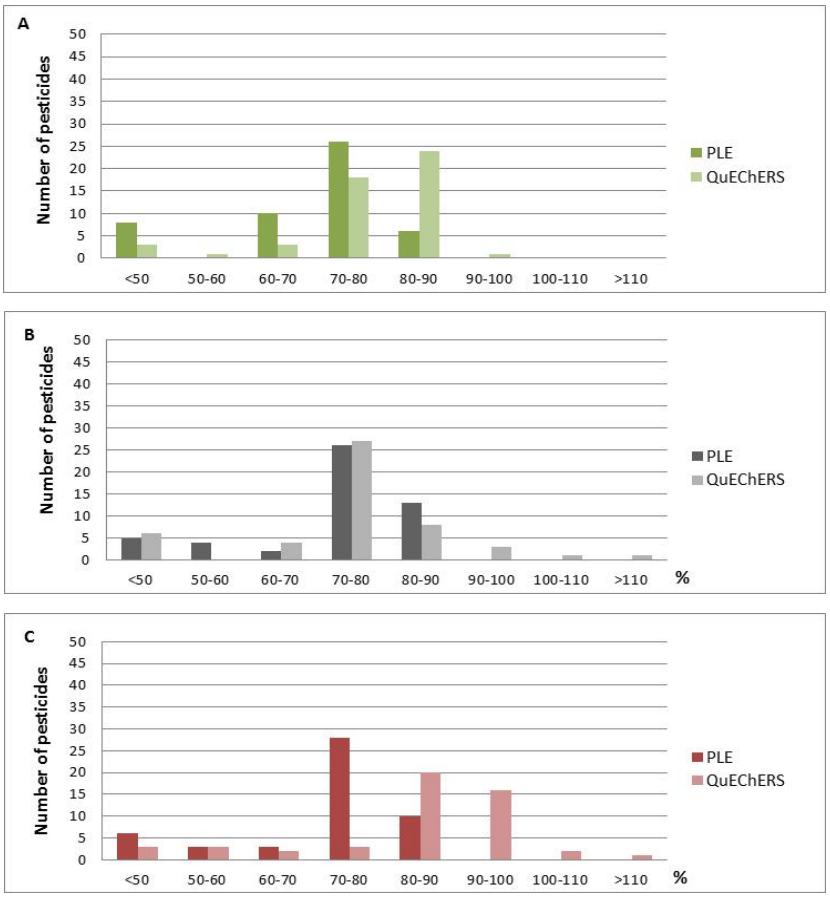


Fig. 5.3 Summary of recoveries obtained by PLE and QuEChERS in (A) soils (B) sediment and (C) sludge.

QuEChERS and PLE clean-up methods are commonly used for pesticide analysis. Even so, these methods have advantages and disadvantages. Table 5.3 summarizes some of them. Among the two evaluated methods, QuEChERS is the fastest and cheapest procedure. QuEChERS procedure was able to extract 10–15 samples in 1 h and 30 min, whereas PLE methods took twice as long. The sample clean-up by PLE requires acidic alumina, which is much more expensive than the QuEChERS reactives. PLE also

consumes much more energy and requires an important amount of nitrogen. Although PLE can be automated, the problems of clogging are quite recurrent.

Table 5.3: Comparison of PLE and QuEChERS

Parameters	**PLE**	**QuEChERS**
Sample amount (g)	1	1
Sorbent (g)	---	$MgSO_4$ 6 g NaCl 1 g Na_3Cit 0.5 g Na_2HCit sesq. 1.5 g
Disposable material	2 glass filters	----
Solvent (mL)	H_2O 7.5 mL Acetonitrile 10 mL	Acetonitrile 11 mL
Clean-up	5 g acidic alumina	PSA 50 mg $MgSO_4$ 150 mg C_{18} 50 mg
N_2 (gas)	100 psi (6.9 bar) maz	---
Energy	50 V AC x 12 min	50 Hz 0.3 A x 2 min 3000 rpm x 10 min
Preconcentration	–	–
Time per sample (min)	12 min	6 min
Recoveries	>80%	80-100 %

Summarizing, QuEChERS was selected for further studies in order to take advantage its potential for simultaneous extraction of selected compounds. The data comparison showed that QuEChERS offered acceptable range of recoveries and low RSDs. Furthermore, QuEChERS gave low time consuming during the extraction procedure, as well as, it was easier and cheaper than PLE. QuEChERS was the most efficient and effective extraction procedure evaluated. Thus, this procedure was selected to analyse soil, sediments and sludge samples as it was advantageous in terms of recovery.

Additionally, it presented other advantages such as time consuming, reagent consumption and energy expenditure.

5.3.3 Validation of the QuEChERS procedure

Validation of the method was performed according to following the guidance document on analytical quality control and validation procedures for pesticide residues [40, 41]. The following parameters were studied: confirmation of identity, specificity/selectivity, linearity, limits of quantification (LOQs), precision as repeatability and within-lab reproducibility, and recovery.

Confirmation of identity was based on the following criteria: (i) the retention time of the pesticide in the extract and in the calibration standard were matched with a tolerance of ± 0.2, (ii) two precursor ion → product ion transition and (iii) the relative intensities of the product ions expressed as a ratio relative to the most intense product ion correspond to that of the calibration within the established tolerance.

LOQ was determined as the lowest concentration of the analyte that was validated with acceptable accuracy by applying the complete analytical methods. The LOQs are reported in Table 5.4. LOQs for the target compounds ranged from 1 to 10 ng $g^{-1}$with the exception of alachlor and acetochlor that gave LOQs of 25 ng $g^{-1}$and were generally similar or lower comparing to the currently published procedures for some target compounds [5, 16-19].

Linearity was then evaluated. Peak area was selected as response and good linearity within LOQ and 100 times LOQ (six-point calibration) was found with determination coefficients higher than 0.9922 in all the cases. The use of an MS/MS detector enables the simultaneous detection and distinction of co-eluting analytes. Representative chromatogram of an extract of spiked sediment containing all 50 pesticides (at 50 ng g^{-1} level) is presented in Fig. 5.4. Retention times correspond to the data in Table S5.4.

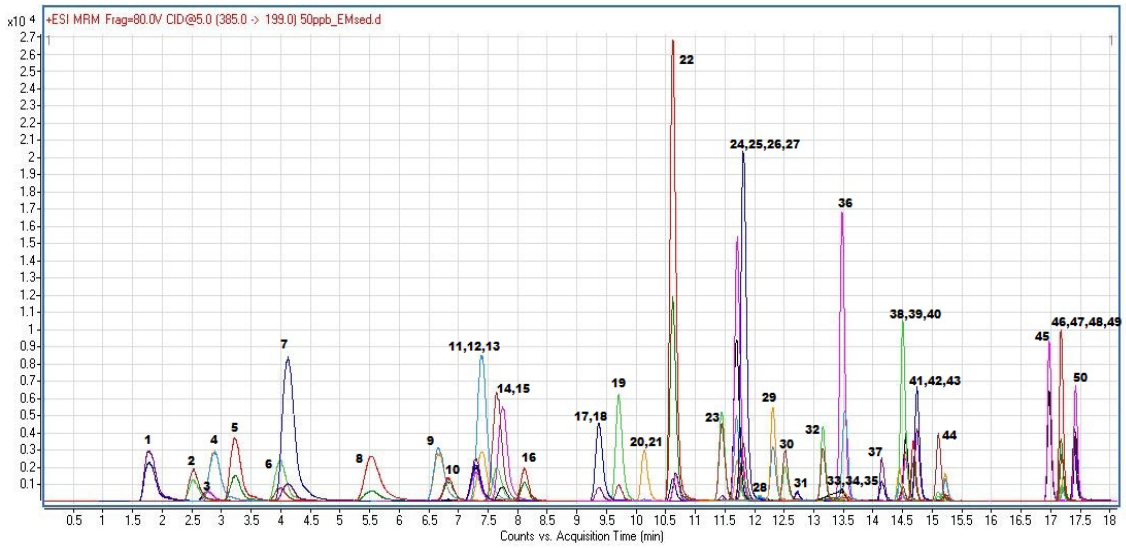


Fig. 5.4 Chromatogram of a sediment sample spiked with all the analytes at 50 ng g-1 (maximum LOQ obtained).

1	**Omethoate**	**11**	**Fenoxon-Sulfone**	**21**	**Fenthion Oxon**	**31**	**Azinphos-ethyl**	**41**	**Chlorfenvinphos**
2	Imidacloprid	12	Fenthion-Sulfoxide	22	Parathion-methyl	32	Methoalachlor	42	Diazinon
3	Atrazine-desisopropyl	13	Terbumeton-desethyl	23	Propazine	33	Acetochlor	43	Prochloraz
4	Carbofuran-3-hydroxy	14	Terbuthylazine-2-hidroxy	24	Methiocarb	34	Alachlor	44	Tolclofos-methyl
5	Dimethoate	15	Terbuthylazine-deethyl	25	Terbumeton	35	Terbutryn	45	Buprofezin
6	Atrazine-desethyl	16	Fenthion-Sulfone	26	Propanil	36	Parathion-ethyl	46	Pyriproxifen
7	Carbendazim	17	Atrazine	27	Terbuthylazine	37	Fenoxon-Sulfoxide	47	Ethion
8	Thiabendazole	18	Isoproturon	28	Molinate	38	Imazalil	48	Chlorpyriphos
9	Carbofuran	19	Diuron	29	Malathion	39	Tebuconazole	49	Dichlofenthion
10	Simazine	20	Azinphos-methyl	30	Fenitrothion	40	Fenthion	50	Hexythiazox

A substantial problem in LC-MS is the presence of matrix components in the extract (co-eluting compounds), which can affect the ionization of the compounds when ESI is used producing the so-called matrix effects (ME). In this way, the linearity in the response was calculated using standard solutions and matrix-matched solutions were prepared by sediments, soils and sludge in triplicate at seven concentrations levels into the analytical range: from LOQ to 100 times this LOQ. Thereby, matrix-matched calibration was used. The ME can be calculated in two ways (i) as the percentage of the matrix-matched calibration slope (B) divided by the slope of the standard calibration in solvent (A), the ratio (B/A×100) is defined as the matrix effect or (ii) as the percentage of the peak area

of the analyte spiked in matrix a fixed concentration (D) divided by the area of the standard at the same concentration in solvent (C) ratio (D/Cx100). The results obtained are outlined in Fig. 5.5. A value of 100% indicates that there is no absolute matrix effect. There is signal enhancement if the value is >100% and signal suppression if the value is <100%. As can be noted ME obtained using both calculation methods are similar demonstrating that their evaluation is accurate. However, the slopes obtained for the matrix-matched and the solvent calibrations are not always parallel and some different cases can be obtained (see supplementary material Fig. S5.2)

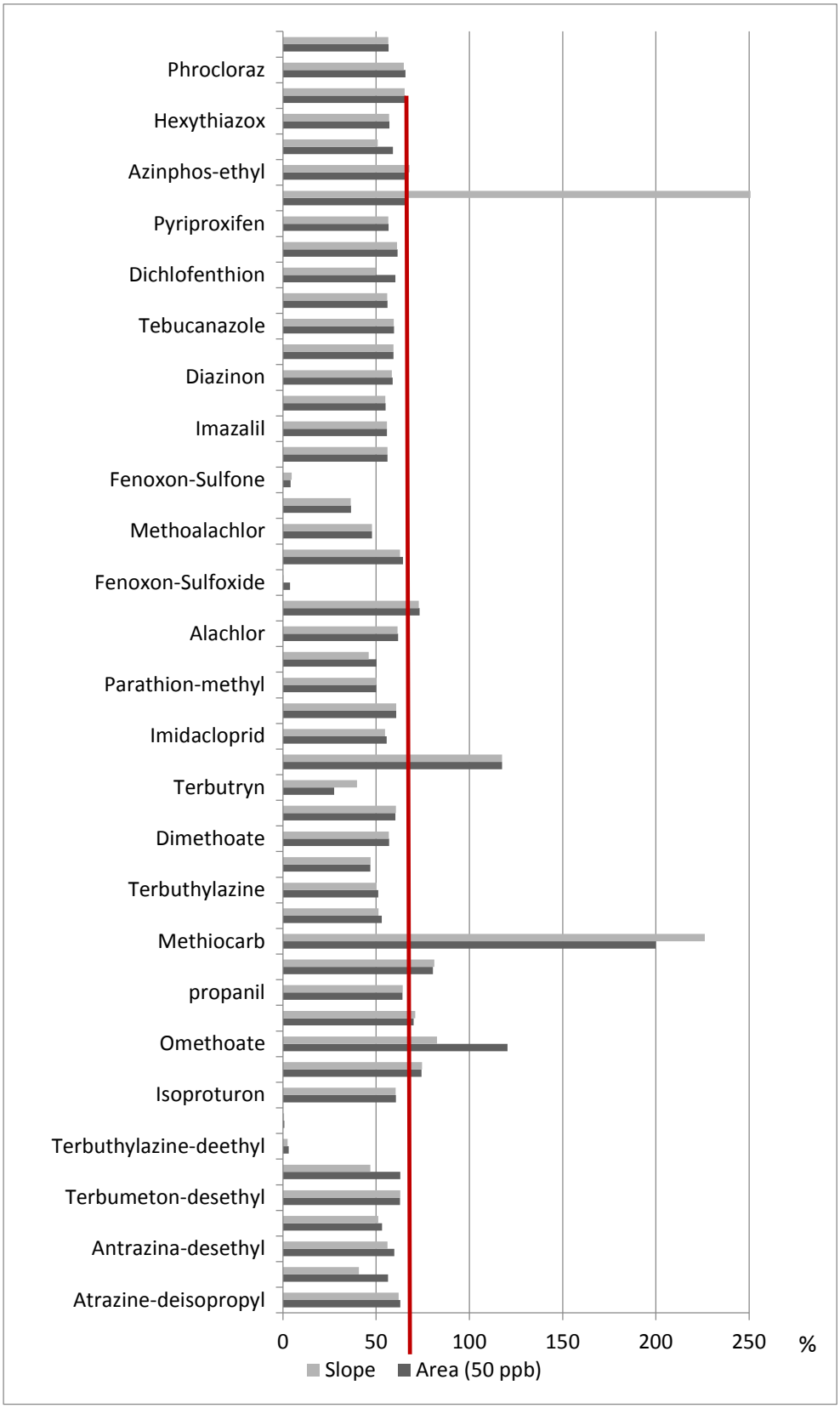


Fig. 5.5 Matrix effects for all the analytes calculate using the slope of the calibration curves and using the area at the concentration of 50 ng g^{-1}

There are different ways, which could be applied to compensate matrix effects. Although the best way to compensate the matrix effect is the use of isotope internal standards, these compounds are not available for some of the studied pesticides, as well as they are expensive for routine analysis. Another more practical form to avoid matrix effects is the use of matrix-matched calibration curves for effective quantitative determinations of pesticides in sediments, soils and sludge; as it was carried out in this study.

Recoveries (*n=5*) were carried out spiking sediment at two levels 5 and 50 ng g^{-1} and soil and sludge at 50 ng g^{-1} (Table 5.4). Recovery ranged from29 to102 % or from 50 to 104 %for sediments at 5 ng g^{-1} and 50 ng g^{-1}, from 40 to 92 % for soils and from 40 to 120 % for sludges. The precision of the method, expressed as relative standard deviation (%RSD), was estimated by the repeated analysis (*n=5*) of a spiked sediment, soil and sludge at these levels during the same day (intra-day) and on different five days (inter-day). Intra-day precision (data not shown) was always better than inter-day (Table 5.4). It can be observed that repeatability, expressed as RSD, for inter-day precision was lower than 26 % for two spiked levels. Thus, the method was successfully validated according to the criteria specified in European guidelines.

Table 5.4 Method LOQ along with recoveries at different concentrations and RSDs for the QuEChERS

Pesticide	**Recovery ± RSD**						**LOQ**
	Soil		**Sediment**		**Sludge**		
	50 ng.g^{-1}		**5 ng.g^{-1}**		**50 ng.g^{-1}**		**ng.g^{-1}**
Acetochlor	47 ± 1		-		50 ± 6	79 ± 11	25
Alachlor	87 ± 2		-		79 ± 25	59 ± 26	25
Atrazine	84 ± 3	48	± 22	78 ± 19	93 ± 4		5
Atrazine-desethyl	77 ± 3	54	± 5	70 ± 16	85 ± 6		5
Atrazine-desisopropyl	75 ± 3	30	± 15	93 ± 1	55 ± 3		3
Azinphos-ethyl	84 ± 4	30	± 3	76 ± 13	93 ± 6		5
Azinphos-methyl	76 ± 5	41	± 16	76 ± 9	89 ± 7		5
Buprofezin	80 ± 3	51	± 4	75 ± 12	78 ± 6		1
Carbendazim	73 ± 4	36	± 2	67 ± 14	82 ± 4		1
Carbofuran	82 ± 6	95	± 17	81 ± 18	93 ± 5		5
Carbofuran-3-hydroxy	80 ± 2		-	96 ± 18	87 ± 5		6
Chlorfenvinphos	87 ± 2	45	± 12	76 ± 15	91 ± 5		5
Chlorpyrifos	92 ± 1	68	± 9	72 ± 15	51 ± 1		0,1
Diazinon	72 ± 3	31	± 3	86 ± 22	79 ± 6		5
Dichlofenthion	75 ± 3		-	83 ± 8	55 ± 4		10
Dimethoate	83 ± 3	55	± 23	97 ± 3	90 ± 3		5
Diuron	80 ± 2		-	99 ± 4	91 ± 4		10
Ethion	83 ± 4	35	± 2	76 ± 10	81 ± 6		0,1
Fenitrothion	81 ± 7		-	104 ± 15	89 ± 4		10
Fenoxon-Sulfone	78 ± 2	29	± 19	86 ± 14	82 ± 2		5
Fenoxon-Sulfoxide	77 ± 2		-	99 ± 22	87 ± 3		10
Fenthion	77 ± 2	50	± 10	99 ± 22	87 ± 3		5
Fenthion Oxon	60 ± 11	51	± 1	83 ± 18	60 ± 13		1
Fenthion-Sulfone	84 ± 1		-	70 ± 19	91 ± 6		6
Fenthion-Sulfoxide	78 ± 2	34	± 19	86 ± 14	82 ± 2		5
Hexythiazox	79 ± 3	37	± 19	70 ± 10	41 ± 14		0,1
Imazalil	54 ± 17		-	92 ± 13	92 ± 7		10
Imidacloprid	79 ± 7	102	± 17	67 ± 12	88 ± 4		0,1

Pesticide	Recovery ± RSD				LOQ
	Soil	Sediment		Sludge	
	50 ng.g^{-1}	5 ng.g^{-1}	50 ng.g^{-1}	50 ng.g^{-1}	ng.g^{-1}
Isoproturon	84 ± 3	51 ± 3	76 ± 16	91 ± 4	5
Malathion	82 ± 2	62 ± 8	68 ± 1	101 ± 5	5
Methiocarb	79 ± 4	72 ± 3	75 ± 14	91 ± 4	5
Methoalachlor	83 ± 2	35 ± 19	70 ± 1	91 ± 5	5
Molinate	81 ± 5	45 ± 19	78 ± 17	85 ± 6	5
Omethoate	67 ± 3	57 ± 17	60 ± 16	67 ± 4	5
Parathion-ethyl	79 ± 4	92 ± 7	68 ± 15	83 ± 10	5
Parathion-methyl	40 ± 8	-	93 ± 23	40 ± 24	10
Prochloraz	79 ± 4	36 ± 4	72 ± 16	84 ± 4	5
Propanil	82 ± 3	45 ± 15	69 ± 6	90 ± 5	5
Propazine	83 ± 3	40 ± 17	78 ± 15	93 ± 2	5
Pyriproxifen	79 ± 4	35 ± 4	79 ± 19	83 ± 7	5
Simazine	74 ± 4	40 ± 16	91 ± 4	120 ± 19	5
Tebuconazole	77 ± 2	-	69 ± 8	84 ± 3	8
Terbumeton	82 ± 3	35 ± 3	72 ± 10	88 ± 7	5
Terbumeton-desethyl	81 ± 3	44 ± 11	75 ± 18	87 ± 3	5
Terbuthylazine	83 ± 3	34 ± 2	72 ± 9	90 ± 4	5
Terbuthylazine-2-hidroxy	60 ± 1	39 ± 23	62 ± 1	61 ± 4	5
Terbuthylazine-deethyl	80 ± 2	30 ± 5	96 ± 6	89 ± 4	5
Terbutryn	82 ± 3	-	73 ± 10	90 ± 3	10
Thiabendazole	60 ± 7	32 ± 20	85 ± 11	102 ± 11	5
Tolclofos-methyl	83 ± 3	-	75 ± 10	91 ± 4	10

5.3.4 Analysis of real samples

The developed analytical method was applied for testing samples from Turia River Basin including soil (31), sediment (54) and dehydrated sludges (21) taking during 2013. Within this monitoring, several pesticides were identified (Table 5.5). Sludges were the most frequently contaminated by pesticides followed by sediments and soils. Chlorpyriphos

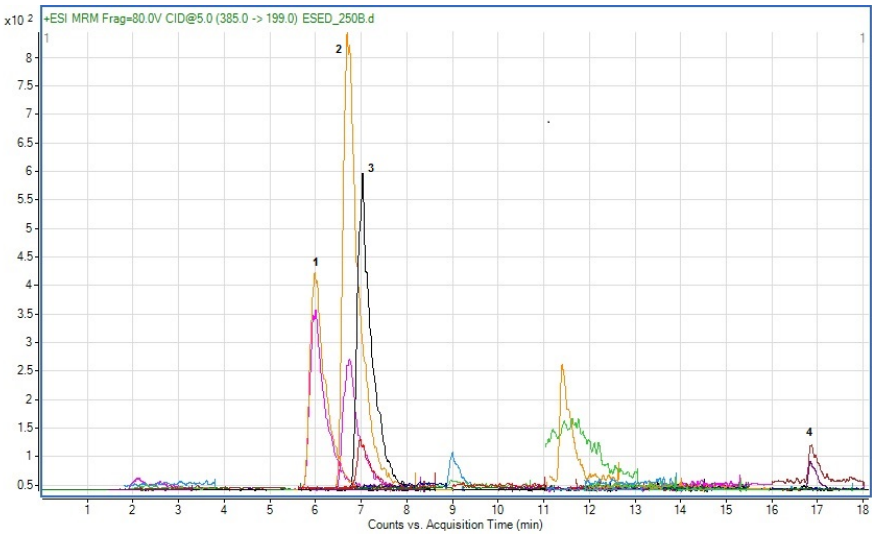
was the most frequent detected in the three types of samples at a concentration up to 65,308 ng g^{-1} in soil (83 % of samples), 560 ng g^{-1} in sediment (36 %) and 703 ng g^{-1} in sludge (90 %). Thiabendazole, imazalil, diazinon, pyriproxyfen and hexythiazox were also commonly detected in sludge. This pattern of pesticides is quite coherent because imazalil and thiabendazole are used as post-harvest fungicides and the others are pesticides widely used in urban areas appearing commonly in the WWTPs. In sediments, carbofuran, isoproturon, and two triazine metabolites-─terbumeton-deethyl and terbuthylazine-deethyl─ were the other pesticides detected. These compounds are extensively used as insecticides and herbicides, they can form deposits in soil and, commonly, reach the river water by runoff and then, they are accumulated in sediments. Soil showed some pesticides (chlorpyriphos, thiabendazole and pyriproxyfen) in common with sludge samples. This can be an indication of the amendment of soils with sludges (a common practice, nowadays). Furthermore, some herbicides that are commonly sprayed near of the soil, such as terbuthylazine and metholachlor were also found.

Table 5.5 Minimum (Min), maximum (Max) and mean concentrations and frequency of detection of the selected pesticides found in the soil, sediment and sludge samples analyzed.

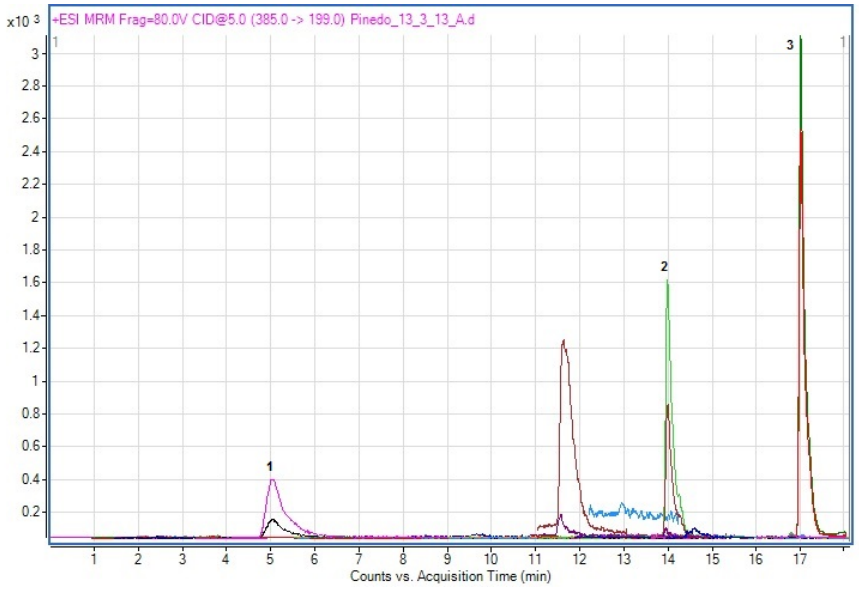
Matrix/Pesticide	**Concentration (ng g^{-1})**			**Frequency**
	Min	**Max**	**Mean**	**(%)**
Soil *(n=30)*				
Chlorpyrifos	20.7	65,308	2,851	83
Metoalachlor	4.43	4.43	4.43	3
Pyriproxifen	3.25	3.25	3.25	3
Terbuthylazine	9.48	9.48	9.48	3
Terbuthylazine-deethyl	3.22	3.22	3.22	3
Thiabendazole	0.29	0.29	0.29	3
Sediment *(n=28)*				
Carbofuran	88.9	336	213	7
Chlorpyrifos	6.33	560	114	39
Isoproturon	6.87	18.9	12.8	7
Terbumeton-deethyl	47.9	163	106	7
Terbuthylazine-deethyl	108	442	275	7
Sludge *(n=10)*				
Chlorpyrifos	0.45	703	140	90
Diazinon	9.59	20.1	16.6	30
Ethion	0.18	0.78	0.58	40
Hexythiazox	2.21	4.84	3.93	40
Imazalil	395	1,082	813	60
Pyriproxyfen	22.2	25.5	24.2	30
Thiabendazole	11.4	186	113	70

Fig. 5.6 shows the chromatograms obtained from one sediment and one sludge contaminated with some of the studied pesticides. The confirmation of positive samples was carried out, according to previous criteria cited above. Furthermore, an internal quality control was carried out for every batch of samples to check the system as well as

to avoid false positive or negatives, and it implied a matrix-matched calibration, a matrix blank and a spiked matrix sample at low concentration (10 ng g^{-1}) level. This quality control was very important to guarantee accuracy of the determination.



1 Carbofuran **2** Terbumeton- desethyl **3** Terbuthylazine deethyl **4** Chlorpyrifos



1 Thiabendazole **2** Imazalil **3** Clorpyrifos

Fig. 5.6 Chromatogram of (A) sediment and (B) sludge containing several pesticides

5.4 Conclusion

The simultaneous extraction of 50 pesticides from soil, sediments and sludges was difficult, because of the great structural variability of these pesticides. The extraction is the critical step because of it has to extract selected compounds and to reach acceptable recoveries. The efficiency and efficacy of optimized QuEChERS demonstrated to be superior to PLE. The developed analytical method could extract selected compounds from soil, sediment and sludges at low cost, reducing time consuming and increasing throughput.

Finally, the validated method was used to analyze soil, sediment and sludge samples from the Túria River basin, detectingchlorpyriphos, thiabendazole, imazalil, diazinon, pyriproxyfen, hexythiazox, carbofuran, isoproturon, terbuthylazine and terbumeton at concentrationsup to 65,308 ng g^{-1}d.w. The applicability of QuEChERS for this type of organic contaminants as well as the excellent sensitivity obtained using LC-MS/MS has been demonstrated.

Acknowledgements

This work has been supported by the Spanish Ministry of Economy and Competitiveness through the projects Consolider-Ingenio 2010 CSD2009, and CGL2011-29703-C02-02.

References

[1] Blasco C, Font G, Pico Y. J Chromatogr A 2002; 970: 201-12.

[2] Pindado Jimenez O, Perez Pastor RM. Anal Chim Acta 2012; 724: 20-9.

[3] Carvalho PN, Rodrigues PN, Alves F, Evangelista R, Basto MC, Vasconcelos M. Talanta 2008; 76: 1124-9.

[4] Salvado V, Alcaide A, Carandell N, Hidalgo M. Int J Environ Anal Chem 2001; 81: 243-56.

[5] Kvicalova M, Doubravova P, Jobanek R, Jokesova M, Ocenaskova V, Suessenbekova H, et al. Bull Environ Contam Toxicol 2012; 89: 21-6.

[6] Vega Moreno D, Sosa Ferrera Z, Santana Rodiguez JJ. Anal Chim Acta 2006; 571: 51-7.

[7] Herbert P, Morais S, Paiga P, Alves A, Santos L. Anal Bioanal Chem 2006; 384: 810-6.

[8] Guo L, Lee HK. J Chromatogr A 2013; 1286: 9-15.

[9] Martinez K, Barcelo D. Fres J Anal Chem 2001; 370: 940-5.

[10] Han M, Gan Z, Liang S, An L, Zhao X. Bull Environ Contam Toxicol 2012; 88: 858-62.

[11] Vagi M, Petsas A, Kostopoulou M, Karamanoli M, Lekkas T. Desalination 2007; 210: 146-56.

[12] Directive 2008/105/EC of the European Parliament and of the council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council. Official Journal of the European Union2008. p. 84-97.

[13] Gerstl Z, Kliger L. J Environ Sci Health Part B-Pestic Contam Agric Wastes 1990; 25: 729-41.

[14] Andreu V, Pico Y. TrAC-Trends Anal Chem 2004; 23: 772-89.

[15] Kalogridi EC, Christophoridis C, Bizani E, Drimaropoulou G, Fytianos K. Environmental science and pollution research international 2014; 21: 7252-62.

[16] Masia A, Campo J, Vazquez-Roig P, Blasco C, Pico Y. J Hazard Mat 2013; 263: 95-104.

[17] Berlioz-Barbier A, Vauchez A, Wiest L, Baudot R, Vulliet E, Cren-Olive C. Anal Bioanal Chem 2014; 406: 1259-66.

[18] Pinto MI, Vale C, Sontag G, Noronha JP. Int J Environ Anal Chem 2013; 93: 1638-59.

[19] Köck-Schulmeyer M, Olmos M, Lopez de Alda M, Barcelo D. J Chromatogr A 2013; 1305: 176-87.

[20] Regueiro J, Matamoros V, Thibaut R, Porte C, Bayona JM. Chemosphere 2013; 91: 1165-75.

[21] Chiaia-Hernandez AC, Krauss M, Hollender J. Environ Sci Technol 2013; 47: 976-86.

[22] Harrison R, Bull I, Michaelides K. Anal Methods 2013; 5: 2053-8.

[23] Lazartigues A, Fratta C, Baudot R, Wiest L, Feidt C, Thomas M, et al. Talanta 2011; 85: 1500-7.

[24] Wu J, Lin Y, Lu J, Wilson C. Sci Total Environ, 2011; 409: 3482-91.

[25] Camino-Sanchez F, Zafra-Gomez A, Perez-Trujillo J, Conde-Gonzalez J, Marques J, Luis Vilchez J. Chemosphere 2011; 84: 869-81.

[26] Naeeni MH, Yamini Y, Rezaee M. J Supercrit Fluids 2011; 57: 219-26.

[27] Brondi S, de Macedo A, Vicente G, Nogueira A. Bull Environ Contam Toxicol 2011; 86: 18-22.

[28] Hassan J, Farahani A, Shamsipur M, Damerchili F. J Hazard Mat 2010; 184: 869-71.

[29] Li H, Wei Y, You J, Lydy MJ. Talanta 2010; 83: 171-7.

[30] Wang D, You J, Lydy MJ. Arch Environ Contam Toxicol 2010; 59: 382-92.

[31] Darwano H, Duy SV, Sauve S. Arch Environ Contam Toxicol 2014; 66: 582-93.

[32] You J, Wang D, Lydy MJ. Talanta 2010; 81: 136-41.

[33] Andreu V, Ferrer E, Rubio JL, Font G, Pico Y. Sci Total Environ, 2007; 378: 124-9.

[34] Pico Y. TrAC-Trends Anal Chem 2013; 43: 84-99.

[35] Moreno D, V, Ferrera Z, Rodriguez J. J Agric Food Chem 2006; 54: 7747-52.

[36] Lu H, Lin Y, Wilson P. Bull Environ Contam Toxicol 2009; 83: 621-5.

[37] Carmona E, Andreu V, Picó Y. Sci Total Environ, 2014; 484: 53-63.

[38] Vazquez-Roig P, Segarra R, Blasco C, Andreu V, Pico Y. J Chromatogr A 2010; 1217: 2471-83.

[39] Anastassiades M, Lehotay SJ, Stajnbaher D, Schenck FJ. J AOAC Int 2003; 86: 412-31.

[40] European Commission. Protection ECDGHaC. Residue analytical methods - for post-registration control and enforcementSANCO/825/00 rev. 8.1, 16/11/2010 updated 2010.

[41] European Commission Directorate-General ECHCP. Method Validation and Quality Control Procedures for Pesticide ResiduesSANCO/12571/2013 – implem. By 01/01/14 upd. 08/01/14 2013.

Suplementary material

Assessment of two extraction methods to determine pesticides in soils, sediments and sludges. Application to the Túria River Basin.

Ana Masiá*, Karina Velasquez, Julián Campo, Yolanda Picó

Food and Environmental Safety Research Group, Department of Medicine Preventive, Faculty of Pharmcy, University of Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia Spain.

* Corresponding author. Tel. +34 963544873; fax: +34 963544954
E-mail address: Ana.Masia@uv.es

Table S5.1 Phisico-chemical properties

This table shows the pesticides and some of their transformation products monitored during 2010 and 2011 campaigns.They were selected according to their extend use, water solubility and amenability to LC-MS analysis. 42 pesticides, with different uses and different physicochemical characteristics and toxicity, were analyzed during the first campaign. 8 more target compounds were introducedto be analyzed in the second year, making a total of 50 pesticides. The increase in the number of target pesticides from first campaign to second one was due to a non-target analysis of the samples from first monitoring in a TOF-MS analyzer revealed the presence of them in most of the samples.

	CAS NUMBER	MOLECULAR FORMULA	PM (g/mol)	log Kow (pH 7, 20 ºC)	GUS INDEX-LEACHABILITY	BIOCIDE ACTION
3-Hydroxycarbofuran	16655-82-6	$C_{12}H_{15}NO_4$	237,25	1.45 (low)	1.45 (low)	Metabolite
Acethochlor	34256-82-1	$C_{14}H_{20}ClNO_2$	269,77	4.14 (high)	1.77 (low)	Herbicide
Alachlor	15972-60-8	$C_{14}H_{20}ClNO_2$	269,77	3.09 (high)	0.80 (low)	Herbicide
Atrazine	1912-24-9	$C_8H_{14}ClN_5$	215,68	2.7 (moderate)	3.30 (high)	Herbicide
Azinphos-ethyl	2642-71-9	$C_{12}H_{16}N_3O_3PS_2$	345,38	3.18 (high)	1.4 (low)	Insecticide, Acaricide
Azinphos-methyl	86-50-0	$C_{10}H_{12}N_3O_3PS_2$	317,32	2.96 (moderate)	0.95 (low)	Insecticide, Acaricide
Buprofezin	69327-76-0	$C_{16}H_{23}N_3OS$	305,44	4.93 (high)	0.46 (low)	Insecticide, Acaricide
Carbendazim	10605-21-7	$C_9H_9N_3O_2$	191,21	1.48 (low)	2.64 (transition state)	Fungicide
Carbofuran	1563-66-2	$C_{12}H_{15}NO_3$	221,26	1.8 (low)	3.02 (high)	Insecticide, Acaricide, Nematicide
Chlofenvinphos	470-90-6	$C_{12}H_{14}Cl_3O_4P$	359,6	3.8 (high)	1.87 (transition state)	Insecticide, Acaricide
Chlorpyriphos	5598-13-0	$C_7H_7Cl_3NO_3PS$	322,53	4 (high)	0.2 (low)	Insecticide, Acaricide
Deisopropylatrazine	1007-28-9	$C_5H_8ClN_5$	173,6	1,15 (low)	-	Metabolite
Deethylatrazine	6190-65-4	$C_6H_{10}ClN_5$	187,63	1.51 (low)	3.54 (high)	Metabolite
Diazinon	333-41-5	$C_{12}H_{21}N_2O_3PS$	304,35	3.69 (high)	1.14 (low)	Insecticide, Acaricide, Repelent

	CAS NUMBER	MOLECULAR FORMULA	PM (g/mol)	log Kow (pH 7, 20 ºC)	GUS INDEX-LEACHABILITY	BIOCIDE ACTION	CHEMICAL FAMILY
Diclofenthion	97-17-6	$C_{10}H_{13}Cl_2O_3PS$	315,15	5.14 (high)	2.14 (transition state)	Insecticide	Organophosphorus
Dimethoate	60-51-5	$C_5H_{12}NO_3PS_2$	229,26	0.704 (low)	1.06 (low)	Insecticide, Acaricide	Organophosphorus
Diuron	330-54-1	$C_9H_{10}Cl_2N_2O$	233,09	2.87 (moderate)	1.83 (transition state)	Herbicide	Urea
Ethion	563-12-2	$C_9H_{22}O_4P_2S_4$	384.48	5.07 (high)	0.00 (low)	Insecticide, Acaricide	Organophosphorus
Fenitrothion	122-14-5	$C_9H_{12}NO_5PS$	277.23	3.32 (high)	0.48 (low)	Insecticide	Organophosphorus
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	-	-	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)	1.11 (low)	Insecticide	Organophosphorus
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)	0.04 (low)	Acaricide	Acaricide
Imazalil	35554-44-0	$C_{14}H_{14}Cl_2N_2O$	297.18	2.56 (low)	0.55 (low)	Fungicide	Azol
Imidacloprid	138261-41-3	$C_9H_{10}ClN_5O_2$	255.66	0.57 (low)	3.76 (high)	Insecticide	Neonicotinoid
Isoproturon	34123-59-6	$C_{12}H_{18}N_2O$	206,3	2.5 (low)	2.07 (transition state)	Herbicide	Urea
Malathion	121-75-5	$C_{10}H_{19}O_6PS_2$	330.36	2.75 (moderate)	(-)1,28 (low)	Insecticide, Acaricide	Organophosphorus
Methiocarb	2032-65-7	$C_{11}H_{15}NO_2S$	225.31	3.18 (high)	0.17 (low)	Insecticide	Carbamates

	CAS NUMBER	MOLECULAR FORMULA	PM (g/mol)	log Kow (pH 7, 20 ºC)	GUS INDEX-LEACHABILITY	BIOCIDE ACTION	CHEMICAL FAMILY
Metolachlor	51218-45-2	$C_{15}H_{22}ClNO_2$	283.8	3.4 (high)	3.49 (high)	Herbicide	Chloroacetanilide
Molinate	2212-67-1	$C_9H_{17}NOS$	187.3	2.86 (moderate)	2.49 (transition state)	Herbicide	Carbamates
Omethoate	1113-02-6	$C_5H_{12}NO_4PS$	213.2	(-) 0.74 (low)	2.73 (transition state)	Insecticide, Acaricide	Organophosphorus
Parathion-ethyl	56-38-2	$C_{10}H_{14}NO_5PS$	291.26	3.83 (high)	2.09 (transition state)	Insecticide, Acaricide	Organophosphorus
Parathion-methyl	298-00-0	$C_8H_{10}NO_5PS$	263.21	3 (moderate)	1.46 (low)	Insecticide	Organophosphorus
Prochloraz	67747-09-5	$C_{15}H_{16}Cl_3N_3O_2$	376.7	3.5 (high)	1.75 (low)	Fungicide	Azol
Propanil	709-98-8	$C_9H_9Cl_2NO$	218.08	2.29 (low)	0.72 (low)	Herbicide	Anilide
Propazine	139-40-2	$C_9H_{16}ClN_5$	229.71	3.95 (high)	3.84 (high)	Herbicide	Triazine
Pyriproxyphen	95737-68-1	$C_{20}H_{19}NO_3$	321.37	5.37 (high)	(-)0.33 (low)	Insecticide	Juvenile Hormone Mimi
Simazine	122-34-9	$C_7H_{12}ClN_5$	201.66	2.3 (low)	2.00 (transition state)	Herbicide	Triazine
Tebuconazole	107534-96-3	$C_{16}H_{22}ClN_3O$	307.82	3.7 (high)	2.00 (transition state)	Fungicide	Triazole
Terbumeton	33693-04-8	$C_{10}H_{19}N_5O$	225.29	3.04 (high)	3.79 (high)	Herbicide	Triazine
Terbumeton-deethyl							
Terbuthylazine	5915-41-3	$C_9H_{16}ClN_5$	229.71	3.4 (high)	3.07 (high)	Herbicide, Microbiocide, Algicide	Triazine
Terbuthylazine-2-hydroxy	66753-07-9	$C_9H_{17}N_5O$	211.33	-	4.59 (high)	Metabolite	Triazine
Terbutryn	886-50-0			3,34			Triazine

	CAS NUMBER	MOLECULAR FORMULA	PM (g/mol)	log Kow (pH 7, 20 ºC)	GUS INDEX-LEACHABILITY	BIOCIDE ACTION	CHEMICAL FAMILY
Thiabendazole	148-79-8	$C_{10}H_7N_3S$	201.25	2.39 (low)	0.36 (low)	Fungicide	Bencimidazole
Tolclophos-methyl	57018-04-9	$C_9H_{11}Cl_2O_3PS$	301.13	4.56 (high)	0.25 (low)	Fungicide	Organophosphorus

Table S5.2 Location of the sampling points

Sample code	Lanscape unit	X_ED50	Y_ED50
201	Villalba Alta-Alfambra	671693	4498425
202	Villalba Alta-Alfambra	667613	4488523
252	Villalba Alta-Alfambra	661934	4471408
255	Villalba Alta-Alfambra	673254	4500252
203	Tramacastilla	620888	4475214
204	Tramacastilla	622260	4476432
250	Tramacastilla-Noguera de A.	619076	4480720
251	Tramacastilla-Torres de A	626379	4474966
205	Gea de Albarracín	639198	4475192
206	Gea de Albarracín	642259	4474336
207	Vega de Teruel-Arquillo de San Blas	654871	4468880
253	Vega de Teruel-Arquillo de San Blas	653932	4469355
208	Vega de Teruel-Teruel	660519	4467637
209	Vega de Teruel-Teruel	659555	4468805
210	Vega de Teruel-Teruel	659792	4464765
254	Vega de Teruel-Teruel	659152	4463449
256	Zagra -Pantano	658749	4411574
213	Pantano Benagéber	661934	4403828
214	Pantano Benagéber	661834	4400271
9	Calles	682987	4388327
10	Calles	674513	4398960
11	Chulilla	679408	4392645
12	Chulilla	682987	4388327
13	Bugarra	690694	4386474
14	Bugarra	692418	4386992
17	Ribarroja-Huerta Valencia	711436	4380129
16	Parque Turia-Huerta Valencia	713695	4378176
15	La Presa-Huerta Valencia	717356	4376399
18	QuartBenáger-Huerta Valencia	722748	4370462
22	Massarojos-Huerta Valencia	723241	4379704
23	Alfara Patriarca-Huerta Valencia	724835	4380286
21	Alqueria del Pou-Huerta Valencia	728133	4370093
20	El Brosquil-Huerta Valencia	728692	4367111
19	Port Catarroja-Huerta Valencia	726311	4363694

Table S5.3 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 S5.4 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-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)
Chlorpyriphos	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)
FenthionOxon	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)
Metholachlor	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)

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)]
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-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

Figure S5.1 Extract sludges, sediment and soil (a) after extraction with acetonitrile only and (b) after dSPE clean-up

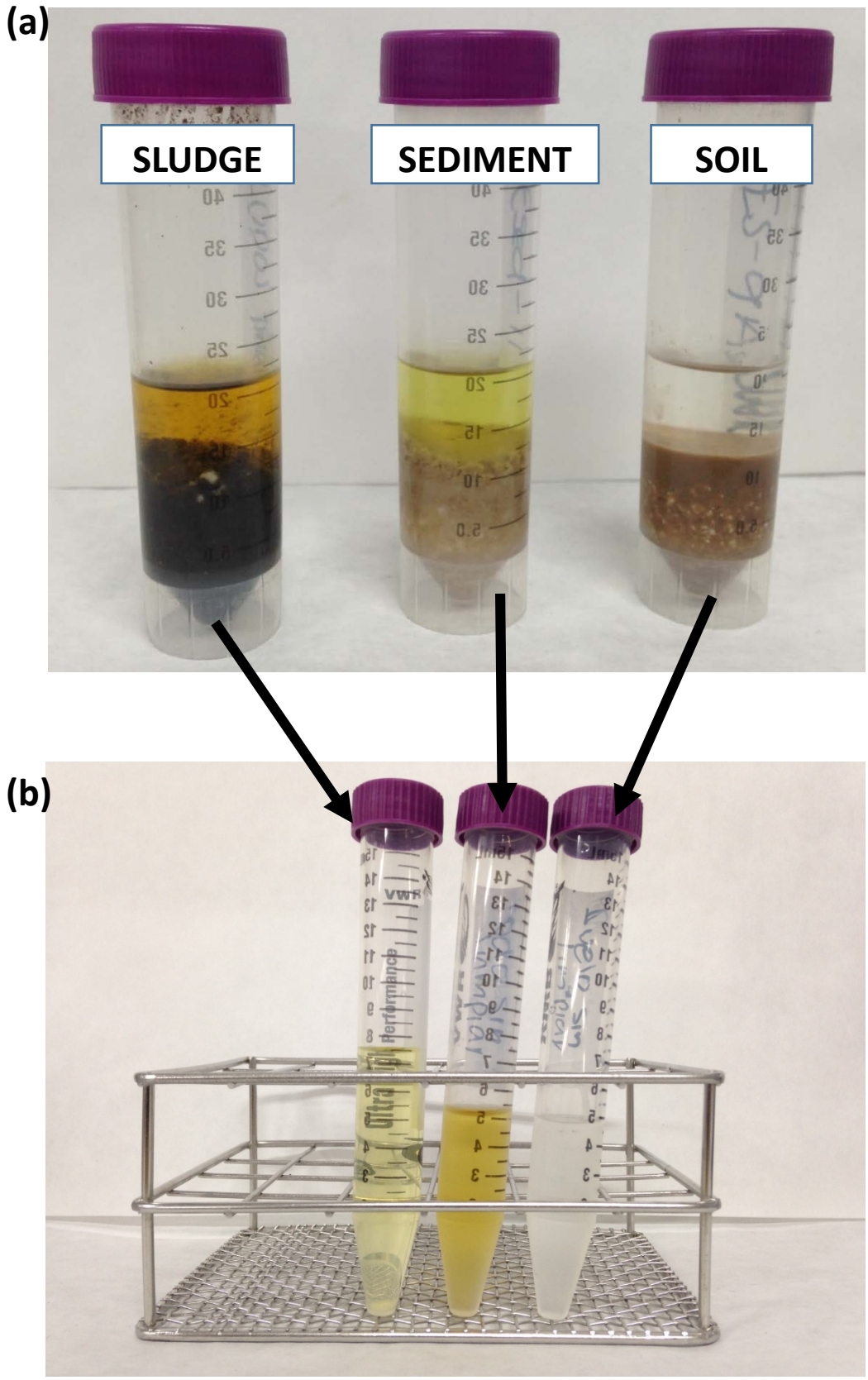
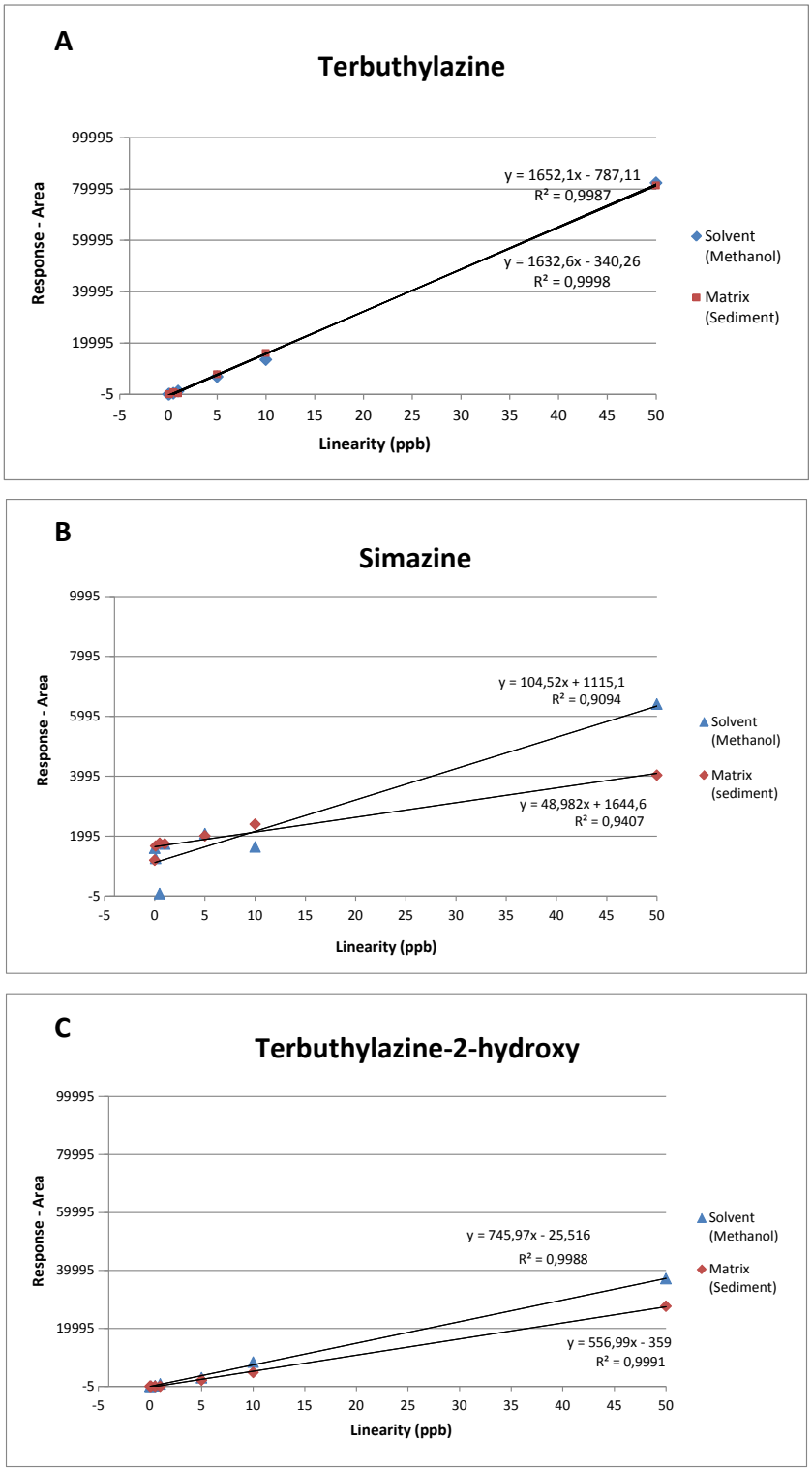


Figure S5.2 Examples of the different calibrations obtained with standards prepared in methanol and in matrix extracts (A) terbuthylazine, (B) Simazine and (C) Terbuthylazine-2-hydroxy



CAPÍTOL 6

Incidència i eficiència de l'eliminació de plaguicides en Plantes de Tractament d'Aigües Residuals (EDARs) en quatre conques mediterrànies

Publicació científica 6

Occurrence and removal efficiency of pesticides in sewage treatment plants of four Mediterranean River Basins

J. Campo, A. Masiá, C. Blasco, Y. Picó

Journal of Hazardous Materials, 263P (2013) 146-157

Journal of Hazardous Materials 263P (2013) 146–157

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Occurrence and removal efficiency of pesticides in sewage treatment plants of four Mediterranean River Basins

Julian Campo*, Ana Masiá, Cristina Blasco, Yolanda Picó

Food and Environmental Safety Research Group (SAMA-UV), Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain

HIGHLIGHTS

- Thirty-four currently used pesticides were quantified in influents and effluents.
- Up to 24 pesticides were present in dehydrated sludge samples.
- Pesticide removal efficiencies in STPs confirm that pesticides are only partially eliminated.
- STPs could be a focal point of pesticide contamination to the Rivers.

ARTICLE INFO

Article history:
Received 20 March 2013
Received in revised form 31 July 2013
Accepted 26 September 2013
Available online 3 October 2013

Keywords:
Wastewater
Sludge
LC–MS/MS
Pesticides
Removal efficiency

ABSTRACT

Removal of contaminants in the sewage treatment plants (STPs) can be incomplete causing their release to the environment. In this paper, the results of an extensive survey on more than 40 pesticides carried out in 2010 and 2011 in 16 STPs of Ebro, Guadalquivir, Jucar and Llobregat Rivers (Spain) are presented. In 2010, of 43 analytes screened, 29 were detected in influent and 28 in effluent samples, meanwhile in 2011, of 50 analytes, 33 and 34 were detected, respectively. Pesticides were in the range of 0.33 ng L^{-1} (terbumeton, 2011)–2526.05 ng L^{-1} (diuron, 2010) for influent and 0.25 ng L^{-1} (terbumeton, 2011)–2821.12 ng L^{-1} (carbendazim, 2011) for effluent. Regarding the sludge samples, 11 pesticides were detected in 2010 and 24 in 2011 at concentrations up to 25667.34 ng g^{-1} dry weight (dw). Removal efficiencies showed that, in 2010, the elimination ranged from −810% (chlorfenvinphos) to 93% (dimethoate), and in 2011, from −4575% (diazinon) to 97% (chlorfenvinphos). All these data confirm that most of the pesticides are only partially eliminated during the secondary and even tertiary treatments, commonly used in STPs, suggesting that they can be a focal point of contamination to the rivers.

1. Introduction

The re-use of sewage treatment plant (STP) effluents is currently one of the most employed strategies in several countries to deal with the water shortage problem. This water can be recycled in agricultural irrigation, for municipal and industrial purposes, to maintain the ecological flow, to recharge aquifers or it can be directly discharged into rivers or the sea [1–3]. Removal of contaminants by STPs is, in some cases, not complete and, accordingly, the occurrence of contaminants and residues in this water is of concern, because of their likely entry into the environment [4]. Currently, the European Water Framework Directive (WFD) [5] has established bases to regulate the water resources with the objective of preserving, protecting and improving their quality and sustainable use, through a list of 33 priority substances (that is now enlarging) to be controlled, the third part of which are pesticides [6].

Pesticides – widely applied to protect plants from diseases, weeds and insects damage – are also bio-accumulative and due to their vertebrate and non-vertebrate toxicity, they can affect non-target organisms [7], especially in the aquatic ecosystems [4,8]. As widely recognized and because of the extensive crop treatments, surface water pollution by pesticides mostly results from water runoff, agricultural storm-water discharges and return flows from irrigated agriculture. As the runoff moves, it picks up and carries away pesticides, finally depositing them into lakes, rivers, wetlands, coastal waters and ground waters. Polar and highly soluble pesticides have been frequently detected mainly in surface and wastewaters at ng L^{-1} concentration range [1,9–16]. However, much of this uncontrolled runoff finishes also in the STPs. There are few peer-reviewed articles that analyse concentrations of pesticides in Spanish STPs [1,17] and these have been reported mainly in those located along the Llobregat River [4,8,10], making clear a lack of research in other rivers. Additionally, due to the lack of

* Corresponding author. Tel.: +34 963543092; fax: +34 963544954.
E-mail address: julian.campo@uv.es (J. Campo).

0304-3894/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jhazmat.2013.09.061

reference levels in wastewaters, the removal efficiency of pesticides in such waters has been scarcely determined.

This work presents the results of, to our knowledge, the first extensive monitoring survey that was carried out in 2010 and 2011 in STPs from Ebro, Guadalquivir, Jucar and Llobregat Rivers in Spain. More than 40 currently used pesticides, belonging to different chemical classes, have been monitored (differently to other studies that screened a smaller number of pesticides, ca. 17–25) [18–20]. Pesticide concentrations have been analysed in the influent, effluent and dehydrated sludge from main STPs located along the rivers. With these data, removal efficiencies of such STPs have been calculated and reported. The final objective of this study is to improve the knowledge about the causes of aquatic environments contamination considering the STPs as point sources of contaminants such as pesticides.

2. Materials and methods

2.1. Description of the study area

This study covers 16 STPs that dump their effluents to Ebro (6), Guadalquivir (5), Jucar (2) and Llobregat (3) Rivers, which are between the 15 longest rivers in Spain (910, 657, 498 and 156 km). These areas were selected because of their economic and environmental importance [21]. The Ebro River is the most important river in Spain with a drainage basin of 85534 km^2. Its delta is in intensive agricultural use for rice, fruit (in particular citrus), and vegetables. The Guadalquivir River is, together with its tributaries, the main water source of the Andalusian region (more than 7 million inhabitants). Approximately 7000 km^2 of its basin (57,527 km^2) are devoted to agriculture, with very high production of rice, olives and fruits. The Jucar River basin has an area of 21,632 km^2, serving 1,030,979 people. Urbanized, industrial, and agricultural uses are located mainly in its lowest part with and irrigated area of 1879 km^2. The Llobregat River is the second longest river in Catalonia, and it is one of Barcelona's major drinking water resources. Its basin of 4957 km^2 receives extensive urban and industrial (e.g. tannery, food products, textile, pulp and paper) wastewater discharges as well as surface runoff from agricultural areas (vineyards).

2.2. Sampling

The location of the STPs monitored in the four River Basins is shown in Fig. 6.1. The STPs analysed in the Ebro River were Logro ño, Pamplona, Tudela, Zaragoza, Lleida and Tortosa; in the Jucar River, Cuenca and Alzira; in the Llobregat River, Igualada and Manresa (in 2011 Abrera STP was added); and in the Guadalquivir River, Cordoba, Loja, Moron de la Frontera, Ranilla and Copero (these last two treat the wastewaters from Sevilla city, 4th largest city in Spain). Their characteristics are summarized in Table 6.1.

Influent, effluent and dehydrated sludge samples were collected in two campaigns carried out in October of 2010 and 2011 in each STP (15 in 2010 and 16 in 2011). Integrated samples of both influent and effluent were taken using automatic 24-h volume-proportional composite sampling (the device takes a constant sample volume at variable time intervals after a certain volume of sewage has passed the sampling point). In 2010, punctual influent and effluent samples were analysed in Alzira, Loja and Cuenca STPs meanwhile in 2011, only the samples of Cuenca STP were punctual. Dehydrate sludge samples were provided by the STPs operators and they were transferred to aluminium foil. Before the analysis, both wastewaters were vacuum filtered through 1 μm glass fiber filters followed by 0.45 μm nylon membrane filters (VWR, Barcelona, Spain). It is important to state that during the studied periods the influent and effluent flows did not change significantly.

Table 6.1
Description of Sewage Treatment Plants in the different basins (quality parameters are representative of the whole year).

Basin	STP name	Equivalent people[a]	Served people (inhabitants)	Flow (m^3 day^{-1})	Treatments	Influent quality (mg L^{-1})	Effluent quality (mg L^{-1})
Ebro	Pamplona	776190	349104 (2011)	86141 (2011)	Secondary[b]	BOD_5[e] = 334; SS[f] = 298	BOD_5 = 19; SS = 13 (2000)
	Logroño	466560	242374 (2011)	51387 (2011)	Secondary[b]	BOD_5 = 283; SS = 217	BOD_5 = 9.9; SS = 8.4 (2011)
	Tudela	46237	38969 (2010)	18573 (2010)	Secondary[c]	BOD_5 = 223; SS = 214	BOD_5 = 11; SS = 18 (2010)
	Zaragoza	1200000	652472 (2011)	259200 (2011)	Secondary[b]	BOD_5 = 288	BOD_5 = 23 (2011)
	Lleida	186000	140000 (2009)	59706 (2009)	Secondary[b]	BOD_5 = 170; SS = 120	BOD_5 = 8.5; SS = 6 (2011)
	Tortosa	46847	34642 (2011)	6670 (2011)	Secondary[c]	BOD_5 = 355; SS = 343	BOD_5 = 6; SS = 11 (2011)
Guadalquivir	Cordoba	522000	516128 (2012)	108000 (design)	Secondary[c]	BOD_5 = 1540; SS = 344	BOD_5 = 77; SS = 20 (2013)
	Loja	30480	30480 (design)	–	Secondary[c]	–	–
	Moron de la Frontera	30000	30000 (design)	90000 (design)	Secondary[c]	BOD_5 = 100	BOD_5 = 3.5 (2010)
	Copero (Sevilla)	1487500	711000	135000 (2010)	Secondary[b]	BOD_5 = 244; SS = 254	BOD_5 = 156; SS = 95 (2013)
	Ranilla (Sevilla)	555000	555000 (design)	90000 (design)	Tertiary[d]	BOD_5 = 370; SS = 400	BOD_5 = 25; SS = 35 (design)
Llobregat	Manresa	196167	196167 (design)	53500 (design)	Secondary[b]	BOD_5 = 220; SS = 310	BOD_5 = 11; SS = 24.8 (2011)
	Igualada	285666	80000 (design)	20000 (design)	Secondary[c]	BOD_5 = 1118; SS = 875	BOD_5 = 56; SS = 87.5 (2011)
	Abrera	80000	285666 (design)	23000 (design)	Secondary[b]	BOD_5 = 200; SS = 225	BOD_5 = 4; SS = 4.5 (2011)
Jucar	Cuenca	80000	57000 (2012)	15000 (2012)	Secondary[c]	–	–
	Alzira	232656	96230 (2012)	33584 (2012)	Tertiary[d]	BOD_5 = 220; SS = 220	BOD_5 = 11; SS = 4.4 (2012)

[a] Equivalent people: design values.
[b] Pre-treatment: screening, sand and fat-free/Biological treatment: aeration, flocculation and settling, denitrification.
[c] Pre-treatment: screening, sand and fat-free/Biological treatment: aeration, flocculation and settling.
[d] Pre-treatment: screening, sand and fat-free/Biological treatment: aeration, flocculation and settling/Tertiary treatment: microfiltration, blending, deodorization.
[e] BOD_5: Biochemical oxygen demand (five days).
[f] SS: Suspended sediments.

Fig. 6.1 Location of the STPs along the course of the different Rivers.

2.3. Chemicals and reagents

Selected pesticides (43 in 2010 and 50 in 2011) belong to different chemical families, with a variety of uses as well as different physicochemical characteristics and toxicity. Among them, there are chloroacetanilides, carbamates, triazines, organophosphorus, etc. Exhaustive description of selected target pesticides is provided in Supplementary content Table S6.1.

2.4. Sample preparation and instrumental analysis

2.4.1. Water extraction

An off-line solid-phase extraction (SPE) procedure was used for the pre-concentration of water samples [22]. Very briefly, water samples (250 mL) were vacuum passed through STRATA cartridges (Phenomenex SPE cartridge 200 mg sorbent/6 mL cartridge, Torrance, CA, USA), previously preconditioned (5 mL methanol–dichloromethane (50:50, v/v) and 10 mL water). The cartridges were air-dried and, then, analytes were eluted with 10 mL of dichloromethane–methanol (50:50, v/v) drop by drop. Extracts were evaporated to dryness and re-constituted with 1 mL of methanol.

2.4.2. Sludge extraction

The QuEchERS method was applied to 1 g of lyophilized sludge sample, which was weighed in a 50 mL falcon tube, and homogenized with 7.5 mL water and 10 mL acetonitrile. Then, $MgSO_4$ (6 g), NaCl (1.5 g), tri-sodium citrate dehydrate [$Na_3C_6H_5O_7 + 2H_2O$ (1.5 g)] and disodium hydrogen citrate sesquihydrate [$HOC(COOH)(CH_2COONa)_2 \cdot 1.5H_2O$ (0.75 g)] were added. The mixture was agitated intensively in a vortex for 1 min and centrifuged at 3000 rpm for 5 min.

An aliquot (1 mL) of the upper organic phase was cleaned up by dispersive solid phase extraction (d-SPE) clean-up using PSA (50 mg), $MgSO_4$ (150 mg) and C_{18} (50 mg). This mixture was shaken in a vortex for 1 min and centrifuged at 3000 rpm for 5 min. The supernatant was analysed.

2.4.3. LC–MS/MS determination

The chromatographic instrument was an HP1200 series LC – with 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). Data were processed using a MassHunter Workstation Software for qualitative and quantitative (external standard methodology based on peak areas) analysis (A GL Sciences, Tokyo, Japan). Detailed information relating to instrumental determination is listed in Tables S6.2 and S6.3 (supplementary information).

2.4.4. Quality assurance/quality control

To monitor the results a strong quality control was performed. The analytical methods were carefully validated. In water samples, recoveries ranging from 48% to 70%, with relative standard deviations between 2% and 19%; and low limits of quantification were achieved for all selected pesticides. For dehydrated sludge samples, recoveries were between 40% and 105% and, relative standard deviation was in all cases below 20% at the limits of quantification (LOQs). These limits were 0.1–5.0 ng g^{-1} for sediments and 0.01–5 ng L^{-1} for influent and effluent waters (Detailed information for the recovery of each pesticide, and its standard deviation, for both matrixes is available in Supplementary information Table S6.4). Calibration curves were prepared daily obtaining $R^2 > 0.98$.

Prior and after each sampling batch, the calibration curve was injected. Each 15 samples, one procedural blank and one positive control were analysed.

3. Results and discussion

3.1. Occurrence of selected pesticides in wastewater samples

Of the 43 analytes included in 2010, 29 were found in influent and 28 in effluent samples (Table 6.2). In 2011, of the 50 analytes screened, 33 and 34 were detected in at least one occasion in influent and effluent samples, respectively. Therefore, there are not apparent differences in the number of pesticides found at the inlet and at the outlet of STPs considered.

The use of pesticides could be seasonal. In the case of this survey, the different locations of the STPs cover the national territory of Spain (5,05,992 km^2), thus the climatic conditions were highly different and the seasonal compartmentalization, at least, complicated. Taken into consideration the flow of the surrounding rivers,

Table 6.2
Concentration and occurrence frequency (in percentage) of each pesticide in the wastewater samples analysed in 2010 and 2011. Concentrations are given as range (minimum–maximum), while mean concentrations are reported in parenthesis.

Family/compound	2010		2011	
	Mean (ng L^{-1})	Frequency (%)	Mean (ng L^{-1})	Frequency (%)
Anilide	2.01–49.76 (14.60)	10.00	n.d.	0
Propanil	2.01–49.76 (14.60)	10.00	n.d.	0
Azole	2.87–2120.84 (158.06)	90.91	2.62–229.08 (23.84)	37.50
Imazalil	5.22–2120.84 (292.32)	80.00	2.62–229.08 (35.88)	68.75
Prochloraz	2.87–63.15 (23.81)	86.67	11.81–11.81 (11.81)	6.25
Benzimidazole	n.a.	n.a.	0.59–2821.12 (154.71)	92.19
Carbendazim	n.a.	n.a.	4.89–2821.12 (268.87)	46.88
Thiabendazole	n.a.	n.a.	0.59–505.36 (40.56)	90.63
Carbamate	2.79–140.40 (24.72)	16.67	1.26–105.31 (7.43)	10.16
3-Hydroxycarbofuran	4.48–140.40 (72.62)	10.00	4.05–4.05 (4.05)	3.13[b]
Carbofuran	2.79–42.10 (9.00)	26.67	2.76–4.46 (3.61)	6.25
Methiocarb	3.77–5.74 (4.73)	20.00	1.26–105.31 (14.92)	31.25
Molinate	5.76–19.16 (12.54)	10.00	n.d.	0.00
Carboxamide	0.46–15.71 (4.97)	90.00	1.34–2.03 (1.78)	12.50
Hexythiazox	0.46–15.71 (4.97)	90.00	1.34–2.03 (1.78)	12.50
Chloroacetanilide	34.27–313.51 (129.21)	11.11	1.15–42.59 (23.50)	8.33
Acetochlor	n.d.	0.00	11.93–35.17 (28.86)	9.38
Alachlor	n.d.	0.00	n.d.	0.00
Metolachlor	34.27–313.51 (129.21)	33.33	1.15–42.59 (18.14)	15.63
J hormone mimic	0.48–75.46 (17.92)	90.00	1.58–1.58 (1.58)	3.13
Pyriproxyfen	0.48–75.46 (17.92)	90.00	1.58–1.58 (1.58)	3.13[a]
Neonicotinoid	2.00–6.75 (3.39)	66.67	1.39–165.66 (34.44)	59.38
Imidacloprid	2.00–6.75 (3.39)	66.67	1.39–165.66 (34.44)	59.38
Organophosphorus	1.02–848.00 (78.11)	23.65	0.69–640.16 (30.84)	14.06
Azinphos-Ethyl	52.85–65.49 (59.17)	6.67[b]	38.18–135.50 (86.84)	6.25
Azinphos-Methyl	n.d.	0.00	n.d.	0.00
Chlorfenvinphos	3.07–268.10 (52.59)	80.00	0.69–78.16 (18.06)	43.75
Chlorpyriphos	1.02–163.72 (19.09)	90.00	0.86–108.68 (14.08)	71.88
Diazinon	3.58–315.97 (73.87)	96.67	0.72–75.34 (15.88)	84.38
Dichlofenthion	7.48–34.86 (14.59)	53.33	n.d.	0.00
Dimethoate	2.82–620.56 (101.09)	93.33	1.98–640.16 (88.13)	50.00
Ethion	n.d.	0.00	4.22–12.80 (8.62)	9.38
Fenchlorphos	n.d.	0.00	n.a.	n.a.
Fenitrothion	23.79–23.79 (23.79)	3.33[a]	n.d.	0.00
Fenoxon	n.d.	0.00	n.d.	0.00
Fenoxon Sulfone	n.d.	0.00	16.76–16.76 (16.76)	3.13[b]
Fenoxon Sulfoxide	5.14–50.36 (17.07)	20.00	n.d.	0.00
Fenthion	n.d.	0.00	n.d.	0.00
Fenthion Sulfone	10.07–35.34 (15.96)	33.33	13.22–13.22 (13.22)	3.13[a]
Fenthion Sulfoxide	n.d.	0.00	14.53–16.76 (15.65)	6.25
Malathion	848.00–848.00 (848.00)	3.33[a]	n.d.	0.00
Omethoate	2.42–5.64 (3.98)	16.67	2.80–2.80 (2.80)	3.13[b]
Parathion-Ethyl	n.d.	0.00	n.d.	0.00
Parathion-Methyl	n.d.	0.00	n.d.	0.00
Tolclofos-Methyl	n.d.	0.00	n.d.	0.00
Triazine	3.90–277.44 (27.20)	35.38	0.25–236.83 (20.93)	35.23
Atrazine	3.90–27.44 (12.82)	46.67	7.20–36.90 (20.88)	12.50
Atrazine-Deisopropyl	14.55–41.79 (21.97)	20.00	31.60–34.63 (33.12)	6.25
Atrazine-Desethyl	15.99–158.37 (56.77)	56.67	6.42–24.74 (19.79)	9.38
Propazine	6.74–277.40 (42.82)	26.67	1.53–5.70 (3.66)	9.38
Simazine	5.00–5.00 (5.00)	6.67	4.55–37.79 (20.25)	21.88
Terbumeton	n.a.	n.a.	0.25–49.92 (11.78)	28.13
Terbumeton-Desethyl	n.a.	n.a.	0.46–236.83 (45.94)	28.13
Terbuthylazine	n.a.	n.a.	2.15–35.54 (12.44)	50.00
Terbuthylazine-2 Hydroxy	n.a.	n.a.	0.94–176.79 (24.47)	78.13
Terbuthylazine-Desethyl	n.a.	n.a.	3.04–80.77 (19.90)	59.38
Terbutryn	5.00–182.87 (23.81)	73.33	0.82–73.46 (18.05)	84.38
Triazole	n.a.	n.a.	0.52–261.63 (22.99)	34.38
Tebuconazole	n.a.	n.a.	0.52–261.63 (22.99)	34.38
Unclassified	1.59–9.03 (5.68)	43.33	n.d.	0.00
Buprofezin	1.59–9.03 (5.68)	43.33	n.d.	0.00
Urea	1.00–2526.05 (168.64)	76.67	1.08–1217.94 (84.60)	68.75
Diuron	19.10–2526.05 (321.66)	76.67	5.56–1217.94 (158.72)	81.25
Isoproturon	1.00–101.76 (15.63)	76.67	1.08–34.22 (10.48)	56.25

n.d.: not detected; n.a.: not analysed.
[a] Only in the influent sample.
[b] Only in the effluent sample.

Guadalquivir and Llobregat Rivers were at high flow in 2010, and at medium flow in 2011. Ebro and Jucar Rivers were at medium flow in 2010 and at low-medium flow in 2011. However, in the fortnight previous to the sampling there were not rains in the four river basins, thus runoff could not be expected in the wastewater influent. Results of previous study developed in STPs from Catalonia (NE-Spain), comprising 8 different periods during almost 2 years, did not show significant differences related to cold, warm and hot

seasons [23]. Similarly, in a survey on the presence of pharmaceuticals in urban wastewater of a Spanish Mediterranean area, seasonal variation was not clearly observed [24]. This fairly constant pattern of contamination is consistent with the primary urban origin of the wastewaters.

Wastewater samples of all STPs were contaminated, in both years, with at least one pesticide. The frequency of detection (in percentage) of the different pesticide families for each River Basin in both campaigns is shown in Fig. 6.2. In 2010, organophosphorus and azole pesticides were the most frequently detected, followed by carboxamides, juvenile hormone mimics and triazines. This pattern changed the next year, and the most common screened families of pesticides were organophosphorus and triazines followed by benzimidazoles, azoles and ureas (present in more than 80% of STPs in both years). The pesticide residues pattern has to be considered having in mind that the pesticide families with higher number of analytes were the most frequently detected in samples. In both years, in the Guadalquivir STPs the frequencies of insecticides and herbicides (i.e. organophosphorus and triazines) were higher than that of fungicides (azoles and benzimidazoles). On the contrary, fungicides were more frequent in the Ebro, Jucar and Llobregat STPs. This could be related to different agricultural practices because the fungicides selected for this study are those used in the post-harvest treatment of apples, pears and citric very abundant in these three catchments.

In 2010 and 2011, almost all samples were polluted by organophosphorus as diazinon (mainly those of Ebro and Llobregat rivers) and dimethoate followed by chlorfenvinphos and chlorpyriphos (very common in the Guadalquivir STPs). Fenthion sulfone was identified in the 10 samples of 2010 in the Guadalquivir basin. Regarding the azoles, prochloraz (mainly in 2010) and imazalil (both years) were present in all the samples of Jucar and Llobregat rivers, and in 22 of the Ebro River (Fig. 6.2A). On the other hand, for triazine herbicides, the highest frequencies of atrazine-desethyl and propazine were both observed in the Guadalquivir River (2010). Terbutryn was usually found in the wastewater of all STPs, especially in those of Jucar and Llobregat rivers. In 2011, terbuthylazine was detected in samples of all rivers but its metabolites (terbuthylazine-2 hydroxy and terbuthylazine-desethyl) showed higher occurrence frequencies followed by terbumeton and terbumeton-desethyl.

The carboxamide (hexythiazox), in 2010, was observed in 29 water samples while in 2011 (Fig. 6.2B), it was only in the wastewaters of 3 STPs located in the Guadalquivir and Jucar catchments. Urea analytes were detected in the STPs of the four river catchments. However, in 2010, the Ebro River samples showed the highest frequency and, in 2011, isoproturon was mainly in those of Guadalquivir and Llobregat rivers. Carbamates were not very common any year. Methiocarb showed its highest concentration in the Ebro STPs. Among the benzimidazoles, analysed only in 2011, carbendazim and thiabendazole were found in all wastewaters analysed in the Ebro, Jucar and Llobregat basins. Acetochlor and metolachlor (chloroacetanilides) were detected in six Guadalquivir samples only during the second sampling. Finally, in 2010, anilides (propanil) were found in three Guadalquivir samples, while buprofezin (unclassified) was detected in Guadalquivir and Llobregat STPs. Some insecticides as pyriproxyfen and imidacloprid were observed in 69 water samples, being this last one more frequently detected in wastewaters of Guadalquivir River sampled in 2011. Summarizing, 8% of pesticides considered in this research were detected in 75–100% of the studied STPs, 22% in 50–75% and 20% in 25–50% (details in Supplementary information, Fig. S6.1).

Fig. 6.3 shows the maximum pesticide concentrations detected by each river catchment in 2010. In the Ebro River, the maximum concentration in the influent samples was observed for imazalil, malathion and diuron, showing up to 2121 ng L^{-1} for imazalil. The concentration of these analytes in the effluent samples was 50% lower than of that of the influents, except for the imazalil whose influent concentration was 1171 ng L^{-1}. In the Guadalquivir STPs, the higher influent concentrations were observed for diuron (2526 ng L^{-1}), dimethoate (621 ng L^{-1}), metolachlor (314 ng L^{-1}), propazine (277 ng L^{-1}) and imazalil (211 ng L^{-1}), meanwhile in the effluents only the diuron (2393 ng L^{-1}), dimethoate (520 ng L^{-1}) and metolachlor (178 ng L^{-1}) kept their high concentrations.

In the Jucar STPs, the higher concentrations in the influent samples were again for imazalil (676 ng L^{-1}), followed by diazinon (316 ng L^{-1}) and chlorfenvinphos (268 ng L^{-1}). The concentration of effluents was also high for imazalil (629 ng L^{-1}) and chlorfenvinphos (259 ng L^{-1}). The STPs samples of Llobregat River presented the lowest pesticides concentration, not surpassing the 100 ng L^{-1} in any case. The highest value detected was also for the imazalil, which reached 87 ng L^{-1} in the influent and 99 ng L^{-1} in the effluent. The diazinon concentration was also high with values of 70 and 79 ng L^{-1}, respectively (Fig. 6.3).

In 2011, several pesticides frequently identified in non-target analyses [22] were added to the screening (Table 6.2). Generally, in the Ebro STPs, analyte concentrations were lower than those observed in 2010, meanwhile in Llobregat and Guadalquivir were higher than in 2010, and in Jucar quite similar. On the other hand, differences in pesticide concentrations between the effluent and the influent were increased in 2011 for Ebro and Llobregat samples, and decreased for Guadalquivir and Jucar catchments. Higher concentrations in influents of the Ebro STPs were found for chlorpyriphos (37 ng L^{-1}), diuron (25 ng L^{-1}) and carbendazim (22 ng L^{-1}) while in the effluents the maximum values were 127 ng L^{-1} for imazalil, 105 ng L^{-1} for methiocarb, 64 ng L^{-1} for diuron and 50 ng L^{-1} for carbendazim (Fig. S6.1). In the Guadalquivir STPs, the higher concentrations in the influent samples were observed again in diuron (1022 ng L^{-1}) and dimethoate (634 ng L^{-1}), and for carbendazim (1083 ng L^{-1}) that was added in the second sampling campaign. In the effluents, diuron (1218 ng L^{-1}), carbendazim (1249 ng L^{-1}) and dimethoate (640 ng L^{-1}), kept their high concentrations, whilst tebuconazole (261 ng L^{-1}) presented and increase of two orders of magnitude with regard to the concentration obtained in the influent samples (detailed information is provided in the supplementary information, Fig. S6.2).

In the Jucar STPs, the higher concentrations in the influent samples were detected in the thiabendazole (311 ng L^{-1}), which was not included in the 2010 analyses, in the terbumeton-desethyl (237 ng L^{-1}) and the imazalil (216 ng L^{-1}). These analytes showed also high concentration in the effluent samples, 505, 161 and 229 ng L^{-1} for thiabendazole, terbumeton-desethyl and imazalil, respectively. Wastewater samples of Llobregat River presented again the lowest pesticide concentrations, except for the carbendazim that reached values of 1449 and 2821 ng L^{-1}, in the influents and effluents, respectively. Diuron concentrations were also high, with 122 ng L^{-1} in the influent and 168 ng L^{-1} in the effluent. Maximum concentrations of the other pesticides did not surpass the 100 ng L^{-1}, only the azinphos-ethyl reached 136 ng L^{-1} in the effluent samples (Supplementary information, Fig. S6.2).

Comparison of these results with data previously published in other STPs (see Table S6.5) shows that most of the concentrations detected here, mainly those of 2010, were higher than those reported previously in Spanish STPs, except those detected in one Almería's STP. In the case of Llobregat River surface waters, historical data are available (see Table S6.6). Some of them have already been compiled in a comprehensive review published by González et al. [4]. Among these, it is possible to highlight the level of simazine (2210 ng L^{-1}), atrazine (463 ng L^{-1}), diuron (239 ng L^{-1}), diazinon (785 ng L^{-1}), isoproturon (800 ng L^{-1}) and dimethoate (200 ng L^{-1}). Frequency and concentrations of common pesticides detected in this study were similar to those found in recent years

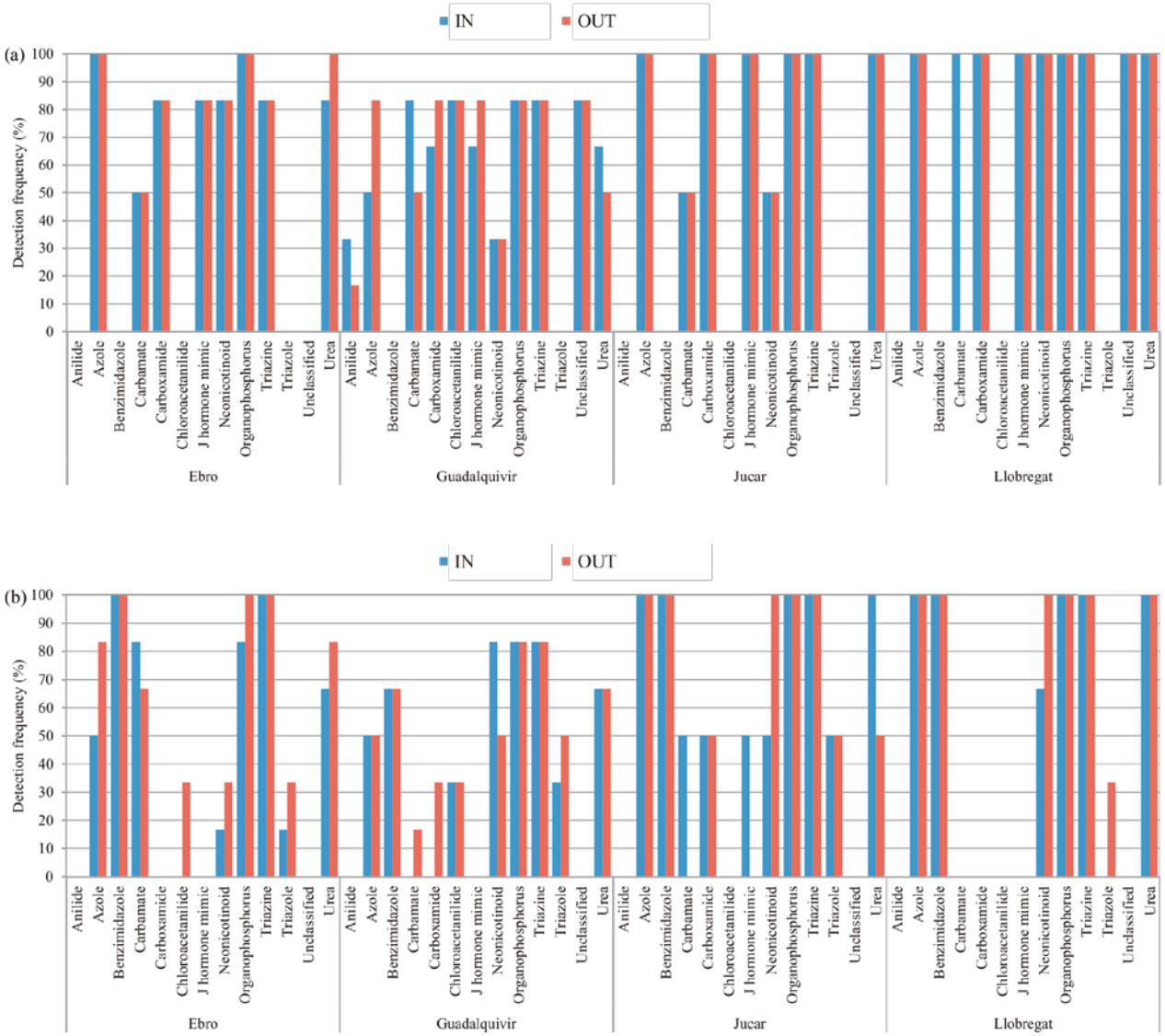


Fig. 6.2 Detection frequency (%) of the different families of pesticides in the STPs of the four river catchments and in (a) 2010 and (b) 2011 sampling campaigns. IN: influent, OUT: effluent.

(2009–2010 and 2008), particularly for atrazine, atrazine-desethyl, malathion, alachlor, metolachlor, and molinate. These concentrations are significantly lower than the ones reported in previous studies (2000–2006), which were of environmental concern. As it can be observed contaminant levels vary not only spatially but also temporally, making difficult a direct comparison among studies (Table S6.6).

Considering the Maximum Allowable Concentrations (MAC) stipulated by the Directive 2008/105/EC for pesticides in inland and other surface waters [6], diuron exceeded these limits (700 ng L^{-1} for alachlor; 2000 ng L^{-1} for atrazine; 1800 ng L^{-1} for diuron; 1000 ng L^{-1} for isoproturon; 4000 ng L^{-1} for simazine), although the limit of 500 ng L^{-1} set for total pesticides [25] was exceeded in many of the STP's effluents (in 2010: Lleida, Tortosa, Tudela, Copero, Cordoba, Ranilla, Alzira and Cuenca 2010; in 2011 Moron, Ranilla, Alzira, Abrera, Igualada). Nevertheless, it is important to emphasize that although the pesticides concentrations measured were relatively low (according to directives); this study analysed just some of them. A wide variety of other compounds, including other pesticides and pesticides transformation products, may contribute to the bad quality of wastewater when re-used, especially having in mind that there is not any legislation or rule in the European Union or United States of America about levels of pesticides in wastewaters [1].

3.2. *Occurrence of selected pesticides in sludge samples*

In the first sampling campaign, 11 pesticides were detected in the sludge, meanwhile in the second one, the number of analytes identified increased up to 24. It is important to remark that in 2011 the sludge of all treatment plants was contaminated, with at least one pesticide, similarly to what happened in 2010 with the samples of Jucar and Ebro rivers. In this year, only and 40% and 50% of the sludge of Guadalquivir and Llobregat STPs, respectively, showed positive pesticides contamination.

With regard to the frequency of the different families, and having in mind the results obtained for the wastewaters, it is possible to observe how the pesticides commonly found in the influent and

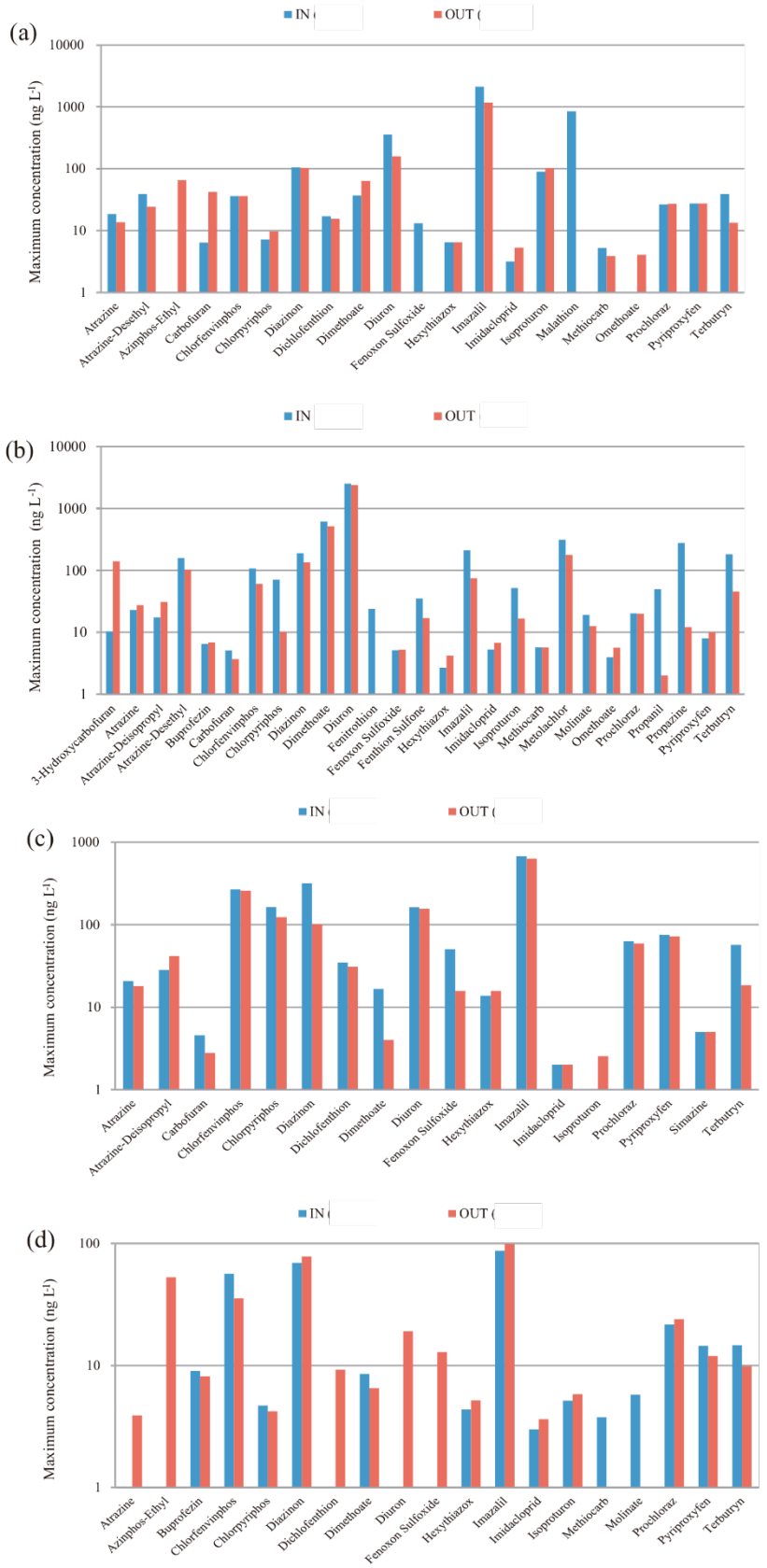


Fig. 6.3 Maximum pesticide concentration detected in (a) Ebro, (b) Guadalquivir, (c) Jucar and (d) Llobregat STPs influent (IN) and effluent (OUT) samples in 2010.

Table 6.3
Concentration and occurrence frequency (in percentage) of each pesticide in the sludge samples analysed in 2010 and 2011. Concentrations are given as range (minimum–maximum), while mean concentrations are reported in parenthesis.

Family/compound	2010		2011	
	Mean (ng g^{-1} dw)	Frequency (%)	Mean (ng g^{-1} dw)	Frequency (%)
Anilide	n.d.	0.00	29.59–29.59 (29.59)	7.14
Propanil	n.d.	0.00	29.59–29.59 (29.59)	7.14
Azole	0.10–1166.06 (120.80)	58.33	0.89–523.33 (51.60)	50.00
Imazalil	0.10–1166.06 (237.38)	91.67	0.89–523.33 (96.89)	85.71
Prochloraz	2.43–9.14 (4.21)	25.00	3.74–8.88 (6.31)	14.29
Benzimidazole	n.a.	n.a.	0.61–198.30 (48.50)	32.14
Carbendazim	n.a.	n.a.	0.61–83.87 (17.14)	42.86
Thiabendazole	n.a.	n.a.	1.16–198.30 (79.87)	21.43
Carbamate	n.d.	0.00	n.d.	0.00
3-Hydroxycarbofuran	n.d.	0.00	n.d.	0.00
Carbofuran	n.d.	0.00	n.d.	0.00
Methiocarb	n.d.	0.00	n.d.	0.00
Molinate	n.d.	0.00	n.d.	0.00
Carboxamide	1.41–7.31 (2.96)	41.67	3.26–7.63 (4.79)	21.43
Hexythiazox	1.41–7.31 (2.96)	41.67	3.26–7.63 (4.79)	21.43
Chloroacetanilide	2.24–2.24 (2.24)	5.56	n.d.	0.00
Acetochlor	n.d.	0.00	n.d.	0.00
Alachlor	n.d.	0.00	n.d.	0.00
Metolachlor	2.24–2.24 (2.24)	16.67	n.d.	0.00
J hormone mimic	4.09–40.92 (12.60)	41.67	2.72–63.04 (20.96)	35.71
Pyriproxyfen	4.09–40.92 (12.60)	41.67	2.72–63.04 (20.96)	35.71
Neonicotinoid	n.d.	0.00	0.79–1.06 (0.86)	28.57
Imidacloprid	n.d.	0.00	0.79–1.06 (0.86)	28.57
Organophosphorus	0.05–77.88 (6.58)	259.92	0.66–25667.34 (1321.27)	11.07
Azinphos-Ethyl	n.d.	0.00	n.d.	0.00
Azinphos-Methyl	n.d.	0.00	n.d.	0.00
Chlorfenvinphos	n.d.	0.00	2.19–2.19 (2.19)	7.14
Chlorpyriphos	1.02–77.88 (22.00)	91.67	1.80–112.55 (26.45)	92.86
Diazinon	0.62–21.66 (4.02)	83.33	0.66–14.62 (4.99)	71.43
Dichlofenthion	n.d.	0.00	4.12–425.90 (145.70)	21.43
Dimethoate	n.d.	0.00	n.d.	0.00
Ethion	0.03–0.03 (0.03)	16.67	1.45–25667.34 (6427.03)	28.57
Fenchlorphos	n.d.	0.00	n.a.	n.a.
Fenitrothion	0.27–0.27 (0.27)	16.67	n.d.	0.00
Fenoxon	n.d.	0.00	n.d.	0.00
Fenoxon Sulfone	n.d.	0.00	n.d.	0.00
Fenoxon Sulfoxide	n.d.	0.00	n.d.	0.00
Fenthion	n.d.	0.00	n.d.	0.00
Fenthion Sulfone	n.d.	0.00	n.d.	0.00
Fenthion Sulfoxide	n.d.	0.00	n.d.	0.00
Malathion	n.d.	0.00	n.d.	0.00
Omethoate	n.d.	0.00	n.d.	0.00
Parathion-Ethyl	n.d.	0.00	n.d.	0.00
Parathion-Methyl	n.d.	0.00	n.d.	0.00
Tolclofos-Methyl	n.d.	0.00	n.d.	0.00
Triazine	2.59–10.61 (4.41)	6.94	0.91–60.35 (6.43)	9.74
Atrazine	n.d.	0.00	n.d.	0.00
Atrazine-Deisopropyl	n.d.	0.00	n.d.	0.00
Atrazine-Desethyl	n.d.	0.00	n.d.	0.00
Propazine	n.d.	0.00	n.d.	0.00
Simazine	n.d.	0.00	n.d.	0.00
Terbumeton	n.a.	n.a.	2.72–2.72 (2.72)	7.14
Terbumeton-Desethyl	n.a.	n.a.	n.d.	0.00
Terbuthylazine	n.a.	n.a.	2.33–5.41 (4.38)	21.43
Terbuthylazine-2 Hydroxy	n.a.	n.a.	4.21–4.21 (4.21)	7.14
Terbuthylazine-Desethyl	n.a.	n.a.	n.d.	0.00
Terbutryn	2.59–10.61 (4.41)	41.67	0.91–60.35 (14.42)	71.43
Triazole	n.a.	n.a.	1.21–16.94 (6.87)	35.71
Tebuconazole	n.a.	n.a.	1.21–16.94 (6.87)	35.71
Unclassified	0.87–3.21 (1.37)	41.67	n.d.	0.00
Buprofezin	0.87–3.21 (1.37)	41.67	n.d.	0.00
Urea	n.d.	0.00	6.29–44.25 (25.27)	14.29
Diuron	n.d.	0.00	44.25–44.25 (44.25)	7.14
Isoproturon	n.d.	0.00	6.29–6.29 (6.29)	7.14

dw: dry weight; n.d.: not detected; n.a.: not analysed.

effluent samples were also present in the sludge. Thus, organophosphorus and azoles were the most frequently detected, followed, in 2010, by triazines and carboxamides and in 2011, by triazines, benzimidazoles, triazoles and the juvenile hormone mimic (Table 6.3).

In 2010, sludge samples of the Ebro STPs were contaminated with imazalil, which showed a concentration of 1166 ng g^{-1} in dry weight (dw), and with chlorpyriphos (78 ng g^{-1} dw) and diazinon (22 ng g^{-1} dw) among others (Fig. 6.4). The very high concentration of the azole in the influent and their possible stronger adsorption may be the reason for its high concentration in the sludge. On the other hand, the pesticide residue concentrations in the sludge samples of Guadalquivir River were low, reaching the highest level at only 18 ng g^{-1} dw (chlorpyriphos).

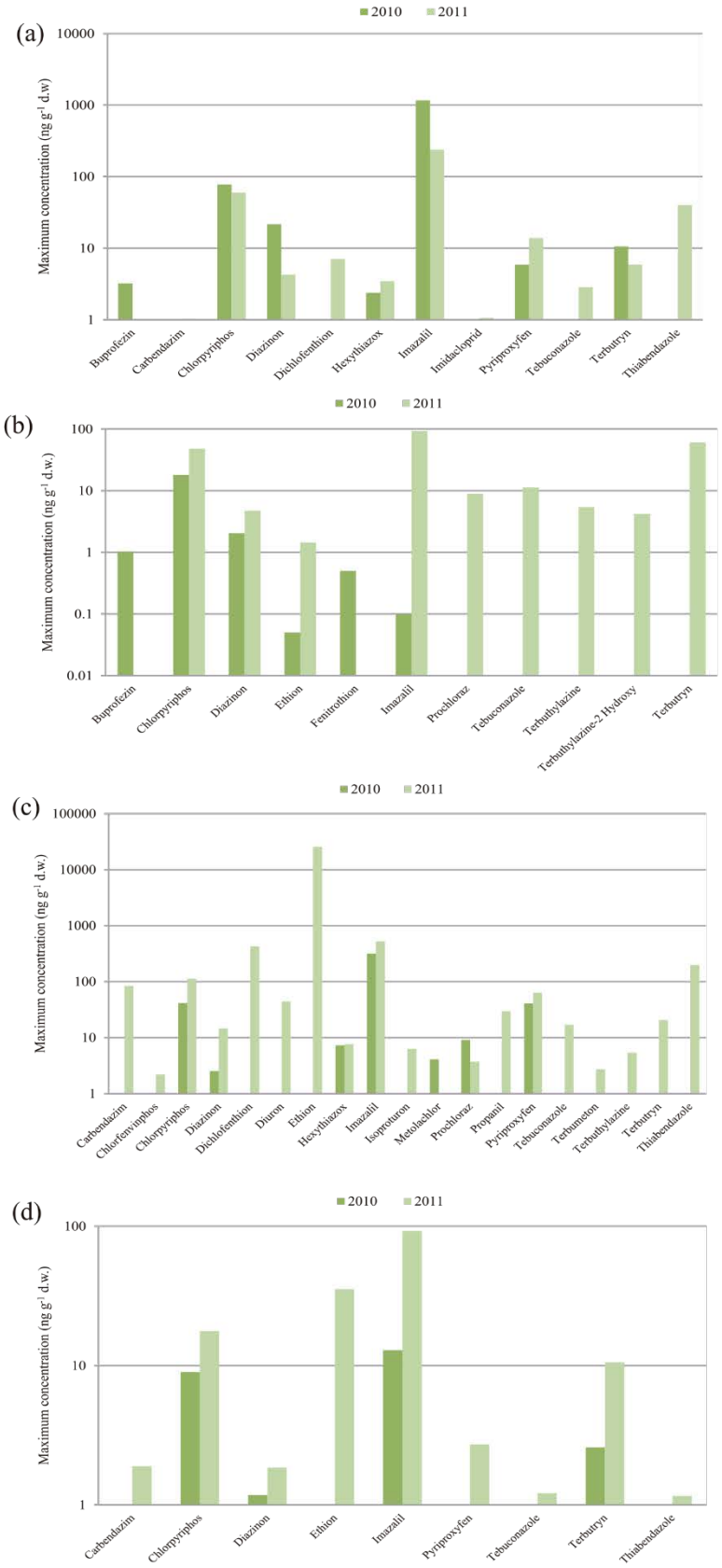


Fig. 6.4 Maximum pesticide concentration detected in (a) Ebro, (b) Guadalquivir, (c) Jucar and (d) Llobregat STPs dehydrated sludge samples in 2010. dw: dry weight

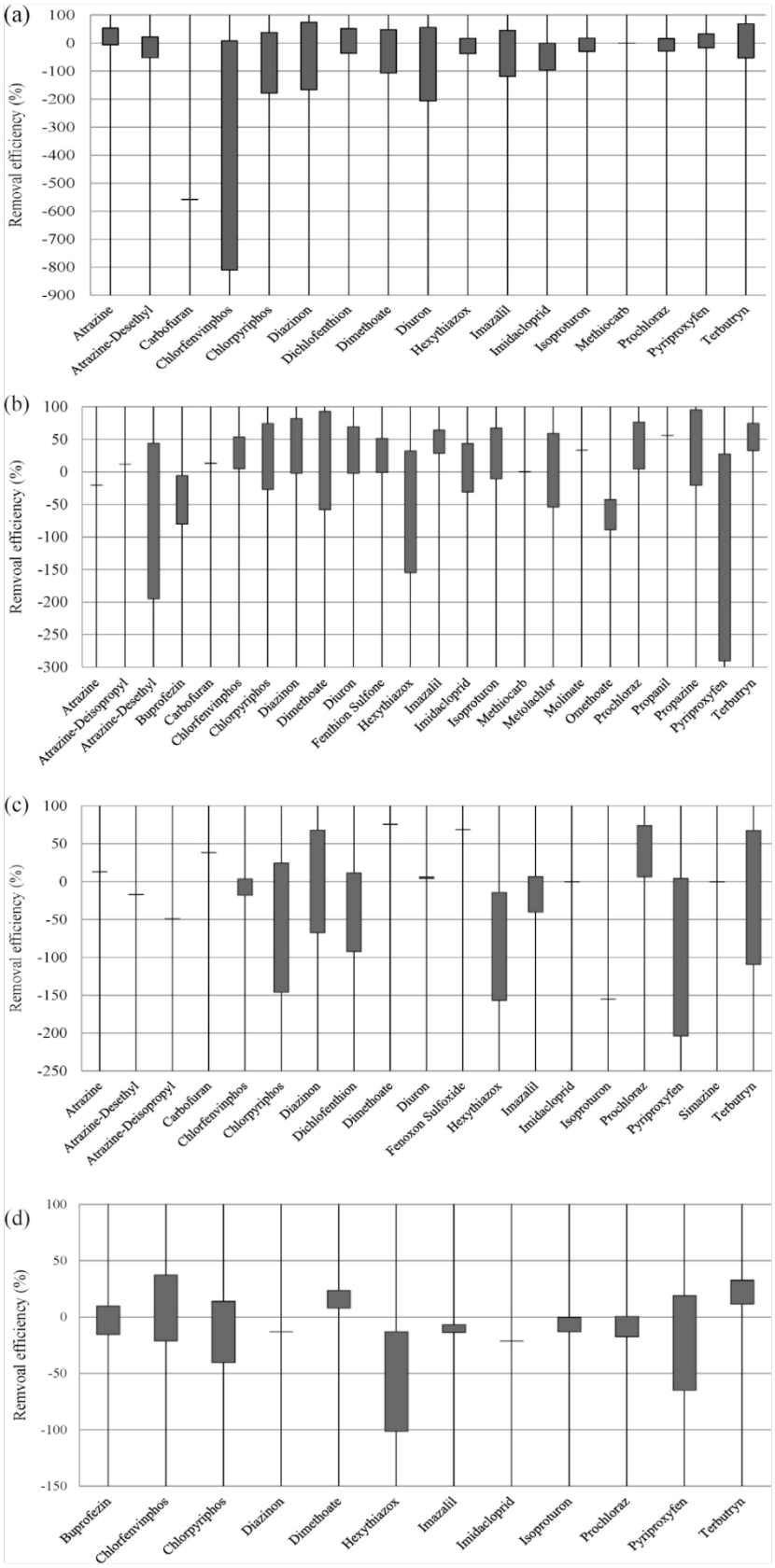


Fig. 6.5 Pesticide removal efficiency of the (a) Ebro, (b) Guadalquivir, (c) Jucar and (d) Llobregat sewage treatment plants in October of 2010.

Table 6.4
Pesticide residue occurrence in the effluent of Tortosa STPs and in the Ebro River before and after this STP.

Pesticide concentrations (ng L^{-1}) in water sample before STP (Ebro 7)[a]	Pesticide concentrations (ng L^{-1}) in STP influent sample	Pesticide concentrations (ng L^{-1}) in STP effluent sample	Pesticide concentrations (ng L^{-1}) in water sample after STP (Ebro 8)
	Atrazine: 11.01	Atrazine: 5.07	Atrazine: 8.95
	Carbofuran: 6.40	Azinphos ethyl: 65.49	Deisopopylatrazi: 4.35
	Chlorfenvinphos: 3.07	Carbofuran: 42.10	Deethylatrazine: 19.95
Chlorpyriphos: 1.22	Chlorpyriphos: 3.48	Chlorpyriphos: 9.70	Chlorpyriphos: 6.26
Diazinon: 0.28	Diazinon: 8.47	Diazinon: 9.38	Diazinon: 2.73
	Dichlofenthion: 8.57	Chlorfenvinphos: 27.96	Buprofezin: 8.04
	Dimethoate: 36.89	Dichlofenthion: 11.68	Chlorfenvnphos: 31.17
	Diuron: 35.63	Dimethoate: 63.49	Fenoxon Sulfox: 3.45
	Fenoxon Sulfox: 13.12	Diuron: 109.68	Hexythiazox: 8.74
	Hexythiazox: 4.03	Hexythiazox: 5.56	Imidacloprid: 2.77
Imazalil: 93.20	Imazalil: 90.17	Imazalil: 108.06	Imazalil: 109.25
	Imidacloprid: 2.70	Imidacloprid: 5.30	Prochloraz: 34.47
	Isoproturon: 3.32	Isoproturon: 3.01	Pyriproxyfen: 33.28
	Prochloraz: 26.53	Prochloraz: 22.17	Dimethoate: 2.33
	Pyriproxyfen: 12.43	Pyriproxyfen: 14.50	Terbuthylazine: 18.26
	Terbutryn: 8.68	Terbutryn: 8.77	Dichlofenthion: 14.84

[a] Ebro 7–8 correspond to sampling points monitored in the Ebro River.

Jucar sludge samples showed pesticide contamination with chlorpyriphos, pyriproxyfen, prochloraz and imazalil. This last one showed again the highest concentration, 318 ng g^{-1} in dry weight. Pesticide concentration in the sludge samples of the Llobregat STPs were also low as those detected in waters. The highest value were for imazalil with only 13 ng g^{-1} dw and chlorpyriphos with 9 ng g-1 dw.

In 2011, sludge samples of the Ebro STPs were also contaminated with imazalil, but with a lower concentration (239 ng g^{-1} dw), and with chlorpyriphos (60 ng g^{-1} dw) and thiabendazole (40 ng g^{-1} dw) among others. In the sludge samples of Guadalquivir, the pesticides concentration were greater than in 2010. The highest values were detected for imazalil (93 ng g^{-1} dw), terbutryn (60 ng g^{-1} dw) and chlorpyriphos (48 ng g^{-1} dw). On the other hand, it was observed a significant increase in the maximum concentration of pesticides in the Jucar sludge samples. In these, ethion was detected at a concentration of 25667 ng g^{-1} dw followed by imazalil (523 ng g^{-1} dw), dichlofenthion (426 ng g^{-1} dw) and thiabendazole (198 ng g^{-1} dw). Finally, the pesticide concentration in the sludge samples of Llobregat STPs were again low – only 93 ng g^{-1} dw of imazalil, 35 ng g^{-1} dw of ethion and 18 ng g^{-1} dw of chlorpyriphos (Fig. 6.4).

3.3. Removal of pesticides by STPs

The removal efficiency of pesticides was calculated from the analyte concentration in influent (C_{in}) and effluent (C_{ef}): $[(C_{in} - C_{ef})/C_{in}] \times 100\%$. According to this equation, and evaluating the removal efficiency of the most ubiquitous families, in 2010, the elimination of organophosphorus ranged from −811% (chlorfenvinphos) to 93% (dimethoate), meanwhile the removal of azoles was in the range −119% (imazalil) to 77% (prochloraz). Similarly, in 2011, the elimination percentage of organophosphorus was between −4575% (diazinon) and 97% (chlorfenvinphos), and in the case of triazines, it ranged from −570% (terbuthylazine) to 91% (terbumeton-desethyl).

Studying the pesticides removal by basin and year, in 2010 (Fig. 6.5), the less efficient STP in the Ebro River was Tortosa, with −811% of chlorfenvinphos elimination, and the most efficient was Pamplona, with 74% of diazinon removal (Fig. 6.5). In the Guadalquivir basin, the removal ranged from −290% of pyriproxyfen to 96% of propazine, both in the STP of Copero. In the Jucar River, the efficiencies were between -204% of pyriproxyfen, in Cuenca, and 76% of dimethoate, in Alzira. Finally, in the Llobregat basin, the STP of Manresa showed the lowest (−101% of hexythiazox) and the highest(37% of chlorfenvinphos) elimination efficiencies.

In the next year, 2011, in the Ebro basin, the removal ranged from −7587% of methiocarb, in Tudela's treatment plant, to 96% of chlorpyriphos, in the STP of Logroño (Fig. S6.3). The less efficient STP in the Guadalquivir River was Ranilla, with −377% of terbuthylazine-desethyl elimination, and the most efficient was Copero, with 91% of chlorpyriphos removal (both STPs located in Sevilla). In the Jucar basin, the STP of Alzira showed the lowest (−2175% of imidacloprid), and the STP of Cuenca the highest (97% of chlorfenvinphos), elimination efficiencies. Finally, in the Llobregat River, the efficiencies were between −581% of isoproturon, in Abrera's STP, and 88% of chlorfenvinphos in Manresa's STP. Summarizing, 64% of the pesticides analysed in this study were not eliminated or even reduced in the treatment plants. Only 13% reached elimination efficiencies between 25% and 75% and none of the efficiencies exceeded the 75% (for detailed data see Fig. S6.4).

Previous works regarding the occurrence and removal of pesticides in WWTP also indicate a quite poor efficiency and high variability, being concentrations in effluents frequently higher than in influents [17,23]. This might be explained by sampling variations due to sampling conditions limitations. Influent, effluent and sludge samples were collected at the same day, while hydraulic retention time (HRT) was between 24 and 72 h, and solid retention time (SRT) was from 7.5 to 25 days depending on the plant. Composite samples of 24 h may be insufficient to determine pesticide removal in STPs.

Secondly, estimated loads were calculated using results from filtered water, which does not take into account the fraction of compounds in the non-aqueous phase, thus could underestimating the total load [1]. Other cause could be the presence of pesticide conjugates and/or metabolites that are reverted back during treatment into its original form, hydrolysis, and desorption from particulate matter during wastewater treatment [23].

The different physical–chemical properties of the compounds (octanol–water partition coefficient, water solubility, and groundwater ubiquity score (GUS) index) did not show any relation with the poor removal efficiency of pesticides. These results are clearly in agreement with those already reported by Köck-Schulmeyer et al. [23], who did not obtain any significant difference after statistical crosschecking these properties with the "in-out balance" of each individual balance.

Based in the removal efficiencies obtained in this study, the contribution of pesticides coming from sewage treatment plants to rivers would be possible. To confirm this hypothesis, and taking as

example the Tortosa STP, surface water samples from the Ebro River were taken prior and after the STPs. The results shown in Table 6.4 demonstrated how before this STP, the water sample analysed only showed positive contamination with chlorpyriphos, diazinon and imazalil, On the contrary, the high number of pesticides and their high concentrations in the sample analysed after the plant could indicate that sewage treatment plants constitute a focal point of contamination to the river. This conclusion is not detrimental to the important role that STPs play in wastewater depuration.

4. Conclusions

All the data obtained over a 2 year period, confirm that most of the pesticides are only partially eliminated during the secondary treatment usually used in STPs, demonstrating their persistence (in 2010, 42% of analytes were present in more than a half of treatment plants considered). Poor removal efficiencies presented in this study (in 2010, 50% of pesticides were not eliminated or even reduced in the treatment plants) could be related to the treatment process used, hydraulic and solid retention times, besides the dilution and temperature of the raw sewage and the plant's configuration, in addition to the different physicochemical properties of each compound. These low efficiencies are responsible of the high pesticides concentration (e.g. diuron) found in some effluents, which may endanger water quality of the ecosystem when they re-used or directly discharged into the river. Accordingly, sewage treatment plants can be considered a focal point of contamination to surface waters. It should be noted that direct dumping of non-treated wastewater to the river is worst.

In view of the precautionary principle, continuous monitoring and stronger quality control of wastewaters are advised in order to gain a more complete knowledge of the environmental status of main Spanish rivers basins. This is very important if a representative picture of rivers water quality is wanted, mainly because pollutants levels vary both spatially and temporally. Additionally, pollutants monitoring should be complemented with studies dealing with the determination of the effects caused by the contamination.

Acknowledgements

This work has been supported by the Spanish Ministry of Economy and Competitiveness through the Projects "Assessing and Predicting Effects on Water Quantity and Quality in Iberian Rivers Caused by Global Change" (SCARCE, CSD-665-2009) and "Evaluation of Emerging Contaminants in the Turia River Basin: From Basic Research to the Application of Environmental Forensics" (CGL2011-29703-C02-02).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2013.09.061.

References

[1] N. Barco-Bonilla, R. Romero-González, P. Plaza-Bolaños, A. Garrido Frenich, J.L. Martínez Vidal, Analysis and study of the distribution of polar and non-polar pesticides in wastewater effluents from modern and conventional treatments, J. Chromatogr. A 1217 (2010) 7817–7825.
[2] C. Blasco, Y. Picó, Prospects for combining chemical and biological methods for integrated environmental assessment, TrAC Trend. Anal. Chem. 28 (2009) 745–757.
[3] M. Petrovic, S. Gonzalez, D. Barceló, Analysis and removal of emerging contaminants in wastewater and drinking water, TrAC Trend. Anal. Chem. 22 (2003) 685–696.
[4] S. González, R. López-Roldán, J.L. Cortina, Presence and biological effects of emerging contaminants in Llobregat River basin: A review, Environ. Pollut. 161 (2012) 83–92.
[5] Directive of the European Parliament and of the council 2000/60/EC establishing a framework for community action in the field of water policy, Official Journal C513, 23/10/2000 (2000).
[6] European Parliament legislative resolution of 16 December 2008 on the proposal for a directive of the European Parliament and of the Council on environmental quality standards in the field of water policy and amending Directive 2008/105/CE, [COM (2007)0765 – C6-0468/2007-2007/0279(COD)] (2008).
[7] S. Moganti, B.J. Richardson, K. McClellan, M. Martin, P.K.S. Lam, G.J. Zheng, Use of the clam *Asaphis deflorata* as a potential indicator of organochlorine bioaccumulation in Hong Kong coastal sediments, Mar. Pollut. Bull. 57 (2008) 672–680.
[8] M. Köck-Schulmeyer, A. Ginebreda, S. González, J.L. Cortina, M.L. de Alda, D. Barceló, Analysis of the occurrence and risk assessment of polar pesticides in the Llobregat River Basin (NE Spain), Chemosphere 86 (2012) 8–16.
[9] A. Belmonte Vega, A. Garrido Frenich, J.L. Martínez Vidal, Monitoring of pesticides in agricultural water and soil samples from Andalusia by liquid chromatography coupled to mass spectrometry, Anal. Chim. Acta 538 (2005) 117–127.
[10] M. Kuster, M.J. López de Alda, M.D. Hernando, M. Petrovic, J. Martín-Alonso, D. Barceló, Analysis and occurrence of pharmaceuticals, estrogens, progestogens and polar pesticides in sewage treatment plant effluents, river water and drinking water in the Llobregat river basin (Barcelona, Spain), J. Hydrol. 358 (2008) 112–123.
[11] A. Lazartigues, C. Fratta, R. Baudot, L. Wiest, C. Feidt, M. Thomas, C. Cren-Olivé, Multiresidue method for the determination of 13 pesticides in three environmental matrices: water, sediments and fish muscle, Talanta 85 (2011) 1500–1507.
[12] M. Ricart, H. Guasch, D. Barceló, R. Brix, M.H. Conceiçao, A. Geiszinger, M.J.L.p.d. Alda, J.C. López-Doval, I. Muñoz, C. Postigo, A.M. Romaní, M. Villagrasa, S. Sabater, Primary and complex stressors in polluted mediterranean rivers: Pesticide effects on biological communities, J. Hydrol. 383 (2010) 52–61.
[13] J. Quintana, I. Martí, F. Ventura, Monitoring of pesticides in drinking and related waters in NE Spain with a multiresidue SPE-GCGÇôMS method including an estimation of the uncertainty of the analytical results, J. Chromatogr. A 938 (2001) 3–13.
[14] S. Rodriguez-Mozaz, M.J. López de Alda, D. Barceló, Monitoring of estrogens, pesticides and bisphenol A in natural waters and drinking water treatment plants by solid-phase extraction–liquid chromatography–mass spectrometry, J. Chromatogr. A 1045 (2004) 85–92.
[15] A. Kampioti, A. Borba da Cunha, M. López de Alda, D. Barceló, Fully automated multianalyte determination of different classes of pesticides, at picogram per litre levels in water, by on-line solid-phase extraction–liquid chromatography–electrospray-tandem mass spectrometry, Anal. Bioanal. Chem. 382 (2005) 1815–1825.
[16] M. Köck-Schulmeyer, A. Ginebreda, C. Postigo, R. López-Serna, S. Pérez, R. Brix, M. Llorca, M.L. de Alda, M. Petrovic, A. Munné, L. Tirapu, D. Barceló, Wastewater reuse in Mediterranean semi-arid areas: The impact of discharges of tertiary treated sewage on the load of polar micro pollutants in the Llobregat river (NE Spain), Chemosphere 82 (2011) 670–678.
[17] M.J.M. Bueno, M.J. Gomez, S. Herrera, M.D. Hernando, A. Agüera, A.R. Fernández-Alba, Occurrence and persistence of organic emerging contaminants and priority pollutants in five sewage treatment plants of Spain: Two years pilot survey monitoring, Environ. Pollut. 164 (2012) 267–273.
[18] D. Fatta, S. Canna-Michaelidou, C. Michael, E. Demetriou Georgiou, M. Christodoulidou, A. Achilleos, M. Vasquez, Organochlorine and organophosphoric insecticides, herbicides and heavy metals residue in industrial wastewaters in Cyprus, J. Hazard. Mater. 145 (2007) 169–179.
[19] R. Loos, G. Hanke, S.J. Eisenreich, Multi-component analysis of polar water pollutants using sequential solid-phase extraction followed by LC–ESI-MS, J. Environ. Monit. 5 (2003) 384–394.
[20] E. Pitarch, C. Medina, T. Portolés, F.J. López, F. Hernández, Determination of priority organic micro-pollutants in water by gas chromatography coupled to triple quadrupole mass spectrometry, Anal. Chim. Acta 583 (2007) 246–258.
[21] A. Navarro-Ortega, V. Acuña, R.J. Batalla, J. Blasco, C. Conde, F.J. Elorza, A. Elosegi, F. Francés, F. La-Roca, I. Muñoz, M. Petrovic, Y. Picó, S. Sabater, X. Sanchez-Vila, M. Schuhmacher, D. Barceló, Assessing and forecasting the impacts of global change on Mediterranean rivers. The SCARCE Consolider project on Iberian basins, Environ. Sci. Pollut. Res. 19 (2012) 918–933.
[22] A. Masía, M. Ibañez, C. Blasco, J.V. Sancho, Y. Picó, F. Hernández, Combined use of liquid chromatography triple quadrupole mass spectrometry and liquid chromatography quadrupole time-of-flight mass spectrometry in systematic screening of pesticides and other contaminants in water samples, Anal. Chim. Acta 761 (2013) 117–127.
[23] M. Köck-Schulmeyer, M. Villagrasa, M. López de Alda, R. Céspedes-Sánchez, F. Ventura, D. Barceló, Occurrence and behavior of pesticides in wastewater treatment plants and their environmental impact. Original Research Article, Sci. Total Environ. 458–460 (2013) 466–476.
[24] E. Gracia-Lor, J.V. Sancho, R. Serrano, F. Hernández, Occurrence and removal of pharmaceuticals in wastewater treatment plants at the Spanish Mediterranean area of Valencia, Chemosphere 87 (2012) 453–462.
[25] Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumptiom., Off. J. Eur. Communities L 330/32 (1998).

Supplementary material

Occurrence and removal efficiency of pesticides in sewage treatment plants of four Mediterranean River Basins.

Julián Campo*, Ana Masiá, Cristina Blasco, Yolanda Picó

Food and Environmental Safety Research Group (SAMA-UV), Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n. 46100, Burjassot, València, Spain.

* Corresponding author. Tel. +34 963543092; fax: +34 963544954
E-mail address: Julian.Campo@uv.es

Table S6.1 Pesticides selected in this study, their family, use and year(s) of sampling campaign(s)

Family	Compound	Use	Sampling campaign
Anilide	Propanil	Herbicide	2010-2011
Azole	Imazalil	Fungicide	2010-2011
	Prochloraz	Fungicide	2010-2011
Benzimidazole	Carbendazim	Fungicide	2011
	Thiabendazole	Fungicide	2011
Carbamate	3-Hydroxycarbofuran	Herbicide	2010-2011
	Carbofuran	Herbicide	2010-2011
	Methiocarb	Herbicide	2010-2011
	Molinate	Herbicide	2010-2011
Carboxamide	Hexythiazox	Acaricide	2010-2011
	Acetochlor	Herbicide	2010-2011
Chloroacetanilide	Alachlor	Herbicide	2010-2011
	Metolachlor	Herbicide	2010-2011
Juvenile hormone mimic	Pyriproxyfen	Insecticide	2010-2011
Neonicotinoid	Imidacloprid	Insecticide	2010-2011
Organophosphorus	Azinphos-Ethyl	Insecticide	2010-2011
	Azinphos-Methyl	Insecticide	2010-2011
	Chlorfenvinphos	Insecticide	2010-2011
	Chlorpyriphos	Insecticide	2010-2011
	Diazinon	Insecticide	2010-2011
	Dichlofenthion	Insecticide	2010-2011
	Dimethoate	Insecticide	2010-2011
	Ethion	Insecticide	2010-2011
	Fenchlorphos	Insecticide	2010
	Fenitrothion	Insecticide	2010-2011
	Fenoxon	Insecticide (Metabolite)	2010-2011
	Fenoxon Sulfone	Insecticide (Metabolite)	2010-2011
	Fenoxon Sulfoxide	Insecticide (Metabolite)	2010-2011
	Fenthion	Insecticide	2010-2011
	Fenthion Sulfone	Insecticide (Metabolite)	2010-2011
	Fenthion Sulfoxide	Insecticide (Metabolite)	2010-2011
	Malathion	Insecticide	2010-2011
	Omethoate	Insecticide (Metabolite)	2010-2011
	Parathion-Ethyl	Insecticide	2010-2011
	Parathion-Methyl	Insecticide	2010-2011
	Tolclofos-Methyl	Fungicide	2010-2011
Triazine	Atrazine	Herbicide	2010-2011
	Atrazine-Deisopropyl	Herbicide (Metabolite)	2010-2011
	Atrazine-Desethyl	Herbicide (Metabolite)	2010-2011
	Propazine	Herbicide	2010-2011
	Simazine	Herbicide	2010-2011
	Terbumeton	Herbicide	2011
	Terbumeton-Desethyl	Herbicide (Metabolite)	2011
	Terbuthylazine	Herbicide	2011
	Terbuthylazine-2 Hydroxy	Herbicide (Metabolite)	2011

Family	Compound	Use	Sampling campaign
	Terbuthylazine-Desethyl	Herbicide (Metabolite)	2011
	Terbutryn	Herbicide	2010-2011
Triazole	Tebuconazole	Fungicide	2011
Unclassified	Buprofezin	Insecticide	2010-2011
Urea	Diuron	Herbicide	2010-2011
	Isoproturon	Herbicide	2010-2011

Table S6.2 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 S6.3 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)
Chlorpyriphos	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)
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)

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]
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)
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

Table S6.4 Recoveries of the selected pesticides and Relative Standard Deviations (RSD %) at a concentration of 25 ng L^{-1} for each pesticide

Target Pesticide	Wastewater		Sludge	
	Recovery	RSD (%)	Recovery	RSD (%)
Acetochlor	66,1	4,1	60,6	12,4
Alachlor	58,3	10,8	75,1	12,8
Atrazine	64,6	16,8	40,0	9,9
Atrazine-desethyl	56,1	6,4	40,0	14,0
Atrazine-desisopropyl	53,6	2,9	98,9	12,1
Azinphos-ethyl	58,3	16,6	97,8	12,0
Azinphos-methyl	50,7	6,7	97,8	14,3
Buprofezin	52,1	19,1	61,7	11,4
Carbendazim	64,6	16,0	40,0	9,5
Carbofuran	58,3	12,8	77,3	10,5
Carbofuran-3-hydroxy	66,8	4,6	42,2	13,2
Chlorfenvinphos	61,4	108	42,2	19,7
Chlorpyriphos	52,1	4,4	44,4	10,9
Diazinon	48,5	6,1	59,5	15,4
Dichlofenthion	65,4	14,6	61,7	12,0
Dimethoate	56,8	4,0	63,9	11,3
Diuron	49,2	10,9	43,3	11,4
Ethion	53,6	4,1	42,2	13,8
Fenitrothion	66,8	12,3	78,4	9,1
Fenoxon-Sulfone	56,8	12,4	105,0	10,0
Fenoxon-Sulfoxide	52,9	4,1	90,6	14,1
Fenthion	50,7	10,0	61,7	12,6
Fenthion-Sulfone	57,5	2,6	62,8	11,7
Fenthion-Sulfoxide	61,4	10,7	44,4	12,3
Hexythiazox	70,0	6,4	78,4	12,1
Imazalil	64,6	19,6	63,9	13,3
Imidacloprid	60,0	8,7	63,9	12,3
Isoproturon	55,8	6,9	63,9	10,1
Malathion	50,7	8,6	43,3	13,0
Methiocarb	66,1	4,7	41,1	11,4
Methoalachlor	52,1	8,3	60,6	12,3
Molinate	61,4	17,0	62,8	14,3
Omethoate	53,6	6,1	91,7	10,4
Parathion-ethyl	60,7	6,5	42,2	11,0
Parathion-methyl	68,3	16,9	40,0	10,6
Prochloraz	52,9	14,4	78,4	10,5
Propanil	56,8	2,7	44,4	11,5
Propazine	62,2	14,0	62,8	13,7
Pyriproxifen	66,8	14	43,3	14,9

Target Pesticide	Wastewater		Sludge	
	Recovery	RSD (%)	Recovery	RSD (%)
Simazine	58,3	8,0	62,8	12,7
Tebuconazole	49,2	10,7	43,3	11,9
Terbumeton	70,0	6,9	78,4	12,0
Terbumeton-desethyl	66,1	4,4	60,6	12,1
Terbuthylazine	66,8	12,6	78,4	9,1
Terbuthylazine-2-hidroxy	64,6	16,7	40,0	9,3
Terbuthylazine-deethyl	52,1	4,8	44,4	10,0
Terbutryn	64,6	12,3	79,5	10,2
Thiabendazole	53,6	4,0	42,2	13,8
Tolclofos-methyl	66,1	12,1	41,1	11,9

Table S6.5 Concentration of pesticides reported in Spain STPs in previous studies

Pesticide	Concentration (ng L^{-1})			
	2010[a]	2012[b]	2013[c] IN	2013[c] OUT
Anilide				
Propanil			9	9
Chloroacetanilide				
Alachlor			2	-
Triazines				
Atrazine		5-1053	1	124
Atrazine-Deisopropyl			14	39
Atrazine-Desethyl			24	23
Simazine		8-980	7	169
Terbuthylazine	81-152		21	20
Terbuthylazine-Desethyl	20-46			
Ureas				
Diuron	118-331	22-2975	93	127
Isoproturon		105-1242	-	13
Organophosphates				
Chlorfenvinphos		11-1774		
Diazinon			133	281
Dimethoate			4	49
Malathion			-	0.5

(a) Barco-Bonilla et al. [1]: Foundation Centre for New Water Technologies in Seville; (b) Martínez Bueno et al. [17]: Almería, Cantabria, Madrid (2) and Barcelona; (c) Köck-Schulmeyer et al. [25]: Catalonia (3)

Table S6.6 Concentration of pesticides reported in the Llobregat River in previous studies (modified from Köck-Schlmeyer et al. [8])

Pesticide	Concentration (ng L^{-1})						
	2000[a]	2002[b]	2003[c]	2005-06[d]	2008[e]	2009-10[f]	2010-11[g]
Triazines							
Atrazine	25–29	5–463	7.8	0.05–1.1	1.0–1.2	n.d.–10.2	<Ldet-3.9
Simazina	25–84	8–2218	9.9	0.1–53.6	1.5–2.2	n.d.–38.4	<Ldet-26.9
Desethyl atrazine	n.d.	n.d.-4	0.6	27.1–27.1	n.d.	n.d.–8.2	<Ldet
Deisopropylatrazine	25–62	n.m.	1.2	0.1–14.4	n.d.	<Ldet	<Ldet
Terbuthylazine	n.d.	n.m.	12	0.1–21.9	18.6–44.6	n.d.–81.5	
Ureas							
Diuron	118-331	64–239	9.5	0.4–99.7	10.5–36.0	n.d.–818.0	19.1-168.0
Isoproturon	n.m.	5–503	0.5	0.5–7.8	n.d.-0.5	n.d.–81.6	1.6-17.0
Organophosphates							
Malathion	n.m.	n.m.	n.m.	n.d.	n.d.	n.d.–18.9	<Ldet
Diazinon	n.m.	n.m.	8.4	0.8–785.0	17–34	n.d.–132.3	1.2-78.6
Dimethoate	60–154	n.m.	42	0.6–87.8	n.d.	n.d.–189.9	4.2.-19.7
Chloroacetanilides							
Alachlor	n.m.	n.m.	n.d.	2.2–17.1	n.d.	n.d.–11.1	<Ldet
Metolachlor	n.d.	n.m.	1.5	7.4	n.d.	n.d.–13.1	<Ldet
Thiocarbamate							
Molinate	n.m.	n.m.	n.d.	1.0–3.8	n.d.	n.d.	<Ldet-5.8

(a) Quintana et al. [13]; (b) Rodriguez-Mozaz et al. [14]; (c) Kampioti et al. [15]; (d) Ricart et al. [12]; (e) Köck-Schulmeyer et al. [16]; (f) Köck-Schulmeyer et al. [8]; (g) this study; n.d.: not detected; n.m.: not measured; <Ldet: below limit of determination.

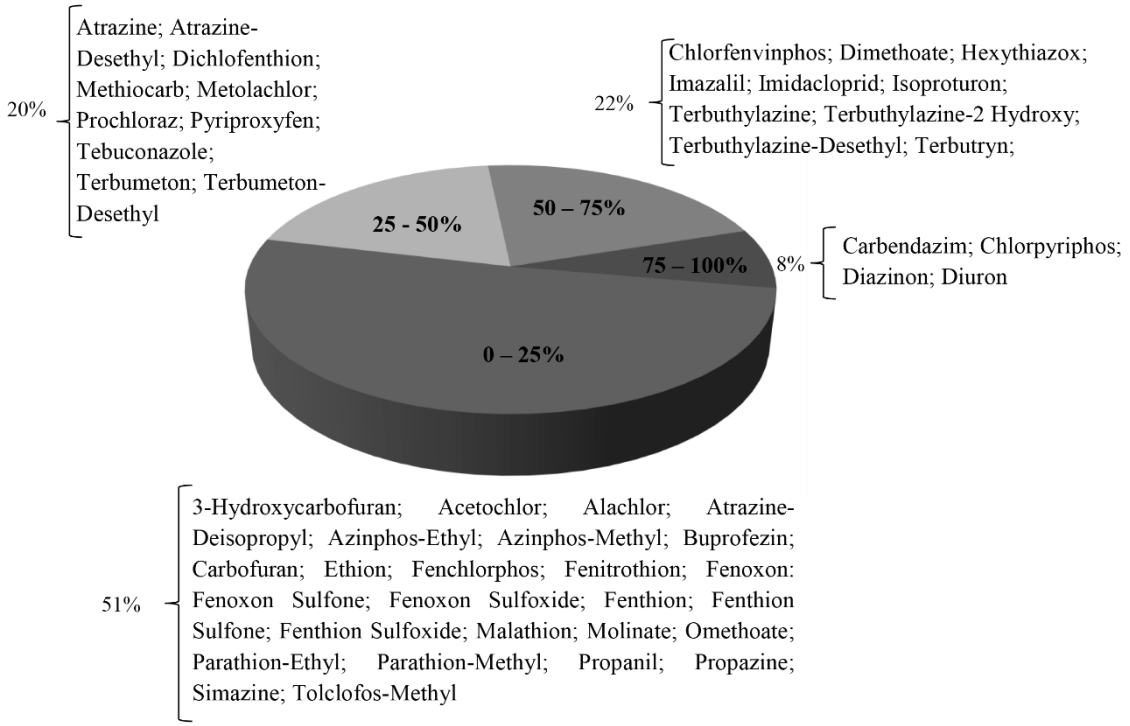


Figure S6.1 Pesticide occurrence frequency in all the STPs considered in this study;

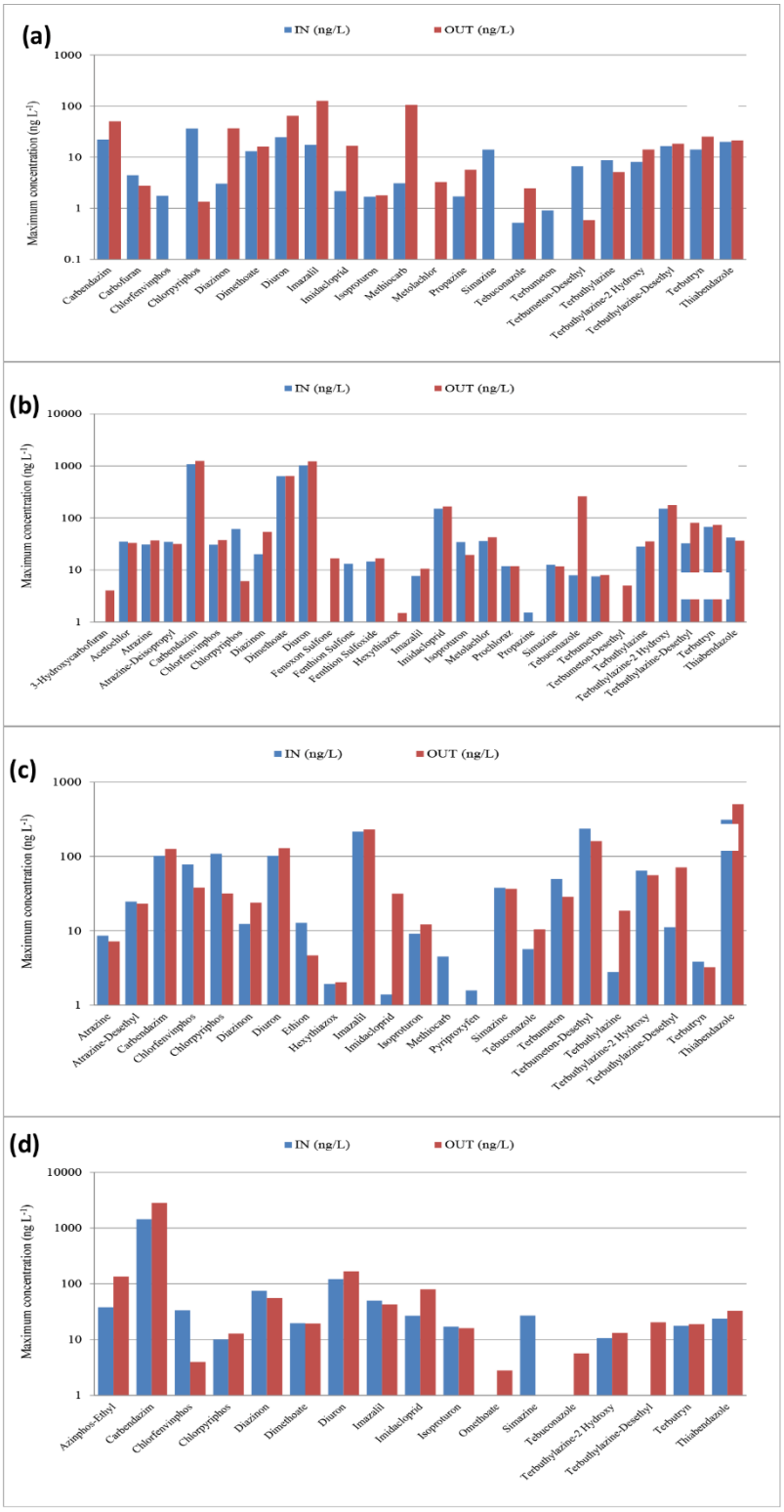
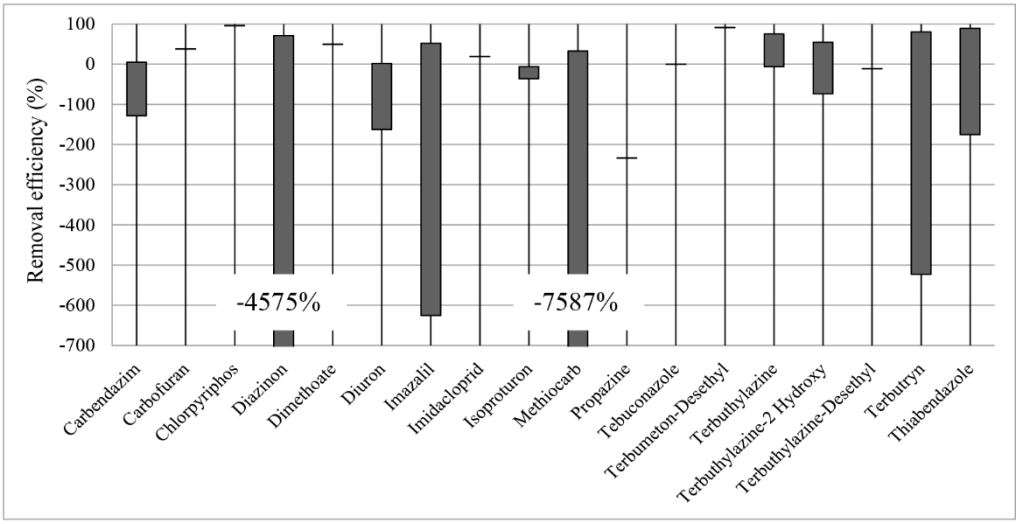
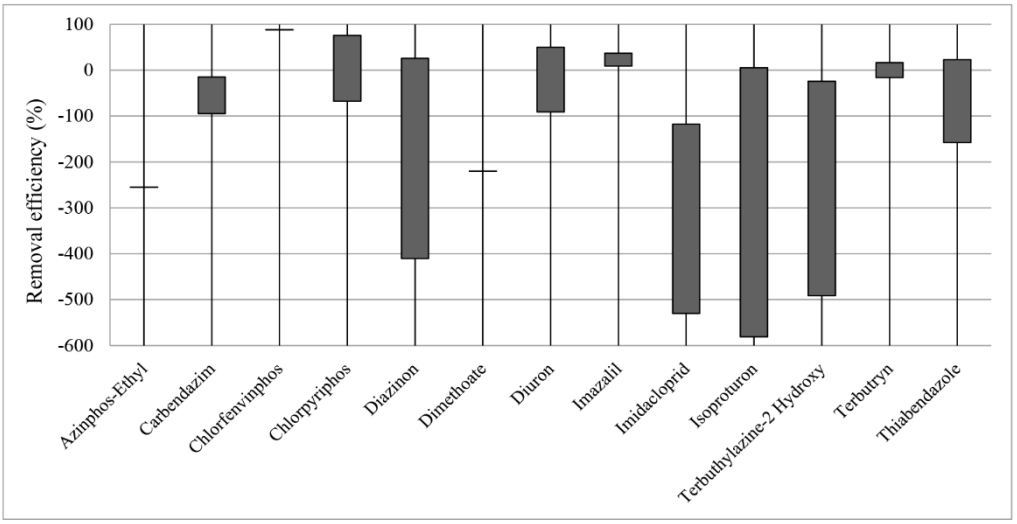


Figure S6.2 Maximum pesticide concentration detected in (a) Ebro, (b) Guadalquivir, (c) Jucar and (d) Llobregat STPs influent (IN) and effluent (OUT) samples in 2011

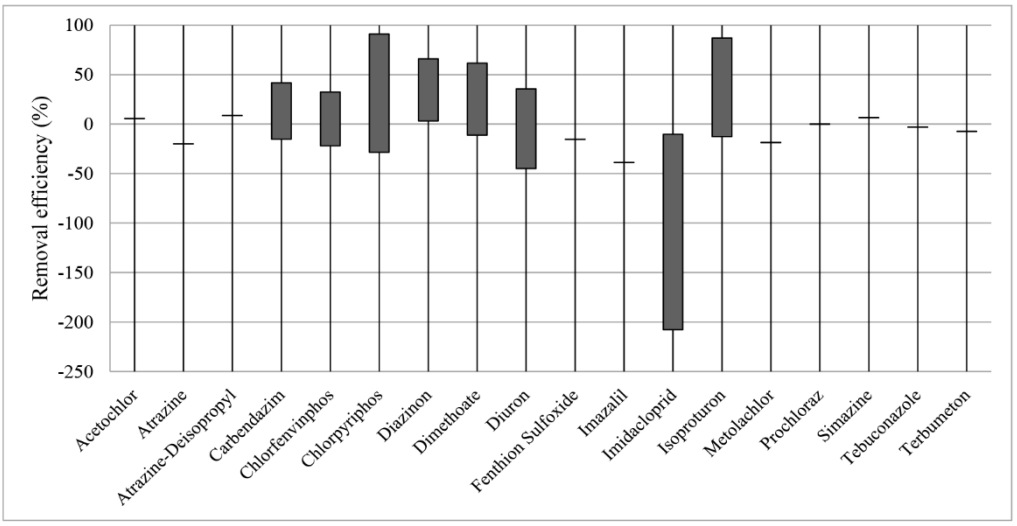
(a)


Removal efficiency (%)
100
0
-100
-200
-300
-400
-500
-600
-700
-4575%
-7587%
Carbendazim
Carbofuran
Chlorpyriphos
Diazinon
Dimethoate
Diuron
Imazalil
Imidacloprid
Isoproturon
Methiocarb
Propazine
Tebuconazole
Terbumeton-Desethyl
Terbuthylazine
Terbuthylazine-2 Hydroxy
Terbuthylazine-Desethyl
Terbutryn
Thiabendazole

(b)


Removal efficiency (%)
100
0
-100
-200
-300
-400
-500
-600
Azinphos-Ethyl
Carbendazim
Chlorfenvinphos
Chlorpyriphos
Diazinon
Dimethoate
Diuron
Imazalil
Imidacloprid
Isoproturon
Terbuthylazine-2 Hydroxy
Terbutryn
Thiabendazole

(c)


Removal efficiency (%)
100
50
0
-50
-100
-150
-200
-250
Acetochlor
Atrazine
Atrazine-Deisopropyl
Carbendazim
Chlorfenvinphos
Chlorpyriphos
Diazinon
Dimethoate
Diuron
Fenthion Sulfoxide
Imazalil
Imidacloprid
Isoproturon
Metolachlor
Prochloraz
Simazine
Tebuconazole
Terbumeton

(d)

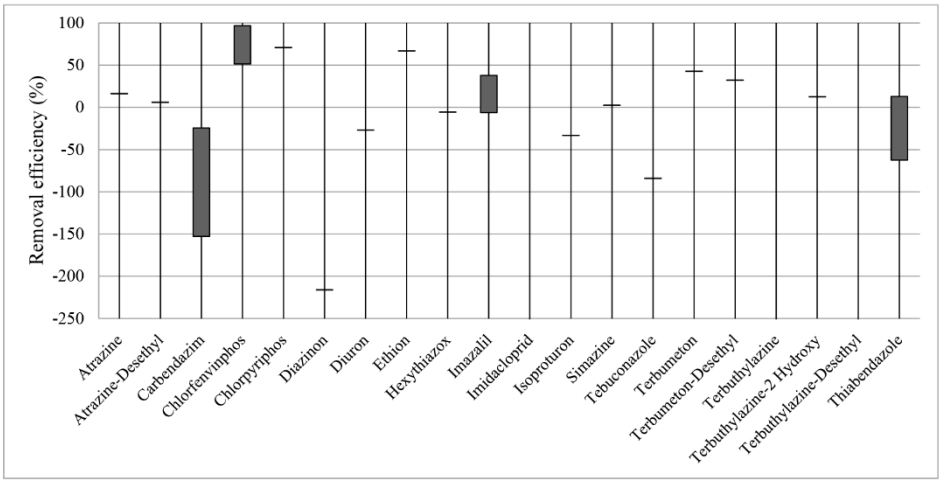


Figure S6.3 Pesticide removal efficiency of the (a) Ebro, (b) Guadalquivir, (c) Jucar and (d) Llobregat sewage treatment plants in October of 2011

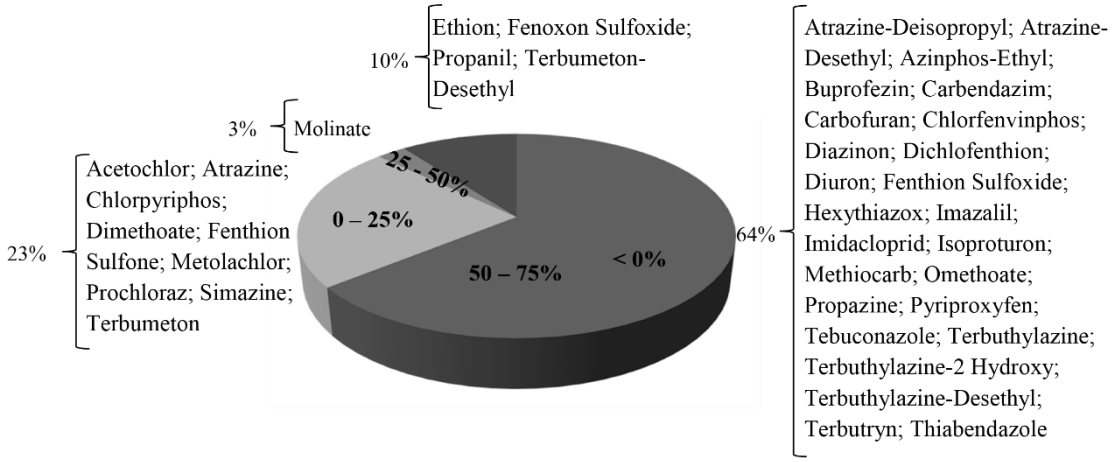


Figure S6.4 Mean pesticide removal efficiency in all the STPs analysed in this work

CAPÍTOL 7

Screening dels plaguicides utilitzats en l'actualitat en aigües, sediments i biota de la conca del Guadalquivir (Espanya)

Publicació científica 7

Screening of currently used pesticides in water, sediments and biota of the Guadalquivir River Basin (Spain)

A. Masiá, J. Campo, P. Vázquez-Roig, C. Blasco, Y. Picó

Journal of Hazardous Materials, 263P (2013) 95-104

Journal of Hazardous Materials 263P (2013) 95–104

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Screening of currently used pesticides in water, sediments and biota of the Guadalquivir River Basin (Spain)

Ana Masiá*, Julián Campo, Pablo Vázquez-Roig, Cristina Blasco, Yolanda Picó

Food and Environmental Safety Research Group, Department of Medicine Preventive, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain

HIGHLIGHTS

- Spatial and temporal distribution of currently used pesticides in the Guadalquivir River Basin.
- Organophosphorus > triazines > carbamates are the most commonly detected.
- Transformation products were found at higher concentrations than parent pesticides.
- WWTP are an important source of pesticide to the environment.
- Low accumulation levels in sediments and biota.

ARTICLE INFO

Article history:
Received 20 May 2013
Received in revised form
11 September 2013
Accepted 17 September 2013
Available online 21 September 2013

Keywords:
Pesticides
Water
Sediment
Fish
Spatial distribution
Temporal distribution

ABSTRACT

The occurrence of 50 currently used pesticides and their transformation products in surface and waste waters, sediment and fish in the Guadalquivir River Basin was determined in 2010 and 2011. After selective sample extraction, pesticides were identified and quantified by liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS). The contamination profile in water and sediments is marked by the presence of organophosphorus and triazines. Transformation products were even at higher concentrations than parent pesticides. A wider range of pesticides was present in water than in sediments but none of them were detected in fish. The mean concentrations ranged from 0.2 to 13.0 ng/L in water and from 0.1 to 13.2 ng/g d.w. in sediment. The spatial distribution of most pesticides was consistent with the agricultural activities of the area or their urban applications. The waste water treatment plant effluents that impact the river are minor sources for few pesticides but for most of them run-off would be the most important contribution. The temporal distribution showed differences between both sampling campaigns related to the river flow. The low-flow produced a pesticide concentration effect, generating higher levels in water and accumulation in sediments. This forecasts a hazard in future scenarios if the current situation of the climate change and water scarcity evolves to more critical conditions highlighting the need of these monitoring studies.

1. Introduction

Water is the primary pathway of pesticides dissemination from their application areas to other parcels of the environment [1,2]. As a result, the presence of pesticides – especially those polar and highly soluble– in surface, waste and groundwaters, typically in the lower ng L^{-1} concentration ranges, have been reported [3–7]. Some of these pesticides are bioaccumulative and due to their vertebrate and non-vertebrate toxicity can affect non-target organisms [8].

In Europe, there are few studies that determine the occurrence of currently used pesticides in environmental compartments other than water and most of them are erratic samplings performed to demonstrate the reliability of an analytical method but not systematic studies evaluating pesticide occurrence and levels in a River Basin [2,9–11]. These studies, without being many, are more prevalent in the United States (US) [12–19].

The Guadalquivir River, chosen as study case, is among the major freshwater sources of the European Basins and the Spain's second longest river. Its natural environment is one of the most varied in Europe, with half of the continent's plant species and nearly all those of the North African region [20]. Thus, maintain a good quality of these ecosystems is of crucial interest. Because of its favorable climate and fertile soils, a wide range of crops are cultivated along the basin and more important, irrigated with its water. Despite its importance and to our knowledge, only few studies have been carried out in this River Basin. These reported the concentrations of

* Corresponding author. Tel.: +34 963544118; fax: +34 963544954.
E-mail address: Ana.Masia@uv.es (A. Masiá).

0304-3894/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jhazmat.2013.09.035

persistent organochlorine pesticides [21,22] and herbicides used in olive groves [23] in waters, the punctual determination of 32 pesticides in water and soil samples [9] and the dissipation of chloridazon and lenacid in a clayey soil of their marshes [24].

The objective of this work is to monitor 50 currently used pesticides in two consecutive years (2010 and 2011) in water, sediment and biota (only 2010) of the Guadalquivir River Basin. The selection of the target pesticides and metabolites was based on extend of use, water solubility and amenability to LC–MS analysis. The list included selected compounds from different families, such as organophosphorus, ureas, phenylureas, azoles, neonicotinoids, carbamates, triazines, chloroacetanilides and acetanilides. This is the first extensive pilot study undertaken in this Spanish River Basin and it intends to improve the knowledge of these pesticides occurrence in the aquatic environment. The concentrations of currently-used pesticides associated with sediments and biota can also ascertain which pesticides are more likely to partition to the suspended sediment phase, or to bioaccumulate in the aquatic trophic chain, and this information will be useful for other watersheds where these compounds are applied.

2. Experimental

2.1. Site description and sampling

The whole course of the Guadalquivir River was studied, from its source in the mountains of the Jaén province, to its mouth into the Atlantic Ocean at Sanlúcar de Barrameda, on the Gulf of Cádiz. The main river and several tributaries (Borosa, Guadiana menor, Cacín, Genil, Gudaira and Guadiamar) were sampled. The river length is 657 km and its drainage area 57,527 km^2. It flows southwest through the region of Andalusia, affecting a population of more than 7 million inhabitants, and passing Córdoba and Seville as major cities.

The hydrological plan, which outlines the general management of the basin, indicates that its average renewable water resources (surface and groundwater) are circa 7000 million m^3 $year^{-1}$. In 2002, the gross consumption of water (for all uses) was estimated at 3583 million m^3 $year^{-1}$ (82% surface and 18% groundwater), which was high compared to the available resources (circa 50% of renewable resources). The rainfall fluctuates and therefore, water allocation to users is not guaranteed. Agriculture is by far the main water use (i.e., 86% of basin's water).

A wide range of crops are cultivated along the basin. In terms of irrigated area, olive trees are the major crop (42.3%), followed by extensive field crops (39.4%), mainly cotton, sugar beet, rice, wheat, maize and sunflower. The area devoted to the cultivation of outdoor vegetables (9.6%), fruit trees (3.8%) and citrus (2.7%) is much lower.

The marshy lowlands at the river's end, used for rice cultivation, are known as "Las Marismas" and border Doñana National Park reserve. The river is navigable to Seville (about 90 km upstream), its major inland port and head of navigation for ocean-going vessels.

The sampling was designed to perform large-scale and long-term (the complete basin, two consecutive years) monitoring to assess temporal trends of contamination. The influence of seasonal variability was reduced monitoring the same period both years. September–November period was selected for several reasons (i) coincides with the end of the growing season, which is appropriate for monitoring sediments and fish, and (ii) there are not recent applications of pesticides, which allow to establish what pesticides are constantly present in the environment because their capacity of accumulation and/or persistence.

Water and sediments were collected at 24 points along the Guadalquivir River and its tributaries (Fig. 7.1) for two consecutive years (2010 and 2011). Additionally, pesticide were also analyzed in effluent samples of the 5 waste water treatment plants (WWTPs): Loja (Granada), La Golondrina (Córdoba), Morón (Morón de la Frontera), Copero (Sevilla South) and Ranilla (Sevilla East). Georeferences of all sampling points are shown in Table S7.1. Samples were taken from the last few days of September to the first week of October for first year and from the end of October to the middle of November for the second one. Grab water samples (2 L) were collected in clean amber glass bottles, from the middle of the river width. Before sample collection, each bottle was thoroughly pre-rinsed with MilliQ water at the laboratory and then, rinsed with sample water prior to actual sample collection. WWTP samples were 24 h composite samples provided by the plant operators.

Sediment samples (aprox. 250 g) were taken from irrigation channels and marshes using a Van Veen grab sampler (0.5 L capacity), they were transferred and wrapped into aluminum foil (previously washed with methanol and dried in oven at 100 °C) that was put inside an aluminum box.

Fish samples were only taken in 2010 at five selected sites of the River course: GUA1, GUA2, GUA3, GUA4 and GUA5. Fish samples were collected using electro-fishing by personnel of Institute of Marine Science of Andalucía (ICMAN), Cadiz, Spain. Collected fish included five Andalusian barbel (*Lucio barbussclateri*) taken one in each one of the sampling points and one Common carp (*Cyprinus carpus*) taken at GUA 5. The fish species were selected as much as possible following the Environmental Quality Standards Directive 2008/105/EC (EQSD) [25]. However, due to scarcity of fish in the river was only possible to base the selection of the species in its abundance, thus, the ease of capture was the most decisive factor. The fish was only sampled in 2010 because the complexity of the basin, the difficulties to perform electrofishing, the small sample size obtained and the lack of positive results.

All samples were transported in hermetic boxes refrigerated with ice upon arrival at the laboratory (located in Valencia, Spain). Then, water samples were stored at 4 °C within 24 h to avoid any degradation and were 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 and fish samples were frozen, lyophilized (Hetosicc CD4, Birkerod, Denmark), pulverized, thoroughly mixed and then passed through a 2 mm Ø sieve.

2.2. Chemicals and reagents

For this study, a group of 42 pesticides including some of their transformation products were selected for 2010 campaign. Eight compounds were added the next year, to make a total of 50 among pesticides and metabolites. These pesticides belong to different chemical families, with a variety of uses as well as different physico-chemical characteristics and toxicity (see Table S7.2). The other reactive used were, at least, of analytical grade.

2.3. Sample preparation and instrumental analysis: water, sediment and fish extraction

The method used for water extraction was already published [26]. Very briefly, water samples (200 mL) were vacuum passed through a SPE column (Oasis HLB SPE cartridge 200 mg sorbent/6 mL cartridge, Waters, Milford, MA, USA). The cartridges were dried under vacuum for 10 min and the analytes eluted with 10 mL of dichloromethane–methanol (50:50, v/v). Extracts were evaporated to dryness and reconstituted with 1 mL of methanol.

QuEchERS method, also previously reported in a variety of formats [3], was applied to 1 g lyophilized sediment and 1 g lyophilized fish sample and it is described in the literature [27]. For sediment samples, $MgSO_4$ (6 g), NaCl (1.5 g), tri-sodium citrate dehydrate [$Na_3C_6H_5O_7{\cdot}2H_2O$ (1.5 g) and disodium hydrogen citrate sesquihydrate [$HOC(COOH)(CH_2COONa)_2{\cdot}1.5H_2O$ (0.75 g)] were used for

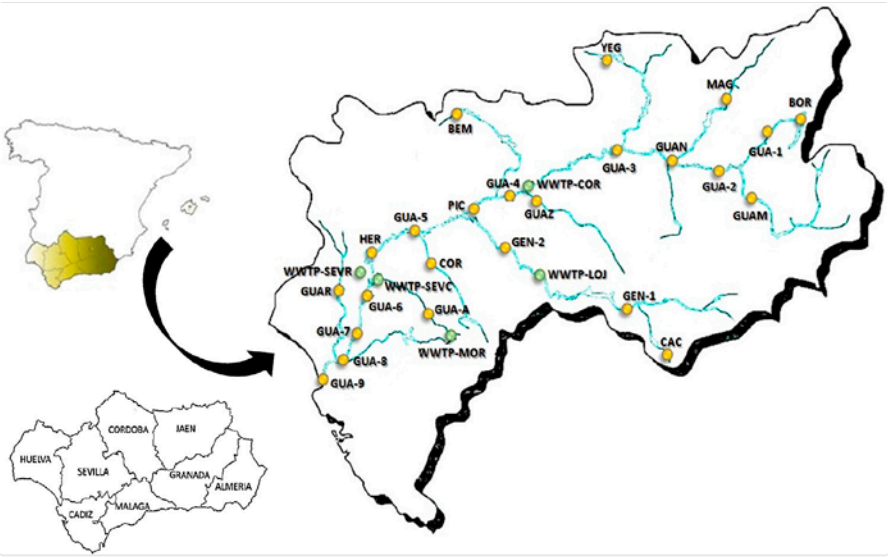


Fig. 7.1 Sampling points.

phase-separation adjustment, and PSA (50 mg), $MgSO_4$ (150 mg) and C_{18} (50 mg) for clean-up step. For fish samples, $MgSO_4$ (6 g) and NaCl (1.5 g) were used for extraction, and PSA (50 mg), $MgSO_4$ (150 mg), C_{18} (50 mg) and active coal (15 mg) for clean-up.

2.4. LC–MS/MS determination

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 (ESI) interface (Agilent Technologies, Waldbronn, Germany). Data were processed using a MassHunter Workstation Software for qualitative and quantitative analysis (A GL Sciences, Tokio, Japan). The conditions are shown in the Supplementary material (see Tables S7.3 and S7.4).

2.5. Quality assurance and quality control

The method developed for water, sediment and biota samples was evaluated through recoveries, precision, linearity and sensitivity. The results are presented in Table S7.5 of the Supplementary material. 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, from 0.03 to 1.67 ng/g for sediment and from 0.08 to 3.75 ng/g for biota.

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 and biota 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 below 20% for all pesticide. In sediment and biota samples, recoveries were higher than 40% (see Table S7.5).

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.

3. Results and discussion

Pesticide residues were detected in water and sediments but not in fish. The results obtained during the two sampling campaigns are summarized in Tables 7.1 and 7.2 for those compounds detected in more than 10% of the samples. The minimum, maximum and mean values together with the frequency of detection are presented. The absence of pesticides in fish could be related to the polarity of the studied pesticides and metabolites. Most of them are highly polar and consequently little bioaccumulative. Furthermore, fish swim in the river stream and could not be exposed to the pesticides found in water for a long period time.

3.1. Occurrence in river water

Of the 50 compounds monitored, 24 were detected in one or more samples with higher detection frequency in 2010 than 2011. Regarding to its frequency, the most detected compounds are outlined in Table 7.1. Prochloraz, thiabendazole, carbofuran and its metabolite, azinphos methyl, ethion, fenthion and its metabolites, malation, propazine, simazine, tebuconazole and isoproturon were also detected sporadically, even though a few of them at high concentrations. Most of target compounds were detected in both sampling campaigns (Table 7.1).

Table 7.1
Minimum, maximum and mean concentration and frequency of detection of the studied pesticides in water.

Class/Pesticide	2010				2011			
	Concentration (ng/L)			Frequency[c] (%)	Concentration (ng/L)			Frequency[c] (%)
	Min[a]	Max	Mean[b]		Min[a]	Max	Mean[b]	
Azole								
Imazalil	0.59	8.05	0.6	3 (13)	4.00	5.56	0.3	4 (17)
Benzimidazole								
Carbendazim	na	na	na		0.57	11.44	1.06	4 (17)
Juvenile hormone mimics								
Pyriproxyfen	0.27	3.04	1.4	21 (88)	–	–	0.3	–
Neonicotinoid								
Imidacloprid	2.34	6.14	1.8	14 (58)	7.04	19.20	2.2	4 (17)
Organophosphorus								
Chlorfenvinphos	0.07	26.48	3.2	17 (71)	1.86	2.01	0.3	2 (8)
ChlorpyriphoS	0.67	11.11	3.8	23 (96)	3.28	14.80	1.8	6 (25)
Diazinon	1.81	23.75	6.3	22 (92)	0.66	456.72	19.3	4 (17)
Dimethoate	4.46	69.26	10.7	17 (71)	2.70	30.60	3.0	4 (17)
Omethoate	7.71	11.71	1.3	3 (13)	2.23	2.23	0.2	1 (4)
Other pesticides								
Buprofezin	1.77	9.28	2.6	23 (96)	–	–	0.3	–
Hexythiazox	0.83	1.94	1.3	23 (96)	1.40	1.40	0.2	1 (4)
Triazines								
Atrazine	4.61	18.63	2.3	6 (25)	–	–	0.7	–
Deisopropylatrazine	7.67	30.18	9.8	14 (58)	43.40	43.40	1.0	1 (4)
Deethylatrazine	4.35	97.21	5.3	3 (13)	–	–	2.8	–
Terbuthylazine	na	na	na	na	1.84	728.00	36.78	7 (29)
Terbuthylazine deethyl	na	na	na	na	3.61	23.80	2.65	5 (21)
Terbuthylazine-2 hydroxy	na	na	na	na	6.70	64.40	4.65	4 (17)
Terbutryn	4.22	12.71	1.5	5 (21)	3.12	3.12	0.2	1 (4)
Ureas								
Diuron	23.05	67.69	13.0	8 (33)	26.76	57.92	6.4	3 (13)

[a] Minimum concentration detected.
[b] Mean value of those samples that presented the pesticide. Non detected were considered at 1/2 LOD.
[c] Number of finding (percentage of positive samples).

All pesticides added in 2011 were detected with the exception of terbumeton and its metabolite.

The relatively constant concentrations of diazinon and chlorpyriphos could be indicative of an urban signal and could be coming not only from agriculture but also from the developed areas (e.g., Sevilla and Cordoba). In a recent study that evaluated the significance of the urban and agricultural land use on the dynamics of the pesticides in surface waters, Wittmer et al. [28] identified five different patterns and classified diazinon within the group of compounds that show elevated background concentrations throughout the year due to constant household sources, which comes to support our findings.

The levels of the analytes detected varied considerably, showing the maximum concentrations for herbicides such as metholachlor (124.43 ng/L in 2010) and terbuthyalzine (Table 7.1), and for the insecticides methiocarb (391.44 ng/L in 2011), diazinon (Table 7.1) and malathion (116.06 ng/L in 2010). These concentrations surpassed 100 ng/L, limit established for individual concentrations in drinking water according to EU legislation (2006/118/EC) [29]. Average concentrations for individual pesticides were basically below 100 ng/L.

Fig. 7.2a summarizes the number of pesticides detected in water samples. The maximum number of compounds detected in a single sample was 16 (HER and GEN-2, in 2010 and 2011 respectively) while five or more compounds co-occurred in 100% of the samples in

Table 7.2
Minimum, maximum and mean concentration and frequency of detection of the studied pesticides in sediments.

Class/pesticide	2010				2011			
	Concentration ng/g dw			Frequency[c] (%)	Concentration ng/g dw			Frequency[c] (%)
	Min[a]	Max	Mean[b]		Min[a]	Max	Mean[b]	
Azole								
Imazalil	–	–	0.1	–	1.4	5.9	1.9	12 (50)
Organophosphorus								
Chlorpyriphos	0.7	2.5	0.3	5 (21)	2.4	15.9	4.8	20 (83)
Diazinon	0.2	175.5	13.2	17 (71)	0.5	8.8	2.9	17 (71)
Ethion	–	–	0.2	–	0.5	0.8	0.2	5 (21)
Triazole								
Tebuconazole	na	na	na	na	2.6	12.7	1.1	3 (13)

[a] Minimum concentration detected.
[b] Mean value of those samples that presented the pesticide. Non detected were considered at 1/2 LOD.
[c] Number of finding (percentage of positive samples).

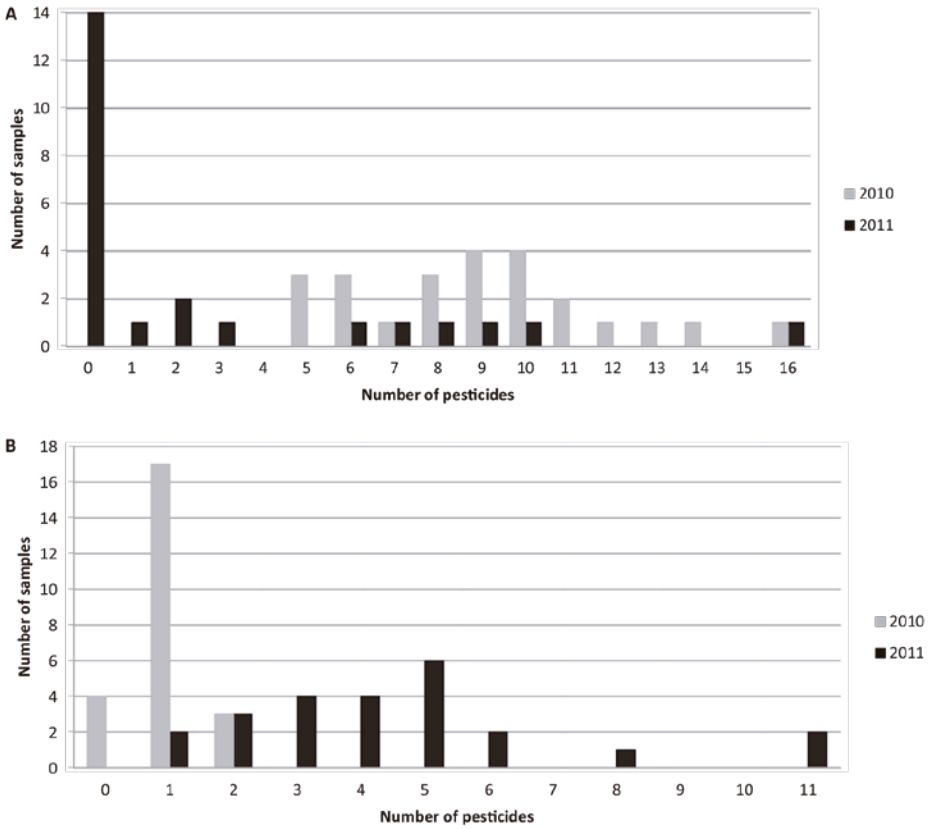


Fig. 7.2 Number of pesticides per samples in (A) water and (B) sediments.

2010 and one or more pesticides co-occurred in 41% of samples in 2011.

Fig. 7.3 offers a general view of the distribution of all pesticide families studied for water and sediment samples in both campaigns. Unlike Table S7.2 and to make it more visual, pesticides have been classified in organophosphorus, triazines and others. This last group encompasses anilides, azoles, benzimidazoles, carbamates, chloroacetanilides, neonicotinoids, thiocarbamates, triazoles, ureas, juvenile hormone mimics and others. It can be observed that the contamination profile was mainly marked by the presence of triazines and organophosphorus followed by others, mostly carbamates and ureas.

Specifically for water samples, occurrence of organophosphorus was higher than triazines in 2010 in contrast to 2011 in which the concentration of triazines was the highest (see Fig. 7.3). This is illustrated in detail in Figs. S7.1 and S7.2. In relation to organophosphorus family, malathion and diazinon were the most

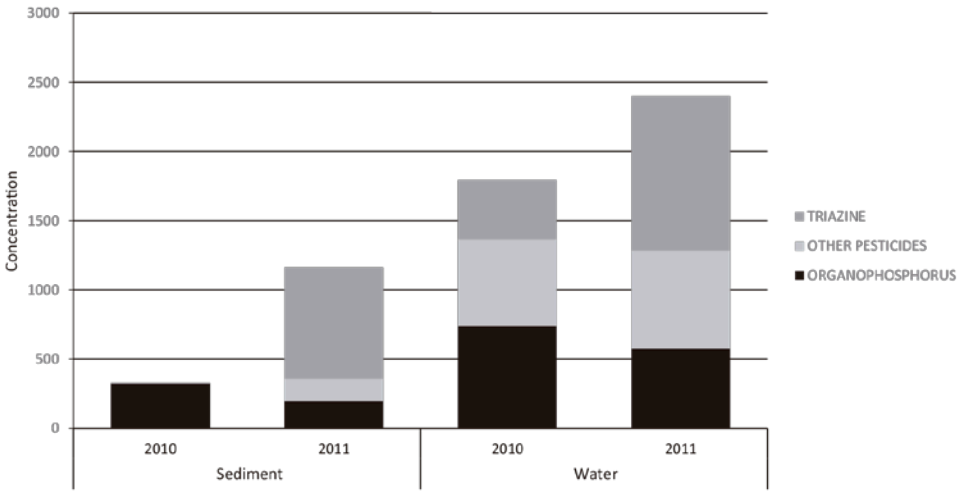


Fig. 7.3 Families detected in water and sediment samples for 2010 and 2011 campaign.

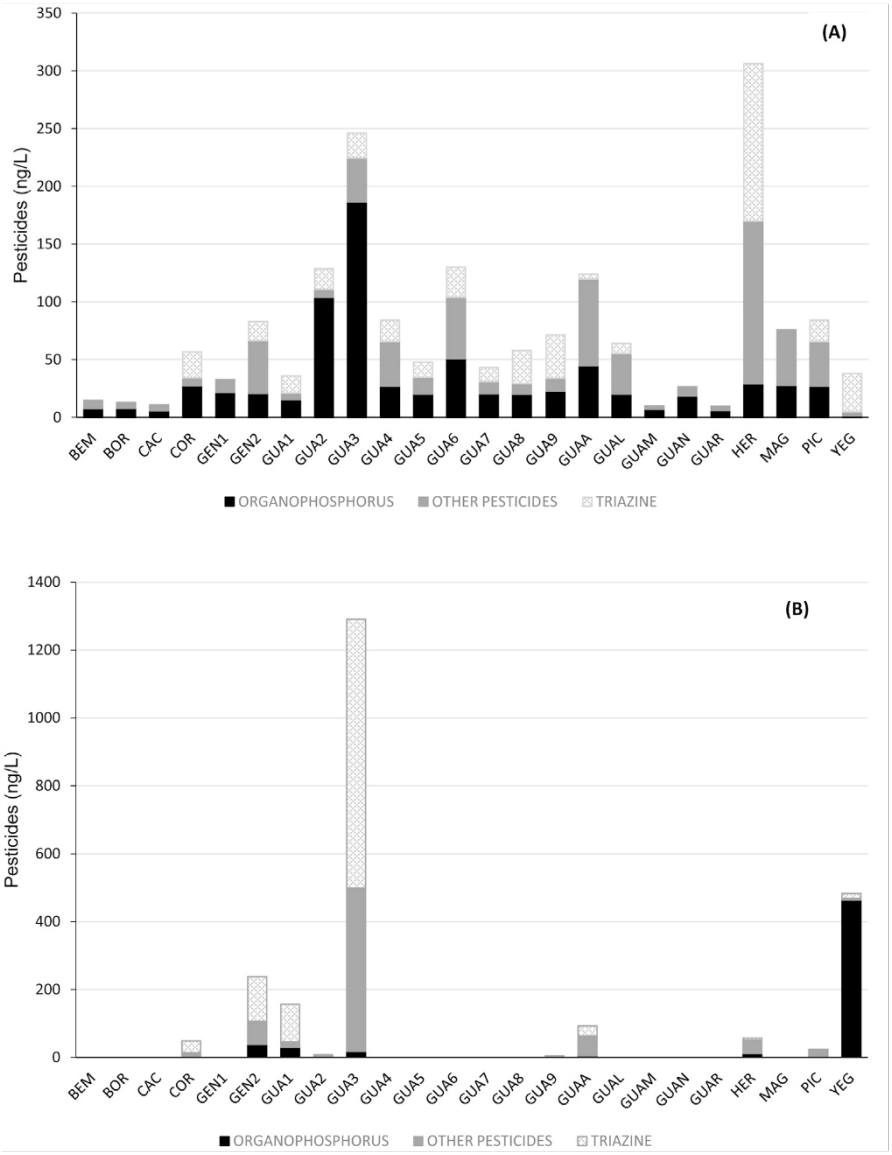


Fig. 7.4 Cumulative distribution of each family pesticide in waters at each samplig point (A) 2010 and (B) 2011.

detected in 2010 and 2011, respectively. However, the highest concentrations were for atrazine, terbuthylazine and their degradation products in both campaigns. In 2010, atrazine metabolites were at higher concentrations than the parent compound. The use of atrazine was banned in EU in 2004, and finally retired from the market in 2007 (Decision 2004/248/CE) [30], so the presence of this pesticide shows either its illegal use or more probably, its slow propagation from a reservoir to the water system. This last hypothesis could be supported by its metabolite ratio and the absence of the compound in 2011.

3.2. Occurrence in sediments

In 2010, 83% of the sediments presented one or more pesticides whereas in 2011 the percentage increased to 100% (see Fig. 7.2b). Table 7.2 shows the compounds detected at frequency higher than 10% of samples. In contrast with water, sediment samples of 2011 showed higher frequency and concentration of pesticides than those of 2010. In 2010, terbutryn was detected in one sample whereas in 2011, prochloraz, carbendazim, thiabendazole, methiocarb, metolachlor, pyriproxyfen, imidacloprid, chlorphenvinphos, fention and its metabolites, hexythiazox, terbumeton and its metabolites, terbuthylazine and its metabolites, terbutryn and diuron were found occasionally.

For sediment samples, chlorpyriphos and diazinon were detected in samples of both years. The co-occurrence of pesticides in each sampling site is shown in Fig. 7.2b, in which is possible to observe that the maximum number of pesticides co-occurring in one sample was eleven (GUA-6), which receives WW from Sevilla city.

The highest concentrations were in 2010 for diazinon (also frequent in water and with high log K_{ow}) and in 2011 for terbuthylazine (451.88 ng/g dw) and its metabolites deethyl-terbuthylazine (200.92 ng/g dw) and terbuthylazine-2-hydroxy (142.80 ng/g dw) in 2011. For more detail see Figs. S7.4 and S7.5 of the Supplementary material. The increased use of terbuthylazine as substitute of atrazine, as well as its detection in groundwaters of Spain and Portu-

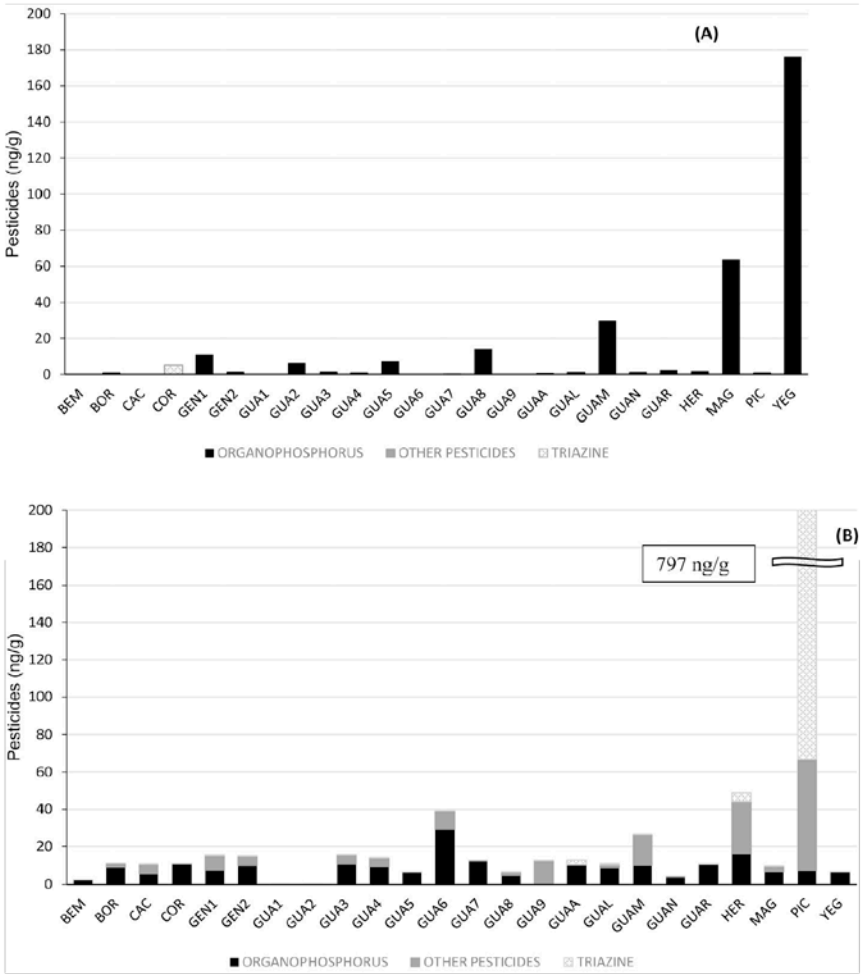


Fig. 7.5 Cumulative distribution of each family pesticide in sediments at each sampling point (A) 2010 and (B) 2011.

gal have been reported recently [31]. Olive trees were harvested in the middle of September just before the sampling campaigns. However, in intensified-traditional and modern olive plantations, these chemicals can remain highly concentrated in the top 5–15 cm of soil, even after several months, and can be washed into streams, rivers and reservoirs with the soil that is eroded by intense heavy rains. Average concentrations for individual pesticides were below 10 ng/g dw, with the exception of diazinon and terbuthylazine, which were at levels between 10 and 20 ng/g d.w.). All pesticides detected in sediment samples present a high log K_{ow} (see Table S7.2 Supplementary material). It means this compounds are relatively hydrophobic and with low solubility in water, therefore, they present a tendency to accumulate in sediment. This parameter allows to predict the distribution of organic compounds between water and sediment, becoming a physical–chemical property relevant for their accumulation. However, chlorfenvinphos, buprofezin, atrazine and propazine, which have also high log K_{ow}, were detected in water but not in sediment. Hladik et al. [15] demonstrated that while the physical–chemical properties of the compounds contribute to their partitioning between dissolved and sediment phases, timing of application and how much time elapses before the next major storm even were also important to pesticide accumulate in sediments. Chlorpyriphos and diazinon are pesticides that have application through the year not only in agriculture but also in urban areas [15,28].

On the other hand, GUS index (groundwater ubiquity score) [32] is an indicator of the potential risk of water pollution and it allows classifying pesticides into potential leachers (GUS > 2.8), non-leachers (GUS < 1.8) and transient leachers (1.8 < GUS < 68 2.8). Pesticides detected in sediment in highest concentrations, except diazinon, present high GUS index (see Table S7.2), i.e., they are contemplated as potential leachers and consequently, they can move easily from surface water to groundwater via the drainage systems.

3.3. Spatial distribution in 2010 and 2011

Figs. 7.4 and 7.5 include a cumulative distribution of the selected pesticide families in each sample point for waters and sediments, respectively. As it can be observed, the distribution of pesticides between water and sediments in both sampling campaigns is quite erratic.

In water samples, the most polluted sites both, in 2010 and 2011, were GUA-3 and GUA-A. The former was located in the upper part of the river, in Jaén province where a high olive crop density exists. The highest concentrations were detected for malathion in 2010 and methiocarb and terbuthylazine in 2011. These three pesticides are widely used in olive crops. The point GUA-3 is related

Table 7.3
Comparison between concentrations in WWTP effluents and receiving waters in 2010.

Target pesticide	Córdoba		Morón		Sevilla			Loja	
	WWTP	Downstream GUA-4	WWTP	Downstream GUA-A	WWTPcopero	WWTPranilla	Downstream GUA-6	WWTP	Downstream GEN-1
3-Hydroxycarbofuran	nd	nd	nd	nd	nd	140.4	nd	nd	nd
Atrazine	nd	nd	nd	nd	nd	27.4	4.7	nd	nd
Atrazine-deethyl	59.6	nd	68.4	nd	74.3	88.2	nd	68.4	nd
Atrazine-deisopropyl	nd	18.7	14.6	nd	nd	30.9	15.8	14.6	nd
Buprofezin	6.9	9.3	6.8	1.9	2.9	3.0	2.4	6.8	2.1
Carbofuran	nd	nd	nd	nd	nd	3.7	nd	nd	nd
Chlorfenvinphos	21.4	nd	nd	26.5	11.2	60.7	8.5	nd	2.8
Chlorpyriphos	1.0	3.5	nd	2.2	10.1	7.6	8.0	nd	4.2
Diazinon	95.4	10.3	12.5	9.7	128.2	135.4	23.8	12.5	7.7
Dimethoate	421.4	13.2	6.3	6.2	67.1	519.7	10.5	6.3	7.1
Diuron	61.1	23.1	nd	67.7	123.9	2393.1	43.6	nd	nd
Fenoxon sulfoxide	nd	nd	nd	nd	nd	5.2	nd	nd	nd
Fenthion sulfone	11.2	nd	10.1	nd	16.9	17.0	nd	10.1	nd
Hexythiazox	1.1	0.8	4.2	1.9	0.9	1.7	1.4	4.2	1.1
Imazalil	nd	nd	nd	nd	5.2	74.9	2.1	nd	nd
Imidacloprid	nd	2.4	nd	2.6	2.9	6.8	2.5	nd	2.7
Isoproturon	12.6	nd	nd	nd	16.7	9.7	nd	nd	nd
Methiocarb	nd	nd	nd	nd	5.7	nd	nd	nd	nd
Metolachlor	79.2	nd	52.7	nd	126.9	178.2	nd	52.7	nd
Molinate	12.6	nd	nd	nd	nd	nd	nd	nd	nd
Omethoate	nd	nd	nd	nd	4.6	5.6	nd	nd	nd
Prochloraz	19.2	nd	19.8	nd	2.9	nd	nd	19.8	4.7
Propanil	2.0	nd	nd	nd	nd	nd	nd	nd	nd
Propazine	8.9	nd	8.1	nd	12.1	nd	nd	8.1	nd
Pyriproxyfen	2.6	3.0	9.3	0.9	1.9	2.4	1.3	9.3	0.6
Terbutryn	nd	nd	nd	4.2	45.8	11.1	5.5	nd	nd

to the other much polluted sites in the upper part of the river, GUA-2 in 2010 and GUA-1 in 2011, both located in Jaén province. Very high concentrations in this area were also detected for other compounds such as dimethoate, deisopropylatrazine or simazine, mostly used to protect olive crops. A concentration gradient that increases from GUA-1 to GUA-3 was observed. In GUA-3 site, the sum of the compounds detected surpassed in 2011 the tolerance threshold of 0.5 $\mu g\,L^{-1}$ for the sum of pesticide residues established by the European Union Commission in order to guarantee water quality [25].

On the other hand, in the middle part of the river, the most contaminated point was GEN-2, located in the Sevilla province and, mainly affected by extensive agricultural farmlands (called "cortijos") in greenhouses.

GUA-A also located in Seville province, is characterized by extensive agricultural around the Guadalquivir River and by the influence of Cordoba WWTP. Some compounds such as chlorpyriphos, diuron and chlorfenvinphos were detected at high concentrations. Chlorpyriphos and chlorfenvinphos are insecticides used for pests control in olive, cereals, citrus, vine, fruit tree and diuron is an herbicide applied to the same sort of crops.

There was less contamination at the lowest part of the river covered by marshes devoted to rice cultivation. Rice is harvested in September and planted in March. Consequently, it was not an active crop for the sampling campaigns.

Some of the studied points, GUA-4, GUA-A, GUA-6 and GEN-1, are located after WWTPs. Concentrations in WWTP effluent samples (Tables 7.3 and 7.4) were high in comparison to surface waters for atrazine and their metabolites, chlorfenvinphos, diazinon, metholachlor, carbendazim, diuron pointing out that these WWTPs could be a source of contamination for receiving waters. Pesticides, probably, are from fruits and vegetable processing plants. On the contrary, other pesticides as chlorpyriphos have a homogeneous concentration in WWTPs and in water indicating non-point punctual contamination, probably, by runoff.

These results appear to be consistent with the agricultural activities because all of them are commonly used in olive, orchards, cotton groves, cereal, corn and grapevine groves typical from this area. The olive groves affect mainly to Córdoba and Jáen provinces. In Seville province, there are diverse extensive agriculture (cereals, cotton, sugar beet, etc.) and rice fields along the river borders. Granada and Malaga provinces are mainly affected by intensive agricultural in greenhouses (tomatoes, strawberries, lettuces, etc.). The last part of the river is a marshy land devoted also to rice field.

The ratio of degradates to pesticides can help to explain the pattern of contamination. Dimethoate is degraded in the environment to the more toxic omethoate. In 2010, dimethoate was widespread in the river. However, both compounds are only simultaneously present in GUA-2 and GUA-3 where the metabolite ratio is 0.16 and the concentration of dimethoate high (>50 ng/L) indicating a new source with recently applied pesticide. The concentration is almost the same in both sites. Upstream in MAG shows a ratio of 0.52 and the concentration of dimethoate is low (15 ng/L). The appearance of deisopropylatrazine and deethylatrazine could be from at least three triazines covered in this study simazine, propazine and atrazine. In 2010, deisopropylatrazine shows a constant background level through the river and a slight decrease of concentration downstream that could indicate its different origin. In 2011, it was detected in only one sample. However, deethylatrazine and atrazine only were detected in several points. In HER, the ratio was the highest (5.2). This high concentration of deethylatrazine could indicate that it come from soil deposits were atrazine is already partly degradate. The higher water solubility of the metabolite could explain its behavior. Both compounds were detected in two consecutive points GUA 8 and GUA 9 located in the marsh area. In this points, the ratio of metabolites decrease downstream from 1.0 to 0.7. This could indicates that runoff of freshly applied herbicides with less degradation or could also be due to the high environmental persistence of atrazine. In the case of fenthion, only the metabolites were scarcely detected. In 2011, terbuthylazine and its deethyl and hydroxylate metabolites were thoroughly detected. However, it was difficult to find a clear transportation pattern downstream because a clear punctual input of this compounds appears in GUA-3 (parent compound at high

Table 7.4
Comparison between concentrations in WWTP effluents and receiving waters in 2011.

Target pesticide	Córdoba		Morón		Sevilla			Loja	
	WWTP	Downstream GUA-4	WWTP	Downstream GUA-A	WWTPcopero	WWTPranilla	Downstream GUA-6	WWTP	Downstream GEN-1
3-Hydroxycarbofuran	nd	nd	nd	nd	4.1	nd	nd	nd	nd
Acetochlor	11.9	nd	nd	nd	nd	33.1	nd	nd	nd
Atrazine	nd	nd	nd	nd	nd	36.9	nd	nd	nd
Atrazine-deisopropyl	nd	nd	nd	nd	nd	31.6	nd	nd	nd
Carbendazim	27.0	nd	18.1	0.6	44.6	1249.4	nd	nd	nd
Chlorfenvinphos	2.7	nd	37.5	nd	nd	0.7	nd	nd	nd
Chlorpyriphos	2.1	nd	3.8	4.2	5.5	5.3	nd	6.1	nd
Diazinon	4.9	nd	4.3	0.7	54.2	19.5	nd	nd	nd
Dimethoate	7.2	nd	8.4	nd	2.2	640.2	nd	nd	nd
Diuron	44.6	nd	334.2	57.4	62.1	1217.9	nd	nd	nd
Fenoxon sulfone	nd	nd	nd	nd	nd	16.8	nd	nd	nd
Fenthion sulfoxide	nd	nd	nd	nd	nd	16.8	nd	nd	nd
Hexythiazox	nd	nd	1.3	nd	nd	nd	nd	1.5	nd
Imazalil	10.6	nd	6.5	nd	nd	nd	nd	nd	nd
Imidacloprid	nd	nd	13.9	nd	11.8	165.7	nd	nd	nd
Isoproturon	17.4	nd	1.5	nd	6.6	19.5	nd	nd	nd
Metolachlor	nd	nd	nd	nd	nd	42.6	nd	nd	nd
Prochloraz	nd	nd	nd	nd	nd	11.8	nd	nd	nd
Simazine	11.7	nd	nd	nd	4.6	nd	nd	nd	nd
Tebuconazole	8.2	nd	4.7	nd	nd	261.6	nd	nd	nd
Terbumeton	3.3	nd	0.3	nd	nd	8.1	nd	nd	nd
Terbumeton-desethyl	1.1	nd	nd	nd	nd	5.0	nd	nd	nd
Terbuthylazine	27.2	nd	35.5	171084.0	18.9	nd	nd	9.5	nd
Terbuthylazine-2 hydroxy	18.8	nd	10.6	nd	14.0	176.8	nd	3.6	nd
Terbuthylazine-desethyl	19.2	nd	17.9	126904.0	18.3	80.8	nd	9.7	nd
Terbutryn	70.3	nd	4.3	nd	23.0	73.5	nd	nd	nd
Thiabendazole	7.7	nd	12.2	nd	12.0	36.5	nd	nd	nd

concentration and proportional low amount of hydroxyl metabolite) and the other points are mostly located at different tributaries without connection, to central part of the basin.

In sediments in 2010, the most ubiquitous pesticides were organophosphorus. Higher concentrations were in the headwaters tributaries of the river (YEG, MAG, GUA) that decrease downstream the main course of the river. In YEG, diazinon was detected at the highest concentration, which could be explained because it is located in the Córdoba province in a zone polluted by "purines". This pesticide, in addition to its urban use, is still used in concentrates for dipping and spraying, in insecticide-impregnated ear-tags and in dressings for pigs.

In 2011, it is observed a uniform appearance of several families of pesticides in almost all points, with the exception of PIC site, where the pollution increased due to the sum of high concentrations of terbuthylazine and their degradation products, which were not monitored in the previous year. However, in the water sample of this location, there is not terbuthylazine. This point is downstream GUA-3 where a high concentration of parent terbuthylazine was detected only in water.

3.4. Temporal distribution in water and sediments

In 2010 all sample locations appeared contaminated by at least one pesticide family, but the sum of pesticide residues did not surpassed the tolerance of 0.5 $\mu g\,L^{-1}$ at any sampling point. In 2011, only ten sampling points were contaminated (see Fig. 7.4b) but at higher concentrations than in 2010. As opposed to water, Table 7.2 and Fig. 7.5 show that sediments taken in 2010 were polluted with less pesticides but with the exception of terbuthylazine at higher concentrations than 2011. These differences observed between both sampling campaigns in water and sediment could be related to the river flow. In 2010, the river presented high flow, and in 2011 it presented low-medium flow.

At least apparently, low-flow produced a pesticide concentration effect showing higher levels in water and accumulation in sediments. This higher concentration in sediments probably was favored by the lower water flow. The higher the flow, the greater the frequency and number of co-occurring pesticides because probably high river flow is related to heavy rains that produces an increment of runoff and the pesticides drag to the water, linked, at the same to the dilution effect. This results in wide range of pesticides present at low concentrations.

4. Conclusions

Water quality of the Guadalquivir River Basin has been established by a monitoring study of pesticides carried out for 2010 and 2011. The results reveal that pesticides are widespread in surface waters and sediments. The main influences to the river probably come from agriculture and industrial wastewaters and run-off. WWTPs located in zones with higher urban influence mean an important source of pesticides to the environment.

The river and its tributaries appear as a high-risk scenario for organophosphorus, triazines and carbamates, which were the families detected with highest frequency and concentration in both periods. Moreover, atrazine and terbuthylazine transformation products were found at higher concentrations than parent pesticides, as a result of their long degradation process.

The spatial distribution of pesticides in the Guadalquivir River Basin showed that sampling sites located in Jaén and Córdoba were dominated by insecticides and herbicides. Sampling sites in the other provinces contained a complex mixture all of herbicides, fungicides and insecticides, which reflect differences in crop patterns.

The temporal distribution could be related to the river flow. Low-medium flow compared to high one produced an increasing of the concentration in water and sediments. Several pesticides exceeded the limit of 0.1 $\mu g\,L^{-1}$ for an individual compounds and

one sampling point, (GUA-3) the limit of 0.5 $\mu g\,L^{-1}$ for the sum of pesticides with low-medium flow.

Acknowledgements

This work has been supported by the Spanish Ministry of Economy and Competitiveness through the projects Consolider-Ingenio 2010 CSD2009, and CGL2011-29703-C02-02. We would also like to thank the persons of the ICMAN research group for taking the samples. P. Vazquez Roig is holder of FPI grant from the Ministry of Science and Innovation (MICINN, Spain).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2013.09.035.

References

[1] C. Blasco, Y. Pico, TrAC Trends Anal. Chem. 28 (2009) 745.
[2] A. Navarro-Ortega, V. Acuña, R.J. Batalla, J. Blasco, C. Conde, F.J. Elorza, A. Elosegi, F. Francés, F. La-Roca, I. Muñoz, M. Petrovic, Y. Picó, S. Sabater, X. Sanchez-Vila, M. Schuhmacher, D. Barceló, Environ. Sci. Pollut. Res. 19 (2012) 918.
[3] A. Navarro, R. Tauler, S. Lacorte, D. Barceló, J. Hydrol. 383 (2010) 18.
[4] J.M. Maron, E.Gracia-Lor, J.V. Sancho, F.J. Lopez, F. Hernandez, J. Chromatogr. A 1216 (2009) 1410.
[5] A. Giordano, M. Fernandez-Franzon, M.J. Ruiz, G. Font, Y. Pico, Anal. Bioanal. Chem. 393 (2009) 1733.
[6] J. Fenoll, P. Hellin, C.M. Martinez, P. Flores, S. Navarro, Talanta 85 (2011) 975.
[7] C. Postigo, M.J. López de Alda, D. Barceló, A. Ginebreda, T. Garrido, J. Fraile, J. Hydrol. 383 (2010) 83.
[8] S. Moganti, B.J. Richardson, K. McClellan, M. Martin, P.K.S. Lam, G.J. Zheng, Mar. Pollut. Bull. 57 (2008) 672.
[9] A. Belmonte Vega, A. Garrido Frenich, J.L. Martínez Vidal, Anal. Chim. Acta 538 (2005) 117.
[10] A. Lazartigues, C. Fratta, R. Baudot, L. Wiest, C. Feidt, M. Thomas, C. Cren-Olive, Talanta 85 (2011) 1500.
[11] Y.B. Li, F.S. Dong, X.G. Liu, J. Xu, J. Li, Z.Q. Kong, X. Chen, W.C. Song, Y.H. Wang, Y.Q. Zheng, J. Chromatogr. A 1218 (2011) 6667.
[12] M.P. Ensminger, R. Budd, K.C. Kelley, K.S. Goh, Environ. Monit. Assess. 185 (2013) 3697.
[13] K.L. Smalling, T.J. Reilly, M.W. Sandstrom, K.M. Kuivila, Sci. Total Environ. 447 (2013) 179.
[14] L.H. Nowell, P.W. Moran, R.J. Gilliom, D.L. Calhoun, C.G. Ingersoll, N.E. Kemble, K.M. Kuivila, P.J. Phillips, Arch. Environ. Contam. Toxicol. 64 (2013) 32.
[15] M.L. Hladik, J.L. Domagalski, K.M. Kuivila, Sci. Total Environ. 408 (2009) 356.
[16] L.A. Knight, M.K. Christenson, A.J. Trease, P.H. Davis, A.S. Kolok, Environ. Toxicol. Chem. 32 (2013) 1544.
[17] N.E. Kemble, D.K. Hardesty, C.G. Ingersoll, J.L. Kunz, P.K. Sibley, D.L. Calhoun, R.J. Gilliom, K.M. Kuivila, L.H. Nowell, P.W. Moran, Arch. Environ. Contam. Toxicol. 64 (2013) 52.
[18] S.J. Kalkhoff, A.V. Vecchia, P.D. Capel, M.T. Meyer, J. Environ. Qual. 41 (2012) 1566.
[19] A.V. Vecchia, R.J. Gilliom, D.J. Sullivan, D.L. Lorenz, J.D. Martin, Environ. Sci. Technol. 43 (2009) 9096.
[20] UNEP, Freshwater in Europe, UNEP/DEWA Europe, Châtelaine, 2004.
[21] J.A. Lopez-Lopez, M. Garcia-Vargas, C. Moreno, Environ. Pollut. 159 (2011) 1136.
[22] C. Mendiguchía, C. Moreno, M.D. Galindo-Riaño, M. García-Vargas, Anal. Chim. Acta 515 (2004) 143.
[23] D.P. Oliver, R. Kookana, J.S. Anderson, J.W. Cox, N. Waller, L.H. Smith, Agric. Water Manage. 106 (2012) 78.
[24] M.V. Cuevas, L. Cox, M.J. Calderón, M.C. Hermosín, J.E. Fernández, Agric. Ecosyst. Environ. 124 (2008) 245.
[25] European Parliament and Council, Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council, Off. J. Eur. Comm. L 348 (2008) 84.
[26] A. Masiá, M. Ibañez, C. Blasco, J.V. Sancho, Y. Picó, F. Hernández, Anal. Chim. Acta 761 (2013) 117.
[27] M. Anastassiades, S.J. Lehotay, D. Bátajnbaher, F.J. Chenck, J. AOAC Int. 86 (2003) 412.
[28] I.K. Wittmer, H.P. Bader, R. Scheidegger, H. Singer, A. Lück, I. Hanke, C. Carlsson, C. Stamm, Water Res. 44 (2010) 2850.
[29] European Commission, Directive 2006/118/EC of the European Parliament and of the Council on the protection of groundwater against pollution and deterioration (2006), Off. J. Eur. Comm. L 372 (2006).
[30] European Commission, Commission Decision concerning the non-inclusion of atrazine in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing this active substance (2004), Off. J. Eur. Comm. L 78 (2004) 53.
[31] E.Herrero-Hernández, M.S. Andrades, A. Álvarez-Martín, E. Pose-Juan, M.S. Rodríguez-Cruz, M.J. Sánchez-Martín, J. Hydrol. 486 (2013) 234.
[32] D.I. Gustafson, Environ. Toxicol. Chem. 8 (1989) 339.

Suplementary Material

Screening of currently used pesticides in water, sediments and biota of the Guadalquivir River Basin

Ana Masiá*, Julián Campo, Pablo Vázquez-Roig, Cristina Blasco, Yolanda Picó

Food and Environmental Safety Research Group, Department of Medicine Preventive, Faculty of Pharmcy, University of Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia Spain.

* Corresponding author. Tel. +34 963544873; fax: +34 963544954
E-mail address: Ana.Masia@uv.es

Table S7.1 Sampling points georeferences

RIVER OR TRIBUTARY	ABREV	LOCATION	COORDINATES UTM		
			ZONE	X	Y
Borosa	BOR	La Iruela	30	512435	4207084
Guadalquivir	GUA-1	Villacarrilo	30	497027	4214205
Guadiana Menor	GUAM	Úbeda	30	481267	4192450
Guadalquivir	GUA-2	Puente del Obispo (Baeza)	30	452771	4200519
Magaña	MAG	Santa Elena	30	456338	4242400
Guadabullón	GUAN	Mengibar	30	431850	4199348
Guadalquivir	GUA-3	Marmolejo	30	395434	4207864
Yeguas	YEG	Cardeña	30	384664	4246754
Guadalmoral	GUAL	Baena	30	375522	4166800
Guadalquivir	GUA-4	Córdoba	30	334794	4189933
Picachos	PIC	Fuente palmera	30	315008	4180807
Bembézar	BEM	Hornachuelos	30	279446	4224770
Cacín	CAC	Arenas del rey	30	423215	4086558
Genil	GEN-1	Loja	30	396109	4116460
Genil	GEN-2	Écija	30	314734	4161417
Guadalquivir	GUA-5	Peñaflor	30	294339	4174415
Corbones	COR	Carmona	30	272990	4153778
Herreros	HER	Alcalá del Río	30	235090	4156650
Guadaira	GUA-A	Morón	30	267816	4123619
Guadalquivir	GUA-6	Coria del Río	29	761545	4129986
Guadalquivir	GUA-7	Brazo del Este	29	759226	4107247
Guadalquivir	GUA-8	Rancho de Barzoques (Lebrija)	29	751405	4094029
Guadiamar	GUAR	Alnalcázar	29	742717	4130496
Guadalquivir	GUA-9	Sanlucar de Barrameda	29	736448	4084204
WWTP					
La Golondrina (Córdoba)	WWTP-COR	Guadalquivir	30	336060	4190361
Loja (Loja-Granada)	WWTP-LOJ	Genil	30	396586	4114676
Morón (Morón de la Frontera-Sevilla)	WWTP-MOR	Arroyo del Cuerno (Guadaira)	30	279678	4113069
Copero (Sevilla-South)	WWTP-SEVC	Guadalquivir	29	762808	4133586
Ranilla (Sevilla-East)	WWTP-SEVR	Guadaira	30	240515	4139452

Table S7.2 Phisico-chemical properties

	CAS NUMBER	MOLECULAR FORMULA	PM (g/mol)	log Kow (pH 7, 20 ºC)	GUS INDEX-LEACHABILITY	BIOCIDE ACTION
3-Hydroxycarbofuran	16655-82-6	$C_{12}H_{15}NO_4$	237,25	1.45 (low)	1.45 (low)	Metabolite
Acethochlor	34256-82-1	$C_{14}H_{20}ClNO_2$	269,77	4.14 (high)	1.77 (low)	Herbicide
Alachlor	15972-60-8	$C_{14}H_{20}ClNO_2$	269,77	3.09 (high)	0.80 (low)	Herbicide
Atrazine	1912-24-9	$C_8H_{14}ClN_5$	215,68	2.7 (moderate)	3.30 (high)	Herbicide
Azinphos-ethyl	2642-71-9	$C_{12}H_{16}N_3O_3PS_2$	345,38	3.18 (high)	1.4 (low)	Insecticide, Acaricide
Azinphos-methyl	86-50-0	$C_{10}H_{12}N_3O_3PS_2$	317,32	2.96 (moderate)	0.95 (low)	Insecticide, Acaricide
Buprofezin	69327-76-0	$C_{16}H_{23}N_3OS$	305,44	4.93 (high)	0.46 (low)	Insecticide, Acaricide
Carbendazim	10605-21-7	$C_9H_9N_3O_2$	191,21	1.48 (low)	2.64 (transition state)	Fungicide
Carbofuran	1563-66-2	$C_{12}H_{15}NO_3$	221,26	1.8 (low)	3.02 (high)	Insecticide, Acaricide, Nematicide
Chlofenvinphos	470-90-6	$C_{12}H_{14}Cl_3O_4P$	359,6	3.8 (high)	1.87 (transition state)	Insecticide, Acaricide
Chlorpyriphos	5598-13-0	$C_7H_7Cl_3NO_3PS$	322,53	4 (high)	0.2 (low)	Insecticide, Acaricide
Deisopropylatrazine	1007-28-9	$C_5H_8ClN_5$	173,6	1,15 (low)	-	Metabolite
Deethylatrazine	6190-65-4	$C_6H_{10}ClN_5$	187,63	1.51 (low)	3.54 (high)	Metabolite
Diazinon	333-41-5	$C_{12}H_{21}N_2O_3PS$	304,35	3.69 (high)	1.14 (low)	Insecticide, Acaricide, Repelent

	CAS NUMBER	MOLECULAR FORMULA	PM (g/mol)	log Kow (pH 7, 20 ºC)	GUS INDEX-LEACHABILITY	BIOCIDE ACTION	CHEMICAL FAMILY
Diclofenthion	97-17-6	$C_{10}H_{13}Cl_2O_3PS$	315,15	5.14 (high)	2.14 (transition state)	Insecticide	Organophosphorus
Dimethoate	60-51-5	$C_5H_{12}NO_3PS_2$	229,26	0.704 (low)	1.06 (low)	Insecticide, Acaricide	Organophosphorus
Diuron	330-54-1	$C_9H_{10}Cl_2N_2O$	233,09	2.87 (moderate)	1.83 (transition state)	Herbicide	Urea
Ethion	563-12-2	$C_9H_{22}O_4P_2S_4$	384.48	5.07 (high)	0.00 (low)	Insecticide, Acaricide	Organophosphorus
Fenitrothion	122-14-5	$C_9H_{12}NO_5PS$	277.23	3.32 (high)	0.48 (low)	Insecticide	Organophosphorus
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	-	-	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)	1.11 (low)	Insecticide	Organophosphorus
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)	0.04 (low)	Acaricide	Acaricide
Imazalil	35554-44-0	$C_{14}H_{14}Cl_2N_2O$	297.18	2.56 (low)	0.55 (low)	Fungicide	Azol
Imidacloprid	138261-41-3	$C_9H_{10}ClN_5O_2$	255.66	0.57 (low)	3.76 (high)	Insecticide	Neonicotinoid
Isoproturon	34123-59-6	$C_{12}H_{18}N_2O$	206,3	2.5 (low)	2.07 (transition state)	Herbicide	Urea
Malathion	121-75-5	$C_{10}H_{19}O_6PS_2$	330.36	2.75 (moderate)	(-)1,28 (low)	Insecticide, Acaricide	Organophosphorus
Methiocarb	2032-65-7	$C_{11}H_{15}NO_2S$	225.31	3.18 (high)	0.17 (low)	Insecticide	Carbamates

	CAS NUMBER	MOLECULAR FORMULA	PM (g/mol)	log Kow (pH 7, 20 ºC)	GUS INDEX-LEACHABILITY	BIOCIDE ACTION	CHEMICAL FAMILY
Metolachlor	51218-45-2	$C_{15}H_{22}ClNO_2$	283.8	3.4 (high)	3.49 (high)	Herbicide	Chloroacetanilide
Molinate	2212-67-1	$C_9H_{17}NOS$	187.3	2.86 (moderate)	2.49 (transition state)	Herbicide	Carbamates
Omethoate	1113-02-6	$C_5H_{12}NO_4PS$	213.2	(-) 0.74 (low)	2.73 (transition state)	Insecticide, Acaricide	Organophosphorus
Parathion-ethyl	56-38-2	$C_{10}H_{14}NO_5PS$	291.26	3.83 (high)	2.09 (transition state)	Insecticide, Acaricide	Organophosphorus
Parathion-methyl	298-00-0	$C_8H_{10}NO_5PS$	263.21	3 (moderate)	1.46 (low)	Insecticide	Organophosphorus
Prochloraz	67747-09-5	$C_{15}H_{16}Cl_3N_3O_2$	376.7	3.5 (high)	1.75 (low)	Fungicide	Azol
Propanil	709-98-8	$C_9H_9Cl_2NO$	218.08	2.29 (low)	0.72 (low)	Herbicide	Anilide
Propazine	139-40-2	$C_9H_{16}ClN_5$	229.71	3.95 (high)	3.84 (high)	Herbicide	Triazine
Pyriproxyphen	95737-68-1	$C_{20}H_{19}NO_3$	321.37	5.37 (high)	(-)0.33 (low)	Insecticide	Juvenile Hormone Mimi
Simazine	122-34-9	$C_7H_{12}ClN_5$	201.66	2.3 (low)	2.00 (transition state)	Herbicide	Triazine
Tebuconazole	107534-96-3	$C_{16}H_{22}ClN_3O$	307.82	3.7 (high)	2.00 (transition state)	Fungicide	Triazole
Terbumeton	33693-04-8	$C_{10}H_{19}N_5O$	225.29	3.04 (high)	3.79 (high)	Herbicide	Triazine
Terbumeton-deethyl							
Terbuthylazine	5915-41-3	$C_9H_{16}ClN_5$	229.71	3.4 (high)	3.07 (high)	Herbicide, Microbiocide, Algicide	Triazine
Terbuthylazine-2-hydroxy	66753-07-9	$C_9H_{17}N_5O$	211.33	-	4.59 (high)	Metabolite	Triazine
Terbutryn	886-50-0			3,34			Triazine

	CAS NUMBER	MOLECULAR FORMULA	PM (g/mol)	log Kow (pH 7, 20 ºC)	GUS INDEX-LEACHABILITY	BIOCIDE ACTION	CHEMICAL FAMILY
Thiabendazole	148-79-8	$C_{10}H_7N_3S$	201.25	2.39 (low)	0.36 (low)	Fungicide	Bencimidazole
Tolclophos-methyl	57018-04-9	$C_9H_{11}Cl_2O_3PS$	301.13	4.56 (high)	0.25 (low)	Fungicide	Organophosphorus

Table S7.3 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 S7.4 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-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)
Chlorpyriphos	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)
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)

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)]
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)
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

Table S7.5 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-Sulfone	57	12	0,20	1,00	105	10	0,17	0,50	89	13	0,38	1,13

	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
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		

	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
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

Fig. S7.1 Triazines detected in water samples 2010-2011

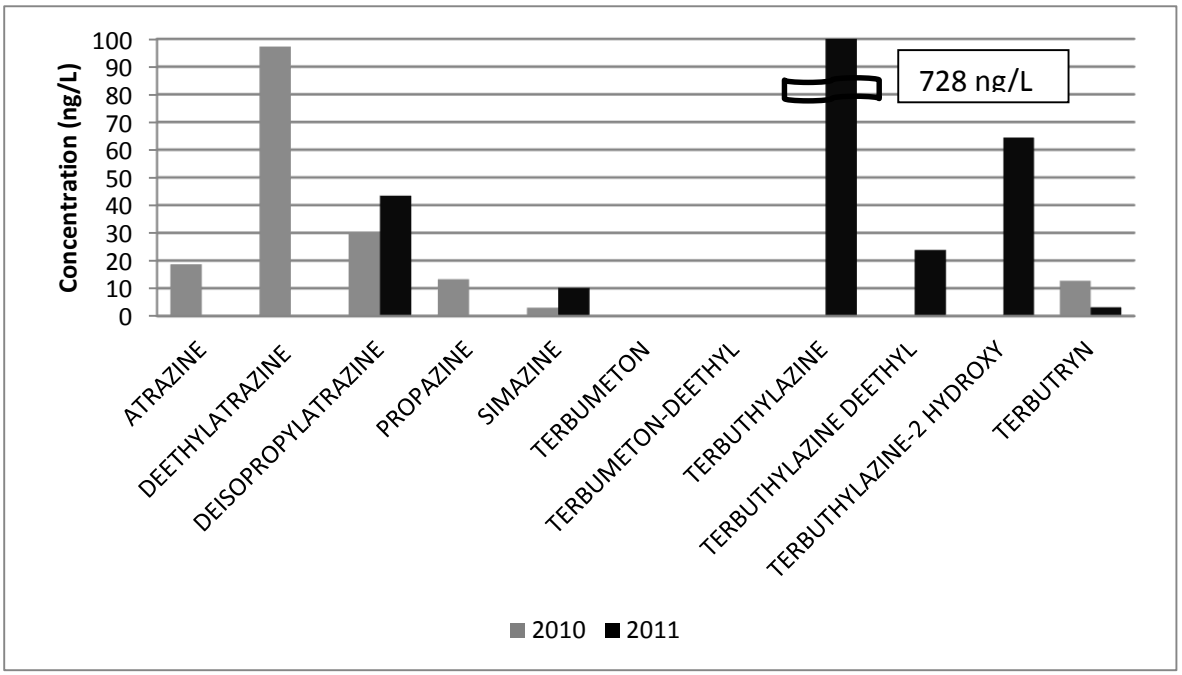


Fig. S7.2 Organophosphorus detected in water samples 2010-2011

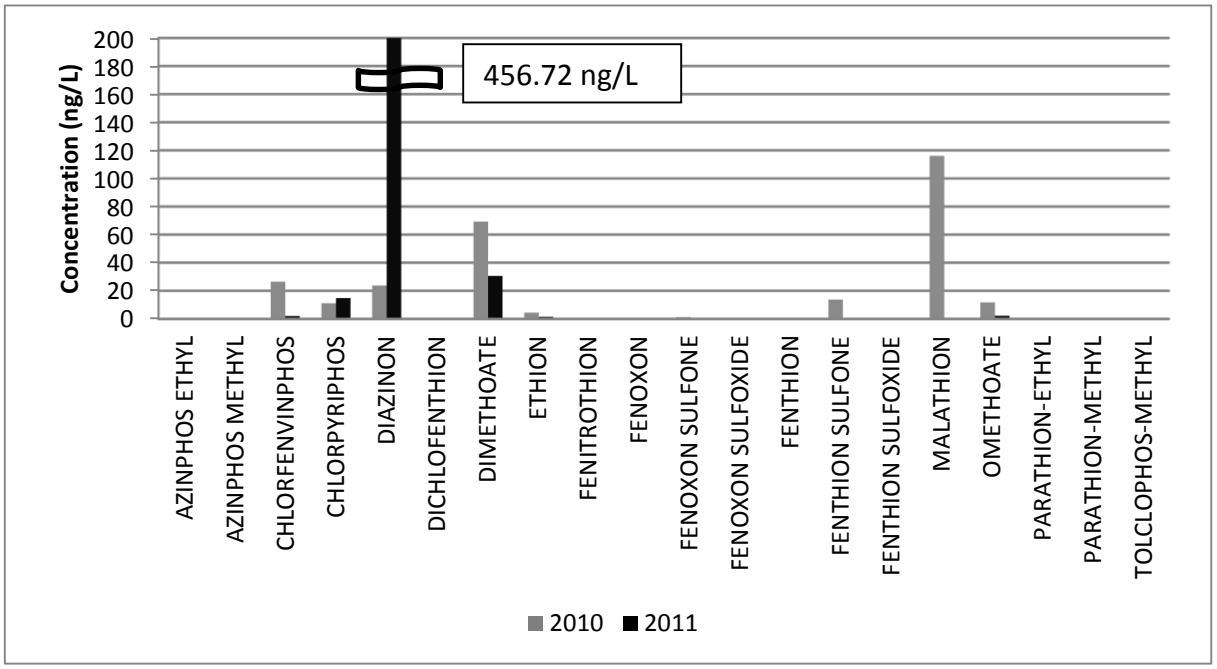


Fig. S7.3 Families appeared in sediment samples 2010-2011

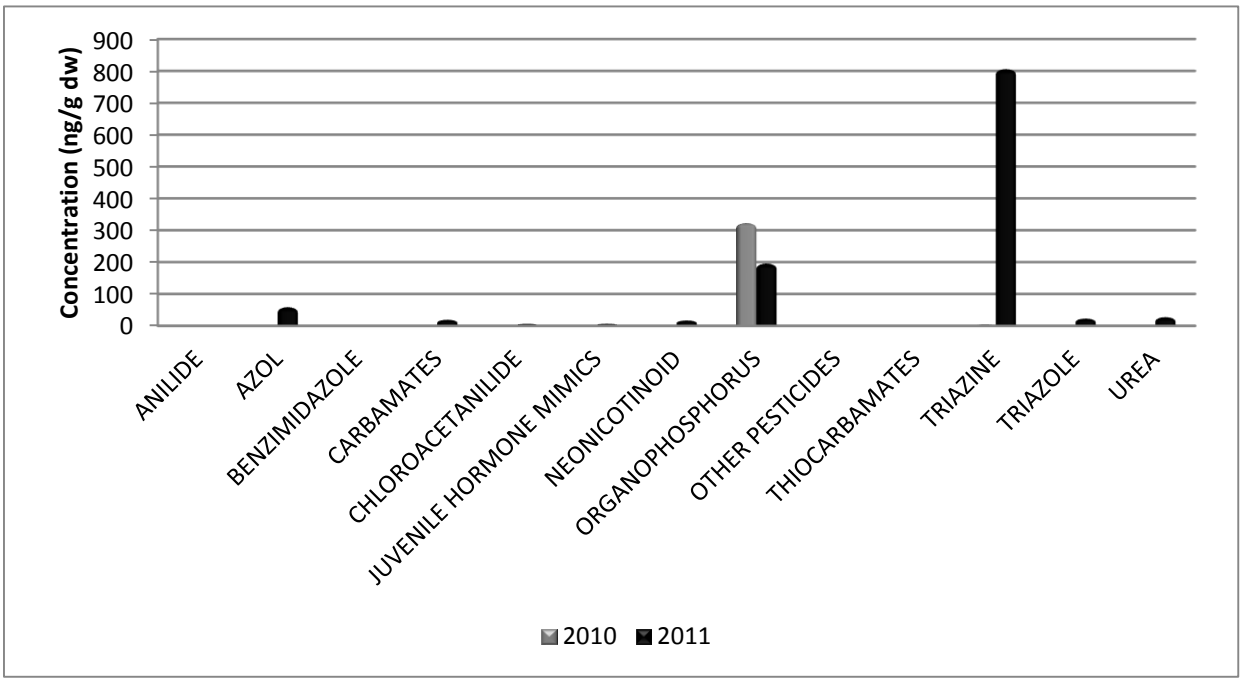


Fig. S7.4 Triazines detected in sediment samples 2010-2011

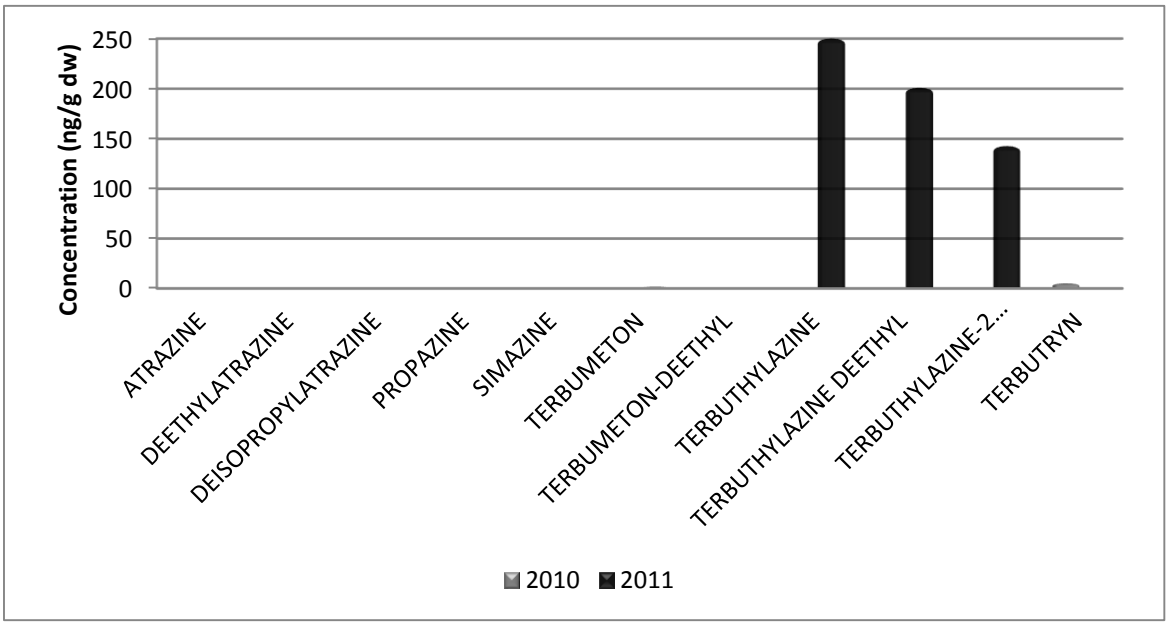
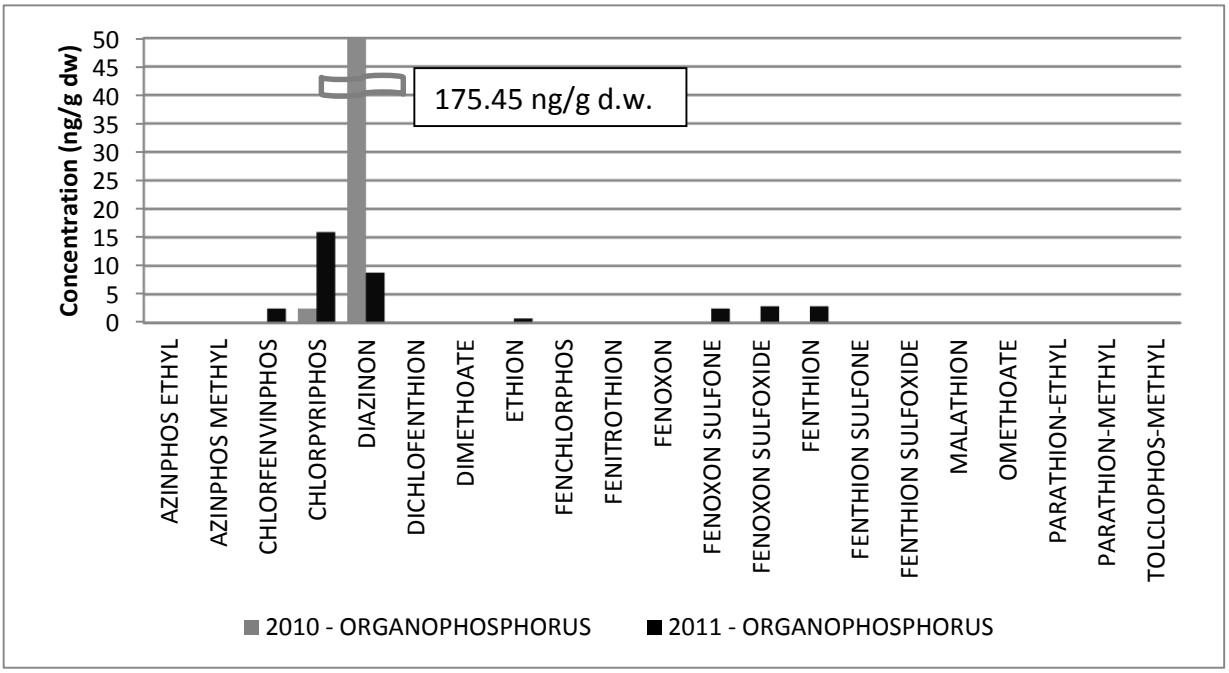


Fig. S7.5 Organophosphorus detected in sediment samples 2010-2011



CAPÍTOL 8

Seguiment de plaguicides en la conca del Llobregat (Catalunya, Espanya) i comparació amb dades històriques

Publicació científica 8

Pesticide monitoring in the basin of Llobregat River (Catalonia, Spain) and comparison with historical data

A. Masiá, J. Campo, A. Navarro-Ortega, D. Barceló, Y. Picó

Science of Total Environment-*En premsa*

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Pesticide monitoring in the basin of Llobregat River (Catalonia, Spain) and comparison with historical data

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

[a] Food and Environmental Safety Research Group, Department of Medicine Preventive, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain
[b] Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research, IDAEA-CSIC, C/ Jordi Girona 18-26, 08034 Barcelona, Spain
[c] Catalan Institute for Water Research (ICRA), C/ Emili Grahit, 101, Edifici H2O, Parc Científic i Tecnològic de la Universitat de Girona, 17003 Girona, Spain

HIGHLIGHTS

- Occurrence of currently used pesticides was detected in the Llobregat River basin.
- Benzimidazoles, organophosphorus and ureas appeared frequently at high levels.
- Sediments and biota were contaminated primarily by organophosphorus (higher K_{ow}).
- Risk evaluation showed low chronic risk to algae and fish from pesticide residues.
- Historical data confirmed a background contamination in the last 20 years.

ARTICLE INFO

Article history:
Received 16 April 2014
Received in revised form 17 June 2014
Accepted 20 June 2014
Available online xxxx

Keywords:
Water
Sediment
Fish
Spatial distribution
Temporal distribution

ABSTRACT

Through an extensive sampling in the Llobregat River basin, the presence of 50 currently used pesticides in water, sediment, and biota was assessed. Pesticides were detected primarily in water (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}) (chlorpyrifos 93% of sediments up to 131 ng g^{-1}). According to the results of this study, pesticide residues in the Llobregat River basin do not seem to represent a high risk to biota, even though some algae and fish can be affected. Nevertheless, the monitoring program can be very useful to control the contamination of the river basin, as the availability of historical data on the basin confirmed background contamination in the last 20 years.

1. Introduction

The application of pesticides and other plant protection products can maintain high product quality by controlling insects and other plant pathogens that harm crops (Cooper and Dobson, 2007). On the other hand, water catchments are highly susceptible to contamination, because these chemicals have high potential to reach the aquatic environment after application, via runoff, agricultural storm-water discharges, and return flows from irrigated fields (Campo et al., 2013; Damasio et al., 2011). As a result, pesticides can remain in water, accumulate in sediment or bioaccumulate in biota, depending on their solubility, with potential to cause adverse effects on human health and the environment, even at low concentrations (Kuster et al., 2008).

To improve this situation, the European Union (EU) Water Framework Directive (briefly WFD) (Directive 2000/60/EC) establishes the bases to regulate the water resources with the objective of preserving, protecting, and improving their quality and sustainable use. The adopted Decision No 2001/2455/EC, sets up a list of 33 priority substances to be controlled, the third part of which are pesticides. Directive 2008/105/EC defines environmental quality standards (EQS), annual average (AA), and maximum allowable concentrations (MAC) in surface waters. Although various pesticides are currently included in the list of priority substances in the EU regulations, many others are still unregulated (Proia et al., 2013).

The Llobregat River is subjected to extensive urban, industrial, and agricultural activities, becoming a damaged river basin, where relevant concentrations of a broad range of organic pollutants occur in surface water and related compartments (Gonzalez et al., 2012; Koeck-Schulmeyer et al., 2011). At the same time, this river is also one of the most important sources of water in the area, serving several million inhabitants living in

* Corresponding author. Tel.: +34 963544118; fax: +34 963544954.
E-mail address: Ana.Masia@uv.es (A. Masiá).

http://dx.doi.org/10.1016/j.scitotenv.2014.06.095
0048-9697/

Barcelona and in the metropolitan area (Paune et al., 1998). The Llobregat River basin has been the object of many published studies. According to a search carried out in ISI Web of Science, using the keyword 'Llobregat', there are more than 600 publications in the past year (2013). Most of these articles focused on surface and groundwater of the lower and medium parts of the river, which are the areas with the worst water quality. Many of them reveal the presence of organic micro-pollutants in wastewaters, surface and groundwater, and ultimately drinking water (Cespedes et al., 2005; Ginebreda et al., 2010; Guerra et al., 2010; Koeck-Schulmeyer et al., 2011, 2012; Kuster et al., 2008; Proia et al., 2013).

An extensive monitoring (including 50 pesticides) has been developed in two consecutive campaigns (2010–2011) in surface water, wastewater effluents, sediment, and biota samples along the entire course of the Llobregat River and its tributaries, with the purpose of establishing pesticide occurrence and distribution. The results obtained by this monitoring were compared with historical data gathered during other monitoring programs in this river basin to describe trends in water quality status and determine potential risks for human health. The added values of this study are the following: (i) although data on the occurrence of some of the studied pesticides in the Llobregat River are available, this is the first time that a so extensive number of pesticides (i.e. 50 active ingredients) is determined in the basin; (ii) three environmental compartments (i.e. water/sediment/biota) were studied for the first time, because most of the previous studies were restricted to water, and (iii) the contamination levels of the Llobregat River were reviewed only once, dealing with the presence and biological effects of any type of emerging contaminants (Gonzalez et al., 2012). Thus, it is also the first time that data related to pesticide occurrence in this river have been assembled and compared in a detailed way.

Furthermore, the results of this study can be widely applicable to other basins that follow the hydrological pattern of the Mediterranean rivers and suffer the increasing effect of climate change. The most representative areas with typical characteristics of the Mediterranean climate are countries bordering the Mediterranean Sea (Spain, Greece, Turkey, Morocco, Algeria, and Italy) as well as countries that have similar climate, such as South Africa, Chile, California, and Australia (Oliver et al., 2012; Delmotte et al., 2011; Daam et al., 2011; Kookana et al., 2010).

2. Material and methods

2.1. Site description

The Llobregat River (northeast of Catalonia, Spain), emerges in the pre-Pyrenees mountains at an altitude of 1400 m a.s.l. and flows along 150 km until its mouth in the Mediterranean Sea, 10 km south of Barcelona, draining an area of 4948 km^2 (Gonzalez et al., 2012). As a Mediterranean river, it presents flow fluctuations highly dependent on climatic conditions, including periodic floods and droughts related to seasons, in which the flow can range from several $m^3\ s^{-1}$ in periods of storms (i.e. normally in spring and autumn) to few $m^3\ s^{-1}$ during summer (dry period). It is also subjected to discharges from more than 30 wastewater treatment plants (WWTPs), which increase the flow river in dry periods, but reduce the dilution factor, compromising clearly water quality (Gonzalez et al., 2012). The river has around 40 tributaries, but the main ones are the Cardener (CAR) and Anoia (ANO) rivers. Both constitute a focus of pollution because of the important agricultural area (mainly vineyards in the Anoia River) and dense population with important demands of water (Gonzalez et al., 2012; Cespedes et al., 2005; Gonzalez et al., 2012; Proia et al., 2013). Close to its mouth, the Llobregat Delta, considered internationally important by the EU, represents the most valuable area from an ecological point of view because it constitutes the migratory route between Europe and Africa of more than 300 species of birds. The Delta is also a fertile area with an important agricultural activity focused on the cultivation of crops such as artichokes, lettuce, and tomatoes (Koeck-Schulmeyer et al., 2012).

2.2. Sampling and sample analysis

The sampling campaign was performed during 15 days in September/October 2010 and October/November 2011. For this purpose, a group of pesticides (as well as some of their common transformation products), from different chemical families were selected according to their extent of use in the studied area, water solubility, and amenability to liquid chromatography–mass spectrometry (LC–MS) analysis (see Table S8.1). In the first campaign, 42 pesticides were determined, while in the second, 50. The pesticides added in the second campaign were frequently detected in some non-target analysis of water samples, revealing their constant presence (Masiá et al., 2013a).

Water and sediments were collected at 14 selected sites along the Llobregat River and its tributaries Anoia and Cardener (Fig. 8.1). Grab water samples (2 L) were collected in clean amber glass bottles, from the middle of the river width. Sediment samples (approx. 250 g) were taken in the same point as the water samples using a Van Veen grab sampler (0.5 L capacity); they were transferred and wrapped into an aluminum foil. Additionally, pesticides were analyzed in influent, effluent, and sludge samples from three waste water treatment plants (WWTPs), which flow into the river, to study the influence of the effluent discharges in the final water quality of the studied rivers. The selected WWTPs were Igualada (located in the Anoia River), Manresa (located in the Cardener River, close to its confluence with the Llobregat River) and Abrera (located in the lower part of the Llobregat River). WWTP samples were 24 h composite samples provided by the plant operators. Georeferences of all sampling points are shown in Table S8.2.

Fish samples were taken only in 2010, according to the EQS (Directive, 2008/105/EC) at five selected sites of the river course: LLO3, LLO4, LLO5, LLO6, and LLO7. Fish samples were collected using electro-fishing by the personnel of Institute of Environmental Assessment and Water Research (IDAEA) in Barcelona, Spain and Catalan Institute for Water Research (ICRA) in Girona, Spain. The collected fish included adult and young barbus (*Barbus guiraonis*) taken at LLO3, LLO4 and LLO6 sites; black bass (*Mycropterus salmoides*) adult taken at LLO3 and common carp (*Cyprinus carpus*) taken in each biota sampling point. Concerning the extraction techniques, an already published SPE method (Oasis HLB SPE cartridge 200 mg sorbent/6 mL cartridge, Waters, Milford, MA, USA) was used for water samples (Masiá et al., 2013a). QuEChERS method (Anastassiades et al., 2003), also described in the literature, was applied in the sediment (Campo et al., 2013) a nd biota (Belenguer et al., 2014) samples. Detailed information on the methods is provided in Figs. S-1 and S-2 of the Supplementary material. Pesticides were determined by liquid chromatography–tandem mass spectrometry, using an HP1200 series LC system, equipped with automatic injector, degasser, quaternary pump and column oven, which was interfaced to an Agilent 6410 triple Quad (QQQ) mass spectrometer, with an electrospray ionization (ESI) interface (Agilent Technologies, Waldbronn, Germany) operating in positive ionization mode. Data were processed using a MassHunter Workstation Software for qualitative and quantitative analyses (A GL Sciences, Tokyo, Japan). Detailed instrumental and dynamic MRM conditions are provided in Tables S8.3 and S8.4 of the Supplementary material. The validation of the method is also fully described in the Supplementary material (see validation of the method and Table S8.5). The limits of detection ranged from 0.01 to 2 ng L^{-1} for water, 0.03 to 1.67 ng g^{-1} for sediment, and 0.08 to 3.75 ng g^{-1} for fish. Recoveries at different concentrations were between 49 and 112% and RSDs <25%.

2.3. Quality control (QC)

Regarding the QC procedures, parameters such as laboratory and field blanks, matrix spikes, and triplicate samples were evaluated. 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,

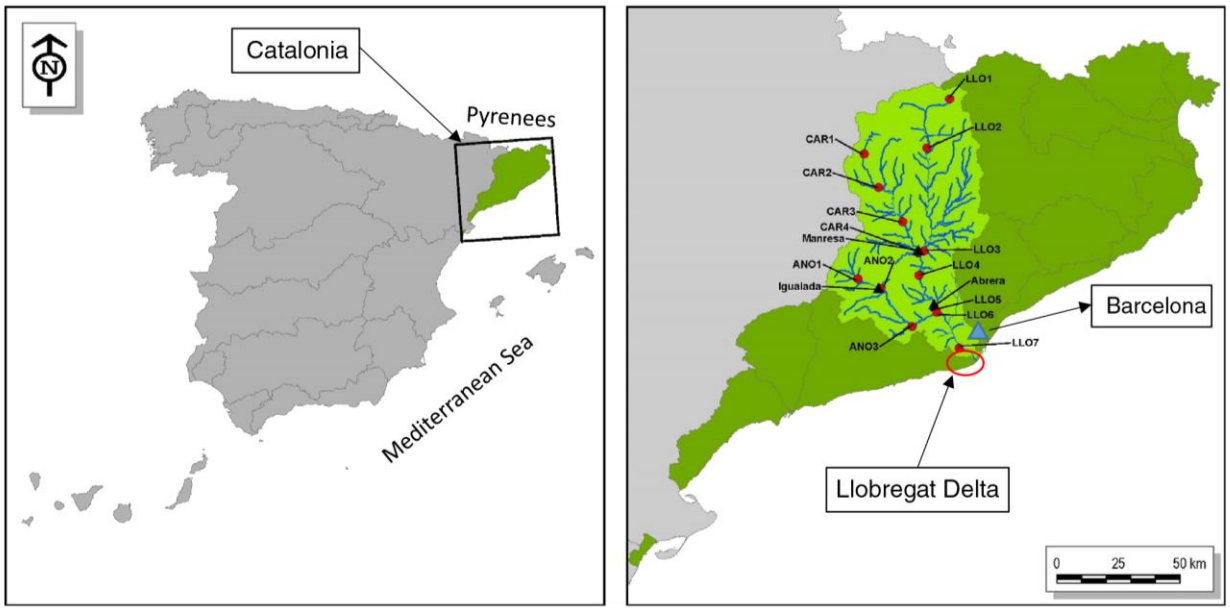


Fig. 8.1 Geographic location of the sampling zone and sampling points.

and glassware. Blank assays were performed employing MilliQ water samples, to check for laboratory background levels of the studied compounds. Although the detected amounts of the target compounds were low (less than 5 ng L^{-1}), it was considered necessary to subtract the quantitative values of the compounds found in the blanks (only ethion and pyriproxyfen). To assure the quality of the results, field blanks were processed with the samples. They consisted of deionized water put down in the same conditions than samples during the sampling process. For each batch of the 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.

2.4. Risk assessment

The Risk Quotient (RQ) for each pesticide 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 L(E)C50 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 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. It means that the potential of the contaminant to cause any ecotoxicological damage by itself is unlikely.

To cover all food chain in the water and following the Water Framework Directive (WFD) (Directive 2000/60/EC) suggestions, the RQ must be calculated at least at three representative taxons (algae, *Dapnnia magna*, and fish) of three trophic levels in the ecosystem. For most compounds, short and long-term values were obtained from the website http://sitem.herts.ac.uk/aeru/ppdb/en/atoz.htm. Values of any compounds not available in this site were calculated using the ECOSAR™ v. 1.11 (ECOlogical Structure Activity Relationship) (2012), in which the lowest toxicity prediction for each taxon was chosen to set in the worst-case scenario. Acute and chronic toxicity values were not available for terbuthylazine metabolites and chronic toxicity was not accessible for terbumeton-deethyl.

3. Results and discussion

3.1. Occurrence in water samples

Results obtained for waters in 2010 and 2011 (minimum and maximum concentrations, mean levels, and frequency of detection) are summarized in Table 8.1. Of the 43 pesticides analyzed during 2010, 24 were detected at the concentrations over the limits of detection (LODs) (ap-proximately 56% of analytes) and of the 50 monitored in 2011, 18 (ap-proximately 36%). Organophosphorus (chlorfenvinphos, chlorpyrifos, diazinon, dimethoate), triazines (atrazine, simazine, terbutryn), ureas (diuron, isoproturon), and others (imazalil, metolachlor, imidacloprid) were detected in both sampling campaigns. These compounds are employed for agricultural and urban uses. In 2010, imazalil, chlorpyrifos, and diazinon were present in 100% of the samples. In 2011, chlorpyrifos and terbuthylazine-2-hydroxy were the most frequent, occurring in 80%and 70% of the samples analyzed, respectively. Chlorpyrifos was the most frequent pesticide in 2010 and 2011, probably because it has gen-eralized urban and agricultural uses, being applied over all types of crops and even as soil powder for insect control. It is especially used as a substitute of other organophosphate pesticides (such as azinphos-methyl, azinphos-ethyl, chlorfenvinphos, diazinon, ethion, fenitrothion, fenthion, omethoate, and parathion-methyl and parathion-ethyl) banned by EU (Regulation EC No 2009/1107) (Terrado et al., 2009). Probably, the continuous presence of these pesticides in every water sample is associated with their widespread and frequent use mainly in agriculture, coupled with their mobility and half-life periods. Moreover, 5 of 8 new pesticides introduced in the 2011 sampling campaign were detected. In the case of terbumeton and terbuthylazine, the parent compounds were not identified, but their transformation products were detected, probably because of photodegradation, since the parent compound is quite resistant to hydrolysis.

Table 8.1
Minimum, maximum, mean concentrations, and frequency of detection of the studied pesticides in water, sediment, and biota.

Pesticides	2010					2011				
	Concentration				Frequency no.[d] (%)	Concentration				Frequency[d] no. (%)
	Min[a]	Max	Mean[b]	Mean[c]		Min[a]	Max	Mean[b]	Mean[c]	
Water samples (ng L^{-1})										
Atrazine	2.63	6.44	0.65	4.53	2 (14)	3.87	6.29	0.73	5.08	2 (14)
Azinphos-ethyl	0.03	3.43	0.96	1.49	9 (64)	–	–	–	–	–
Azinphos-methyl	6.67	8.69	1.10	7.68	2 (14)	–	–	–	–	–
Buprofezin	2.28	4.38	0.48	3.33	2 (14)	–	–	–	–	–
Carbendazim	na[e]	na[e]	na[e]	na[e]	na[e]	10.82	**697.39**	58.41	272.57	3 (21)
Carbofuran	1.91	6.75	2.54	2.73	13 (93)	–	–	–	–	–
Chlorfenvinphos	1.92	3.48	0.39	2.7	2 (14)	1.42	1.42	0.1	1.42	1 (7)
Chlorpyrifos	2.01	6.23	3.95	3.95	14 (100)	0.22	13.65	5.31	6.19	12 (85)
Diazinon	2.6	13.61	4.99	4.99	14 (100)	0.47	35.77	6.45	10.03	9 (64)
Dimethoate	6.62	6.62	0.47	6.62	1 (7)	5.08	71.91	6.22	29.01	3 (21)
Diuron	3.45	23.86	6.06	7.07	12 (86)	43.3	**159.53**	23.29	108.69	3 (21)
Ethion	–	–	–	–	–	0.52	7.1	0.91	4.26	3 (21)
Fenitrothion	35	47.39	5.88	41.19	2 (14)	–	–	–	–	–
Fenoxon-sulfone	1.62	1.76	0.24	1.69	2 (14)	–	–	–	–	–
Hexythiazox	1.97	24	1.85	12.98	2 (14)	–	–	–	–	–
Imazalil	0.18	4.8	2.24	2.24	14 (100)	1.52	6.33	0.94	3.31	4 (28)
Imidacloprid	0.31	5.39	2.13	2.71	11 (78)	2.71	66.53	15.94	24.8	9 (64)
Isoproturon	2.05	3.55	1.48	2.58	8 (57)	0.22	9.6	1.65	2.89	8 (57)
Malathion	3.01	**320.35**	24.68	57.58	6	–	–	–	–	–
Methiocarb	2.58	3.23	0.41	2.9	2 (14)	–	–	–	–	–
Metolachlor	8.34	12.96	1.52	10.65	2 (14)	1.57	5.15	0.48	3.36	2 (14)
Prochloraz	9.87	9.87	0.7	9.87	1 (7)	–	–	–	–	–
Propazine	4.90	8.77	2.14	6.00	5 (36%)	–	–	–	–	–
Pyriproxyphen	1.72	1.72	0.12	1.72	1 (7)	–	–	–	–	–
Simazine	1.79	3.33	1.94	2.47	11 (78)	2	45.77	8.03	14.05	8 (57)
Terbumeton-deethyl	na[e]	na[e]	na[e]	na[e]	na[e]	1.84	1.84	0.13	1.84	1 (7)
Terbuthylazine-deethyl	na[e]	na[e]	na[e]	na[e]	na[e]	7.66	7.66	0.55	7.66	1 (7)
Terbuthylazine-2-hydroxy	na[e]	na[e]	na[e]	na[e]	na[e]	0.44	50.21	9.24	12.94	10 (70)
Terbutryn	9.60	9.60	0.69	9.60	1 (7)	1.04	23.37	3.71	13	4 (28)
Thiabendazole	na[e]	na[e]	na[e]	na[e]	na[e]	4.26	20.48	3.2	11.19	4 (28)
Sediment (ng g^{-1})										
Carbendazim	na[e]	na[e]	na[e]	na[e]	na[e]	1.21	16.55	1.27	8.88	2 (14)
Chlorpyrifos	0.39	0.39	0.03	0.39	1 (7)	2.63	**130.97**	24.26	26.13	13 (93)
Diazinon	0.04	1.32	0.14	0.49	4 (28)	0.12	2.53	0.68	1.19	8 (57)
Diclofenthion	–	–	–	–	–	1.83	1.83	0.13	1.83	1 (7)
Diuron	0.02	0.02	0	0.02	1 (7)	–	–	–	–	–
Metolachlor	0.47	0.47	0.03	0.47	1 (7)	–	–	–	–	–
Tebuconazole						2.29	11.42	2.53	7.09	5
Terbumeton	na[e]	na[e]	na[e]	na[e]	na[e]	0.69	0.69	0.05	0.69	1 (7)
Terbuthylazine-2 hydroxy	na[e]	na[e]	na[e]	na[e]	na[e]	5.4	5.4	0.39	5.4	1 (7)
Terbutryn	–	–	–	–	–	5.41	5.41	0.39	5.41	1 (7)
Thiabendazole	na[e]	na[e]	na[e]	na[e]	na[e]	1.96	1.96	0.14	1.96	1 (7)
Fish (ng g^{-1})										
Azinphos-ethyl										
Common carp (*Cyprinus carpus*): Adult	105.81	105.81	21.162	105.81	1	na[e]	na[e]	na[e]	na[e]	na[e]
Chlorpyrifos						na[e]	na[e]	na[e]	na[e]	na[e]
Barbus (*Barbus guiraonis*): adult	6.32	6.32	2.11	6.32	1	na[e]	na[e]	na[e]	na[e]	na[e]
Barbus (*Barbus guiraonis*): young	1.43	1.44	0.48	1.44	1	na[e]	na[e]	na[e]	na[e]	na[e]
Comon carp (*Cyprinus carpus*): adult	1.17	44.75	9.94	16.57	3	na[e]	na[e]	na[e]	na[e]	na[e]
Diazinon						na[e]	na[e]	na[e]	na[e]	na[e]
Barbus (*Barbus guiraonis*): adult	5.47	5.47	1.82	5.474	1	na[e]	na[e]	na[e]	na[e]	na[e]
Barbus (*Barbus guiraonis*): young	5.43	5.43	1.81	5.43	1	na[e]	na[e]	na[e]	na[e]	na[e]
Comon carp (*Cyprinus carpus*): adult	7.49	14.82	6.83	11.38	3	na[e]	na[e]	na[e]	na[e]	na[e]

Numbers in bold are those results higher than 100.
[a] Minimum concentration detected.
[b] Mean value considering not detected as zero.
[c] Mean value of those samples that presented the pesticide.
[d] Number of finding (percentage of positive samples).
[e] Not analyzed.

Chlorfenvinphos, terbutryn and metolachlor (Regulation EC No 2002/2076), atrazine (Decision 2004/248/EC), and simazine (Decision 2004/247/EC), were detected in both sampling campaigns, despite being withdrawn from the EU (Regulation EC No 2009/1107). 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 soil deposits or eventual contribution of groundwater to surface waters.

Fig. 8.2A shows the total charge of pesticides detected in water along the course of the river basin, in both sampling campaigns (Lopez-Roldan et al., 2010). Most pesticides detected in 2010 were organophos-phorus, whereas in 2011 they were benzimidazoles, followed by ureas,

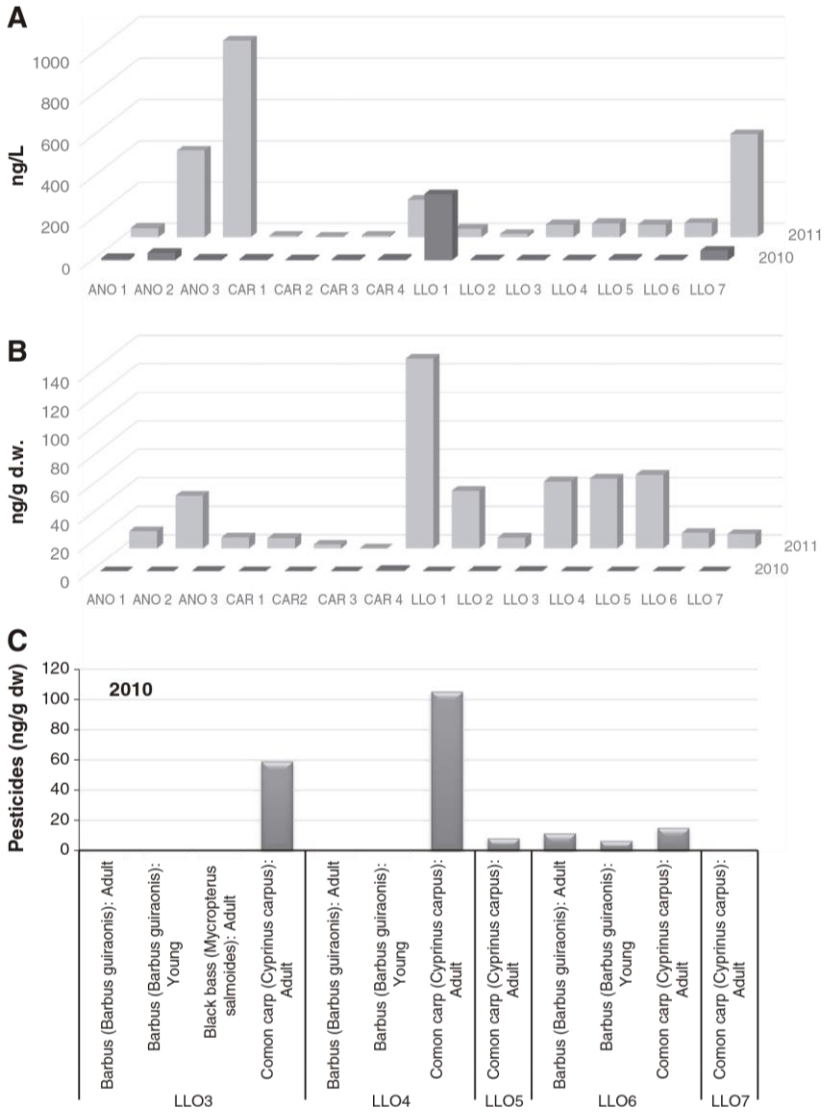


Fig. 8.2 Pesticide families detected in A) water, B) sediment and C) fish samples in 2010 and 2011 according to the sampling point.

triazines, organophosphorus, and neonicotinoids (see Fig. S8.3 Supplementary material). Regarding spatial distribution, the highest concentration in 2010 was found near the source in LLO-1. On the contrary, in 2011, there was a concentration gradient of pesticides from the source to the mouth that was particularly marked in the tributary Anoia. In both sampling campaigns, ANO-1 was the least polluted site. This site is located in the headwater of the Anoia River. Downstream, it flows through the ANO-2 site, highly polluted in both sampling campaigns. It is located downstream Igualada WWTP that receives waste water with an important contribution from urban areas (García-Galan et al., 2010). These results show how the influence of pesticides in urban waters can-not be dismissed. ANO-3, located close to the confluence with the Llobregat River, where the main land use is vineyards (García-Galan et al., 2010), appeared as a high-polluted site in 2011. This was due to a high concentration of carbendazim, a common fungicide widely used to control fungal infections in vineyards (García-Galan et al., 2010), for which the harvest season typically falls between August and October. Besides, some triazine herbicides such as atrazine, terbumeton-deethyl, and terbuthylazine-2-hydroxy, were detected in ANO-3, but not in ANO-2. These triazines were applied on cereal crops a few years ago (Terrado et al., 2009) and they can appear in water from soil deposits or ground water flows, as explained earlier.

By contrast, pesticide contamination had less contribution to the Cardener tributary. In 2010, a constant concentration throughout the river was observed, but in 2011, an increase of different chemical families, such as organophosphorus, neonicotinoids, and benzimidazoles were detected in the convergence with the main river at site CAR-4. This was due to the fact that this sampling site is located downstream of Manresa WWTP and is influenced by its wastewater effluents.

In both sampling campaigns, there was an increase of pollution at the mouth of the river, explained by the agricultural activity along with the large cities located in the area (Barcelona and its surroundings) (Ricart et al., 2010), and due to the cumulative effect throughout the water course, following a pollution gradient (Muñoz et al., 2009).

Council Directive 1998/83/EC establishes in water intended for human consumption a maximum acceptable limit of 100 ng L^{-1} for individual pesticides and 500 ng/L for the sum of all pesticides. Considering that Llobregat is an important drinking water resource, some

compounds were at concentrations up to 100 ng L^{-1}. The highest concentrations were registered as 'punctual inputs' only in the following sites: malathion in LLO-1 (320.3 ng L^{-1}) in 2010, which was not found in samples in 2011 campaign; carbendazim in ANO-3 (up to 697 ng L^{-1}), diuron in LLO-7 (up to 160 ng L^{-1}) and ANO-2 (up to 123 ng L^{-1}) in 2011 (see Fig. S8.4 Supplementary material). However, the average concentrations for individual pesticides did not surpass 100 ng L^{-1} in any case and the average concentration for the sum of all pesticides in each sampling point only exceed the established limit of 500 ng L^{-1} in the sample ANO-3 of the second campaign.

Fig. 8.3A illustrates also the co-occurrence of different pesticides in the water samples. In 2010, 100% of the samples contained at least 6 pesti-cides and 36% of the samples contained more than 11 pesticides. This in-dicates that even though concentrations were low and did not exceed the European threshold for drinking water, the number of pesticides in each sample was high. In 2011, also 100% of the water samples were contaminated with pesticides. However, in 65% of the samples, there was only one pesticide, but detection of a high number of pesti-cides in the same sample was still frequent. The exposure to multiple pesticides is the usual case, which is an additional risk for the aquatic biota that cannot be discharged.

These differences between both sampling campaigns can be explained taking into account the river flow (see details of flow dur-ing sampling in Table S8.6). In 2010, the water flow ranged from 0.12 m^3 s^{-1} (ANO1) to 26.5 m^3 s^{-1} (LLO6). Considering all the flow measurements in the last ten years in each point where there are data available and normalizing them to 100, these values will be in the percentile from 54 to 87%, which can be considered in general as high flow. Lower water flows (−20 to −48% of those of the first campaign) characterized the second campaign (2011) with flows ranging from 0.39 m^3 s^{-1} (ANO3) to 7.33 m^3 s^{-1} (LLO4). Apparently, the low flow produced a pesticide concentration effect, showing higher levels in water (mean levels up to 272 ng L^{-1} in 2011 in front of 57 ng L^{-1} in 2010). The higher the flow, the greater the frequency and number of co-occurring pesticides (36% of the samples had 11 pesticides and 100% of samples were contaminated in 2010) probably because high river flow is related to heavy rains that produces an increment of runoff with more pesticides dragged into the water, but at low concentrations, because they are diluted in a high volume of water (Masiá et al., 2013b).

3.2. Occurrence in sediment samples

Pesticides detected in sediment samples in both sampling campaigns are outlined in Table 8.1. The number of pesticides also increased from the first campaign to the second and the concentrations were also higher in 2011. In the 2010 campaign, 4 pesticides (metolachlor, chlorpyrifos, diazinon, and diuron) comprising 9% of the analytes, were detected at low levels in terms of ng g^{-1} of dry weight (d.w.). Dif-ferently, in 2011, carbendazim, thiabendazole, chlorpyrifos, diazinon, dichlofenthion, terbumeton, terbuthylazine-2-hydroxy, terbutryn, and tebuconazole were found, comprising 18% of all the analytes. It should be taken into account that some compounds detected in the second year were not analyzed in the previous campaign. Regarding the fre-quency of appearance, diazinon was the most prevalent compound in 2010, which appeared in 36% of the samples. In 2011, chlorpyrifos (93%) together with diazinon (57%) were the most frequently detected compounds. Both compounds were the only ones detected in both sam-pling campaigns.

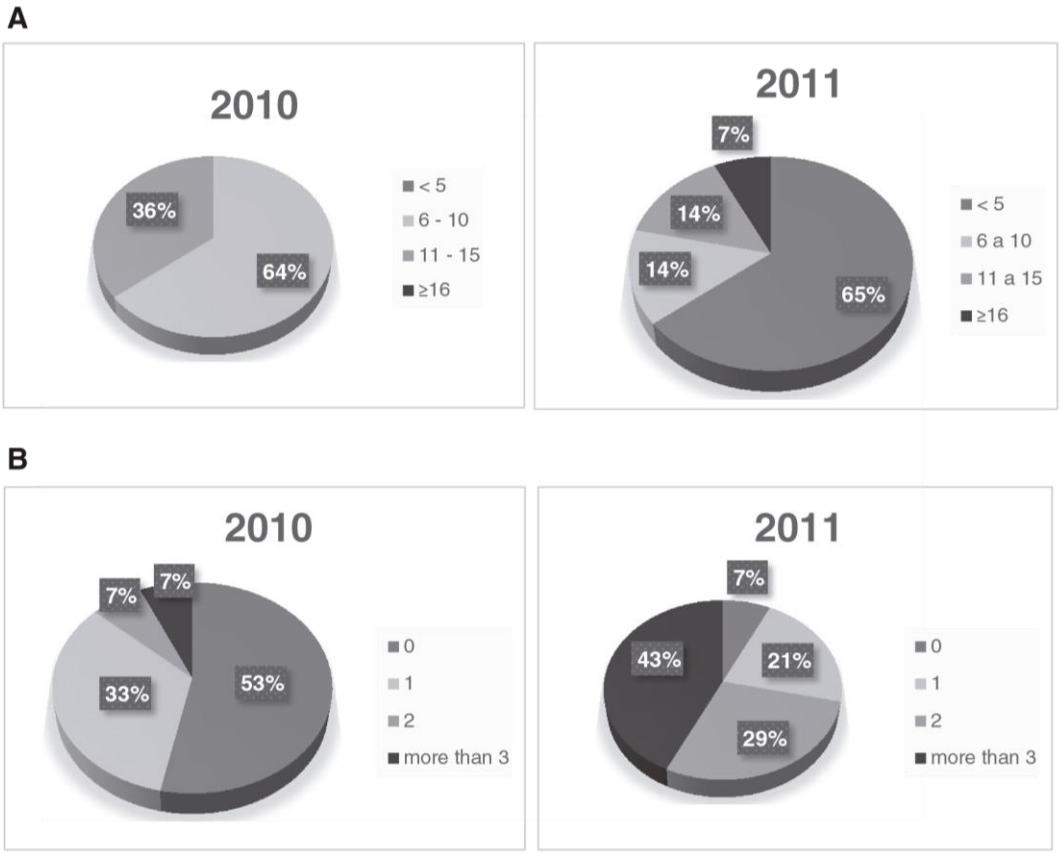


Fig. 8.3 Co-occurrence of pesticides in A) waters and B) sediments in 2010 and 2011.

Cumulative concentration of the different pesticides in each sampling point for both campaigns is shown in Fig. 8.2B. The highest concentration was for chlorpyrifos (130 ng g^{-1} d.w.) at the CAR4 (where the Cardener River flows into Llobregat) in 2011. The concentration of the other pesticides was also lower than 10 ng g^{-1}d.w. in both years, except for carbendazim and tebuconazole, which were below 20 ng g^{-1}d.w. in the last campaign. The average concentrations were also below 10 ng g^{-1}d.w., except for chlorpyrifos (24.26 ng g^{-1} d.w.) in 2011 (see Fig. S8.5 Supplementary material). The constant and high frequency of presence of chlorpyrifos and diazi-non in the sediment samples could be explained by their high octanol/water partition coefficient, log K_{ow} = 4 and 3.96, respectively (see Fig. S8.6 Supplementary material). Both are relatively hydrophobic compounds with low solubility in water, properties which confer them a tendency to accumulate in sediment (Masiá et al., 2013b). Hladik et al. (2009) demonstrated that also other factors such as the timing of application and how much time elapses before the next major storm event are also important for pesticide accumulation in sediments. Both pesticides can be applied throughout the year and not only in agriculture, but also in urban areas (Masiá et al., 2013b). On the other hand, chlorpyrifos was the most prevalent compound both in 2010 and 2011, because of its generalised use, as already commented earlier (Terrado et al., 2009). In the first sampling campaign, pesticides were not detected in 53% of the samples, one was detected in 33%, while two or more pesticides co-occurred in 7% of the samples. On the contrary, in almost 50% of the samples in 2011 more than three pesticides co-occurred, whereas in the remaining samples two or less pesticides occurred (see Fig. 8.3B).

3.3. Occurrence in biota samples and risk assessment

Fish samples were taken only at five points in 2010. The collected fish species included barbus (*B. guiraonis*), black bass (*M. salmoides*) and common carp (*C. carpus*). Chlorpyrifos (K_{ow} = 4), azinphos-methyl (K_{ow} = 3.16), and diazinon (K_{ow} = 3.95) were detected in barbus and common carp. The highest concentration of chlorpyrifos (44.75 ng g^{-1}dw) was found in LLO-3 and azinphos-ethyl (105.81 ng g^{-1} dw) in LLO-4 (see Fig. 8.2C). These data indicated possible bioaccumulation of these pesti-cides in fish. However, it is difficult to evaluate their real significance be-cause the available toxicity data mostly reports EC_{50} and LC_{50} values in water. Then, to complete these data, a risk assessment was carried out by determining RQ. Since pesticide concentrations in water is low, eco-toxicological long-term data are preferred to short-term data. Table 8.2 shows the PNEC and RQ values for each compound calculated for mean and maximum concentrations in both sampling campaigns for algae, *Daphnia*, a n d fish. Anyway, PNEC and RQ values have been also calculated using short-term data (see Table S8.7 Supplementary material).

Few pesticides (carbendazim, metolachlor, ethion and carbendazim) presented a RQ > 1, indicating a potential to cause harmful effects, but the sensibility was different in each trophic level. The highest RQ were obtained for metolachlor in algae and fish (RQ = 21.97 in 2010) and ethion on *D. magna* (RQ = 59.17 in 2011). Then, considering these RQ values, pesticide levels found in the Llobregat basin fulfill the EQS (Directive 2008/105/EC).

Although the environmental concentrations of pesticides do not seem to present a risk according to these standards, the risk for the biota, particularly for fish, which bioaccumulate some apolar pesticides, cannot be neglected. Particularly, taken into account that some previous studies established certain effects of pesticides on the biofilm community, diatomaceous distribution or shellfish impairment (Ricart et al., 2010; Kock et al., 2010), even though none of the targeted pesticides investigated in those studies surpassed the EQS. Furthermore, in our study, most of the detected compounds were organophosphorus cholinesterase inhibitors and thus they share a common mechanism of toxicological action. Therefore, they can produce significant additive or synergistic effects and thus may be lethal even at low concentrations (Damasio et al., 2008; Ginebreda et al., 2010; Yan et al., 2014).

3.4. Comparison with historical data

Previous studies have already shown the quantity and incidence of pesticide residues in the Llobregat River since 1998. The main problem for comparison is that the pesticides analyzed in those studies are not the same and the analytical techniques have greatly increased in sensitivity since then. Table 8.3 shows the chronology of previous studies to re-view trends in average levels of some of the studied pesticides in the Llobregat River basin. In this table the correspondence of these data with the sampling points selected for this study is also identified (as much as possible). Twenty-two of the pesticides reported in the present study have already been previously analyzed. The concentrations found in previous studies carried out in the Llobregat River are of a similar order of magnitude to those found in the present study. However, a full parallelism cannot be established successfully because the concen-tration of pesticides may vary largely, depending on the season of the year in which the samples are taken. Terbuthylazine has been continuously detected in the Llobregat River since 1998. Already in that year, Paune et al. (1998) reported concentrations of 13 μg L^{-1} in the effluent of WWTP release to the river and Lacorte et al. (1998) found concentra-tions ranging from 0.09 to 1.2 ng L^{-1} at the entrance of one of the drink-ing water treatment plant (DWTP) located in the river. Herbicides —mostly triazines and ureas, including also some of their metabolites —and organophosphorus insecticides (e.g. dimethoate) are reported since 2000 (Kampioti et al., 2005; Quintana et al., 2001; Rodriguez-Mozaz et al., 2004).

On the distribution of pesticides through the Llobregat River course, a more recent study carried out in spring and summer of 2006 (Damasio et al., 2008) showed that most pesticides increased towards downstream sites (Table 1). Pesticides, except diazinon, alachlor and metolachlor, showed relatively low environmental levels, which in most occasions were close to their respective detection limits. Mean pesticide residue levels in the middle and downstream stations were higher than 300 ng L^{-1}, whereas those of organophosphorus (mainly diazinon) increased from 50 to over 400 ng L^{-1} from upper to downstream.

In recent studies (Damasio et al., 2011; Ginebreda et al., 2014; Koeck-Schulmeyer et al., 2011, 2012; Proia et al., 2013; Ricart et al., 2010; Terrado et al., 2009), phenylureas such as diuron and the organophosphorus diazinon have been identified abundantly in all the examined samples, confirming the presence of pesticides coming both from urban and agricultural activities. Although, through the year, the maximum concentrations in the Llobregat River did not surpass the EU limit of 0.5 μg L^{-1}, the continuous occurrence of these pesticides at a detectable level requires monitoring programs as well as measures to diminish the levels found in water.

Other environmental matrices different than water have been much less studied. There is only one study reporting the presence of 13 pesticides (including 9 analyzed in this study) in sediment (Ricart et al., 2010). Phenylureas and chloroacetanilides were the compounds detected at high levels, but organophosphorus, such as diazinon, were also detected. These results do not agree completely with the current data. However, it should be taken into account that the target pesticides were not always the same.

To our knowledge there is no study that determines residues of these pesticides in biota. However, a previous study (Ricart et al., 2010) made an attempt to establish the effects of these pesticides on benthic biological communities (invertebrate and diatoms). Multivariate analyses revealed a potential relationship between triazines and the distribution of the diatom community, although no evidence of disruption in the invertebrate community distribution was found. These rare studies pinpoint the weaknesses of the previously used

Table 8.2
PNEC and RQ values calculated for mean and maximum environmental concentrations of pesticides in water, according to chronic toxicity dates measured in algae, *Daphnia*, and fish.

	Algae					Aquatic invertebrates (*Daphnia magna*)					Fish				
	Chronic 96/72 h NOEC					Chronic 21 days NOEC					Chronic 21 days NOEC				
	PNEC (ng L^{-1})	2010		2011		PNEC (ng L^{-1})	2010		2011		PNEC (ng L^{-1})	2010		2011	
Compound		RQ_mean	RQ_max	RQ_mean	RQ_max		RQ_mean	RQ_max	RQ_mean	RQ_max		RQ_mean	RQ_max	RQ_mean	RQ_max
Azol															
Imazalil	92[a]	<0.1	<0.1	<0.1	<0.1	15[a]	0.15	0.32	<0.1	0.42	43,000	<0.1	<0.1	<0.1	<0.1
Prochloraz	10,000	<0.1	<0.1			18[a]	<0.1	0.55	<0.1		49,000	<0.1	<0.1		
Bencimidazole															
Carbendazim	302[a]			0.19	2.31	1500			<0.1	0.46	3200			<0.1	0.22
Thiabendazole	3,200,000			<0.1	<0.1	42,000			<0.1	<0.1	12,000			<0.1	<0.1
Carbamates															
Carbofuran	3,200,000	<0.1	<0.1			8000	<0.1	<0.1			2200	<0.1	<0.1		
Methiocarb	3,200,000	<0.1	<0.1			100	<0.1	<0.1			50,000	<0.1	<0.1		
Chloroacetanilide															
Metolachlor	0.59[a]	2.58	21.97	0.81	8.73	707,000	<0.1	<0.1	<0.1	<0.1	373[a]	<0.1	<0.1	<0.1	<0.1
Juvenile hormone mimics															<0.1
Pyriproxyphen	213[a]	<0.1	<0.1			15	<0.1	0.11			4300	<0.1	<0.1		
Neonicotinoid															
Imidacloprid	10,000,000	<0.1	<0.1	<0.1	<0.1	1,800,000	<0.1	<0.1	<0.1	<0.1	9,020,000	<0.1	<0.1	<0.1	<0.1
Organophosphorus															
Azinphos-ethyl	446[a]	<0.1	<0.1			0.418[a]	2.30	8.21			21[a]	<0.1	<0.1		
Azinphos-methyl	1000[a]	<0.1	<0.1			400	<0.1	<0.1			170	<0.1	<0.1		
Chlorfenvinphos	1,000,000	<0.1	<0.1	<0.1	<0.1	100	<0.1	<0.1	<0.1	<0.1	30,000	<0.1	<0.1	<0.1	<0.1
Chlorpyrifos	43,000	<0.1	<0.1	<0.1	<0.1	4600	<0.1	<0.1	<0.1	<0.1	140	<0.1	<0.1	<0.1	<0.1
Diazinon	10,000,000	<0.1	<0.1	<0.1	<0.1	560	<0.1	<0.1	<0.1	<0.1	700,000	<0.1	<0.1	<0.1	<0.1
Diclofenthion	204[a]					0.0417[a]					4[a]				
Dimethoate	32,000,000	<0.1	<0.1	<0.1	<0.1	40,000	<0.1	<0.1	<0.1	<0.1	400,000	<0.1	<0.1	<0.1	<0.1
Ethion	129[a]			<0.1	<0.1	0.12[a]			7.58	59.17	12[a]			<0.1	0.59
Fenitrothion	100,000	<0.1	<0.1			87	<0.1	0.54			88,000	<0.1	<0.1		
Fenoxon-sulfone	81,113[a]	<0.1	<0.1			256,022[a]	<0.1	<0.1			23[a]	<0.1	<0.1		
Malathion	14,993[a]	<0.1	<0.1			60	0.41	5.34			91,000	<0.1	<0.1		
Other pesticides															
Buprofezin	1146[a]	<0.1	<0.1			80,000	<0.1	<0.1			52,000	<0.1	<0.1		
Hexythiazox	7[a]	0.26	3.43			6100	<0.1	<0.1			40,000	<0.1	<0.1		
Triazines															
Atrazine	100,000	<0.1	<0.1	<0.1	<0.1	250,000	<0.1	<0.1	<0.1	<0.1	2,000,000	<0.1	<0.1	<0.1	<0.1
Propazine	40[a]	<0.1	0.22			420[a]	<0.1	<0.1			277[a]	<0.1	<0.1		
Simazine	600,000	<0.1	<0.1	<0.1	<0.1	2,500,000	<0.1	<0.1	<0.1	<0.1	700,000	<0.1	<0.1	<0.1	<0.1
Terbumeton-deethyl															
Terbuthylazine-deethyl															
Terbuthylazine-2-hydroxy															
Terbutryn	28[a]	<0.1	0.34	0.13	0.83	205[a]	<0.1	<0.1	<0.1	0.11	104[a]	<0.1	<0.1	<0.1	0.22
Ureas															
Diuron	93[a]	<0.1	0.26	0.25	1.72	96,000	<0.1	<0.1	<0.1	<0.1	410,000	<0.1	<0.1	<0.1	<0.1
Isoproturon	52,000	<0.1		<0.1	<0.1	120,000	<0.1	<0.1	<0.1	<0.1	1,000,000	<0.1	<0.1	<0.1	<0.1

[a] Chronic toxicity value calculated by ECOSAR.

Table 8.3
Comparison water and sediment Llobregat dates with historical data.

Sampling point	Correlation to this work	Pesticides	Concentrations (min–max)	Remarks	Ref.
Water (ng L^{-1})					
Mouth	LLO6–LLO7	Terbuthylazine	0.09–1.2	1998 At the entrance of a DWWP	Paune et al. (1998)
Upstream	LLO-1 LLO-2	Terbuthylazine	13,000	1998	Lacorte et al. (1998)
Mouth	LLO-5 LLO-6 LLO-7	Simazine	25–84	2000	Quintana et al. (2001)
		Atrazine	25–29	River, groundwater and treated waters of Barcelona	
		Deisopropylatrazine	25–62		
		Terbutryn	40–70		
		Dimethoate	60-154		
Mouth	LLO-7	Atrazine	5–463	2002 (February to August)	Kampioti et al. (2005)
		Simazine	8–2218	Water from river. groundwater and waterworks	
		Deethylazine	nd–4		
		Diuron	64–239		
		Isoproturon	5–503		
Mouth	LLO-7	Atrazine	0.05–1.1	2003 (June)	Rodriguez-Mozaz et al. (2004)
		Simazine	0.1–53.6	River and groundwater have been analyzed	
		Deethylatrazine	27.1–27.1		
		Deisopropylatrazine	0.1–14.4		
		Terbuthylazine	0.1–21.9		
		Diuron	0.4–99.7		
		Isoproturon	0.5–7.8		
		Diazinon	0.8–785.0		
		Dimethoate	0.6–87.8		
		Alachlor	2.2–17.1		
		Metolachlor	7.4		
		Molinate	1.0–3.8		
Upstream middle downstream	LLO-1 LLO-2 LLO-3 LLO-4 LLO-5 to LLO-7	Upstream	–	2006 (April and July) determination of lindane, PAH. metals and physicochemical water quality parameters	Damasio et al. (2008)
		Atrazine	4–19		
		Terbuthylazine	4–7		
		Diazinon	28–52		
		Chlorpyrifos	<10–11		
		Alachlor	5–7		
		Metolachlor	7–12		
		Middle	–		
		Atrazine	<4–40		
		Simazine	6–33		
		Terbutryn	4–14		
		Terbuthylazine	24–535		
		Diazinon	<10–124		
		Alachlor	6–14		
		Downstream	–		
		Atrazine	4–181		
		Simazine	<4–131		
		Terbutryn	5–57		
		Terbuthylazine	6–2140		
		Diazinon	13–2826		
		Chlorpyrifos	<10–89		
		Alachlor	<4–174		
		Metolachlor	<4–309		
All the river	LLO-1 to LLO-7 ANO-1 to ANO-3 CAR-1 to CAR-4	Atrazine	2.00–91.5	2003–2006	Terrado et al. (2009)
		Terbutryn	2.00–343	(June and December)	
		Simazine	2.00–153	Determination of Lindane and Endosulfan	
		Chlorpyrifos	5.00–1085		
		Diazinon	5.00–376		
Anoia River mid-lower part Llobregat	ANO-1 to ANO-3 LLO-3 to LLO-7	Atrazine	0.05–1.08	Sampling in Spring and autumn 2005 and 2006	Ricart et al. (2010)
		Simazine	0.14–53.60		
		Deethylatrazine	27.10–27.10		
		Terbuthylazine	0.13–21.90		
		Deisopropylatrazine	0.10–14.40		
		Diazinon	0.83–785.00		
		Dimethoate	0.65–87.80		
		Fenitrothion	0.90–3.43		
		Diuron	0.40–99.70		
		Isoproturon	0.46–7.85		
		Alachlor	2.17–17.10		
		Metolachlor	7.37–7.37		
		Molinate	0.96–3.78		
		Propanil	0.03–0.39		
Middle and lower course of the river	LLO-3 to LLO-7	Deethylatrazine	0.45–0.85	Sampling in July 2008	Damasio et al. (2011)
		Dimethoate	0.16–9.83		
		Simazine	0.05–2.57		
		Atrazine	0.44–1.72		
		Diuron	0.15–21.6		

(continued on next page)

Table 8.3 (*continued*)

Sampling point	Correlation to this work	Pesticides	Concentrations (min–max)	Remarks	Ref.
Middle and lower course of the river	LLO-3 to LLO-7	Terbuthylazine	0.12–141		
		Deisopropylatrazine	1.91–151		
		Metholachlor	0.66–12.3		
		Diazinon	0.86–26.2		
Mouth	LLO-4 to LLO-7	Atrazine	nd–10.2	C1: 23 November to 18 December 2009	Koeck-Schulmeyer et al. (2012)
		Simazine	nd–38.4	C2: 10 March to 12 April	
		Deethylatrazine	nd–8.2	C3: 9 June to 12 July	
		Deisopropylatrazine	<Ldet		
		Terbuthylazine	nd–81.5		
		Diuron	nd–818.0		
		Isoproturon	nd–81.6		
		Malathion	nd–18.9		
		Diazinon	nd–132.3		
		Dimethoate	nd–189.9		
		Alachlor	nd–11.1		
		Metolachlor	nd–13.1		
Mouth	LLO-6 to LLO-7	Propanil	1.2–2.5	2008 (summer and fall)	Koeck-Schulmeyer et al. (2011)
		Diazinon	17–74.5	Samples from WWTP tertiary effluents release to the river	
		Diuron	10.5–142		
		Isoproturon	bld–0.5		
		Atrazine	1.0–2.4		
		Simazine	1.5–4.6		
		Terbuthylazine	18.6–215		
Middle-lower part of the river	LLO-4 to LLO-7	Atrazine	0.02–3.12	River water collected three times a week between 16th October and 19th November. Three sampling sites: Castellbell. Mina de Terrassa and Sant Joan Despí	Proia et al. (2013)
		Deisopropylatrazine	3.57–36.89		
		Deethylatrazine	0.17–2.73		
		Simazine	0.04–4.07		
		Terbuthylazine	0.04–82.84		
		Diuron	1.12–31.13		
		Isoproturon	0.01–0.81		
		Malathion	0.20–4.63		
		Diazinon	0.78–79.03		
		Dimethoate	0.49–3.52		
		Alachlor	0.66–0.90		
		Metholachlor	0.13–1.31		
		Molinate	0.50–4.22		
Anoia River mid-lower part Llobregat	ANO-1 to ANO-3 LLO-3 to LLO-7	Propanil	0.13–0.0375	-	Ginebreda et al. (2014)
		Fenitrothion	0.228–1.13		
		Isoproturon	0.154–1.96		
		Atrazine	bld–0.297		
		Diuron	0.225–54		
		Alachlor	1.98–5.62		
		Diazinon	bld–310		
		Dimethoate	bdl–29.3		
		Metolachlor	bld–1.84		
		Molinate	0.243–1.20		
		Simazine	bld–14.3		
		Terbuthylazine	0.170–6.05		
Sediment (ng g^{-1})					
Anoia River mid-lower part Llobregat	ANO-1 to ANO-3 LLO-3 to LLO-7	Atrazine	0.03–0.86	Sampling in spring and autumn 2005 and 2006	Ricart et al. (2010)
		Simazine	0.81–0.81		
		Deisopropylatrazine	0.62–0.70		
		Diazinon	0.09–1.29		
		Fenitrothion	1.51–3.00		
		Diuron	0.16–31.80		
		Isoproturon	0.08–0.73		
		Metolachlor	7.59–43.20		
		Propanil	0.11–24.70		

RQ approach and the necessity of more field data on pesticide toxicity.

4. Conclusions

Pesticides were detected primarily in water, where they appeared almost continuously. Their presence in sediments was much more intermittent and in biota it was rather scarce. The most prevalent pesticides that appeared in high concentrations were the benzimidazoles (carbendazim), the organophosphorus (malathion, chlorpyrifos), the triazines (terbuthylazine-2-hydroxy, simazine), the neonicotinoids (imidacloprid) and the triazoles (tebuconazole). However, different patterns of pesticide contamination in different flow conditions were revealed that can affect toxicity to biota. Furthermore, historical data confirmed a background contamination in the area of the Llobregat River in the last 20 years. Periodic monitoring on the environmental quality status of the river in combination with previous data will assist in controlling the contamination of the river basin and ascertain the effect of different physicochemical parameters, such as flow.

Although the RQ values showed low risk of toxicity from the presence of pesticides in water, the bioaccumulation of some lipophilic pesticides in fish together with some previous results that describe adverse effects of pesticides on biota highlight the need of alternative and complementary approaches to that provided by the simple compliance with the EQS. Field data on pesticide levels in different trophic levels will be very helpful to enhance the knowledge on this topic.

Acknowledgements

This work has been supported by the Spanish Ministry of Economy and Competitiveness through the projects Consolider-Ingenio 2010 CSD2009, and CGL2011-29703-C02-02. We would also like to thank the staff of the IDAEA and ICRA research groups for taking the samples. We also would like to acknowledge the anonymous reviewers for their helpful comments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2014.06.095.

References

Anastassiades M, Lehotay SJ, Stajnbaher D, Schenck FJ. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and 'dispersive solid-phase extraction' for the determination of pesticide residues in produce. J AOAC Int 2003;86:412–31.

Belenguer V, Martinez-Capel F, Masiá A, Picó Y. Patterns of presence and concentration of pesticides in fish and waters of the Júcar River (eastern Spain). J Hazard Mater 2014;265:271–9.

Campo J, Masiá A, Blasco C, Picó Y. Occurrence and removal efficiency of pesticides in sewage treatment plants of four Mediterranean River basins. J Hazard Mater 2013;263:146–57.

Cespedes R, Lacorte S, Raldua D, Ginebreda A, Barceló D, Pina B. Distribution of endocrine disruptors in the Llobregat River basin (Catalonia, NE Spain). Chemosphere 2005;61:1710–9.

Cooper J, Dobson H. The benefits of pesticides to mankind and the environment. Crop Prot 2007;26:1337–48.

Council Directive 1998/83/EC of 3 November 1998 on the quality of water intended for human consumption. Off J Eur Communities 1998 A.D.; L330: 32–54.

Daam MA, Cerejeira MJ, Van den Brink PJ, Brock TC. Is it possible to extrapolate results of aquatic microcosm and mesocosm experiments with pesticides between climate zones in Europe? Environ Sci Pollut Res Int 2011;18:123–6.

Damasio J, Tauler R, Teixido E, Rieradevall M, Prat N, Riva MC, et al. Combined use of Daphnia magna in situ bioassays, biomarkers and biological indices to diagnose and identify environmental pressures on invertebrate communities in two Mediterranean urbanized and industrialized rivers (NE Spain). Aquat Toxicol 2008;87:310–20.

Damasio J, Barceló D, Brix R, Postigo C, Gros M, Petrovic M, et al. Are pharmaceuticals more harmful than other pollutants to aquatic invertebrate species: a hypothesis tested using multi-biomarker and multi-species responses in field collected and transplanted organisms. Chemosphere 2011;85:1548–54.

Decision 2001/2455/EC of the European Parliament and of the Council of 20 November 2001 establishing the list of priority substances in th field of water policy and ammending Directive 2000/60/EC. Off J Eur Communities 2001;331:1–5.

Decision 2004/247/EC concerning the non-inclusion of simazine in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing this active substance. Off J Eur Union 2004;78:50–2.

Decision 2004/248/EC concerning the non-inclusion of atrazine in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing this active substance. Off J Eur Union 2004;78:53–5.

Delmotte S, Tittonell P, Mouret J, Hammond R, Lopez-Ridaura S. On farm assessment of rice yield variability and productivity gaps between organic and conventional cropping systems under Mediterranean climate. Eur J Agron 2011;35:223–36.

Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. Off J Eur Communities 2000;L37:1–72.

Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council. Off J Eur Union 2008;348:84–97.

Ecosar™ v. 1.11 (ECOlogical Structure Activity Relationship), USA Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention. Available at http://www.epa.gov/oppt/newchems/tools/21ecosar.htm, 2012.

García-Galan MJ, Villagrasa M, Silvia Diaz-Cruz M, Barceló D. LC-QqLIT MS analysis of nine sulfonamides and one of their acetylated metabolites in the Llobregat River basin. Quantitative determination and qualitative evaluation by IDA experiments. Anal Bioanal Chem 2010;397:1325–34.

Ginebreda A, Muñoz I, Lopez de Alda M, Brix R, Lopez-Doval J, Barceló D. Environmental risk assessment of pharmaceuticals in rivers: relationships between hazard indexes and aquatic macroinvertebrate diversity indexes in the Llobregat River (NE Spain). Environ Int 2010;36:153–62.

Ginebreda A, Kuzmanovic M, Guasch H, Lopez de Alda M, Lopez-Doval JC, Muñoz I, et al. Assessment of multi-chemical pollution in aquatic ecosystems using toxic units: compound prioritization, mixture characterization and relationships with biological descriptors. Sci Total Environ 2014;468–469:715–23.

Gonzalez S, Lopez-Roldan R, Cortina JL. Presence and biological effects of emerging contaminants in Llobregat River basin: a review. Environ Pollut 2012;161:83–92.

Guerra P, Eljarrat E, Barceló D. Analysis and occurrence of emerging brominated flame retardants in the Llobregat River basin. J Hydrol 2010;383:39–43.

Hladik ML, Domagalski JL, Kuivila KM. Concentrations and loads of suspended sediment-associated pesticides in the San Joaquin River, California and tributaries during storm events. Sci Total Environ 2009;408:356–64.

Kampioti AA, da Cunha ACB, Lopez de Alda M, Barceló D. Fully automated multianalyte determination of different classes of pesticides, at picogram per litre levels in water, by on-line solid-phase extraction-liquid chromatography–electrospray-tandem mass spectrometry. Anal Bioanal Chem 2005;382:1815–25.

Kock M, Farre M, Martinez E, Gajda-Schrantz K, Ginebreda A, Navarro A, et al. Integrated ecotoxicological and chemical approach for the assessment of pesticide pollution in the Ebro River Delta (Spain). J Hydrol 2010;383:73–82.

Koeck-Schulmeyer M, Ginebreda A, Postigo C, Lopez-Serna R, Perez S, Brix R, et al. Wastewater reuse in Mediterranean semi-arid areas: the impact of discharges of tertiary treated sewage on the load of polar micro pollutants in the Llobregat River (NE Spain). Chemosphere 2011;82:670–8.

Koeck-Schulmeyer M, Ginebreda A, Gonzalez S, Cortina J, Lopez de Alda M, Barceló D. Analysis of the occurrence and risk assessment of polar pesticides in the Llobregat River basin (NE Spain). Chemosphere 2012;86:8–16.

Kookana R, Holz G, Barnes C, Bubb K, Boardman B. Impact of climatic and soil conditions on environmental fate of atrazine used under plantation forestry in Australia. J Environ Manage 2010;91:2649–56.

Kuster M, Lopez de Alda M, Hernando MD, Petrovic M, Martin-Alonso J, Barceló D. Analysis and occurrence of pharmaceuticals, estrogens, progestogens and polar pesticides in sewage treatment plant effluents, river water and drinking water in the Llobregat River basin (Barcelona, Spain). J Hydrol 2008;358:112–23.

Lacorte S, Vreuls JJ, Salau JS, Ventura F, Barceló D. Monitoring of pesticides in river water using fully automated on-line solid-phase extraction and liquid chromatography with diode array detection with a novel filtration device. J Chromatogr A 1998;795:71–82.

Lopez-Roldan R, Lopez de Alda M, Gros M, Petrovic M, Martin-Alonso J, Barceló D. Advanced monitoring of pharmaceuticals and estrogens in the Llobregat River basin (Spain) by liquid chromatography–triple quadrupole-tandem mass spectrometry in combination with ultra performance liquid chromatography–time of flight-mass spectrometry. Chemosphere 2010;80:1337–44.

Masiá A, Ibañez M, Blasco C, Sancho JV, Picó Y, Hernandez F. Combined use of liquid chromatography triple quadrupole mass spectrometry and liquid chromatography quadrupole time-of-flight mass spectrometry in systematic screening of pesticides and other contaminants in water samples. Anal Chim Acta 2013a;761:117–27.

Masiá A, Campo J, Vazquez-Roig P, Blasco C, Picó Y. Screening of currently used pesticides in water, sediments and biota of the Guadalquivir River basin (Spain). J Hazard Mater 2013b;263:95–104.

Muñoz I, Lopez-Doval JC, Ricart M, Villagrasa M, Brix R, Geiszinger A, et al. Bridging levels of pharmaceuticals in river water with biological community structure in the Llobregat River basin (northeast Spain). Environ Toxicol Chem 2009;28:2706–14.

Oliver D, Kookana RS, Anderson JS, Cox J, Waller N, Smith L. The off-site transport of pesticide loads from two land uses in relation to hydrological events in the Mt. Lofty Ranges, South Australia. Agric Water Manag 2012;106:70–7.

Paune F, Caixach J, Espadaler I, Om J, Rivera J. Assessment on the removal of organic chemicals from raw and drinking water at a Llobregat River water works plant using GAC. Water Res 1998;32:3313–24.

Proia L, Osorio V, Soley S, Koeck-Schulmeyer M, Perez S, Barceló D, et al. Effects of pesticides and pharmaceuticals on biofilms in a highly impacted river. Environ Pollut 2013;178:220–8.

Quintana J, Marti I, Ventura F. Monitoring of pesticides in drinking and related waters in NE Spain with a multiresidue SPE-GC–MS method including an estimation of the uncertainty of the analytical results. J Chromatogr A 2001;938:3–13.

Regulation (EC) No 2002/2076 of 20 November 2002 extending the time period referred to in Article 8(2) of Council Directive 91/414/EEC and concerning the non-inclusion of certain active substances in Annex I to that Directive and the withdrawal of authorisations for plant protection products containing these substances (text with EEA relevance). Off J Eur Communities 2002;319:3–11.

Regulation (EC) No 2009/1107 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Off J Eur Union 2009;309:1–50.

Ricart M, Guasch H, Barceló D, Brix R, Conceicao MH, Geiszinger A, et al. Primary and complex stressors in polluted mediterranean rivers: pesticide effects on biological communities. J Hydrol 2010;383:52–61.

Rodriguez-Mozaz S, Lopez de Alda M, Barceló D. Monitoring of estrogens, pesticides and bisphenol A in natural waters and drinking water treatment plants by solid-phase extraction-liquid chromatography–mass spectrometry. J Chromatogr A 2004;1045:85–92.

Terrado M, Lavigne MP, Tremblay S, Duchesne S, Villeneuve JP, Rousseau AN, et al. Distribution and assessment of surface water contamination by application of chemometric and deterministic models. J Hydrol 2009;369:416–26.

Yan Z, Yang X, Lu G, Liu J, Xie Z, Wu D. Potential environmental implications of emerging organic contaminants in Taihu Lake, China: comparison of two ecotoxicological assessment approaches. Sci Total Environ 2014;470–471:171–9.

Suplementary material

Monitoring and correlation with historical dates of pesticides in Llobregat River Basin (Catalonia, Spain)

Ana Masiá*, Julián Campo, Alicia Navarro-Ortega, Damià Barceló, Yolanda Picó

Food and Environmental Safety Research Group, Department of Medicine Preventive, Faculty of Pharmcy, University of Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia Spain.

* Corresponding author. Tel. +34 963544873; fax: +34 963544954
E-mail address: Ana.Masia@uv.es

Table S8.1 Phisico-chemical properties

This table shows the pesticides and some of their transformation products monitored during 2010 and 2011 campaigns. They were selected according to their extend use, water solubility and amenability to LC-MS analysis. 42 pesticides, with different uses and different physicochemical characteristics and toxicity, were analyzed during the first campaign. 8 more target compounds were introduced to be analyzed in the second year, making a total of 50 pesticides. The increase in the number of target pesticides from first campaign to second one was due to a non-target analysis of the samples from first monitoring in a TOF-MS analyzer revealed the presence of them in most of the samples.

	CAS NUMBER	MOLECULAR FORMULA	PM (g/mol)	log Kow (pH 7, 20 ºC)	GUS INDEX-LEACHABILITY	BIOCIDE ACTION
3-Hydroxycarbofuran	16655-82-6	$C_{12}H_{15}NO_4$	237,25	1.45 (low)	1.45 (low)	Metabolite
Acethochlor	34256-82-1	$C_{14}H_{20}ClNO_2$	269,77	4.14 (high)	1.77 (low)	Herbicide
Alachlor	15972-60-8	$C_{14}H_{20}ClNO_2$	269,77	3.09 (high)	0.80 (low)	Herbicide
Atrazine	1912-24-9	$C_8H_{14}ClN_5$	215,68	2.7 (moderate)	3.30 (high)	Herbicide
Azinphos-ethyl	2642-71-9	$C_{12}H_{16}N_3O_3PS_2$	345,38	3.18 (high)	1.4 (low)	Insecticide, Acaricide
Azinphos-methyl	86-50-0	$C_{10}H_{12}N_3O_3PS_2$	317,32	2.96 (moderate)	0.95 (low)	Insecticide, Acaricide
Buprofezin	69327-76-0	$C_{16}H_{23}N_3OS$	305,44	4.93 (high)	0.46 (low)	Insecticide, Acaricide
Carbendazim	10605-21-7	$C_9H_9N_3O_2$	191,21	1.48 (low)	2.64 (transition state)	Fungicide
Carbofuran	1563-66-2	$C_{12}H_{15}NO_3$	221,26	1.8 (low)	3.02 (high)	Insecticide, Acaricide, Nematicide
Chlofenvinphos	470-90-6	$C_{12}H_{14}Cl_3O_4P$	359,6	3.8 (high)	1.87 (transition state)	Insecticide, Acaricide
Chlorpyriphos	5598-13-0	$C_7H_7Cl_3NO_3PS$	322,53	4 (high)	0.2 (low)	Insecticide, Acaricide
Deisopropylatrazine	1007-28-9	$C_5H_8ClN_5$	173,6	1,15 (low)	-	Metabolite
Deethylatrazine	6190-65-4	$C_6H_{10}ClN_5$	187,63	1.51 (low)	3.54 (high)	Metabolite
Diazinon	333-41-5	$C_{12}H_{21}N_2O_3PS$	304,35	3.69 (high)	1.14 (low)	Insecticide, Acaricide, Repelent

	CAS NUMBER	MOLECULAR FORMULA	PM (g/mol)	log Kow (pH 7, 20 ºC)	GUS INDEX-LEACHABILITY	BIOCIDE ACTION	CHEMICAL FAMILY
Diclofenthion	97-17-6	$C_{10}H_{13}Cl_2O_3PS$	315,15	5.14 (high)	2.14 (transition state)	Insecticide	Organophosphorus
Dimethoate	60-51-5	$C_5H_{12}NO_3PS_2$	229,26	0.704 (low)	1.06 (low)	Insecticide, Acaricide	Organophosphorus
Diuron	330-54-1	$C_9H_{10}Cl_2N_2O$	233,09	2.87 (moderate)	1.83 (transition state)	Herbicide	Urea
Ethion	563-12-2	$C_9H_{22}O_4P_2S_4$	384.48	5.07 (high)	0.00 (low)	Insecticide, Acaricide	Organophosphorus
Fenitrothion	122-14-5	$C_9H_{12}NO_5PS$	277.23	3.32 (high)	0.48 (low)	Insecticide	Organophosphorus
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	-	-	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)	1.11 (low)	Insecticide	Organophosphorus
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)	0.04 (low)	Acaricide	Acaricide
Imazalil	35554-44-0	$C_{14}H_{14}Cl_2N_2O$	297.18	2.56 (low)	0.55 (low)	Fungicide	Azol
Imidacloprid	138261-41-3	$C_9H_{10}ClN_5O_2$	255.66	0.57 (low)	3.76 (high)	Insecticide	Neonicotinoid
Isoproturon	34123-59-6	$C_{12}H_{18}N_2O$	206,3	2.5 (low)	2.07 (transition state)	Herbicide	Urea
Malathion	121-75-5	$C_{10}H_{19}O_6PS_2$	330.36	2.75 (moderate)	(-)1,28 (low)	Insecticide, Acaricide	Organophosphorus
Methiocarb	2032-65-7	$C_{11}H_{15}NO_2S$	225.31	3.18 (high)	0.17 (low)	Insecticide	Carbamates

	CAS NUMBER	MOLECULAR FORMULA	PM (g/mol)	log Kow (pH 7, 20 ºC)	GUS INDEX-LEACHABILITY	BIOCIDE ACTION	CHEMICAL FAMILY
Metolachlor	51218-45-2	$C_{15}H_{22}ClNO_2$	283.8	3.4 (high)	3.49 (high)	Herbicide	Chloroacetanilide
Molinate	2212-67-1	$C_9H_{17}NOS$	187.3	2.86 (moderate)	2.49 (transition state)	Herbicide	Carbamates
Omethoate	1113-02-6	$C_5H_{12}NO_4PS$	213.2	(-) 0.74 (low)	2.73 (transition state)	Insecticide, Acaricide	Organophosphorus
Parathion-ethyl	56-38-2	$C_{10}H_{14}NO_5PS$	291.26	3.83 (high)	2.09 (transition state)	Insecticide, Acaricide	Organophosphorus
Parathion-methyl	298-00-0	$C_8H_{10}NO_5PS$	263.21	3 (moderate)	1.46 (low)	Insecticide	Organophosphorus
Prochloraz	67747-09-5	$C_{15}H_{16}Cl_3N_3O_2$	376.7	3.5 (high)	1.75 (low)	Fungicide	Azol
Propanil	709-98-8	$C_9H_9Cl_2NO$	218.08	2.29 (low)	0.72 (low)	Herbicide	Anilide
Propazine	139-40-2	$C_9H_{16}ClN_5$	229.71	3.95 (high)	3.84 (high)	Herbicide	Triazine
Pyriproxyphen	95737-68-1	$C_{20}H_{19}NO_3$	321.37	5.37 (high)	(-)0.33 (low)	Insecticide	Juvenile Hormone Mimi
Simazine	122-34-9	$C_7H_{12}ClN_5$	201.66	2.3 (low)	2.00 (transition state)	Herbicide	Triazine
Tebuconazole	107534-96-3	$C_{16}H_{22}ClN_3O$	307.82	3.7 (high)	2.00 (transition state)	Fungicide	Triazole
Terbumeton	33693-04-8	$C_{10}H_{19}N_5O$	225.29	3.04 (high)	3.79 (high)	Herbicide	Triazine
Terbumeton-deethyl							
Terbuthylazine	5915-41-3	$C_9H_{16}ClN_5$	229.71	3.4 (high)	3.07 (high)	Herbicide, Microbiocide, Algicide	Triazine
Terbuthylazine-2-hydroxy	66753-07-9	$C_9H_{17}N_5O$	211.33	-	4.59 (high)	Metabolite	Triazine
Terbutryn	886-50-0			3,34			Triazine

	CAS NUMBER	MOLECULAR FORMULA	PM (g/mol)	log Kow (pH 7, 20 ºC)	GUS INDEX-LEACHABILITY	BIOCIDE ACTION	CHEMICAL FAMILY
Thiabendazole	148-79-8	$C_{10}H_7N_3S$	201.25	2.39 (low)	0.36 (low)	Fungicide	Bencimidazole
Tolclophos-methyl	57018-04-9	$C_9H_{11}Cl_2O_3PS$	301.13	4.56 (high)	0.25 (low)	Fungicide	Organophosphorus

Table S8.2 Sampling points georeferences

RIVER OR TRIBUTARY	ABREV	LOCATION		COORDINATES UTM	
			ZONE	X	Y
Anoia	ANO-1	Jorba	31	378856	4207084
Anoia	ANO-2	La Pobla de Claramunt	31	388339	4602206
Anoia	ANO-3	Sant Sadurní d´Anoia	31	401051	4586728
Cardener	CAR-1	Olius	31	381484	4656936
Cardener	CAR-2	Clariana de Cardener	31	387429	4643298
Cardener	CAR-3	Súria	31	397127	4629284
Cardener	CAR-4	Manresa	31	403881	4616871
Llobregat	LLO-1	La Pobla de Lillet	31	416304	4679359
Llobregat	LLO-2	Colònia Rosal	31	407020	4659392
Llobregat	LLO-3	Pont de Vilomara	31	405907	4617415
Llobregat	LLO-4	Castellbell i el Vilar	31	403792	4607459
Llobregat	LLO-5	Abrera	31	410078	4594291
Llobregat	LLO-6	Martorell	31	411036	4592524
Llobregat	LLO-7	Sant Joan D´Espí	31	420247	4577928
WWTP					
Anoia	WWTP-IGU	Vilanova del Camí (Barcelona)	31	387448	4602419
Cardener	WWTP-MAN	Manresa (Barcelona)	31	403666	4617759
Llobregat	WWTP-ABR	Abrera (Barcelona)	31	410063	4595868

Table S8.3 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 S8.4 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-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)
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

Validation of the method

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 (2012) (see Table S-8.5).

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.

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, from 0.03 to 1.67 ng/g for sediment and from 0.08 to 3.75 ng/g for biota.

Recovery tests were carried out by spiking a pure water sample at 10 ng L^{-1} (low spike) and 100 ng L^{-1} (high spike) of each pesticide. For sediment and biota the spiked levels were of 25 ng g^{-1} (low spike) and 100 ng g^{-1} (high spike) of each pesticide. Five replicates were done in order to evaluate the precision of the method. In water samples, recoveries varied from 48.50 % to 70 % and precision was below 20 % for all pesticide. In sediment and biota samples, recoveries were higher than 40 %. (See Table S-8.5).

Table S8.5 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-Sulfone	57	12	0,20	1,00	105	10	0,17	0,50	89	13	0,38	1,13

	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
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		

	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
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

Table S8.6 Llobregat medium flow during sampling period

CODE	1st CAMPAIGN			2nd CAMPAIGN			% variation
	date	flow	Percentile	date	flow	Percentile	
LLO1	23/09/2010	0,52	56%	12/09/2011	-	-	-
LLO2	23/09/2010	-	-	12/09/2011	-	-	-
LLO3	23/09/2010	-	-	14/09/2011	-	-	-
CAR1	23/09/2010	-	-	12/09/2011	-	-	-
CAR2	22/09/2010	3,64	83%	12/09/2011	2,64	63%	-20%
CAR3	22/09/2010	3,63	80%	14/09/2011	1,86	32%	-48%
CAR4	23/09/2010	3,88(22/09/2010)	54%	14/09/2011	-	-	-
LLO4	23/09/2010	14,77	86%	14/09/2011	7,33	47%	-39%
LLO5	21/09/2010	15,23	84%	14/09/2011	-	-	-
ANO1	22/09/2010	0,12	70%	13/09/2011	-	-	-
ANO2	22/09/2010	-	-	13/09/2011	-	-	-
ANO3	21/09/2010	0,96	66%	13/09/2010	0,39	27%	-39%
LLO6	21/09/2010	26,5	89%	13/09/2011	-	-	-
LLO7	21/09/2010	21,47	89%	13/09/2011	4,99	46%	-43%

Table S8.7 PNEC and RQ values calculated for mean and maximum environmental concentrations of pesticides in water, according to accute toxicity dates measured in algae, daphnia and fish.

	Algae					Aquatic invertebrates (Daphnia magna)					Fish				
	Accute 72/96 h E C50					Accute 48 h E C50					Accute 96 h LC50				
Compound	PNEC (ng L^{-1})	*2010* RQ_Mean	*2010* RQ_Max	*2011* RQ_Mean	*2011* RQ_Max	PNEC (ng L^{-1})	*2010* RQ_Mean	*2010* RQ_Max	*2011* RQ_Mean	*2011* RQ_Max	PNEC (ng L^{-1})	*2010* RQ_Mean	*2010* RQ_Max	*2011* RQ_Mean	*2011* RQ_M
Azol															
imazalil	870	<0,1	<0,1	<0,1	<0,1	3500	<0,1	<0,1	<0,1	<0,1	1480	<0,1	<0,1	<0,1	<0,
prochloraz	5,5	0,13	1,79			734[a]	<0,1	<0,1	<0,1	<0,1	1500	<0,1	<0,1		<0,
Bencimidazole															
carbendazim	7700			<0,1	<0,1	150			0,39	4,65	190			0,31	3,6
thiabendazole	9000			<0,1	<0,1	810			<0,1	<0,1	550			<0,1	<0,
Carbamates															
carbofuran	6500	<0,1	<0,1			9,4	0,27	0,72			180	<0,1	<0,1		
methiocarb	2200	<0,1	<0,1			3[a]	0,14	1,08			650	<0,1	<0,1		
Chloroacetanilide															
metolachlor	57100	<0,1	<0,1	<0,1	<0,1	23500	<0,1	<0,1	<0,1	<0,1	3900	<0,1	<0,1	<0,1	<0,
Juvenile Hormone Mimics															
pyriproxyphen	150	<0,1	<0,1			400	<0,1	<0,1			270	<0,1	<0,1		
Neonicotinoid															
imidacloprid	10000	<0,1	<0,1	<0,1	<0,1	85000	<0,1	<0,1	<0,1	<0,1	211000	<0,1	<0,1	<0,1	<0,
Organophosphorus															
azinphos ethyl	372[a]	<0,1	<0,1			0,2	4,80	17,15			80	<0,1	<0,1		
azinphos methyl	7150	<0,1	<0,1			1,1	1,00	7,90			20	<0,1	0,43		
chlorfenvinphos	1360	<0,1	<0,1	<0,1	<0,1	0,25	1,56	13,92	0,40	5,68	1100	<0,1	<0,1	<0,1	<0,
chlorpyrifos	480	<0,1	<0,1	<0,1	<0,1	0,1	39,50	62,30	53,10	136,50	1,3	3,04	4,79	4,08	10,5

	Algae					Aquatic invertebrates (Daphnia magna)					Fish				
	Accute 72/96 h EC50					Accute 48 h EC50					Accute 96 h LC50				
Compound	PNEC (ng L^{-1})	2010		2011		PNEC (ng L^{-1})	2010		2011		PNEC (ng L^{-1})	2010		2011	
		RQ_Mean	RQ_Max	RQ_Mean	RQ_Max		RQ_Mean	RQ_Max	RQ_Mean	RQ_Max		RQ_Mean	RQ_Max	RQ_Mean	RQ_M
diazinon	6400	<0,1	<0,1	<0,1	<0,1	1	4,99	13,61	6,45	35,77	3100	<0,1	<0,1	<0,1	<0,
diclofenthion	420					1,1					1,25				
dimethoate	90400	<0,1	<0,1	<0,1	<0,1	2000	<0,1	<0,1	<0,1	<0,1	30200	<0,1	<0,1	<0,1	<0,
ethion	326[a]			<0,1	<0,1	0,056			16,25	126,79	500			<0,1	<0,
fenitrothion	1300	<0,1	<0,1			8,6	0,68	5,51			1300	<0,1	<0,1		
fenoxon sulfone	552587[a]	<0,1	<0,1			53[a]	<0,1	<0,1			25533[a]	<0,1	<0,1		
malathion	13000	<0,1	<0,1			0,7	35,26	457,64			18	1,37	17,80		
Other pesticides															
buprofezin	2100	<0,1	<0,1			420	<0,1	<0,1			330	<0,1	<0,1		
hexythiazox	400	<0,1	<0,1			470	<0,1	<0,1			3200	<0,1	<0,1		
Triazines															
atrazine	59	<0,1	0,11	<0,1	0,11	85000	<0,1	<0,1	<0,1	<0,1	4500	<0,1	<0,1	<0,1	<0,
propazine	180	<0,1	<0,1			17700	<0,1	<0,1			17500	<0,1	<0,1		
simazine	40	<0,1	<0,1	0,20	1,14	1100	<0,1	<0,1	<0,1	<0,1	90000	<0,1	<0,1	<0,1	<0,
terbumeton-deethyl															
terbuthylazine deethyl	140			<0,1	<0,1	42000			<0,1	<0,1	18000			<0,1	<0,
terbuthylazine-2 hydroxy	3800			<0,1	<0,1	2800			<0,1	<0,1	2500			<0,1	<0,
terbutryn	2,4	0,29	4,00	1,55	9,74	2660	<0,1	<0,1	<0,1	<0,1	1100	<0,1	<0,1	<0,1	<0,
Ureas															
diuron	2,7	2,24	8,84	8,63	59,09	5700	<0,1	<0,1	<0,1	<0,1	6700	<0,1	<0,1	<0,1	<0,

Figure S8.1 Water Sample Extraction

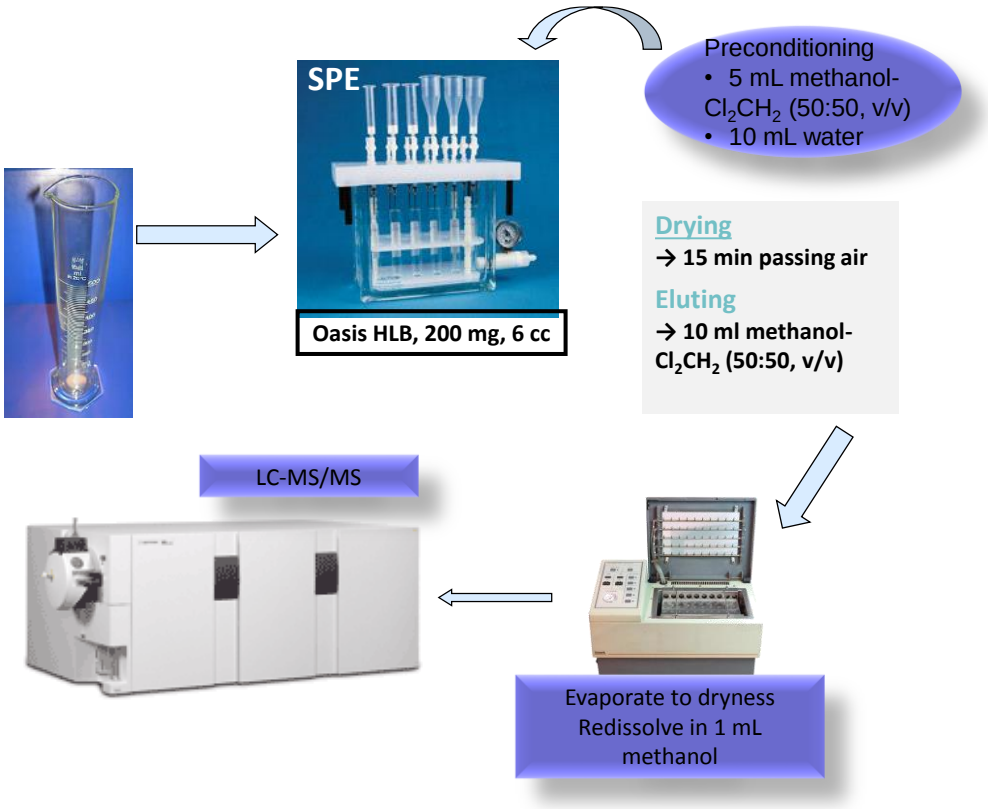


Figure S8.2 Sediment and Biota samples extraction

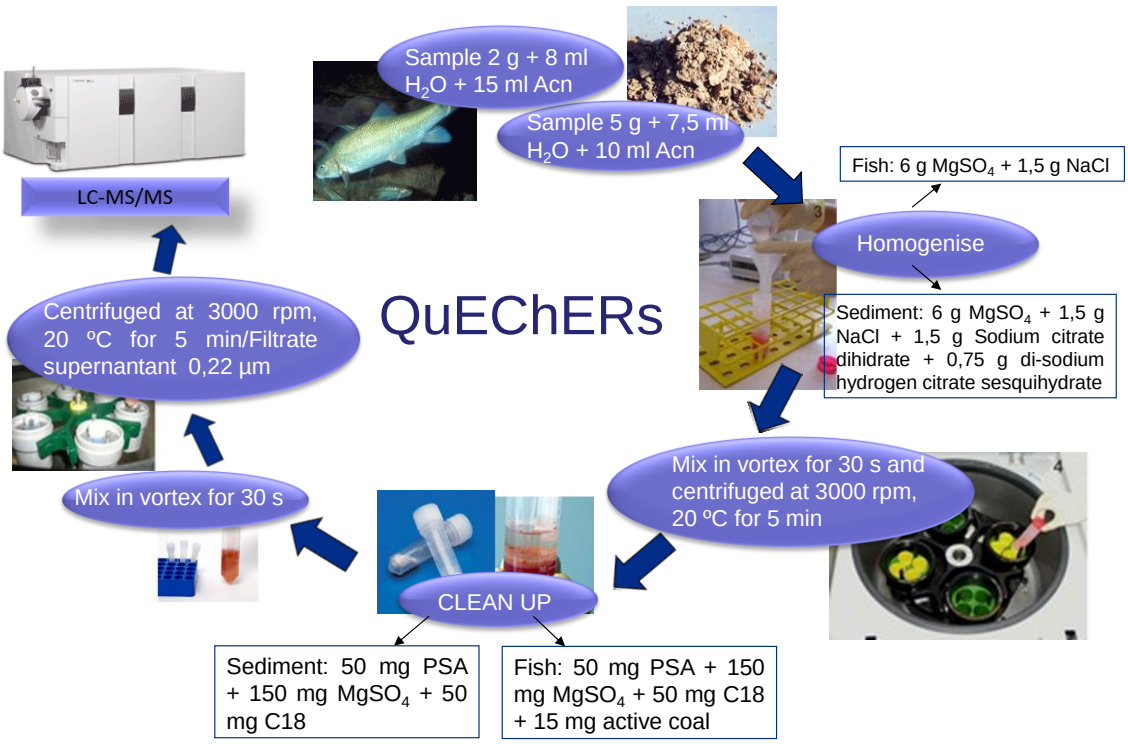


Figure S8.3 Cummulative distribution of each family pesticide in waters at each sampling site (A) 2010 and (B) 2011

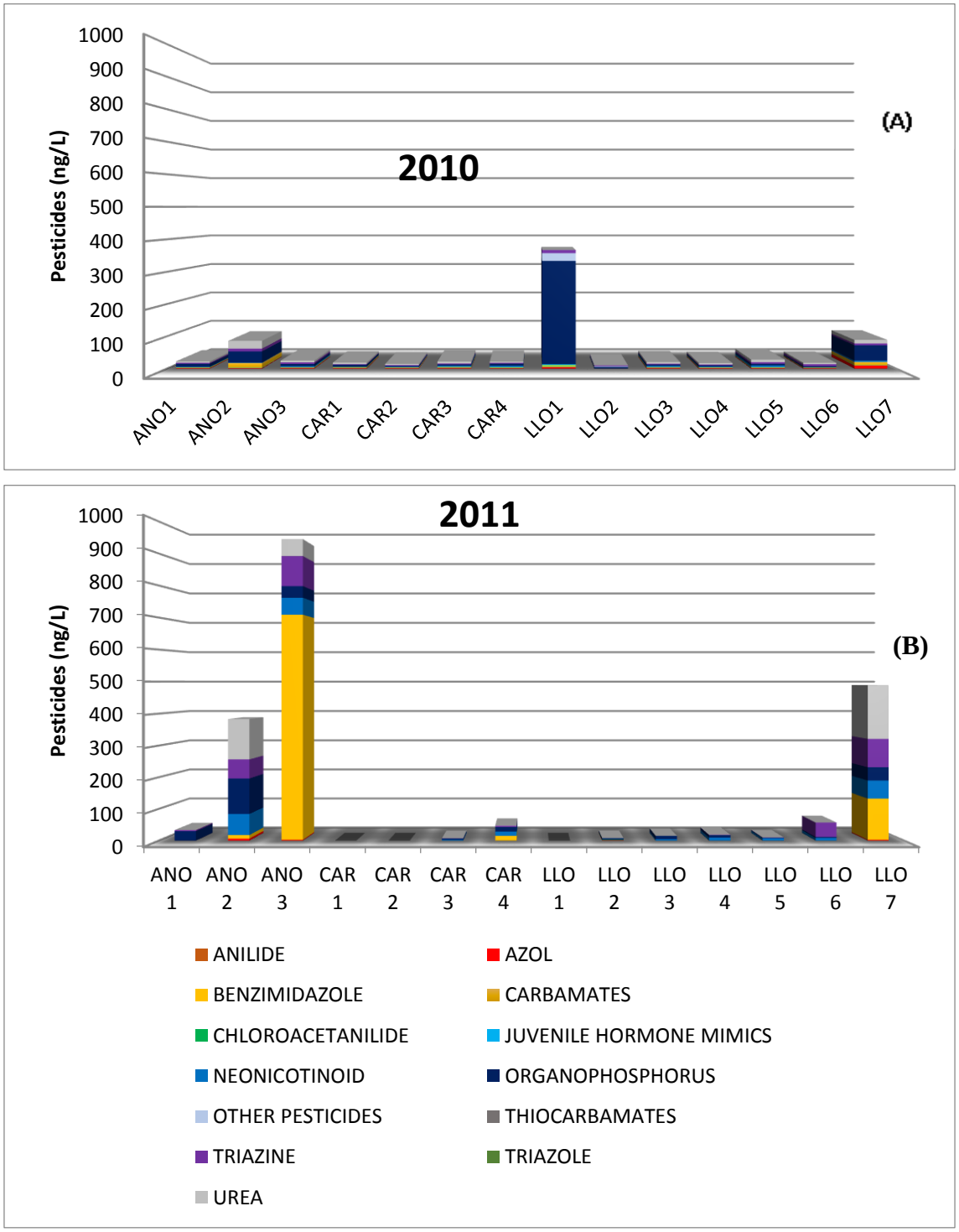


Figure S8.4 Maximum concentrations detected in water during 2010 and 2011

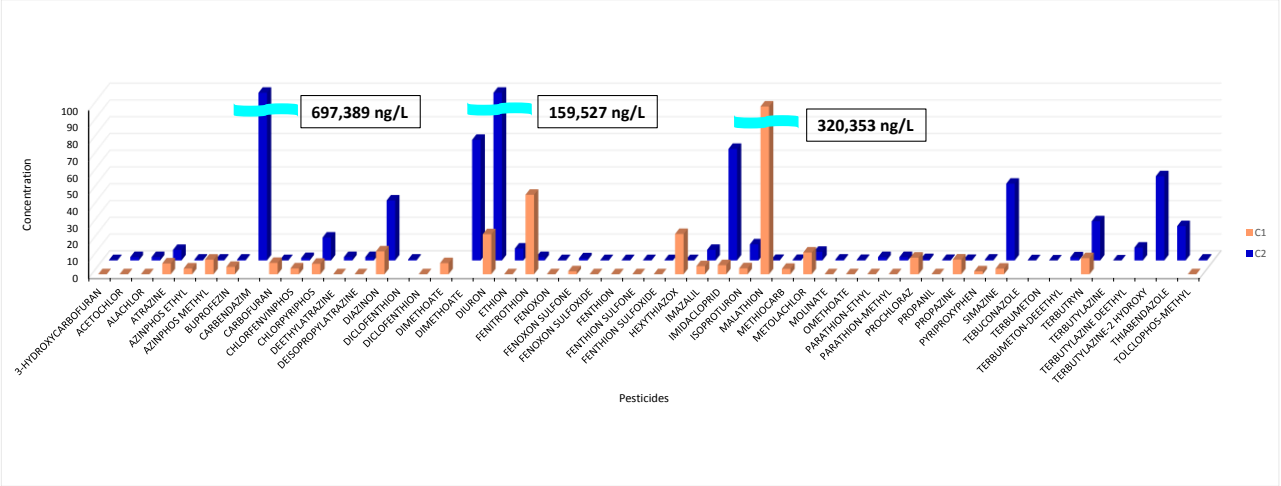


Figure S8.5 Maximum concentrations of pesticides detected in sediments during 2010 and 2011

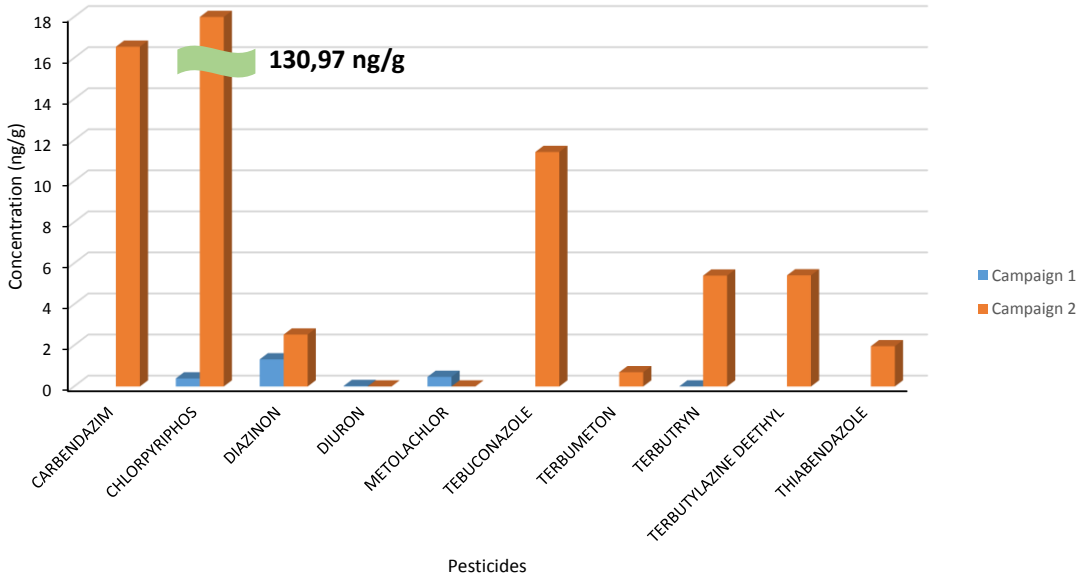
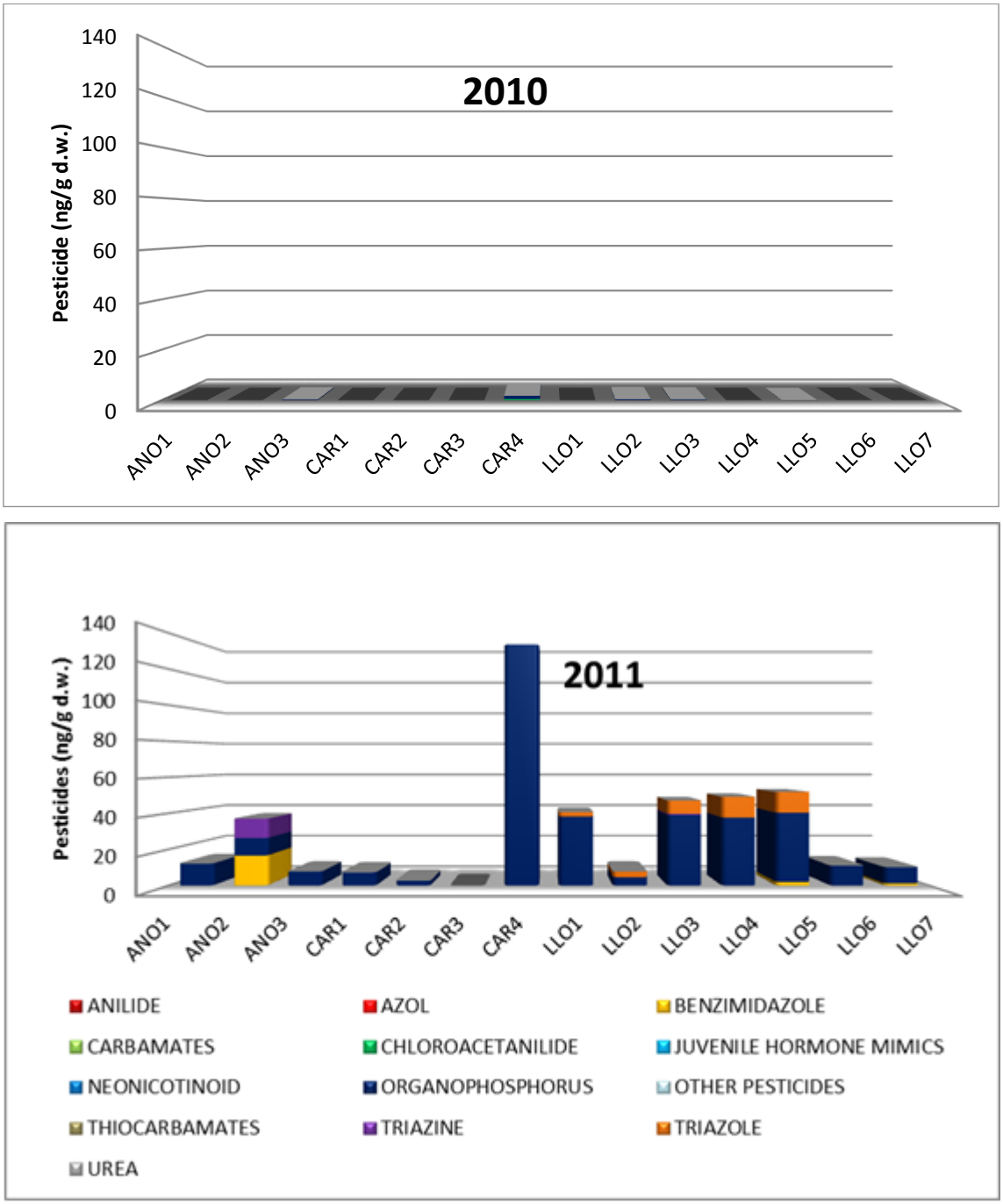


Figure S8.6 Cumulative distribution of each family pesticide in sediments at each sampling site (A) 2010 and (B) 2011.



References

Method validation and quality control procedures for pesticide residues analysis in food and feed. Document N° SANCO/12495/2011 2012.

CAPÍTOL 9

Integració de diferents models predictius per a calcular la concentració de plaguicides i la informació derivada de les campanyes de seguiment

Publicació científica 9

Integrate different forecasting models for pesticide concentration calculation as well as information derived from environmental monitoring campaigns. The example of glyphosate in Italy and several other herbicides in Spain

A. Masiá, Y. Picó, M. Calliera, L. Lamastra, F. Ferrari

Revista de L´Alguer

Integrate different forecasting models for pesticide concentration calculation as well as information derived from environmental monitoring campaigns. The example of glyphosate in Italy and several other herbicides in Spain.

Ana Masia[1], Yolanda Picó[1], Maura Calliera[2], Lucrezia Lamastra[2], Federico Ferrari[3]

[1] *Food and Environmental Safety Research Group, Department of Medicine Preventive, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés, s/n*

[2]*Institute of Agricultural and Environmental Chemistry (ICAA), Università Cattolica del Sacro Cuore, Piacenza, Italy*

3 Aeiforia srl, Fidenza, Italy

The integration of agri-environmental databases, mathematical model and GIS allows the production of maps of potential vulnerability of soils to the leaching of plant protection products. However, sometimes, these forecasting are not corroborate by monitoring data. Glyphosate in the Lombardia area of Italy and triazine herbicides in the Valencian Community were taken as study case. Glyphosate was found in groundwater of the Lombardia area despite the modelling results clearly indicate non potential risk of groundwater contamination. Among the triazine herbicides, simazine, even though present in surface waters was not found in groundwaters contrarily to its expected behaviour because it is considered a potential leacher. Glyphosate behaviour discrepancy can be explained by infiltration and point contamination sources and the lack of simazine can be justified by its facility of degradation. Results of this study remarks the importance of the integration of the two approaches to improve knowledge, and to obtain data of quality.

Keywords: Pesticides; Glyphosate; Urea; Triazines; Geographical Information Systems (GIS); monitoring data.

9.1 Introduction

The new regulation 1107/2009 on the placing of plant protection products on the market that replace the Directive 91/414 provide a comprehensive risk assessment procedure before each active substance can be authorised for the use and marketing.

On the other side, the Groundwater Daughter Directive (2006/118/EC) and the Water Framework Directive (WFD 2000/60/EC) establishes a legal framework to protect and restore clean water in sufficient quantity across Europe. In detail, in accordance with Article 17, WFD establishes EU-wide quality standards for nitrates and pesticides that must be meet to comply with "good groundwater chemical status". The goal of WFD is to reach a coherent and integrated approach to water management across the EU. This also include surface waters. Directive 2008/105/EC and most recently directive 2013/39/EU sets limits on concentrations of priority substances in surface waters, including atrazine, diuron, isoproturon and simazine.

For the evaluation of the chemical status of water bodies, the WFD introduced criteria for establishing a list of priority substances and priority hazardous substances, for which specific measures such as quality standards and emission controls must be taken in order to reduce or eliminate emissions, discharges and losses. Plant Protection Products (PPPs) have been an obvious target for monitoring activities, given that they are released directly into the environment.

One of the shortcomings of the legal framework concerning pesticides is that the actual use phase, which is a key element for the determination of the overall risks that

they pose, was not sufficiently addressed. To achieve a more sustainable use of pesticides, in the year 2009 the European Parliament approved the Directive 128 for the Sustainable Use of Pesticides (SUD)(2009/128/EC) and proposed measures for the determination of the overall risks that pesticides pose with regard to the use-phase in the life-cycle of pesticides. The purpose is to improve methods and tools, and to develop innovative approaches which will inform the policy-making process for sustainable development and therefore to address this deficiency.

In this legal framework monitoring might assume an important role in environmental risk management for chemicals. However, there are a number of different drivers that make difficult the planning of PPPs monitoring campaigns.

In Italy monitoring and environmental control is guaranteed by a large number of institutional bodies. Each region (through its environmental agency- ARPAx), applies its monitoring plan: does sampling, analysis, collect information at local level and delivers every year the data to the national authority who coordinates the overall monitoring plans providing technical protocols, data processing, statistical assessment. The systematic pesticide monitoring, for environmental purposes, was carried at the time of present study, in 85% of the Italian regions. In general there was a gradual increase in the coverage and the significance of the surveys. However, the national framework on the presence of pesticides in the water is still incomplete There are still some differences between regions, both as regards the extension of the monitoring network and frequency of sampling, both in terms of the number of substances are looking for, but a good process of harmonisation is ongoing. Differently, in Spain the occurrence of pesticides in surface and groundwater is monitored by the Hydrographic Confederations of each River Basin that depends on the Ministry of Agriculture, Food and Environment (Magrama, 2014). Commonly, there is a well-established network, with well-set up protocol that monitors

all the pesticides included in the WFD. However, in both Spain and Italy, these nertworks present several limitations in terms of sampling frequency and period of the year. Unless many samples were taken over a period of time, the results might be not representative of typical conditions. On the other hand, tests reflecting typical conditions may also miss evidence of problems that only occur infrequently. Relating to PPPs the trend today is to manage the risk not only at the stage of registration and control (monitoring of residues) but also at the territory level. This is clearly evident in the WFD when referring to the management of chemical risks at the level of river basins (an example is the identification of vulnerable areas) or in the Directive for the sustainable use of plant protection products where the main objective is the reduction of risk during the use phase of these substances. To overcome these problematic aspects in the last years the use of simulation models coupled with a geographical information system(GIS) have been proposed such a valuable tools to predict pollution risk and enable the prevention of contamination (Ackbar, 2011; Cornelis, 2009; de Paz & Rubio, 2006). They are an effective approach in evaluating herbicides leaching into groundwater at a regional scale, and can be helpful in making decisions regarding protection from and prevention of pollution.

Some Italian and Spanish Regions started different projects to identify the driving forces of the processes involved in pesticide movement and developed tools to simulate the behaviour of pesticide at different scales with the aims to defining pesticide use permissions (or restrictions) at a regional level, planning monitoring programs, optimizing the study budget by focusing sampling in the areas where higher pesticide concentrations are likely to be found.

9.2 Material and methods

9.2.1 Studied areas

The plains area of Lombardy, north Italy have developed one of the most intensive agriculture in the world. Preserving water quality, and aquatic ecosystems together, has become one of priority problems.

An early definition of critical environmental areas was carried out through a preliminary recognition scale 1:250.000 survey by the combined analysis of the environmental factors that can contribute to contamination of groundwater (intrinsic vulnerability of aquifers), the loads of anthropogenic origin imposed on the territory (pressure) and the status of groundwater quality (chemical quality).

Regarding the pressure exerted by pesticides, already in 1997, ERSAF (Ente Regionale Servizi Agricoltura e Foreste) has developed the system SuSAP (Supplying Sustainable Agriculture Production), a decision support system, developed in a GIS environment, that take into account the protective capacity of the soil, the loads distributed, the spatial distribution of crops, irrigation practices and the chemical and physical properties of the molecules under investigation. The integration of agri-environmental database, a mathematical model and a geographic information system allows the production of maps of the potential vulnerability of soils to the leaching of plant protection products.

The Valencian Community (Spain) produces the majority of the oranges and tangerines consumed locally and much of those exported to Europe and third countries. During the 2010-2011 season, citrus production was greater of 3.000.000 tons, of which

approximately 2.800.000 tons were destined for export. This intensive agricultural activity constitute a non-point pollution source that threats surface and groundwater quality, both of them used to supply the population with drinking water. In the Valencian Community, 65 % of the population is supplied with groundwater and the rest with surface water. The inappropriate use of herbicides in this area results in the contamination of surface and groundwater. Although herbicides are normally less toxic for humans than other pesticide families, their inclusion in priority lists of monitoring programmes is of great importance to obtain more comprehensive knowledge of groundwater pollution, as these compounds have been the main pesticide contaminants in this type of water sample. Herbicides such as diuron, atrazine, simazine, terbuthylazine and terbumeton have been detected in surface waters as well as in some wells in the Valencian Community as well as in other countries (Hernandez et al. 2008; Bottoni et al. 2013; García-Galan et al. 2010; Jurado et al. 2012 and ref. cited therein) being necessary a study that starts at the regional level.

Several models integrated in an Arc/Infor GIS has been applied in citrus growing areas of the Valencian Community to evaluate and ranking the potential leaching risk of the most frequently applied herbicides (de Paz & Rubio, 2006; Ferrer et al. 2012; de Paz et al. 2006).

The integration of agri-environmental database, a mathematical model and a GIS allows the production of maps of the potential vulnerability of soils to the leaching of plant protection products.

The limits of these maps should however, be emphasized. Even if they constitute an important contribution to identify areas where the potential risk of leaching is higher, they assumed that a treatment with one active ingredient is modelled on a single crop

throughout the area under investigation. Therefore, at present, there is no real mapping of vulnerabilities to pesticides but good maps of intrinsic vulnerability made for the evaluation of nitrate vulnerability. However, these maps can provide useful information for a preliminary investigation.

This raises the need to develop decision support systems that integrate different forecasting models for calculation of surface water or groundwater pesticide concentration as well as information derived from environmental monitoring campaigns.

A discrepancy between the model predictions and the concentration detected by the action of monitoring may call the attention of the decision maker towards the identification of the causes, such contamination point, but also incorrect modelling calculations or insufficient quality of the input data, and then make the most appropriate choices such as greater investment in the knowledge of the area and quality production data or application of appropriate mitigation measures.

An interesting example could be the active ingredient glyphosate. As a result of many studies and reports glyphosate is characterised as having little propensity to leach to groundwater. Despite being one of the best-selling substances at national level in Italy and set in Italy as priority substance for both surface and groundwater, glyphosate monitoring was initiated for the first time in 2005, and only in the Lombardy region by the regional environmental agency (ARPA) despite the modelling result clears indicate non potential risk of groundwater contamination.

Other interesting example could be the triazines (terbumeton, atrazine, propazine, simazine and terbuthylazine) and ureas (diuron and isoproturon), which are the most used herbicides in Spain, together with glyphosate and diquat. Triazines and ureas are among the most frequently detected due to their high mobility in the soil-water environment.

Once the contact between pesticides and the soil-water environment is made, they can be degraded in different ways to a variety of transformation products (TPs). Although TPs are usually less active and harmless than their parents, they can still have a certain degree of toxicity. As a consequence of their polarity, they normally have a higher mobility in the soil-water environment and can reach groundwater more easily than their parent compounds. Therefore, the inclusion of relevant TPs in analytical methodology applied in water monitoring programmes is necessary to provide a realistic overview of pesticide pollution.

Although these herbicides are frequently monitored because modelling results noted some leaching potential. According to the modelling terbumeton, and simazine are very mobile and the pollution risk of these herbicides is high. The highest value of simulated attenuation factor was recorded for terbumeton, with up to 58% of the applied herbicide being leached. On the contrary, herbicides that are strongly adsorbed by the soil, such as terbuthylazine and diuron, present a lower risk than the minimum value across 99% of the study area. A theoretical ranking obtained from this modelling and ordered from highest to lowest risk, is as follows: terbumeton > propazine> simazine > atrazine > terbuthylazine > diuron > isoproturon > glyphosate. However, the experimental data obtained from monitoring studies are not always in agreement with the established ranking. Table 9.1 shows some physico-chemical characteristics of all the herbicides covered in these examples and that condition their leaching potential.

Table 9.1 Herbicides properties

Herbicide	$T_{1/2}^{*}$ (days)	K_{oc}^{**} (ml g^{-1})	GUS	Leaching risk
Terbumeton	300	158	4.46	High
Propazine	35-231	8910	3.84	High
Simazine	60	130	3.35	High
Atrazine	17-271	501	3.30	High
Terbuthylazine	60	250	2.85	Moderate
Diuron	90	480	2.58	Moderate
Isoproturon	30	316	2.07	Moderate
Glyphosate	47	24,000	− 0.64	Low

$^{*}T_{1/2}$: pesticide half-life, $^{**}K_{oc}$: pesticide sorption coefficient, GUS: Groundwater Ubiquity Score

9.2.2 Investigation Of The Potential Glyphosate Groundwater Contamination In Lombardia Region (North Italy)

(N-(phosphonomethyl)glycine, $C_3H_8NO_5P$)is the world's biggest-selling chemical used as a herbicide in agriculture and forestry, orchards, viniculture, horticulture in private gardens and on non-cultivated areas as railway tracks, yards, lanes or places. The agricultural use comprises, besides the pre-emergence herbicide control, the pre-harvest applications in order to facilitate the harvest, as well as the post-harvest use to control volunteer crops. The application rates of glyphosate for weed control range from few hundred g ha^{-1} to several kg ha^{-1}(Baylis, 2000).

Mobility, and hence leachability, of glyphosate in soil depends on its inactivation in soil by its relatively fast degradation and its sorption. These two features can be very different from soil to soil, but nevertheless if compared with other pesticides, glyphosate possesses unique sorption characteristics in soil. (Borggaard et al., 2008).

Studies on the glyphosate mobility in soil discuss the state of knowledge with respect to the mobility and leaching of glyphosate from agricultural soils and concluded

that it is mainly governed by macropore flow (Vereecken, 2005, Borggaard and Gimsing, 2008). Since soil particle or colloidal transport of strongly adsorbed pesticides, such as glyphosate, through macropores (preferential flow) has been demonstrated, it could be predicted a slight increase of the low glyphosate leaching potential in soils where preferential flow is a significant process, such as in many structured clayey soils. Decisive condition is a heavy rainfall event shortly after glyphosate application while vegetation, tillage, phosphate concentration have a little or no effect in influencing the transport of glyphosate to drainage system by preferential flows. The leaching could be considered limited in uniform, non-structured soils, without macropores, as in sandy soil. The risk of contamination, however, exists in some sandy oxide-poor soils in which there is a shallow groundwater table (Borggaard and Gimsing, 2008).

Consequently to the glyphosate application for weed control and also in new agricultural systems, notably glyphosate-tolerant crops (Baylis, 2000; Kannan et al., 2006), glyphosate and its degradation product AMPA were often detected in surface waters and partially in groundwater (Botta et al ,2010; Sanchis et al, 2012).

In Lombardy region Glyphosate was found, during 2007, in 65 surface water samples on 154 collected in 53 monitoring sites, 33,8 % of the positive samples have concentration over 0,1 μgL^{-1}, during 2008, in 37 surface water samples on 205 collected in 48 monitoring sites, 34,1 % of the positive samples present concentration above 0,1 μgL^{-1}. Otherwise monitoring data of groundwater in the region of Lombardy from 2005 to 2009 generally do not present positive data (over 0,1 µg/L; the limit prescribed by law). Glyphosate concentrations above the drinking water limit were detected only in some groundwater monitoring sites, all collected in the May 2007 monitoring campaign in which 84 samples were collected from 57 selected wells. As shown in the Table 9.2, all

the contaminated wells are located in the south east part of the region as shown in the Fig. 9.1.

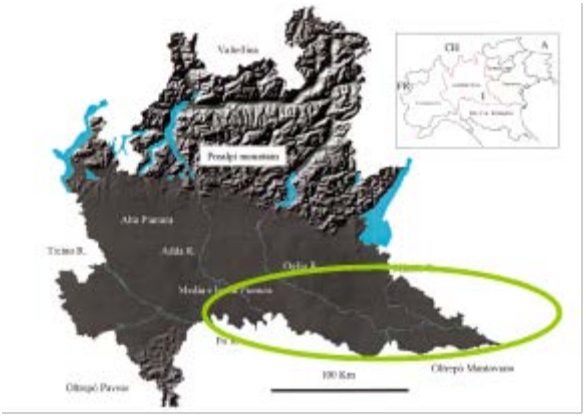


Fig. 9.1 Map of the studied area

The finding of the active ingredient glyphosate could be grouped in several causes/hypothesis such as point source contamination, chemical-physical properties of the soil, competition with inorganic phosphate for sorption sites, the influence of phosphate content in soil on its ability to adsorb glyphosate is very soil dependent, macropore flow;(shallow groundwater), inflow of surface water or bank filtrate.

However, contamination by glyphosate has covered only the 4%of the wells analyzed in the three provinces of Lodi, Cremona, Mantova in which are situated the resulted contaminated wells. These results show an extremely localized contamination. The main goal of this study was to investigate the findings in the groundwater at concentrations ≥ 0,1μg/L and to clarify the causes trough the site inspection and evaluation and the water sample collection and analysis.

Parallel samples from contaminated sites, and from surrounding areas where piezometers were available, were collected to assess residue concentrations of glyphosate, to characterize the water, and to investigate possible different analytical methods .In general, the groundwater-flow direction was known, or deducible on the basis of scientific

expert judgement. However, generally the hydrological connectivity between treated areas and the aquifer accessed by a well and the solute travel time from the surface to the aquifer were not known.

Sampling campaigns were organized from November 2010 to January 2011 in order to collect groundwater from the contaminated wells. All the monitoring wells are part of the monitoring network of ARPA Lombardia. Samples were collected in polypropylene bottles (1000 mL) and immediately stored in an insulated container chilled using ice packs for travel to the laboratory. All samples were received by the laboratory within 12 h, stored in freezer for maximum two weeks and then extracted accordingly the selected analytical method.

The method is based on Hanake et al. (2008). The instrument HPLC-MS Thermo MSQ (single quadrupole ESI/APCI) with autosampler was used to confirm the identity of samples that were analyzed and quantified with a HPLC/FD system.

Due to low recovery obtained in natural water, a purification step using a strong anion exchange (SAX) chromatographic technique was taken into account.

9.2.3 Monitoring of herbicides in waters of the Valencian Community.

Triazine compounds are often used as the basis for various herbicides such as atrazine, propazine, simazine, terbuthylazine and tebumeton. As herbicides, the triazines may be used alone or in combination with other herbicide active ingredients to increase the weed control spectrum. They are inhibitors of electron transport in photosynthesis. Tolerant plants are capable of metabolizing the active ingredient, whereas susceptible plants do not. Triazines are some of the oldest herbicides, with research initiated on their weed control properties during the early 1950s.Some of its uses are classified as restricted because of ground and surface water concerns.

Urea herbicides are generally used in weed control in agricultural and non-agricultural practices and work by inhibiting photosynthesis. Some of the urea herbicides can be very unrelenting in the environment and there is the danger that they are present in the drinking water, for instance.

The topography of the Valencian Community is largely flat, and the climate is mainly Mediterranean semiarid and mesothermic. Average annual precipitation ranges from 391 to 584 mm, with intensive rain during October (80–110 mm) and dry summers (July has 6–13 mm of rain). Temperature ranges from a minimum of 4 °C in January to a maximum of 36 °C in July. Most of the soils are calcareous Fluvisols with heavy textures in deeper horizons. There are also large areas of Calcisols (petric and haplic) with low levels of organic matter, high calcium carbonate content, and textures that are lighter than those of the Fluvisols. Less frequent are the Luvisol soils also used by farmers to grow citrus. The heavy textures in the deeper horizons of these soils protect the aquifers from NPS pollution. Regosols and Arenosols with light textures and low organic matter contents are also found in the region, although they are not important in the citrus-growing area. The covered area is showed in Fig 9.2. It was selected because there is the only area in the Valencian Community that has both, vulnerability maps available (de Paz & Rubio, 2006) and data on several monitoring campaigns (Hernandez et al. 2008).

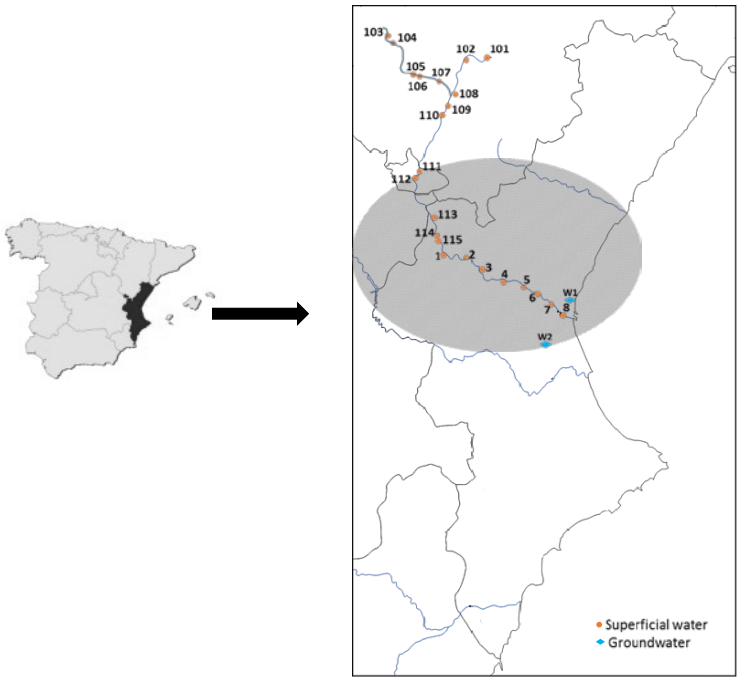


Fig. 9.2 Location of the studied area in Spain

There are several studies based on the presence of herbicides triazines and ureas, as well as, other pesticide families in surface, ground water and also air in Valencian Community area.

Pesticide concentration in groundwater depends on many factors such as crop and soil type, weather, season, degradation rates in the environment, physical and chemical characteristics of the pesticide, application rate and management practices.

There are some interesting overviews on the presence and changes over time of pesticide residues in groundwater from this area of the Valencian Community, one of the most important citrus cultivation sites of Southern Europe. Several wells, representing the different types of aquifers present in this area, were monitored during two sampling periods of a year (2000 and 2003) by Hernandez et al. (2008). Around 50 pesticides and transformation products (TPs) were included in the 2000 monitoring, while 2003´s analyses were focused on the most frequently detected compounds in the previous monitoring, mainly herbicides and their TPs. Simazine, terbuthylazine, terbumeton,

terbutryn and diuron were frequently found at concentrations around 0.1 µg/L or higher in most of the samples collected during both sampling periods. There are also data on the pesticides presents in the inhalable fraction of particulate matter (PM10) in stations located inthis area. The herbicide terbuthylazine appeared in 75% of samples and its metabolites between 31 and 60%. [Coscollà et. al. 2013].

In this study, a large group of triazines and ureas were monitored for 2012 in superficial waters of the Turia River Basin, the most important river of the area and ground waters from two representative wells (well 1 located in Carcaixent which is subject to extensive citrus crop activity and well 2 located in Alboraia). The sampling campaign was carried out from the end of September to the first of October. Atrazine, deisopropylatrazine, deethylatrazine, propazine, simazine, terbumeton, terbumetondeethyl, terbuthylazinedeethyl, terbuthylazine 2-hydroxy, terbutryn, diuron and isoproturon were monitored.

Water samples (2 L) were collected in clean amber glass bottles, from the middle of the river width. Before sample collection, each bottle was thoroughly pre-rinsed with MilliQ water and then with the same water that was going to be collected.

All samples were transported in hermetic boxes refrigerated with ice upon arrival at the laboratory (located in Valencia, Spain). Then, water samples were stored at 4 °C within 24 h to avoid any degradation and were pre-treated in the 5 subsequent days. Before the analysis, waters were vacuum filtered through 1 µm glass fiber filters followed by 0.45 µm nylon membrane filters (VWR, Barcelona, Spain).

The method used for water extraction was based on the off-line SPE procedure already published (Masia et al. 2013). Very briefly, water samples (200 mL) were vacuum passed through the SPE column (Oasis HLB SPE cartridge 200 mg sorbent/6 mL

cartridge, Waters, Milford, MA, USA). The cartridges were dried under vacuum for 10 minutes and the analytes eluted with 10 mL of dichloromethane-methanol (50:50, v/v). Extracts were evaporated to dryness and reconstituted with 1 mL of methanol.

For chromatographic separation and determination, an HP1200 series LC coupled to an Agilent 6410 triple quadrupole (QQQ) mass spectrometer, equipped with an electrospray ionization (ESI) interface were used, operating in multiple reaction monitoring (MRM) which provides higher sensitivity and selectivity. Data were processed using a MassHunter Workstation Software for qualitative and quantitative analysis (A GL Sciences, Tokio, Japan).

9.3. Results

9.3.1 Italy

The obtained results in four wells, summarised in the table 9.2, confirm the findings of ARPA obtained during the 2007 monitoring campaign, highlighting the persistence of the groundwater contamination. The analytical method adopted in this study gave results comparable at the results obtained with the ARPA method, in particular as LOQ and recovery values.

The four wells in which the study was conducted resulted still contaminated by glyphosate at concentration reliable at the concentration found during the ARPA monitoring campaigns in 2007

The data for these stations seem to reflect localized hot spots of non-agricultural contamination that are not reliable to effective groundwater contamination. This assumption was later confirmed, analyzing additional wells in same catchment area in which contamination was not demonstrated.

Table 9.2 Results of the monitoring in comparison with ARPA

Date	Monitoring site	Glyph. (µg/L) ARPA		Glyphosate (µg/L) UCSC	Date
10 may 2007	Site 1	0.9	→	<loq	28 nov 2010
22 may 2007	Site 2	0.2	→	0.252	28 nov 2010
08 may 2007	Site 3	0.2	→	0.163	30 nov 2010
05 june 2007	Site 4	0.7	→	0.525	16 oct 2010
06 june 2007	Site 5	1.2	→	1.375	12 jan 2011

9.3.2 Spain

The obtained results in surface and ground water samples are shown in Tables 9.2 & 9.3. The obtained results confirm the previous findings in the area showing the presence of several triazines and ureas in surface waters (indicative of its use in the area).

Table 9.3: Herbicides detected in surface waters in 2012

Compounds	**Min. (ng/L)**	**Max. (ng/L)**	**Mean1 (ng/L)**	**Mean2 (ng/L)**	**Frecuency (%)**
Triazines					
atrazine	< LOD	< LOD	< LOD	< LOD	-
deisopropylatrazine	< LOD	< LOD	< LOD	< LOD	-
desethylatrazine	1.38	3.95	0.79	2.50	73
propazine	< LOD	< LOD	< LOD	< LOD	-
simazine	13.89	13.89	0.63	13.89	5
terbumeton	< LOD	< LOD	< LOD	< LOD	-
terbumeton-deethyl	1.43	6.76	0.45	3.29	14
terbuthylazine	4.01	8.15	2.12	6.14	41
terbuthylazine deethyl	10.65	14.86	0.48	12.76	9
terbuthylazine-2 hydroxy	1.77	7.83	0.75	3.81	36
terbutryn	4.98	4.98	0.00	4.98	5
Ureas					
diuron	< LOD	< LOD	< LOD	< LOD	-
isoproturon	3.39	3.53	0.15	3.46	9

Table 9.4: Herbicides detected in ground waters

Compounds	Min. (ng/L)	Max. (ng/L)	Mean1 (ng/L)	Mean2 (ng/L)	Frecuency (%)
Triazines					
atrazine	1.25	1.25	0.62	1.25	1
deisopropylatrazine	2.35	6.42	4.35	4.35	2
desethylatrazine	52.5	102.0	77.2	77.2	2
propazine	< LOD	< LOD	< LOD	< LOD	-
simazine	< LOD	< LOD	< LOD	< LOD	-
terbumeton	0.50	15.29	7.82	7.82	2
terbumeton-deethyl	1.29	25.22	13.25	13.25	2
terbuthylazine	7.50	12.5	10.0	10.0	2
terbuthylazine deethyl	102.5	125.1	113.8	113.8	2
terbuthylazine-2 hydroxy	< LOD	< LOD	< LOD	< LOD	-
terbutryn	< LOD	< LOD	< LOD	< LOD	-
Ureas					-
diuron	< LOD	< LOD	< LOD	< LOD	-
isoproturon	< LOD	< LOD	< LOD	< LOD	-

9.4 Discussion

Glyphosate, as showed in different studies, is a strongly sorptive and rapidly degrading compound. In fact, glyphosate could be considered immobile in soil on the basis of its sorption properties, as reflected in high adsorption constants expressed as K_{oc} or K_f values (Vereecken, 2005). The chemical-physical previsions are supported by the groundwater monitoring results in which a low occurrence of glyphosate was obtained in Europe (Vereecken, 2005), however, in Catalonia results reported are higher than 0.1 µg L^{-1} (Sanchis et al, 2012). In addition to the chemical-physical properties of the active ingredient that could affect its leaching and degradation capacity, the results of the monitoring campaign have to be analyzed in order to identify the processes that could be responsible for well contamination. Site inspections and evaluations trough different approaches are useful to define the susceptibility and the vulnerability of the wells to contamination. Vulnerability is the probability of a pollution event occurring, and it is a part of the risk assessment processes. At present, in Lombardia region there is no real

mapping of vulnerabilities to pesticides but good maps of intrinsic vulnerability made for the evaluation of nitrate vulnerability. However, these maps can provide useful information for a preliminary investigation. The intrinsic vulnerability of the selected aquifers are defined using index-based methods in which various parameters are empirically combined to produce a vulnerability index. In Lombardia Region the index is obtained through the integration of hydrogeological vulnerability and soil protective capacity. The protective capacity of the soil towards the groundwater describes the soil ability to control the transport of soluble pollutants in the deep percolation waters into the subsurface water resources and is one of the key elements for assessing the vulnerability of the aquifers. The soil properties that could influence the soil protective capacity are permeability, depth of the shallow groundwater, particle size, pH and cation- exchange capacity (CEC), used as an indicator of the buffering capacity of soils. The hydrogeological vulnerability of an aquifer is essentially linked to the possibility of penetration and propagation of any pollutant in the aquifer itself. The ability of a deposit to be crossed by a possible pollutant is based on several factors including the thickness of the unsaturated level and lithology that characterizes it. All the contaminated sites are situated in the south east of Lombardy, an area characterized by different levels of intrinsic vulnerability, including in some case the high level, but the potential vulnerability maps for glyphosate developed by the regional decision support system, in a GIS environment, and that take into account the protective capacity of the soil, the loads distributed, the spatial distribution of crops, irrigation practices and the chemical and physical properties of the molecules under investigation did not reveal any areas of particular interest or threshold limit exceeding for glyphosate . The hydrological vulnerability and in particular the depth to water table plays a key role in the case of glyfosate, which assumes greater importance than the other parameters.

For surface water samples analysed in Valencia Community, the most detected compounds regarding to their frequency were desethylatrazine, terbuthylazine and terbuthylazine-2-hydroxy. The maximum concentration was detected for simazine (13.89 ng/L) and terbuthylazine-deethyl (14.86 ng/L), but in any case, the concentrations did not surpassed 100 ng/L, limit established for individual concentrations in drinking water according to EU legislation (2006/118/EC). Average concentrations were always below 100 ng/L. It should be noted that transformation products appear in higher concentration than parent compounds as a result of their long degradation process and the increase use of terbuthylazine as substitute of atrazine, because it was banned in European Union (EU) in 2004 because its persistent groundwater contamination. However, due to its long half-life it could still be present.

In Spain, the hydrographic confederations, have not either developed maps of vulnerability for pesticides, but are those existing for nitrates (Magrama, 2014) that can give a general idea of the risk of leaching is influenced by soil type. Furthermore, the application of a GIS-AF/RF model to assess the risk of herbicide leaching has been study in the south part of the studied area, mostly devoted to a citrus orchard (de Paz & Rubio, 2006). The resulting maps identify areas of potential risk in term of herbicide leaching and indicate that terbumeton, bromazil and simazine herbicides have the highest risk of leaching.

However, the results of the monitoring carry out in this study, agree only partly with the simulation. In the groundwater samples analyzed, atrazine, terbumeton and terbuthylazine were always detected. With regard to terbumeton, its degradation product deethylterbumeton was always found at higher concentration that the parent compound. However, simazine which is present in surface water was not detected. This is contrary

to its expected behavior because it is considered a potential leacher. It could be explained because it is quite easily degraded in environmental conditions.

9.5 Conclusions

A study was developed to investigate the findings in the groundwater at concentrations ≥ 0.1μg/L of glyphosate in monitoring sites in the south east of the Lombardy region. To clarify the causes, site inspection, well status and well surround site evaluation have been done and local authorities or the owners of the wells have been contacted.The results of this study showed:

- the findings of glyphosate were confirmed on three of the four previous contaminated wells,are reliable with the results obtained by ARPA during the 2007 monitoring campaign and confirm the persistence of the contamination.. The extreme localization of the three contaminated wells underlines the occurrence of non-agricultural contamination.

- The result in site 4 well was not confirmed, but the well conditions and the adopted management options near to the well could explain the previous contamination. However the analyzed wells seem to be mainly contaminated by point sources originating from losses of herbicide near to the farm houses or from the cleaning of sprayers and trucks in the proximity of the well. Moreover the water could be considerably polluted from surface waters by bankfiltration or infiltration during artificial groundwater recharge with resulted polluted surface water and are unrelated to the active ingredient.

-In addition some of the sampled wells did not meet the requirements for groundwater quality wells. Generally it can be noted that the conditions and the positions

of the wells were not suitable for the collection of groundwater quality samples for the assessment of a possible contamination of plant protection products at trace levels.

A similar study was developed to investigate the findings of triazine and urea herbicides in groundwater of the Valencian Community. Simazine has an unexpected behaviour according to the vulnerability maps because it is a potential leacher. However, it only occurs in surface water and not in groundwater. Less potential leacher compounds as atrazine or terbuthylazine were present in groundwater according to the expected behaviour.

Atrazine metabolites were found in higher concentration than parent compound in surface and ground water. The use of atrazine was banned in EU in 2004 and finally retired from the market in 2007 (Decision 2004/248/CE) [20], so the presence of this pesticide atrazine either represents illegal continuation of use, slow propagation from a reservoir to the water system as well as long persistence. The presences in groundwater could be due to its long persistence as established in the literature.

Vulnerability studies have become more and more an essential part of groundwater protection strategies in the Water Framework Directive and could represent a valuable tool in environmental management.The analysed wells are characterized to have a different vulnerability level. The obtained results appear to be independent of the level of vulnerability of the soil, this fact confirm the point source contamination or the infiltration of contaminated surface water as main cause of the findings in the groundwater and the importance of the integration between monitoring data and model to underline specific problems links to bad agricultural practices and territory specificities.

These works, conducted in two different countries highlight the discrepancy between modelling and monitoring and the importance of the integration of the two

approaches, in order to call the attention of the decision maker towards the identification of the causes, and then make the most appropriate choices such as greater investment in the knowledge of the area and quality production data or application of appropriate mitigation measures.

Acknowledgments

This work was partly supported by the Spanish Ministry of Economy and Competitiveness through the projects SCARCE (Consolider-IngenioCSD2009-00065) and EFAMED (CGL2011-29703-C02-02).

References

Akbar, T.A., H. Lin & J. DeGroote. 2011. Development and evaluation of GIS-based ArcPRZM-3 system for spatial modeling of groundwater vulnerability to pesticide contamination, Computers & Geosciences, 37: 822-830

Baylis, A.D., 2000. Why glyphosate is a global herbicide: strengths, weaknesses and prospects. Pest Management Science 56, 299–308.

Botta, F., Lavison, G., Couturier, G.,Alliot, F., Moreau-Guigon, E., Fauchon, N, Guery, B., Chevreuil, M., Blanchoud, H.,2009 Transfer of glyphosate and its degradate AMPA to surface waters through urban sewerage systems. Chemosphere 77, pp 133–139.

Bottoni, P., P. Grenni, L. Lucentini & A. Barra Caracciolo. 2013. Terbuthylazine and other triazines in Italian water resources. Microchemical Journal, 107: 136-142.

Ole K Borggaard and Anne Louise Gimsing, 2008. Fate of Glyfosate in soil and the possibility of leaching to ground and surface waters: a review. Pest Manag Sci,64: 441-456

Cornelis, C., G. Schoeters, E. Kellen, F. Buntinx& M. Zeegers. 2009.Development of a GIS-based indicator for environmental pesticide exposure and its application to a Belgian case–control study on bladder cancer.International Journal of Hygiene and Environmental Health, 212: 172-185

Coscollà, C., E. Hart, A. Pastor& V. Yusà.LC-MS characterization of contemporary pesticides in PM10 of Valencia Region, Spain. Atmospheric Environment, Volume 77, October 2013, Pages 394-403

de Paz, J.M&J.L. Rubio. 2006.Application of a GIS-AF/RF model to assess the risk of herbicide leaching in a citrus-growing area of the Valencia Community, Spain, Science of the Total Environment 371:44-54.

De Paz, J.M.M., J. Sanchez & F. Visconti. 2006. Combined use of GIS and environmental indicators for assessment of chemical, physical and biological soil degradation in a Spanish Mediterranean region, Journal of Environmental Management, 79: 150-162

Dick RE and Quinn JP, Glyphosate-degrading isolates from environmental samples: occurrence and pathways of degradation. ApplMicrobiolBiotechnol 43:545–550 (1995).

Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy, Official Journal of the European Union L 327, 22.12.2000, p. 1–73

Directive 2006/118/EC of the European Parliament and of the Council of 12 December 2006 on the protection of groundwater against pollution and deterioration (Daughter to 2000/60/EC), Official Journal of the European Union. L372.

Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008
on environmental quality standards in the field of water policy, amending and subsequently
repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council. L 348, 24.12.2008, p. 84-97

Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. L 226, 24.8.2013, p. 1-17.

Directive 2009/128/EC of the European Parliament and of the council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides. L309, 24.11.2009, p. 71-86.

Ferrer, J., M.A. Pérez-Martín, S. Jiménez, T. Estrela& J. Andreu. 2012. GIS-based models for water quantity and quality assessment in the Júcar River Basin, Spain, including climate change effects. Science of The Total Environment, 440: 42-59

García-Galán, M.J., M. S. Díaz-Cruz& D. Barceló. 2010.Determination of triazines and their metabolites in environmental samples using molecularly imprinted polymer extraction, pressurized liquid extraction and LC–tandem mass spectrometry. Journal of Hydrology, 383: 30-38

Hanke I, Singer H, Hollender J (2008) Ultratrace-level determination of glyphosate, aminomethylphosphonic acid and glufosinate in natural waters by solid-phase extraction followed by liquid chromatography–tandem mass spectrometry: performance tuning of derivatization, enrichment and detectionAnalBioanalChem 391:2265–2276

Hernández, F., J.M. Marín, O.J. Pozo, J.V, Sancho, F.J., López& I. Morell. 2008. Pesticide residues and transformation products in groundwater from a Spanish agricultural region on the Mediterranean Coast. International Journal of Environmental Analytical Chemistry 88: 409-424.

ISPRA Definizione delle liste di priorità per la progettazione del monitoraggio delle acque di cui al D. Lgs 152/2006 e s.m.i. (2011).

ISPRA Piano nazionale di controllo degli effetti ambientali dei prodotti fitosanitari Residui di prodottifitosanitari nelle acque 2005-2006 (2008)

ISPRA Monitoraggio nazionale dei pesticidi nelle acque Dati 2007-2008 (2011)

Jurado, A., E. Vàzquez-Suñé, J. Carrera, M. López de Alda, E. Pujade & D.Barceló. 2012. Emerging organic contaminants in groundwater in Spain: A review of sources, recent occurrence and fate in a European context. Science of The Total Environment, 440: 82-94.

Kannan, K., Ridal, J., Struger, J., 2006. Persistent Organic Pollutants in the Great Lakes, Pesticides in the Great Lakes. In: Hites Ronald A. (Ed.), Handbook of Environmental Chemistry, vol. 5. Springer-Verlag Publishers, pp. 151–200.

Magrama (Spanish Ministry of Agriculture, Food and Environment) 2014. Confederaciones Hidrográficas. http://www.magrama.gob.es/es/ministerio/funciones-estructura/organizacion-organismos/organismos-publicos/confederaciones-hidrograficas/default.aspx (last accessed January 31, 2014).

Masiá, A., M. Ibáñez, C. Blasco, J.V. Sancho, Y. Picó& F. Hernández. 2013. Combined use of liquid chromatography triple quadrupole mass spectrometry and liquid chromatography quadrupole time-of-flight mass spectrometry in systematic screening of pesticides and other contaminants in water samples. Analytica Chimica Acta 761: 117-127

Regione Lombardia – D. G. Reti e Servizi di Pubblica Utilità – U. O. Regolazione del Mercato e Programmazione Programma di Tutela e Uso delle Acque – Allegato 10 *"Definizione delle zone vulnerabili da nitrati di origine agricola e da prodotti fitosanitari"*

Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC, Official Journal of the European Union L 309, 24.11.2009, p. 1–50

Sanchis, J.; Kantiani, L.; Llorca, M.; Rubio, F.; Ginebreda, A.; Fraile, J.; Garrido, T.; Farre, M. 2012. Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry. Analytical and Bioanalytical Chemistry 402: 2335-2345

Shinabarger DL and Braymer HD, Glyphosate catabolism by Pseudomonas sp. strain PG2982. J Bacteriol 168:702–707 (1986).

Skark, C., Zullei-Seibert, N., Wilme, U., Gatzemann, U., Schlett, C., 2004. Contribution of non agricultural pesticides to pesticides load in surface water. Pest Management Science 60, 525–530.

Suoli e paesaggi della provincia di Cremona. ERSAF - Ente Regionale per i Servizi all'Agricoltura e alle Foreste. Regione Lombardia. 2004.

Harry Vereecken, 2005, Mobility and leaching of glyphosate: a review. Pesticide Management Science 61:1139–1151

Resum de Resultats i Discussió

1. Desenvolupament de metodologies analítiques

1.1 Determinació de 43 plaguicides per cromatografia líquida-espectrometria de masses utilitzant un analitzador de triple quadrupol.

A causa del caràcter polar dels plaguicides escollits per a aquesta tesi, seleccionàrem la determinació per cromatografia líquida espectrometria de masses en tàndem. L'espectròmetre de masses utilitzàrem en mode de monitorització de reaccions seleccionades (SRM), ja que treballant en aquest mode s'obté la màxima sensibilitat.

Per al mètode SRM, seleccionàrem dues transicions per compost per tal de complir amb els requeriments establits per la UE per a confirmar la identitat de l'analit (Decisió de la Comissió 2002/657/CE o No SANCO/12495/2011) [1, 2]. A més de la detecció de les dues transicions, la UE exigeix com a criteris d'identificació addicionals que la relació entre les dues transicions i la desviació del temps de retenció estiguen dins d'un marge expressat com a RSD. La transició menys intensa (SRM2) es va utilitzar per a la confirmació de l'analit, mentre que l'àrea del pic de la transició més intensa (SRM1) es va considerar per a fins quantitatius. A la Taula 2.1 mostrem les transicions seleccionades per a cada plaguicida i els seus temps de retenció. A la Figura 2.3, presentem els cromatogrames obtinguts per LC-MS/MS de diversos plaguicides en aigües superficials amb les dues transicions monitoritzades.

La segona transició d'alguns compostos, com diuron (5.5%), isoproturon (14.7%), metolaclor (17.8%) i terbutrina (5.9%), van presentar una baixa intensitat, la qual cosa limita la concentració a la qual es pot obtenir una confirmació per a aquests analits.

La cromatografia es va realitzar utilitzant una columna analítica convencional de C18 i una fase mòbil composta de metanol-aigua, ambdós amb formiat amònic10 mM. En la bibliografia s'han descrit dos tipus de fases mòbils per a la determinació de

plaguicides: addicionades amb sals o àcids. Kmellára et al. [3] van avaluar els modificadors més comuns emprats en l'anàlisi de plaguicides i van arribar a la conclusió que eren àcid fòrmic (0.01-0.2%), formiat amònic (2-10 mmol L^{-1}) i acetat amònic (1-20 mmol L^{-1}). Aquests autors també van mostrar que usant la mateixa columna les diferències en el temps de retenció obtingut amb àcid fòrmic com a modificador en lloc de sals d'amoni era al rang de ± 1 min en la majoria dels casos.

La validació del mètode es va dur a terme en una mostra d'aigua real. Els LODs (0.04-2 ng L^{-1}) es van comparar amb els recollits en anteriors estudis, comprovant que eren adequats per determinar plaguicides en aigües superficials i residuals. La precisió interdia (% RSD) va oscil·lar entre 2-19% i la intra-dia va ser inferior a 16%; i les corbes de calibratge van presentar coeficients de correlació (r^2) superiors a 0.99.

Els analits que donen pics cromatogràfics amples tenen LODs més alts i per tant, són més difícils d'identificar i integrar. Aquests pics cromatogràfics són més propensos a crear interferències que els analits que donen pics estrets. Particularment, l'herbicida acetamida sovint produeix formes de pic àmplies o amb cola excessiva fent que sigui més problemàtica la quantificació fiable a nivells baixos.

No obstant això, els resultats de la validació confirmen que es disposa d'un mètode robust i fiable per a la determinació d'un elevat nombre de plaguicides en mostres d'aigua superficial i residual.

1.2 Determinació de plaguicides i altres contaminants per cromatografia liquida -espectrometria de masses utilitzant un analitzador quadrupol temps de vol (QTOF)

Per tal de trobar altres contaminants orgànics potencials no investigats amb el triple quadrupol, els extractes de mostres d'aigües prèviament analitzats per LC-QqQ-MS/MS, es van sotmetre a una anàlisi qualitativa no-dirigida per UHPLC-QTOF MS davant d'una base de dades que incloïa més de 1000 contaminants orgànics de diferents famílies.

L'instrument QTOF va operar en mode MS^E, que implica l'adquisició simultània de l'espectre de masses a baixa (LE, 4 ev) i alta energia (HE, 15-40 ev), així s'obtenien dos espectres simultàniament. D'aquesta manera, s'aconsegueix, en una sola adquisició i sense la necessitat de seleccionar l'ió precursor, la molècula (de) protonada (al LE) i els ions fragment (al MS a HE). Amb l'enfocament MS^E, l'instrument QTOF s'utilitza en mode TOF, però la fragmentació té lloc durant la funció de HE.

Un problema del mode MS^E és que els compostos que coelueixen donen lloc a un espectre a HE en què es solapen els fragments de tots els compostos. En aquest cas, l'única solució possible per distingir el compost és la separació prèvia per UHPLC. Per exemple, a la Figura 2.4 mostrem els cromatogrames UHPLC-TOF MS obtinguts a LE i els espectres de massa exacta per a diversos contaminants detectats en un influent d'una aigua residual. El plaguicida simazina i el producte de transformació terbutilazina-desetil comparteixen la mateixa composició elemental i, per tant, la mateixa massa exacta, però es poden distingir gràcies a la separació cromatogràfica, ja que elueixen de la columna analítica a diferents t_R. No obstant això, s'ha de tenir en compte que la separació cromatogràfica no sempre és possible.

El principal problema d'aquest enfocament rau en la dificultat de confirmar inequívocament que els fragments obtinguts en el MS amb HE provenen de l'ió precursor observat en l'adquisició amb LE, és a dir, els espectres dels fragments poden contenir ions no relacionats amb el compost original.

En aquest estudi, les possibilitats del QqTOF en mode MS^E ,es van avaluar només per a la identificació de compostos utilitzant el screening no-dirigit front a una base de dades que contenia aproximadament 800 contaminants. No obstant això, aquests instruments també ofereixen la possibilitat d'identificar compostos desconeguts. Un dels principals inconvenients del QqTOF és la manca de resposta lineal, la qual cosa dificulta la quantificació. Els nous instruments són cada vegada més precisos, exactes i amb capacitats millorades.

Per això, el següent pas, va ser l'avaluació en un model més actual de UHPLC-QqTOF MS dels modes d'adquisició dels espectres d'ions producte depenent de la informació (IDA), en els quals es fragmenta l'ió precursor, permetent obtenir un espectre d'ions producte inequívoc, la identificació de contaminants desconeguts, així com la quantificació d'un grup de compostos seleccionats com a model, i en els quals es va efectuar una anàlisi dirigida. Aquest estudi ens va permetre establir les possibilitats de l'aplicació de les tècniques "òmiques" en mostres d'aigua.

Fet i fet, en aquesta ocasió, l'adquisició MS la realitzàrem en mode adquisició d'informació-depenent (IDA), que va consistir en dos experiments: un espectre de masses (full scan) entre m/z 100-950 i un scan dels ions producte depenent de la informació (IDA-MS/MS) dels ions precursors que el sistema selecciona automàticament d'acord amb els següents criteris: ions que excedien 100 cps, la tolerància d'ions 50 mDa, energia de col·lisió fixa a 45 V i resta de fons dinàmica activada. A la figura 3.1A es pot veure el

cromatograma total dels ions (TIC) per l'experiment MS i ida-MS realitzat en una aigua de riu addicionada amb els plaguicides seleccionats per a l'estudi (target).

Per a tots els analits, inclosos en l'anàlisi dirigida, la mitjana de la massa exacta va ser <0.5 ppm (i mai més de 3.2 ppm per a un plaguicida individual) i la DS d'aquests errors va oscil·lar entre 0.1-0.9, la qual cosa indica una desviació molt baixa de la resposta de l'instrument respecte de la massa monoisotópica. Per al compost original, el percentatge de diferència entre la relació de l'isòtop teòrica i experimental va ser en tots els casos inferior a 8%. En la identificació de l'espectre MS/MS enfront de la llibreria, es van obtenir valors de percentatge de puresa superiors a 75%, fins i tot en mostres addicionades a baixa concentració (Taula 3.1).

A les mostres d'aigua processades davant la Taula XIC, a més de 27 plaguicides detectats en l'anàlisi dirigida, es van identificar diversos compostos que no eren objecte d'estudi, majoritàriament productes farmacèutics i drogues il·lícites, amb un error de massa <5 ppm, percentatge de diferència en el patró isotòpic ≤ 10% i la recerca de MS/MS a la llibreria amb un percentatge de puresa ≥ 75% (Taula 3.2). A la Figura 3.2 s'observa la identificació del compost no seleccionat tiabendazole, amb un error de massa de -2.8 i una probabilitat de coincidència de l'espectre de MS/MS amb el de la base de dades de 86%. A la figura 3.3 mostrem la identificació positiva de fleicanide en un efluent d'una aigua residual. En ambdós casos els analits es van confirmar mitjançant la injecció dels patrons analítics.

La majoria dels analits identificats en aquest treball van donar resposta a ESI positiu. Per als compostos que es podien ionitzar en ambdues polaritats es va obtenir una confirmació addicional en mode negatiu (Taula 3.2, darrera columna). La figura S3.1 mostra els espectres de masses obtinguts per al paracetamol en mode positiu i negatiu.

Encara que ESI negatiu va proporcionar un resultat sensible gràcies al grup hidroxil àcid del paracetamol (pKa 9,4), la sensibilitat va ser menor que en el mode positiu i, en qualsevol mostra d'aigua, la molècula negativa no va ser fragmentada.

A més dels compostos anteriorment identificats pel XIC manager, les mostres d'aigua contenen milers de pics que corresponen a compostos desconeguts no inclosos a la Taula XIC i la seua identificació pot ser d'interés. Una investigació detallada d'aquests senyals podria ser un procés lent i ineficient ja que la majoria d'aquests senyals deriven de components de la matriu o del soroll de fons. Així, la llista de pics de compostos desconeguts ha de reduir-se i contenir únicament els pics d'interès. Amb aquest objectiu, les dades generades per LC-QqTOF-MS/MS es van processar estadísticament utilitzant Anàlisi de Components Principals (PCA) i Components Principals d 'agrupació de variables (PCPG).

Un dels senyals més alts responsable de les diferències entre les aigües residuals i les aigües naturals corresponen a m/z 323.24 a 16.0 min. Amb ajuda del programa Fórmula Finder i utilitzant la informació disponible sobre massa exacta molecular de l'ió quasi-molecular, el perfil isotòpic, els ions agrupats i els ions fragment resultants de l'espectre MS/MS, es va calcular empíricament la fórmula molecular ($C_{20}H_{34}O_3$) (fig. 3.5). Aquesta fórmula es va buscar en bases de dades en línia com "Chemspider", trobant-se trenta-dos estructures diferents que podien ajustar-se a la fórmula empírica. (Taula S3.3). Entre les estructures inspeccionades la molècula més probable tenia 4 dobles enllaços, el que reduïa les possibles estructures a 7. Aquestes estructures es van comparar amb l'espectre MS/MS utilitzant l'eina de predicció de fragment per tal d'establir la més probable. No obstant això, els resultats no van ser del tot concloents, ja que molts d'aquests compostos són isòmers estructurals amb una estructura i fragmentació molt similar. Encara que les més realistes eren 4 - [3-metoxi-4 (noniloxi) fenil] Butan-2-ol i 1

- [4 - (hexiloxi) -3-metoxifenil] -4,4-dimetylpentan-3-ol, és necessària la confirmació utilitzant estàndards analítics (que en aquest cas no estaven disponibles), ja que la fragmentació d'aquests compostos podria ser similar.

Finalment, es va validar el mètode en termes quantitatius (Taula 3.3) i es va procedir a la quantificació de mostres reals (Taula 3.4). El LOD instrumental es va determinar empíricament injectant una sèrie d'extractes addicionats a concentracions que donaven altures de pic > 1.0 x 10^4. Els LODs van oscil·lar entre 0.02-2 ng L^{-1}. En base a aquests LODs, es va establir un LCL de 5 ng L^{-1} per a tots els plaguicides, dels quals sempre va ser possible obtenir l'espectre MS/MS per a tots els compostos seleccionats, addicionats en qualsevol tipus de matriu, fins i tot els influents de les aigües residuals. Per tant, la combinació de SPE i UHPLC-QqTOF-MS (IDA-MS/MS) aconsegueix suficient sensibilitat per a determinar residus de plaguicides a nivells de ng L^{-1}. Aquests resultats es van comparar amb el mètode UHPLC-QqQ-MS/MS que el seu desenvolupament i validació es descriu al principi d'aquesta discussió per als mateixos plaguicides, en què el LOQ va oscil·lar de 0.2-6 ng $L^{-1.}$ Per a la majoria dels plaguicides els resultats són del mateix ordre.

A la figura 3.6 s'observa supressió del senyal, per a tots els analits excepte els que elueixen al final (procloraz, buprofezin, etió, piriproxifen, clorpirifòs, diclofentió, hexitiazox), en tots els tipus d'aigua analitzada, encara que va ser més notable en els influents. L'efecte matriu es va corregir quantificant amb un calibrat preparat en extractes de matriu, aconseguint reduir-lo per sota del 20% per a tots els tipus d'aigua.

A les mostres d'aigua de riu les recuperacions van ser satisfactòries per a tots els compostos (67-97%) excepte per a diazinon, etió, fentió i malation (recuperacions <70%); amb RSD compreses entre 3-20%, excepte per al molinat (RSD> 20%).

El mètode validat es va aplicar a diversos tipus de mostres que es van analitzar per triplicat i els resultats es van comparar amb els obtinguts en un treball anterior realitzat amb QqQ per als mateixos plaguicides. La bona concordança entre ambdues tècniques és notable.

2. Mètodes d'extracció

2.1 Determinació de plaguicides en aigües per extracció en fase sòlida (SPE)

La preconcentració aplicada a les mostres d'aigua, està basada en la tècnica d'extracció en fase sòlida (SPE) "off-line" descrita per Almeida Azevedo et al. [4]. El procediment va consistir en passar 200 ml de mostra d'aigua a través d'un cartutx Oasis HLB, (prèviament condicionat amb 5 ml de diclorometà-metanol (50:50) (v/v), 5 ml de metanol i 10 ml d'aigua desionitzada). Els cartutxos es van assecar aplicant buit durant 10 minuts, i els analits retinguts en el cartutx s'eluïren amb 10 ml de diclorometà-metanol (50:50, v/v). Els extractes es van evaporar a sequedat sota un corrent de nitrogen i es van reconstituir amb 1 ml de metanol. Finalment, es van filtrar a través de filtres de 0.45 micres de politetrafluoroetilè (PTFE) per a l'anàlisi per LC-MS.

El mètode d'extracció es va validar utilitzant una mostra real d'aigua. Es va triar com a blanc l'influent d'una aigua residual (el cas més difícil), que es va analitzar per tal de determinar la presència dels compostos diana.

Tots els compostos presenten LODs (en mostres de l'influent) molt per sota de 100 ng L^{-1}, que és la tolerància per a plaguicides individuals en l'aigua potable. Els límits de detecció estaven compresos en el rang de 0.04-2 ng L^{-1}, corresponent els més baixos a imidacloprid i diazinon (0.04 ng L^{-1}) i el més alt a acetoclor, alaclor, atrazina-desetil, atrazina-deisopropil, fenitrotion , paratió-etil, paratió-metil i simazina (2 ng L^{-1}). Aquests

límits de detecció són comparables als recollits en estudis previs, i apropiats per a determinar residus de plaguicides en mostres d'aigua superficials i residuals [5, 6-8].

La corba de calibratge es va preparar en extractes d'aigües residuals addicionades a concentracions creixents, compreses entre 0.01-50 ng/ml, de la barreja de plaguicides. La linealitat en el rang estudiat va ser bona, amb coeficients de correlació superiors a 0.99 per a tots els compostos.

Els efectes de matriu, ja siguen com a supressió o increment del senyal, són un gran inconvenient per a l'anàlisi quantitativa de traces per LC-ESI-MS. Trenta-cinc dels plaguicides estudiats no van presentar efecte de matriu rellevant (relació de pendents entre 0.8 i 1.2, el que significa la supressió o augment del senyal < 20%), mentre que vuit dels analits van mostrar efecte de matriu moderat (supressió o augment del senyal en el rang de 20-50%). Acetoclor, atrazina-deetil, atrazina-deisopropil, metolaclor i propazina van mostrar supressió del senyal moderat (relació de les pendents de 0.5 a 0.8), i carbofuran, carbofuran-3-hidroxi i ometoato van mostrar un augment del senyal moderat (de 1.2 a 1.5). No es va observar un fort augment o supressió del senyal. Cal fer l'excepció, que la composició de les aigües, especialment les residuals, pot ser molt diferent, depenent del tipus de mostra l'efecte matriu pot variar. A la Guia europea per al control de qualitat [1,2] es recomana l'ús d'un patró intern (IS). No obstant això, com no apareixen efectes de matriu rellevants, fins i tot en els pitjors tipus de mostres analitzades i a causa de les restriccions econòmiques per adquirir un gran nombre d'estàndards de referència marcats isotòpicament (els efectes de matriu depenen de l'analit i de la matriu), no es van utilitzar IS. La validació del mètode es va realitzar quantificant amb estàndards preparats en extractes de la matriu amb la finalitat d'evitar errors de càlcul a causa de l'efecte matriu.

La precisió intra-dia i inter-dia, (% RSD) va ser acceptable. La RSD va oscil·lar entre 2-19% i inferior al 16% per a les precisions inter-dia i intra-dia, respectivament. Les recuperacions van ser superiors al 70% per a tots els analits, excepte per a l'ometoato i atrazina-deisopropil, que va produir recuperacions de 48% i 52%, respectivament.

2.2 Determinació de 9 contaminants orgànics en aigües per microextracció en fase sòlida en tub (IT-SPME)

El procés d' IT-SPME utilitzat per a la preconcentració dels analits en mostres d'aigües està basat en el desenvolupat per Campins-Falcó et al. [9]. Per a l'acoblament d'IT-SPME-UHPLC-QqQ-MS/MS es va treballar amb un sistema de configuració de vàlvula diferent al que utilitzen altres sistemes de LC. Per a la preparació de la mostra es va emprar un dispositiu compost per dues vàlvules de sis ports acoblades per a prevenir les altes pressions generades per la columna d'UHPLC, que pot afectar negativament el sistema IT-SPME (Figura 4.1).

Es van fer passar 4 ml de mostra pel sistema mitjançant injecció manual amb una xeringa Hamilton de precisió d'1 ml. A continuació, seguint el mateix procediment que amb la mostra, es van injectar 40 ml de metanol a través del capil·lar i es va girar la segona vàlvula manualment de manera que els analits es desordiren del capil·lar de cromatografia de gasos i es van transferir a la columna analítica per a la seua separació i posterior detecció.

Per dur a terme l'optimització de la injecció on-line i amb l'objectiu d'aconseguir el màxim factor de pre-concentració, es va estudiar l'efecte del volum de mostra i d'eluent utilitzats, en mostres addicionades amb 10 ng dels compostos a estudiar i la concentració variava depenent del volum de mostra.

La Figura 4.2 mostra els cromatogrames obtinguts amb diferents volums d'aigua (2,4 i 6 ml) i metanol (30,35,40 i 45 ml) assajats. Per a un determinat volum d'aigua, s'obté un senyal diferent depenent del volum de metanol usat per a eluir els analits. El volum de metanol òptim per a la desorció també pot variar en funció del volum d'aigua que passa a través del capil·lar, ja que els analits poden ser adsorbits en diferents parts del capil·lar, per causes inherents al procés cromatogràfic. Per a la majoria dels volums d'aigua, el senyal més intens correspon a un volum d'elució de 40 µl de metanol. Només es va assajar la desorció amb metanol i acetonitril perquè el DEHP és apolar i l'ús d'altres combinacions d'aigua-metanol o acetonitril-aigua no va desordir completament el DEHP. El metanol i acetonitril van proporcionar resultats similars. Per tant, es va triar metanol que també es va utilitzar per a la separació cromatogràfica. Per a determinar el volum de mostra es van processar diferents volums d'aigua addicionats amb 1 ng de clorpirifòs i 10 ng dels altres compostos i 's'eluïren amb 35, 40 i 45 µl de metanol. Per a la majoria d'analits, s'obté una intensitat de senyal similar amb un volum d'aigua que va oscil·lar entre 1 i 4 ml mentre que el senyal disminueix gradualment per volums més alts perquè es produeix algun sagnat lleu de l'analit (Figs. 4. 1S, 4.2S i 4.3S).

Les recuperacions absolutes per a tots els volums d'aigua assajats i 40 µl de metanol van oscil·lar de 0.34%-67%. Les recuperacions més elevades es van obtenir per a clorfenvinfòs, clorpirifòs, terbutilazina, trifluralina i DEHP. Aquests valors estan d'acord amb els resultats obtinguts en altres estudis per SPME [9]. Encara que per a alguns compostos les recuperacions són baixes, la tècnica proporciona LODs inferiors a causa de l'alt volum processat (4 ml).

Per a la majoria de compostos, les recuperacions més altes s'assoleixen per als volums compresos entre 1 i 4 ml, amb excepció de clorpirifòs i simazina, en què les recuperacions més altes corresponen a 5 i 6 ml d'aigua, respectivament.

La linealitat en el rang de concentració estudiat va ser bona, amb coeficients de correlació superiors a 0.99 per a tots els compostos d'interés, excepte per al DEHP, perquè en la majoria dels casos es van detectar blancs contaminats.

Respecte a la sensibilitat del mètode es va demostrar per a la majoria dels analits que els LODs es redueixen notablement utilitzant SPME "en tub" respecte els obtinguts per injecció directa en l'UHPLC-MS/MS i IT-SPME-CapLC-DAD-MS. Només la simazina no es va poder determinar a nivells inferiors als exigits per la present directiva. Una solució adequada per millorar els LODs seria utilitzar un bucle més llarg en la segona vàlvula, ja que només una fracció de 5 µl dels 40 µl utilitzats per a desordir els analits va ser injectada finalment en el UHPLC. Una estratègia alternativa seria allargar el capil·lar utilitzat per augmentar la quantitat dels analits adsorbits.

En comparació amb altres procediments d'extracció més convencionals com la SPE cal destacar que, tot i que aquest mètode presenta recuperacions molt baixes, permet assolir factors d'enriquiment elevats. Aquest fet el converteix en un mètode analític útil i convenient, ja que permet assolir límits de detecció més baixos, incrementant la sensibilitat 100 vegades aproximadament en comparació amb la preconcentració off-line. La IT-SPME facilita la preparació de la mostra, afavorint la química analítica verda, en comparació amb altres mètodes d'extracció clàssics.

No obstant això, en treballs posteriors es va ampliar el rang de plaguicides a estudiar, incloent compostos pertanyents a diverses famílies amb diferents propietats fisicoquímiques, per als que la polaritat era molt variada. Des del començament de la SPE, s'han desenvolupat fases estacionàries polimèriques i fases lligades amb una bona estabilitat que cobreixen les diferents polaritats d'aquests analits. Per aquesta raó, SPE va ser la tècnica d'elecció per a l'extracció dels plaguicides en totes les mostres d'aigua analitzades en aquesta tesi. Va proporcionar elevades recuperacions dels analits, elevada

selectivitat, senzillesa i rapidesa. A més a més, aquesta tècnica pot usar-se com un procés d'extracció i purificació simultània. Tot això, la converteix en una tècnica molt versàtil i robusta adequada per a la monitorització d'un elevat nombre de compostos i mostres.

2.3 Avaluació de QuEChERS i Extracció amb dissolvents pressuritzats (PLE) per a la determinació de plaguicides en sòls, sediments i fangs

Es van avaluar alguns paràmetres del PLE i del QuEChERS per millorar l'extracció dels plaguicides en les mostres de sediments.

PLE està influenciat pel dissolvent orgànic, quantitat de la mostra, grandària de la cel·la, temperatura, pressió, temps estàtic, nombre de cicles i percentatge de purga, així com el sorbent utilitzat per a la purificació en línia [10]. La influència de la relació sòlid-dissolvent es va estudiar variant la quantitat de sediment (0.5, 1, 2, 5 i 10 g) que s'utilitza en una extracció amb una cel·la d'11 ml. Els millors resultats es van obtenir utilitzant 1 g de mostra, ja que l'eficàcia de l'extracció dels plaguicides per unitat de massa de sediment va disminuir amb l'augment de la matèria particulada en l'extracte. Com més gran era la quantitat de mostra, més gran era la freqüència d'obstrucció del sistema de conducció. De les diferents grandàries de cel·la que es van assajar (5, 11, 22 i 33 ml), es va seleccionar la cel·la d'11 ml que garanteix l'empaquetament fàcil i reproduïble de la mostra i els sorbents, així com la detectabilitat apropiada de l'analit. Els paràmetres que més van influenciar la recuperació van ser el dissolvent, la temperatura i l'addició d'una purificació en línia (figura 5.1). Es van provar acetat d'etil, acetonitril, metanol i una barreja de metanol-aigua. L'acetonitril va produir una eficiència d'extracció acceptable i permet una millor comparació de tots dos procediments ja que també s'utilitza en el QuEChERS. Per avaluar la influència de la temperatura sobre l'eficàcia de l'extracció total i estabilitat dels plaguicides en els sediments, es van seleccionar 3 temperatures

diferents (70, 100 i 130° C), una per sota i dos per sobre del punt d'ebullició del acetonitril (p.e. 81-82º C). La purificació en línia es va avaluar comparant entre la sílice, florisil, alúmina, sílice octadecil (C18) que es col·locaven a la part inferior de la cel·la. La sílice va proporcionar els extractes més transparents i les millors recuperacions. Pel que fa a les quantitats assajades (1, 5 i 10 g), la millor purificació mantenint l'eficiència es va obtenir amb 5 g.

El percentatge de purga (de 50% a 150%) va donar les millors recuperacions al 100% i el nombre de cicles d'extracció (d'1 a 5) proporciona recuperacions constants independentment del nombre de cicles. Per tant, es va utilitzar 1 cicle. Es van provar 3 pressions diferents (1000, 1250 i 1500 psi) que s'apliquen comunament amb PLE per avaluar si la pressió influeix en la capacitat d'extracció dels plaguicides mitjançant l'augment de la difusivitat del solvent d'extracció dins de la matriu. Els millors resultats i més reproduïbles es van obtenir a 1500 psi. Els sediments es van extreure amb quatre temps estàtics diferents (5, 7, 10 i 15 min). Un augment de 5 a 7 min va resultar en un augment en el rendiment de l'extracció, però un augment addicional del temps estàtic no millora les recuperacions.

Del mètode QuEChERS original, basat en l'extracció amb dissolvent (acetonitril) i posterior purificació mitjançant extracció en fase sòlida dispersiva (d-SPE) usant PSA i $MgSO_4$ anhidre per eliminar l'aigua, s'han desenvolupat dues modificacions. Es van avaluar les tres versions del mètode QuEChERS (mètode original sense tampó, versió tamponada amb acetat a pH = 4.8 i la tamponada amb citrat a pH = 5-5.5), utilitzant 1 g d'un blanc de sediment liofilitzat. L'aplicació de citrat de sodi augmenta notablement les recuperacions, el que va conduir a l'adopció d'aquest mètode com el millor (figura 5.2a).

Per a la purificació de la matriu es van examinar tres sorbents àmpliament utilitzats, PSA, C18 i GCB, combinats en diferents proporcions. El sorbent GBC no es va utilitzar per a la purificació pel baix contingut en lípids de la matriu, així com per la presència d'imazalil, plaguicida que al tindre caràcter àcid, pot quedar-se retés en el sorbent. D'altra banda, alguns plaguicides (atrazina, clorfenvinfòs, diuron i imazalil) es retenen al GBC (figura 5.2b).

A continuació, es va avaluar l'eficàcia de cada mètode a través de les recuperacions obtingudes per les dues tècniques (Taula 5.2). Tots els plaguicides seleccionats es van extreure per QuEChERS i PLE en les tres matrius. Les recuperacions obtingudes per PLE van oscil·lar des de 38-85% per al sòl, 35-89% per al sediment i 31-120% per als fangs amb recuperacions mitjanes de 68, 72 i 71%, respectivament. Les recuperacions obtingudes per QuEChERS van oscil·lar del 25 al 92% per al sòl, del 39 al 120% per als sediments i del 31 al 120% per als fangs, amb recuperacions mitjanes de 76, 73 i 82%, respectivament. Les recuperacions obtingudes van ser acceptables -considerant l'àmplia varietat i la polaritat dels plaguicides estudiats i les RSDs van ser inferiors a 20%.

QuEChERS va ser el procediment d'extracció avaluat més eficient i efectiu. Per tant, es va seleccionar per analitzar les mostres de sòl, sediments i fangs, ja que era avantatjós en termes de recuperació. A més a més, presenta altres avantatges, com ara el menor consum de temps, la caiguda en el consum de reactius i la despesa d'energia.

El mètode va ser validat amb èxit d'acord amb els criteris especificats en les directrius europees. El LOQ per als compostos d'interés va oscil·lar entre 1 i 10 ng g^{-1} amb l'excepció de l'alaclor i l'acetoclor que va donar LOQ de 25 ng g^{-1} (Taula 5.4) i van

ser generalment similars o més baixos en comparació amb els procediments publicats en l'actualitat per a alguns compostos [11, 12-15].

Per a compensar els efectes de la matriu es van utilitzar corbes de calibratge en matriu per a obtindre determinacions quantitatives eficaços dels plaguicides en sediments, sòls i fangs (figura 5.5).

Les recuperacions van oscil·lar de 29-102%, o 50-104% per als sediments en 5 i 50 (ng g^{-1}), de 40 al 92% per als sòls i de 40 a 120% per als fangs. La precisió intra-dia (dades no mostrades) va ser sempre millor que la inter-dia (Taula 5.4). Es pot observar que la repetibilitat, expressada com RSD, per a la precisió inter-dia va ser menor que el 26% en els dos nivells de fortificació.

3. Aplicació a diferents casos d'estudi.

3.1 Monitorització de les concentracions de plaguicides en aigües i fangs en les Estacions Depuradores d'Aigües Residuals (EDARs) de 4 conques hidrogràfiques mediterrànies

Durant l'octubre de 2010 i 2011 es van recollir mostres d'influent, d'efluent i de fangs deshidratats de 16 EDARs (Figura 6.1 i Taula 6.1) que aboquen els seus efluents als rius Ebre, Guadalquivir, Xúquer i Llobregat.

3.1.1 Incidència dels plaguicides en mostres d'aigües residuals

Dels 43 analits determinats al 2010, 29 es van detectar en mostres d'influents i 28 en mostres d'efluents (Taula 6.2). Al 2011, dels 50 analits seleccionats, es van detectar 33 i 34 en almenys una ocasió en les mostres d' influent i d'efluent, respectivament. Per tant, no hi ha diferències aparents en el nombre de plaguicides que es troben a l'entrada i a la sortida de les EDARs.

La freqüència de detecció (en percentatge) de les diferents famílies de plaguicides durant les dues campanyes, per a cada conca hidrogràfica, es mostra a la figura 6.2. Organofosforats, triazines i azols van ser les famílies més freqüents, seguit de carboxamides, imitadors d'hormones juvenils i triazines. La comparació d'aquests resultats amb els publicats anteriorment per a altres EDARs (Taula S6.5) mostra que la majoria de les concentracions que hem detectat, especialment les de 2010, són superiors a les obtingudes prèviament en altres EDARs espanyoles, excepte les detectades en una d'Almeria [16].

Tenint en compte les concentracions màximes permissibles (MAC) estipulades en la Directiva 2008/105/CE sobre plaguicides en aigües continentals i superficials [17], només el diuron va superar aquests límits, tot i que el límit de 500 ng L^{-1} establert per a la suma total de plaguicides [18] s'ha superat en molts dels efluents de les EDARs (el 2010: Lleida, Tortosa, Tudela, Copero, Còrdova, Ranilla, Alzira i Conca, i el 2011 Morón, Ranilla, Alzira, Abrera i Igualada). No obstant això, és important destacar que encara que les concentracions dels plaguicides detectats en aquest estudi van ser relativament baixes (d'acord amb les directives); aquest estudi només va analitzar alguns d'ells. Una gran varietat d'altres compostos, incloent altres plaguicides i els seus productes de transformació, poden contribuir a la mala qualitat de les aigües residuals que es van a re-utilitzar, especialment si es té en compte que no existeix cap llei o norma de la UE o d'USA que legisle els nivells màxims de plaguicides en les aigües residuals [19].

3.1.2 Incidència dels plaguicides en mostres de fangs

En la primera campanya de mostreig es van detectar 11 plaguicides, mentre que en la segona, els analits identificats van augmentar fins a 24. És important assenyalar que en 2011 els fangs de totes les plantes de tractament estaven contaminats, amb almenys un plaguicida, de manera similar al que va passar al 2010 amb les mostres de les EDARs del

Xúquer i de l'Ebre. Aquest any, només el 40% i el 50% dels fangs de les EDARs del Guadalquivir i del Llobregat, respectivament, estaven contaminats amb plaguicides.

Pel que fa a la freqüència d'aparició de les diferents famílies, i tenint en compte els resultats obtinguts per a les aigües residuals, és possible observar com els plaguicides que es van detectar comunament en les mostres d'influent i d'efluent també van estar presents en els fangs (Taula 6.3).

3.1.3 Eliminació de plaguicides en les EDARs

L'eficiència d'eliminació de les famílies de major incidència es va calcular a partir de la concentració d'analit en l'influent (Cin) i l'efluent (Cef): [(Cin-Cef)/Cin]×100%. D'acord amb aquesta equació i avaluant l'eficiència de remoció de les famílies de major incidència, al 2010, l'eliminació d'organofosforats va oscil·lar entre -811% (clorfenvinfòs) i 93% (dimetoat), mentre que la dels azols es trobava en el rang de -119% (imazalil) a 77% (procloraz). De la mateixa manera, al 2011, el percentatge d'eliminació d'organofosforats va oscil·lar entre -4.575% (diazinon) i 97% (clorfenvinfòs), i en el cas de les triazines, entre -570% (terbutilazina) i 91% (terbumetona-desetil).

A les figures 6.5 i S6.3 es mostren els resultats de l'estudi d'eliminació per a cada compost per conca i per any. De forma general, el 64% dels plaguicides analitzats en aquest estudi no van ser eliminats o, fins i tot, reduïts en les plantes de tractament. Només el 13% va aconseguir eficiències d'eliminació entre 25% i 75% i cap de les eficiències va superar el 75% (figura S6.4).

Treballs anteriors que van investigar la presència i l'eliminació de plaguicides en altres EDARs també indiquen eficiències de remoció molt baixes i molt variables sent, sovint, les concentracions més altes en els efluents que en els influents [20,21]. Això, és a conseqüència de diverses causes:

1. Les variacions en el mostreig a causa de les limitacions que el condicionen. Les mostres d'influent, d'efluent i de fangs es van recollir en el mateix dia, però, el temps de retenció hidràulica (RTH) oscil·la entre 24 i 72 h, i el dels sòlids (SRT) entre 7.5-25 dies depenent de la planta. Les mostres compostes de 24 hores poden ser insuficients per a determinar l'eliminació de plaguicides en les EDARs.

2. Les càrregues de plaguicides es van estimar utilitzant els resultats d'aigua filtrada, que no tenen en compte la fracció de compostos en la fase no aquosa, pel que es podria subestimar la càrrega total [19].

3. La presència de conjugats i/o metabòlits de plaguicides que tornen de nou a la seua forma original durant el tractament, la hidròlisi, o la desorció del material particulat durant la depuració de les aigües residuals [21].

3.2 Monitorització de les concentracions de plaguicides en aigües, sediments i biota a la conca hidrogràfica del Guadalquivir

Durant el 2010 i 2011 es va dissenyar un mostreig a gran escala que cobria tota la conca del Guadalquivir en què es van detectar residus de plaguicides en l'aigua i sediments, però no en peixos. L'absència de plaguicides en peixos pot estar relacionada amb la polaritat dels plaguicides i metabòlits estudiats, ja que la majoria d'ells són molt polars i per tant poc bioacumulables. A més a més, els peixos naden en el corrent del riu i podrien no estar exposats als plaguicides trobats en l'aigua durant un llarg període de temps.

3.2.1 Distribució espacial

La distribució espacial dels plaguicides entre l'aigua i els sediments en ambdues campanyes de mostreig és bastant irregular (Figura 7.4 i 7.5). A les mostres d'aigua, els punts més contaminats en 2010 i 2011, van ser GUA-3 i GUA-A.

A la part superior del riu, el punt més contaminat va ser GUA-3, juntament amb GUA-2 en 2010 i GUA-1 al 2011, tots ells localitzats a la província de Jaén on hi ha una elevada densitat de cultiu d'oliveres. Les concentracions més altes es van detectar per a plaguicides àmpliament utilitzats en la protecció d'aquests cultius; en el punt GUA-3, malation al 2010 i metiocarb i terbutilazina al 2011, mentre que en els punts GUA-1 i GUA-2 les majors concentracions van correspondre a dimetoat, atrazina-deisopropil o simazina.

A la part mitjana del riu, el punt més contaminat va ser GEN-2 (província de Sevilla), afectat principalment pels extensos cultius agrícoles en hivernacles ("masos"). GUA-A (província de Sevilla), es caracteritza per una agricultura extensa al voltant del riu Guadalquivir i per la influència de l'EDAR de Còrdova. Es van detectar els insecticides clorpirifòs i clorfenvinfòs i l'herbicida diuron en concentracions altes, utilitzats per a controlar plagues en oliveres, cereals, cítrics, vinya i arbres fruiters.

Hi va haver menys contaminació a la part més baixa del riu, cobert per pantans dedicats al cultiu d'arròs. L'arròs es cull al setembre i se sembra al març, de manera que aquest cultiu no estava actiu durant les campanyes de mostreig.

Les concentracions en les mostres d'efluents de les EDAR van ser altes en comparació amb les aigües superficials dels punts localitzats després de les planta de tractament (GUA-4, GUA-A, GUA-6 i GEN-1) per a l'atrazina i els seus metabòlits,

clorfenvinfòs, diazinon, metolaclor, carbendazima i diuron, el que indica que aquestes EDARs podrien ser una font de contaminació per a les aigües receptores.

Aquests resultats semblen estar d'acord amb les activitats agrícoles perquè tots ells s'utilitzen comunament en les oliveres, horts d'arbres fruiters, cereals, dacsa i vinya típica d'aquesta zona.

En els *sediments*, al 2010, els plaguicides que apareixen amb més freqüència van ser els organofosforats. Les concentracions més altes es van trobar en les capçaleres dels afluents (YEG, MAG, GUA) i disminueixen aigües avall del curs principal del riu. En YEG (província de Còrdova), es va detectar la concentració més alta de diazinon. Una possible explicació a la elevada concentració d'aquest plaguicida es que el punt està localitzat en una zona contaminada per "purins". El diazinon, a més del seu ús urbà, s'utilitza en els concentrats per a la desinfecció i polvorització, impregnar la marca auricular i en apòsits per a porcs.

En 2011, s'observa una aparició uniforme de diverses famílies de plaguicides en gairebé tots els punts, amb l'excepció del punt PIC, on la contaminació va augmentar a causa de la suma d'altes concentracions de terbutilazina i els seus productes de degradació, que no es van monitoritzar l'any anterior. No obstant això, a la mostra d'aigua d'aquesta ubicació, no hi ha terbutilazina. Aquest punt està aigües avall de GUA-3 on es va detectar una alta concentració de terbutilazina en aigua.

3.3 Monitorització de les concentracions de plaguicides en aigües, sediments i biota a la conca hidrogràfica del Llobregat

3.3.1 Distribució espacial

En 2010 la concentració més alta es va trobar a la capçalera del riu, prop de LLO-1. Per contra, en 2011, hi va haver un gradient de concentració dels plaguicides des del naixement fins a la desembocadura, particularment marcat en l'afluent Anoia.

En ambdues campanyes de mostreig, ANO-1, localitzat a la capçalera del riu Anoia, va ser el lloc menys contaminat. Aigües avall, flueix a través del punt ANO-2, molt contaminat en ambdues campanyes. Està situat aigües avall de l'EDAR d'Igualada i rep les aigües residuals amb una important contribució de les zones urbanes [22]. Aquests resultats mostren com la influència dels plaguicides en les aigües urbanes no es pot desestimar. ANO-3, situat prop de la confluència amb el riu Llobregat, on l'ús principal del sòl són les vinyes [22], va aparéixer com un punt molt contaminat al 2011. Això va ser a causa d'una alta concentració de carbendazima, un fungicida comú molt utilitzat per a controlar les infeccions per fongs en les vinyes [22], per al qual la temporada de collita normalment cau entre agost i octubre. A més a més, alguns herbicides de triazina com atrazina, terbumetona-desetil, i terbutilazina-2-hidroxi, es van detectar a ANO-3, però no a ANO-2. Aquestes triazines es van aplicar en els cultius de cereals fa uns anys [23] i poden aparèixer a l'aigua perquè procedeixen de dipòsits del sòl o fluxos d'aigua subterrània.

Per contra, la contaminació per plaguicides va tenir menys contribució en l'afluent Cardener. En 2010, es va observar una concentració constant al llarg del riu, però en 2011, es va detectar un augment de diferents famílies químiques (organofosforats, neonicotinoids i benzimidazols) en la convergència amb el riu principal en el punt CAR-

4, pel fet que aquest punt es troba aigües avall de l'EDAR, a Manresa, i està influenciat pels seus efluents d'aigües residuals.

En ambdues campanyes de mostreig, es va produir un augment de la contaminació a la desembocadura del riu, explicat per l'activitat agrícola, juntament amb les grans ciutats situades a la zona (Barcelona i rodalies) [24], i a causa de l'efecte acumulatiu al llarg del curs d'aigua, seguint un gradient de contaminació [25].

3.3.2 Incidència en biota i avaluació del risc

Les mostres de peixos es van prendre només en cinc punts en l'any 2010. Les espècies de peixos recollits inclouen Barbus (*B. guiraonis*), perca americana (*M. salmoides*) i carpa comuna (*C. carpio*). Clorpirifòs (Kow = 4), metil-azinfòs (Kow = 3.16), i diazinon (Kow = 3.95) van aparéixer en Barbus i carpa comuna. La major concentració de clorpirifòs (44.75 ng g^{-1} dw) es va trobar a LLO-3 i etil-azinfòs (105.81 ng g^{-1} dw) a LLO-4 (vegeu la fig. 8.2c). Aquestes dades indiquen una possible bioacumulació d'aquests plaguicides en els peixos. No obstant això, és difícil avaluar la seua importància real, perquè les dades de toxicitat disponibles recullen principalment valors de EC50 i LC50 en aigua. Per tal de completar aquestes dades, duguérem a terme una avaluació de riscos mitjançant la determinació del quocient de risc (RQ) per a les concentracions màximes i mitjanes en les dues campanyes de mostreig per a les algues, *Daphnia* i peixos (Taula 8.2).

Pocs plaguicides (carbendazim, metolaclor, etió i carbendazim) van presentar un RQ> 1, la qual cosa indica un potencial per a causar dany, però la sensibilitat va ser diferent en cada nivell tròfic. El RQ més alt es va obtenir amb el metolaclor en algues i peixos (RQ = 21.97 el 2010) i etió a *D. magna* (RQ = 59.17 el 2011). Tenint en compte

aquests valors de RQ, els nivells de plaguicides que es troben a la conca del Llobregat compleixen les normes de qualitat ambiental (NCA) [17].

Encara que les concentracions de plaguicides en el medi ambient semblen no presentar un risc d'acord amb aquestes normes, el risc per a la biota, especialment per als peixos, no es pot descuidar ja que bioacumulen alguns plaguicides apolars que poden produir efectes additius significatius o sinèrgics, podent ser letals fins i tot a baixes concentracions [26-28].

3.3.3 Comparació amb dades històriques

La taula 8.3 mostra la cronologia dels estudis previs per a examinar les tendències dels nivells mitjans d'alguns dels plaguicides estudiats a la conca del riu Llobregat. També s'identifica, en la mesura que siga possible, la correspondència d'aquestes dades amb els punts de mostreig seleccionats per a aquest estudi. Vint dels plaguicides seleccionats en aquest estudi ja han estat prèviament analitzats en aquest riu i les concentracions trobades són d'un ordre de magnitud similar als trobats en aquest treball. No obstant això, no es pot establir un paral·lelisme complet pel fet que la concentració de plaguicides pot variar àmpliament, depenent de l'estació de l'any en què es prenen les mostres.

Altres matrius ambientals diferents de l'aigua han estat menys estudiades. Només hi ha un estudi que va informar de la presència de 13 plaguicides (incloent 9 analitzats en aquest estudi) en sediments [24]. Aquests resultats no concorden completament amb les dades actuals. No obstant això, cal tenir en compte que els plaguicides seleccionats no sempre eren els mateixos.

Fins on sabem, no hi ha cap estudi que determine residus d'aquestes substàncies en la biota. No obstant això, un estudi previ [24] va fer un intent per establir els efectes

d'aquests plaguicides en les comunitats biològiques bentòniques (invertebrats i diatomees). L'anàlisi multifactorial va revelar una relació potencial entre triazines i la distribució de la comunitat de diatomees, encara que no es va trobar cap evidència d'alteració en la distribució de la comunitat d'invertebrats. Aquests estudis rars assenyalen clarament els punts febles de l'enfocament RQ utilitzat anteriorment i la necessitat d'obtenir més dades de camp sobre la toxicitat dels plaguicides.

3.4 Comparació de l'estudi de les conques hidrogràfiques del Guadalquivir i del Llobregat durant el període 2010-2011

3.4.1 Incidència dels plaguicides en aigües

A la Figura 5 es representen les concentracions mitjanes de les famílies de plaguicides detectades a les conques del Guadalquivir i del Llobregat durant les dues campanyes de mostreig.

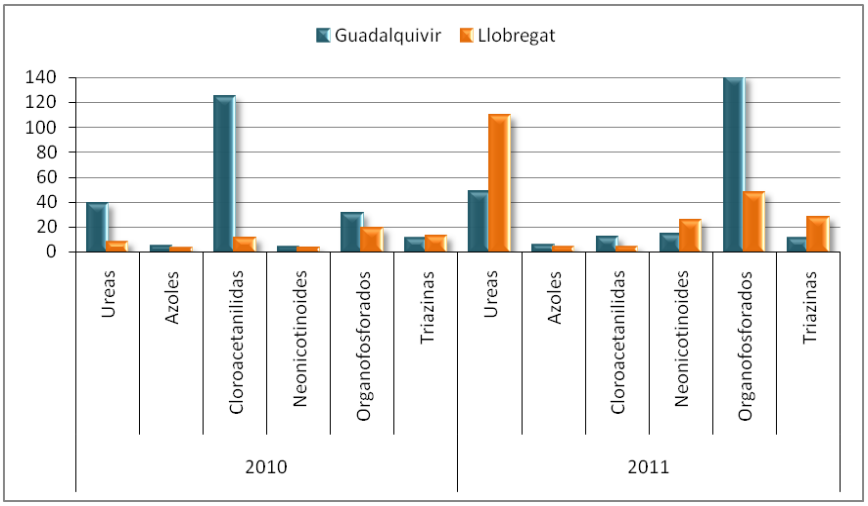


Figura 5. Concentracions mitjanes de les famílies de plaguicides a la conca del Guadalquivir i del Llobregat 2010-2011

En general, les concentracions mitjanes van ser superiors en 2010 en les dues conques hidrogràfiques. Les triazines (simazina, terbutrina) i neonicotinoids (imidacloprid) presenten una concentració similar en ambdues conques hidrogràfiques en 2010, mentre que en 2011 són superiors al Llobregat.

En 2010, el diuron (urea) es detecta en concentracions més altes al Guadalquivir, mentre que l'any següent s'inverteix el patró de contaminació, ja que es registra una entrada puntual d'aquest compost (160 ng L^{-1}) en el punt LLO-7.

Els azols (imazalil), cloroacetanilides (metolaclor) i organofosforats (clorfenvinfòs, clorpirifòs, diazinon, dimetoato) es van detectar en concentracions majors al Guadalquivir en les dos campanyes. La concentració elevada de cloroacetanilides en el 2010 es produeix a conseqüència d'una concentració màxima de metolaclor (124.43 ng L^{-1}) en el punt HER (Guadalquivir).

Probablement, la presència contínua de diazinon i clorpirifòs en ambdues conques hidrogràfiques s'associa al seu ús urbà i agrícola generalitzat i freqüent, juntament amb la seua mobilitat i llarga vida mitja (clorpirifòs fins a un any i diazinon sis setmanes aproximadament).

El clorpirifòs s'aplica a tot tipus de cultius i, fins i tot, com pols al sòl per al control d'insectes. S'utilitza especialment com a substitut d'altres plaguicides organofosforats (com metil-azinfòs, etil-azinfòs, clorfenvinfòs, diazinon, etió, fenitrotion, fentió, ometoato, i paratió-metil i paratió-etil) prohibits per la UE [29, 23].

En un estudi recent que va avaluar la importància de l'ús del sòl urbà i agrícola en la dinàmica dels plaguicides en les aigües superficials, Wittmer et al. [30] van identificar cinc patrons diferents i van classificar el diazinon dins del grup de compostos que mostren

concentracions de fons elevades durant tot l'any a causa de les fonts domèstiques constants, la qual cosa ve a donar suport a les nostres troballes.

Clorfenvinfòs, terbutrina i metolaclor [31], atrazina [32], i simazina [33] es van detectar en ambdues conques hidrogràfiques, tot i estar retirats de la UE [29]. Aquests compostos són resistents a la hidròlisi i persistents com dipòsits ambientals; la seua incidència es manté al dia amb l'activitat agrícola en la zona. La seua presència en l'aigua superficial es pot justificar pel vessament dels dipòsits de sòl o la contribució eventual de les aigües subterrànies a les superficials.

3.4.2 Incidència dels plaguicides en sediments

En ambdues conques hidrogràfiques, els organofosforats clorpirifòs i diazinon es van detectar en les dues campanyes de mostreig, mentre que al 2011 es van detectar les famílies bencimidazols (carbendazim, tiabendazole), triazines (terbumetona, terbutilazina-desetil) i triazols (tebuconazole), que no s'havien analitzat l'any anterior.

La freqüència constant i elevada presència de clorpirifòs i diazinon en les mostres de sediments podria explicar-se pel seu alt coeficient de repartiment octanol/aigua, (log Kow = 4 i 3.96, respectivament). Tots dos plaguicides poden ser aplicats durant tot l'any no només en l'agricultura, sinó també a les zones urbanes. Tots dos són compostos relativament hidròfobs amb una baixa solubilitat en aigua, propietats que els confereixen una tendència a acumular-se en els sediments [12]. Hladik et al. van demostrar que mentre les propietats fisicoquímiques contribueixen al seu repartiment entre la fase dissolta i el sediment, el temps d'aplicació i la quantitat de temps que transcorre abans d'unes pluges intenses també són importants per a l'acumulació de plaguicides en sediments [34].

3.4.3 Distribució temporal

A més de les similituds, en ambdues conques es van trobar diferències relacionades amb la distribució espacial, que poden explicar-se tenint en compte el cabal del riu. En 2010, els dos rius presentaven un cabal elevat probablement a conseqüència de les fortes pluges que produeixen un efecte de dilució i increment del vessament. Els plaguicides són arrossegats a l'aigua i, per tant, s'observa una àmplia gamma de plaguicides presents a baixes concentracions, ja que es dilueixen en un volum alt d'aigua. En canvi, en 2011 els dos rius van presentar un cabal mitjà-baix que produeix un efecte de concentració dels plaguicides, mostrant els nivells més alts en aigües i l'acumulació en sediments. A les aigües del Guadalquivir, es van observar nivells mitjans fins a 10.7 ng L^{-1} en 2010 front a 36.78 ng L^{-1} en 2011, mentre que a les aigües del Llobregat, les concentracions mitjanes van oscil·lar de 57 ng L^{-1} a 272 ng L^{-1} en 2010 i 2011, respectivament.

Aquesta situació pronostica un perill en escenaris futurs si la situació actual del canvi climàtic i l'escassetat d'aigua es desenvolupa en condicions més crítiques, destacant la necessitat dels estudis de seguiment.

3.5 Avaluació del risc potencial de lixiviació dels plaguicides. Estudi de triazines i urees a la Comunitat Valenciana (Espanya)

A la Comunitat Valenciana es va estudiar el cas dels herbicides triazines i urees que es detecten sovint en aquesta zona. Aquests herbicides es controlen amb freqüència perquè els resultats del model indiquen un potencial de lixiviació. D'acord amb el model, el terbumetona i la simazina són molt mòbils i el risc de contaminació d'aquests herbicides és elevat. El valor més alt es va registrar per al terbumetona, ja que fins al 58% de l'herbicida aplicat lixiviava. Per contra, els herbicides fortament adsorbits pel sòl, com

la terbutilazina i diuron, presenten un risc menor. La classificació teòrica obtinguda amb aquest model, de major a menor risc és la següent: terbumetona> propazina> simazina> atrazina> terbutilazina> diuron> isoproturon> glifosat. No obstant això, les dades experimentals obtingudes a partir d'estudis de seguiment no sempre estan d'acord amb aquest ordre.

Entre finals de setembre i principis d'octubre de 2012 es van controlar un ampli grup de triazines (atrazina, atrazina-deisopropil, atrazina-desetil, propazina, simazina, terbumetona, terbumetona-desetil, terbutilazina-desetil, terbutilazina-2-hidroxi, terbutrina) i urees (diuron i isoproturon) a les aigües superficials del riu Túria i les aigües subterrànies de dos pous representatius localitzats a Carcaixent i Alboraia.

Les taules 9.3 i 9.4 mostren els resultats en aigües superficials i subterrànies. Els resultats confirmen les troballes prèvies en l'àrea mostrant la presència de diverses triazines i urees en aigües superficials, el que indica el seu ús en aquesta àrea. A la figura 9.5 es resumeixen les concentracions mitjanes de cada herbicida detectat en les aigües superficials i la seua comparació amb aquells trobats en aigües subterrànies.

L'aplicació d'un model GIS-AF/RF per a avaluar el risc de lixiviació d'herbicides ha estat objecte d'estudi en la part sud de l'àrea d'estudi, majoritàriament dedicada a horts de cítrics [35]. Els mapes resultants identifiquen àrees de risc potencial en termes de lixiviació d'herbicides i indiquen que els herbicides terbumetona, bromazil i simazina tenen el major risc de lixiviació. No obstant això, els resultats del monitoratge que es duen a terme en aquest estudi només coincideixen en part amb la simulació. En les mostres d'aigües subterrànies analitzades, sempre es van detectar atrazina, terbumetona i terbutilazina. Pel que fa al terbumetona, el seu producte de degradació terbumetona-desetil sempre es va trobar en major concentració que el compost original. No obstant això, no es va detectar simazina que està present en l'aigua superficial. Això és contrari

al comportament esperat, ja que la simazina es considera un potencial lixiviador. Això es podria explicar pel fet que es degrada molt fàcilment en condicions ambientals.

La comparació d'aquestes dades amb les obtingudes en zones de la regió de la Llombardia on es van observar discrepàncies entre l'aplicació de models de GIS i els resultats obtinguts en analitzar la presència de glifosat a les aigües subterrànies posa de manifest la necessitat de dur a terme programes d'anàlisi i monitorització de les aigües.

Referències bibliogràfiques:

[1] Decisió de la Comissió de 12 d'agost de 2002 per la qual s'aplica la Directiva 96/23/CE del Consell quant al funcionament dels mètodes analítics i la interpretació dels resultats, Brussel·les, D. Of. Comun. Eur. L221 (Agost) (2002) 8.

[2] Procediments de validació de mètodes i control de qualitat per a l'anàlisi de residus de plaguicides en aliments i pinsos, document n ° SANCO / 12495/2011 implementat l' 01/01/2012.

[3] B. Kmellára, L. Pareja, C. Ferrera, P. Fodorb, A.R. Fernández-Alba, J. Chromatogr. A 84 (2011) 262.

[4] D. Almeida Azevedo, S. Lacorte, T. Vinhas, P. Viana, D. Barceló, J. Chromatogr. A 879 (2000) 13.

[5] A.C. Navarro-Ortega, R. Tauler, S. Lacorte, D. Barceló, J. Hydrol. 383 (2010) 5.

[6] O.J. Pozo, J.V. Sancho, M. Ibañez, F. Hernandez, W.M.A. Niessen, Trends Anal. Chem. 25 (2006) 1030.

[7] A. Giordano, M. Fernández-Franzon, M.J. Ruiz, G. Font, Y. Picó, Anal. Bioanal. Chem. 393 (2009) 1733.

[8] Ministeri català d'Agricultura, Alimentació i Medi Ambient (Eds.), Avanç Anuari d'Estadística de 2011, Ministeri d'Agricultura, Alimentació i Medi Ambient d'Espanya, Madrid, Espanya, 2012. [9] P. Campíns-Falcó, J. Verdú-Andrés, A. Sevillano-Cabeza, R. Herráez-Hernández, C. Molins-Legua, Y. Moliner-Martinez, J. Chromatogr. A 1217 (2010) 2695.

[10] M.H. Naeeni, Y. Yamini, M.J. Rezaee, Supercrit Fluids 57 (2011) 219.

[11] M. Kvicalova, P. Doubravova, R. Jobanek, M. Jokesova, V. Ocenaskova, H. Suessenbekova, et al., Bull Environ CONTAM Toxicol 89 (2012) 21.

[12] A. Masiá, J. Campo, P. Vázquez-Roig, C. Blasco, Y. Picó, J Hazard Mat 263 (2013) 95.

[13] A. Berlioz-Barbier, A. Vauchez, L. Wiest, R. Baudot, E. Vulliet, C. Cren-Olive, Anal Bioanal Chem 406 (2014) 1259.

[14] M.I. Pinto, C. Vale, G. Sontag, J.P. Noronha, Int J Environ Anal Chem 93 (2013) 1638.

[15] M. Köck-Schulmeyer, M. Olmos, M. López de Alda, D. Barceló, J Chromatogr A 1305 (2013) 176.

[16] S. González, R. López-Roldán, J.L. Cortina, Environ. Pollut. 161(2012) 83.

[17] Directiva 2008/105/CE del Paralament Europeu i del Consell de 16 de desembre de 2008 relativa a les normes de qualitat ambiental en l'àmbit de la política d'aigües, per la qual es modifiquen i deroguen ulteriorment les Directives 82/176/CEE, 83/513/CEE, 84/156/CEE, 84/491/CEE i 86/280/CEE del Consell, i per la qual es modifica la Directiva 2000/60/CE, D. Of. Union Eur. L348 (2008) 84.

[18] Directiva 98/83/CE del Consell, de 3 de novembre de 1998 sobre la qualitat de les aigües destinades al consum humà, D. Of. Comun. Eur. L320 (1998) 32.

[19] N. Barco-Bonilla, R. Romero-González, P. Plaza-Bolaños, A. Garrido Frenich, J.L. Martínez Vidal, J. Chromatogr. A 1217 (2010) 7817.

[20] M.J.M Bueno, M.J. Gómez, S. Herrera, M.D. Hernando, A. Agüera, A.R. Fernández-Alba, Environ. Pollut. 164 (2012) 267.

[21] M. Köck-Schulmeyer, M. Villagrasa, M. López de Alda, R. Céspedes-Sánchez, F. Ventura, D. Barceló, Sci. Total Environ. 458-460 (2013) 466.

[22] M.J. García-Galan, M. Villagrasa, M.S. Diaz-Creu, D. Barceló, Anal Bioanal Chem 397 (2010) 1325.

[23] M. Terrado, M.P. Lavigne, S. Tremblay, S. Duchesne, J.P. Villeneuve, A.N. Rousseau, et al., J Hydrol 369 (2009) 416.

[24] M. Ricart, H. Guasch, D. Barceló, R. Brix, M.H. Conceicao, A. Geiszinger, et al., J Hydrol 383 (2010) 52.

[25] I. Muñoz, J.C. Lopez-Doval, M. Ricart, M. Villagrasa, R. Brix, A. Geiszinger, et al., Environ Toxicol Chem 28 (2009) 2706.

[26] J. Damasio, R. Tauler, E. Teixido, M. Rieradevall, N. Prat, MC Riva, et al., Aquat Toxicol 87 (2008) 310.

[27] A. Ginebreda, I. Muñoz, M. Lopez de Alda, R. Brix, J. Lopez-Doval, D. Barceló, Environ Int 36 (2010) 153.

[28] Z. Yan, X. Yang, G. Lu, J. Liu, Z. Xie, D. Wu, Sci Total Environ 470 (2014) 171.

[29] Reglament (CE) No 1107/2009 Del Parlament Europeu i del Consell de 21 d'octubre de 2009 relatiu a la comercialització de productes fitosanitaris i pel qual es deroguen les Directives 79/117/CEE i 91/414/CEE del Consell, D. Of. Union Eur. L309 (2009) 1.

[30] I.K. Wittmer, H.P. Bader, R. Scheidegger, H. Singer, A. Luck, I. Hanke, C. Carlsson, C. Stamm, Water Res. 44 (2010) 2850.

[31] Reglament (CE) No 2076/2002 de la Comissió de 20 de novembre de 2002 pel qual es perllonga el període contemplat en l'apartat 2 de l'article 8 de la Directiva 91/414/CEE del Consell i relatiu a la no inclusió de determinades substàncies actives en l'annex I d'aquesta Directiva, així com a la retirada d'autoritzacions de productes fitosanitaris que continguen aquestes substàncies, D. Of. Comun. Eur. L319 (2002) 3.

[32] Decisió de la Comissió de 10 de març de 2004 relativa a la no inclusió de la atrazina en l'annex I de la Directiva 91/414/CEE del Consell i a la retirada de les autoritzacions dels productes fitosanitaris que continguen aquesta substància activa, D. Of. Union Eur. L78 (2004) 53.

[33] Decisió 2004/247/CE del Consell relativa a la no inclusió de la simazina en l'annex I de la Directiva 91/414/CEE del Consell i la retirada de les autoritzacions dels productes fitosanitaris que continguen aquesta substància activa. D. Of. Union Eur. L78 (2004) 50.

[34] M.L. Hladik, J.L. Domagalski, K.M. Kuivila, Sci Total Environ 408 (2009) 356.

[35] J.M. de Paz, J.L. Rubio, Science of the Total Environment 371 (2006) 44.

Conclusions

D'acord amb els objectius de la present tesi doctoral, la recerca duta a terme i els resultats descrits en els capítols previs, podem destacar les següents conclusions:

1. La combinació de cromatografia líquida espectrometria de masses en tàndem utilitzant un triple quadrupol (LC-QqQ-MS/MS) i un quadrupol temps de vol (UHPLC-QTOF MS) va resultar viable i eficient per a la determinació sistemàtica de residus de plaguicides. L'analitzador de triple quadrupol (QqQ) és capaç de detectar més plaguicides en les mostres a causa de la seua major sensibilitat, mentre que l'analitzador temps de vol (QTOF MS) permet identificar altres contaminants també presents en les mostres, com són els productes farmacèutics (valsartan i irbersartan), no seleccionats a priori.

2. La cromatografia líquida combinada amb un quadrupol temps de vol amb adquisició de l'espectre dels ions producte depenent de la informació (LC-MS-QqTOF en IDA-MS/MS) automatitzada per a l'anàlisi qualitatiu i quantitatiu va permetre la determinació simultània de 43 plaguicides i la identificació d'un gran nombre d'altres plaguicides i compostos farmacèuticament actius no seleccionats en l'anàlisi dirigida en mostres d'aigües residuals i de riu. El mètode és una alternativa simple, ràpida i viable per al control rutinari de contaminants orgànics en aigües, útil per a la detecció d'un nombre il·limitat de contaminants sense necessitat de realitzar diverses anàlisis.

3. La preparació de la mostra mitjançant extracció en fase sòlida és convenient, simple i ràpida i cobreix una gran varietat de substàncies amb diferent polaritat. Totes les mostres analitzades contenien plaguicides, alguns d'ells en concentració superior a 0,5 $\mu g\ L^{-1}$. Aquests resultats posen de manifest la coherència, bona sensibilitat i l'alt poder d'identificació del mètode desenvolupat.

4. L'acoblament de microextracció en fase sòlida "en tub" (IT-SPME) amb cromatografia líquida espectrometria de masses en tàndem (UHPLC-MS/MS) per a la

identificació i quantificació de contaminants orgànics en mostres d'aigua va proporcionar bona precisió i reproductibilitat (RSD < 20%), alt factor d'enriquiment (ca. 15) i resposta lineal (r > 0.99). El sistema permet l'enriquiment en-línia dels anàlits en el rang de ppt (ng L^{-1}), ja que augmenta la sensibilitat aproximadament 100 vegades en comparació de la injecció directa en el cromatògraf. No obstant això, atés que l'acoblament de l'extracció i la determinació s'ha de realitzar manualment, és menys robust que l'extracció en fase sòlida quan es vol aplicar a la determinació d'un ampli rang de plaguicides (>20).

5. La comparació de l'extracció amb QuEChERS i amb líquids pressuritzats (PLE) per a l'extracció simultània de 50 plaguicides en sòls, sediments i fangs va demostrar que l'eficiència i l'eficàcia del mètode QuEChERS optimitzat per a sediments es superior a la de PLE, permetent reduir el temps i el cost de l'anàlisi, així com augmentar el rendiment.

6. L'estudi de la presència de plaguicides en influents, efluents i fangs deshidratats en les plantes de tractament d'aigües residuals de les principals conques hidrogràfiques espanyoles posa de manifest la presència de plaguicides i la baixa eficàcia en la seua eliminació. A causa d'aquestes baixes eficiències d'eliminació les EDARs es poden considerar un focus de contaminació puntual de les aigües superficials a causa d'aquests compostos.

7. Es va monitoritzar la presència de plaguicides en mostres d'aigües, sediments i biota en les conques hidrogràfiques del Guadalquivir i el Llobregat durant dos anys consecutius (2010 i 2011). En ambdues, els pesticides es van detectar principalment en aigua, on van aparéixer quasi contínuament. La seua presència en sediments va ser molt més intermitent i en biota més aviat escassa. Els insecticides organofosforats i els herbicides triazínics van ser els plaguicides predominants en ambdues conques.

8. L'estudi realitzat en el Guadalquivir revela que organofosforats (malation, clorpirifòs, diazinon), carbamats (metiocarb) i triazines (terbutilazina, deisopropilatrazina), van ser les famílies detectades amb major freqüència i concentració en tots dos períodes. Els productes de transformació de la atrazina i la terbutilazina es van trobar en concentracions més altes que els compostos originals, com a resultat del seu llarg procés de degradació. La distribució espacial dels plaguicides va mostrar que els punts de mostreig localitzats a Jaén i Còrdova estan dominats pels insecticides i herbicides utilitzats en el cultiu de l'olivera mentre que els de les altres províncies contenien una mescla complexa de tots els plaguicides estudiats, que reflecteixen les diferències en els patrons de cultiu.

9. Els plaguicides predominants en el Llobregat (en termes de freqüència i concentració) eren bencimidazols (carbendazim), organofosforats (malation, clorpirifòs), triazines (terbutilazina-2-hidroxi, simazina), neonicotinoids (imidacloprid) i triazols (tebuconazole). Els nivells són majors en la confluència dels rius Anoia i Llobregat. Aquesta zona és eminentment agrícola i dedicada al cultiu de la vinya.

10. La comparació dels resultats d'aquest estudi amb dades històriques confirmen l'existència d'una contaminació de fons en la zona del riu Llobregat en els últims 20 anys i demostren que el seguiment periòdic de l'estat de la qualitat ambiental dels rius ajudarà al control de la contaminació i a determinar l'efecte de diferents paràmetres fisicoquímics.

11. L'estudi de la distribució temporal durant els dos anys del seguiment indica que podria estar relacionada amb el cabal del riu. El flux mitjà-baix comparat amb un flux alt produeix un augment de la concentració en l'aigua i els sediments. En ambdues conques hidrogràfiques diversos plaguicides excedien el límit de 0,1 µg L^{-1} per als compostos individuals. Els punts de mostreig GUA-3 i ANO-3, localitzats en els rius

Guadalquivir i Llobregat respectivament, van excedir el límit de 0,5 µg L^{-1} per a la suma dels plaguicides en règim de cabal baix-mitjà.

12. Els diferents patrons de contaminació per plaguicides en diferents condicions de flux van revelar que pot afectar a la toxicitat per a la biota. Encara que els valors del quocient de risc (RQ) van mostrar un baix risc de toxicitat per la presència de plaguicides en l'aigua, la bioacumulació de clorpirifòs i diazinon, plaguicides lipofíls (Kow> 3) en peixos, destaca la necessitat d'enfocaments alternatius i complementaris als previstos pel simple compliment de les normes de qualitat ambiental (NQA).

13. Es va realitzar un estudi en el riu Túria sobre la possible infiltració de les triazines i urees de les aigües superficials a les aigües subterrànies. Aquestes dades es van confrontar amb els mapes de vulnerabilitat obtinguts pel Sistema d'Informació Geogràfica (GIS) en la zona per a aquests mateixos herbicides. Els pous analitzats es caracteritzen per tenir un nivell de vulnerabilitat diferent a l'establert mitjançant l'aplicació de models GIS. Els resultats obtinguts apunten a l'existència de factors addicionals al nivell de vulnerabilitat dels sòls. Aquests resultats es van comparar amb els obtinguts en diverses zones d'Itàlia sobre la vulnerabilitat dels aqüífers subterranis al glifosat (herbicida que d'acord amb els models de GIS no hauria de lixiviar). Tots dos estudis confirmen la importància de la integració entre les dades de la vigilància i el model per a subratllar els casos en els quals el comportament no s'ajusta al predit pel model i els problemes específics vinculats a males pràctiques agrícoles i a les especificitats territorials.

Resums

RESUMEN EN CASTELLANO

El continuo crecimiento demográfico de la población en el último siglo ha creado la necesidad de incrementar la producción de alimentos. Sin embargo, las plagas y enfermedades destruyen cerca de la tercera parte de las cosechas durante su producción, transporte y almacenamiento. Ante estas circunstancias la utilización de plaguicidas que controlan la acción de las plagas es imprescindible. [1]

El empleo de productos fitosanitarios ha aportado indiscutibles beneficios económicos puesto que aumentan la productividad agrícola, y disminuyen la mano de obra así como también el coste de los alimentos. Junto a las ventajas derivadas de su uso agrícola, el empleo de plaguicidas también ha beneficiado al campo de la sanidad, donde han contribuido al control e, incluso, erradicación en algunas zonas, de enfermedades transmitidas por vectores [2].

Todo ello, unido a que su síntesis es económica, su estabilidad química excelente y su riesgo toxicológico para la salud humana y el medio ambiente se ha evidenciado relativamente tarde, ha provocado su uso masivo e indiscriminado. [3]

Los plaguicidas no son selectivos a la plaga que pretenden controlar, y afectan no sólo a los vectores relacionados con esta, sino que también a otras especies, provocando desequilibrios en los ecosistemas. Según estudios realizados en EE.UU., de los 500 millones de kilos de plaguicidas utilizados anualmente, sólo el 1% de los productos alcanza a los organismos nocivos a los que van destinados. El 99% restante permanece en los ecosistemas, se transfieren a la atmósfera por volatilización, al suelo y a los acuíferos [4]. Si bien los productos fitosanitarios aumentan la producción de alimentos, los efectos negativos en el medio ambiente son indiscutibles. Desde las aguas superficiales, los plaguicidas entran en los niveles más bajos de la cadena alimentaria en

concentraciones bajas, se bioacumulan en cada nivel trófico y se biomagnifican sucesivamente hasta llegar a la parte más alta de la cadena trófica (aves rapaces, peces o mamíferos depredadores), donde alcanzan concentraciones entre 10 y 100 veces más altas que las originales.

Esta bioacumulación afecta la biodiversidad ocasionando una disminución en sus poblaciones, ya que todos los integrantes de la cadena trófica están expuestos a concentraciones subletales que provocan efectos en los individuos que pueden ir desde indetectables hasta serios daños que afectan a su reproducción y supervivencia.

La Figura 1 muestra cómo los contaminantes llegan a los ecosistemas acuáticos. Los productos químicos que se encuentran en los fitosanitarios penetran en las vías fluviales por efecto de la difusión de la pulverización. El grado y extensión de la difusión depende de factores climáticos externos como la temperatura ambiente y el régimen de lluvias y de vientos.

La lluvia moviliza los plaguicidas del lugar donde se aplicaron por fenómenos de escorrentía superficial o por infiltración a través del suelo hacia las aguas subterráneas. Al igual que la lluvia, el riego incrementa el grado de lixiviación disminuyendo la cantidad de plaguicida que se volatiliza desde el suelo. Un exceso de riego puede percolar los plaguicidas directamente en el acuífero. Parte de los plaguicidas se evaporan pasando al aire o uniéndose a las partículas del suelo, como vapor o como polvo. Pueden ser transportados grandes distancias y nuevamente ser depositados sobre la tierra o las aguas superficiales a través de las lluvias.

En las últimas décadas, ha aumentado la preocupación por la conservación de los recursos naturales desde una perspectiva sostenible y la disponibilidad del agua se ha convertido en una cuestión importante que preocupa a todos los gobiernos.

Esta contaminación generalizada de los ecosistemas acuáticos y del suelo ha obligado a la adopción de medidas legislativas restrictivas por parte de la Unión Europea:

• Directiva Marco del Agua (DMA) (Directiva 2000/60/CE), establece las bases para regular los recursos hídricos con el objetivo de preservar, proteger y mejorar su calidad y su uso sostenible [5].

• Decisión 2455/2001/CE, establece una lista de 33 sustancias prioritarias que deben ser controladas, la tercera parte de las cuales son plaguicidas [6].

• Directiva 2008/105/CE define las normas de calidad ambiental (NCA), la media anual (MA) y las concentraciones máximas admisibles (CMA) en las aguas superficiales para la lista de sustancias prioritarias antes mencionadas. Aunque diversos plaguicidas están incluidos actualmente en esta lista en los reglamentos de la Unión Europea (UE), muchos otros todavía no están regulados [7].

• Directiva 98/83/CE, establece límites para los plaguicidas en las aguas destinadas al consumo humano (100 ng/L para los plaguicidas individuales y 500 ng/L para la suma de todos los plaguicidas) [8].

Las cuencas hidrológicas mediterráneas están sometidas principalmente a presiones que provienen de la expansión y el desarrollo humano en sus diferentes expresiones (agrícola, industrial, urbano...). Los modelos de cambio climático concluyen que las regiones mediterráneas serán las más impactadas en los próximos años y las consecuencias del cambio global afectarán tanto a la disponibilidad como la calidad del agua. Aunque estos contaminantes se encuentran generalmente a nivel de traza en los ambientes acuáticos, es importante conocer su distribución y evolución en el medio ambiente para su control y evolución, ya que la exposición crónica a estas dosis bajas puede suponer una amenaza para en la biota acuática y la salud humana [9].

Todo lo dicho anteriormente pone de manifiesto que el análisis de residuos de plaguicidas representa un instrumento fundamental para la protección humana y del medio ambiente. Esta circunstancia, crea la necesidad de realizar un mayor esfuerzo en el desarrollo de metodologías rápidas y versátiles que permiten la detección de contaminantes emergentes y sus posibles metabolitos en matrices medioambientales [10].

Los estudios acerca de la incidencia de los contaminantes emergentes en la Península Ibérica son escasos [11-16]. En general, en Europa hay pocos estudios que determinan la aparición de los plaguicidas utilizados en la actualidad en los distintos compartimentos ambientales diferentes del agua y la mayoría de ellos son muestreos erráticos realizados para demostrar la fiabilidad de un método de análisis, pero no estudios sistemáticos que evalúan la incidencia y los niveles de plaguicidas en una cuenca hidrográfica [17-20]. Estos estudios, aunque no son muchos, son más frecuentes en los EE.UU. [21-28].

El escaso número de publicaciones relacionadas con la presencia de plaguicidas en sedimentos y biota se debe, en parte, a la falta de NCA para los contaminantes orgánicos en estas matrices, incluidos los plaguicidas y a la dificultad de su extracción. Los sedimentos y los peces son matrices variables y muy complejas, debido a las fuertes interacciones que enlazan los analitos con los diferentes constituyentes de la muestra (materia orgánica y arcillas en sedimentos; grasas y proteínas en los peces), convirtiendo a estas dos matrices en un depósito de estos compuestos y dificultando su extracción [29].

Atendiendo a las necesidades medioambientales existentes se definió el objetivo global de esta tesis doctoral, basado en ofrecer una visión general de la calidad de las aguas de las cuencas mediterráneas representativas de la Península Ibérica, así como la evaluación del riesgo toxicológico de los plaguicidas sobre la biota (algas y bacterias, macroinvertebrados y peces). Con todo ello, esta tesis doctoral pretende contribuir al

conocimiento de la funcionalidad de las cuencas mediterráneas, y de su capacidad de recuperación frente al impacto de las actividades humanas, además, de ser útil como una base sólida para el desarrollo y la planificación de estrategias y metodologías más adecuadas para la recuperación y protección de los ecosistemas mediterráneos distribuidos por todo el planeta, tomando como caso de estudio la presencia de residuos de plaguicidas.

Por tanto, la metodología utilizada en la elaboración de esta tesis fue:

1. Desarrollar y validar las metodologías analíticas basadas en la extracción en fase sólida (SPE), la metodología QuEChERS (acrónimo de Quick, Easy, Cheap, Effective, Rugged, Safe) y la extracción con líquidos presurizados (PLE) seguida de cromatografía líquida acoplada a espectrometría de masas en tándem (LC-MS/MS), utilizando un analizador de triple cuadrupolo así como cuadrupolo tiempo de vuelo, para la determinación de plaguicidas en muestras de agua, sedimentos y peces.

2. Estudiar las concentraciones, distribución y destino de estos contaminantes en ecosistemas fluviales mediterráneos de la Península Ibérica (Guadalquivir, Llobregat y Turia) tratando de determinar el origen y el patrón de distribución de estos compuestos.

3. Evaluar la amenaza que suponen estos compuestos para la fauna acuática, basado en los datos toxicológicos disponibles de plaguicidas. Los organismos diana pertenecían a los tres niveles de la cadena trófica, para obtener una imagen completa del potencial de impacto de estas sustancias para el medio acuático.

Para su desarrollo, las publicaciones que conforman esta tesis se han distribuido en 9 capítulos que se pueden agrupar en tres bloques:

- Capítulos 1-5, enfocados a la puesta a punto de los métodos analíticos que resuelvan los retos que dificultan el análisis de residuos de plaguicidas.

- Capítulos 6-8, basados en la aplicación de la metodología anteriormente desarrollada sobre varios ecosistemas fluviales mediterráneos de la Península Ibérica, a través de una encuesta de seguimiento realizada en las campañas 2010 y 2011, en que se analizaron más de 40 plaguicidas empleados en la actualidad.

  Para evaluar los principales factores de estrés de estas cuencas se estableció una red de puntos de muestreo representativos que cubrían las zonas más afectadas desde el punto de vista de la industria química, hidrológico, morfológico y ecológico, así como los sitios de referencia en los que se esperaba mayor calidad.

- Capítulo 9 se centra en el estudio de modelos sobre el comportamiento de los plaguicidas en las cuencas mediterráneas.

A continuación, detallamos el objetivo de cada capítulo:

El capítulo 1 es una introducción general en la que se abordan diferentes aspectos. En primer lugar se ofrece una visión crítica del actual esquema de trabajo dentro del análisis de residuos de plaguicidas por LC-MS, ya que muchas de las revisiones que tratan este tema son más generales y se centran en otros tipos de técnicas, además de LC-MS. También se presta especial atención a proporcionar una cobertura completa de las últimas innovaciones dentro de este campo. Finalmente, se analizan brevemente las posibles tendencias futuras y desarrollos en esta área.

De los capítulos 2 al 9 se recoge el trabajo experimental realizado durante el doctorado, recopilado en forma de publicaciones, el cual se planificó y se diseñó para conseguir los objetivos propuestos.

En el capítulo 2 se analizan más de 60 muestras de aguas superficiales y residuales por LC-triple cuadrupolo (QqQ) -MS/MS y LC-cuadrupolo tiempo de vuelo (QqTOF)-MS/MS después de una SPE convencional, para investigar si el uso combinado de ambos sistemas es útil en la realización rutinaria de la determinación sistemática de residuos de plaguicidas.

En el capítulo 3 se da un paso adelante en las estrategias analíticas desarrolladas para el análisis no dirigido cuantitativo y no cuantitativo de contaminantes utilizando la última generación LC-QqTOF-MS (ABSciex TripleTOFTM5600) mediante la extracción de los iones con una exactitud de masa de 20 mDa de acuerdo a una base de datos que contiene más de 2.000 compuestos con información de masa exacta y, si están disponibles, los tiempos de retención. Un método de información de adquisición dependiente (IDA) permite obtener automáticamente espectros MS/MS de los iones precursores más intensos (sin selección previa). Por otra parte, por primera vez, y con el fin de identificar contaminantes relevantes inesperados para los sistemas de agua y definir cambios en las huellas dactilares del agua, análisis de datos estadísticos utilizando el análisis de componentes principales (PCA) y los componentes principales de agrupación de variables (PCVG), se combina con el cálculo de la fórmula empírica, buscada en bases de datos en línea (chemspider u otras bases de datos de Internet), y la interpretación de fragmentos de iones MS/MS para detectar con éxito contaminantes desconocidos como una visión dentro de la forensia medioambiental. Finalmente, se exploran las capacidades cuantitativas del sistema para 42 plaguicidas utilizados en la actualidad, cuya determinación se valida de acuerdo con las directrices europeas [30]. La posibilidad de

limitar el análisis a un solo instrumento proporcionará ventajas en términos de ahorro de tiempo y de costes.

En el capítulo 4, se desarrolla un método analítico por microextracción en fase sólida "en tubo" (IT-SPME) acoplado a cromatografía de ultra alta presión- electrospray-espectrometría de masas en tándem (UHPLC-QqQ-MS/MS) para el análisis multiresiduo de nueve sustancias prioritarias incluidas en la Directiva 2008/105/CE en aguas superficiales continentales. Se muestra por primera vez un procedimiento que permite conseguir la selectividad y la sensibilidad necesaria para determinar contaminantes orgánicos en muestras de agua, mediante el acoplamiento de una técnica que necesita una presión relativamente baja (IT-SPME), y otra que opera a una presión relativamente alta (UHPLC). El nuevo acoplamiento se compara con el establecido previamente IT-SPME y Cromatografía Líquida capilar (CapLC).

En el capítulo 5 se describen, se evalúan y se comparan los procedimientos PLE y QuEChERS para la extracción de 50 pesticidas en suelos, sedimentos y lodos y su posterior determinación mediante LC-MS/MS. La combinación QuEChERS con LC-MS/MS, fue la más ventajosa y se aplicó por primera vez para determinar los residuos de plaguicidas en suelos, sedimentos y lodos de la cuenca del río Turia.

En el capítulo 6 se analizan las concentraciones de plaguicidas en el influente, el efluente y los lodos deshidratados de las principales estaciones depuradoras de aguas residuales (EDARs) situadas a lo largo de los ríos Ebro, Guadalquivir, Júcar y Llobregat en España. Con estos datos, se calculan y relatan las eficiencias de eliminación de estas EDARs. El objetivo final de este estudio es mejorar el conocimiento sobre las causas de la contaminación de los ambientes acuáticos considerando las EDARs como fuentes puntuales de contaminantes como son los plaguicidas.

En el capítulo 7 se monitoriza la concentración de plaguicidas en agua, sedimentos y biota (sólo 2010) de la Cuenca Hidrográfica del Guadalquivir. Este es el primer estudio piloto llevado a cabo en esta extensa cuenca hidrográfica española y tiene la intención de mejorar el conocimiento sobre la incidencia de estos plaguicidas en el medio ambiente acuático. Las concentraciones de los plaguicidas utilizados en la actualidad asociados a los sedimentos y la biota también pueden determinar qué plaguicidas tienen más probabilidad de repartirse en la fase de sedimentos en suspensión, o de bioacumularse en la cadena trófica acuática, y esta información será útil para otras cuencas donde se aplican estos compuestos.

En el capítulo 8 se analizan las concentraciones de plaguicidas en muestras de agua superficial, efluentes de aguas residuales, sedimentos y biota (sólo 2010) a lo largo de todo el curso del río Llobregat y sus afluentes, con el fin de establecer la incidencia los plaguicidas y su distribución. Los resultados obtenidos en este seguimiento se compararon con los datos históricos recopilados durante otros programas de monitoreo en esta cuenca para describir las tendencias en el estado de la calidad del agua y determinar los riesgos potenciales para la salud humana.

Esta es la primera vez que (i) se determina un número tan elevado de plaguicidas en la cuenca, (ii) se estudian tres compartimentos ambientales (agua / sedimentos / biota) y (iii) los datos relacionados con la incidencia de los plaguicidas en este río se han recopilado y comparado de una manera detallada. Además, los resultados de este estudio son ampliamente aplicables a otras cuencas que siguen el patrón hidrológico de los ríos mediterráneos y que sufren el efecto del cambio climático en aumento.

Finalmente, en el capítulo 9 se identifican las fuerzas impulsoras de los procesos involucrados en el movimiento de plaguicidas en algunas regiones italianas y españolas. Además, se desarrollan herramientas para simular el comportamiento de los plaguicidas

a diferentes escalas con el objetivo de definir los permisos de uso de plaguicidas (o restricciones) a nivel regional, planificando programas de seguimiento, optimizando el presupuesto de los estudios al centrar el muestreo en las áreas donde es probable que se encuentren las concentraciones de plaguicidas más altas.

Los resultados de esta tesis han contribuido al desarrollo de métodos analíticos más sensibles y fiables, así como, al avance en el conocimiento sobre la distribución de estos compuestos tóxicos en el medio ambiente.

De acuerdo con los objetivos de la presente tesis doctoral, la investigación llevada a cabo y los resultados descritos en los capítulos previos, podemos destacar las siguientes conclusiones:

1. La combinación de cromatografía líquida espectrometría de masas en tándem utilizando un triple cuadrupolo (LC-QqQ-MS/MS) y un cuadrupolo tiempo de vuelo (UHPLC-QTOF MS) resultó viable y eficiente para la determinación sistemática de residuos de plaguicidas. El analizador de triple cuadrupolo (QqQ) es capaz de detectar más plaguicidas en las muestras debido a su mayor sensibilidad, mientras que el analizador tiempo de vuelo (QTOF MS) permite identificar otros contaminantes también presentes en las muestras, como son los productos farmacéuticos (valsartán e irbersartán), no seleccionados a priori.

2. La cromatografía líquida combinada con un cuadrupolo tiempo de vuelo con adquisición del espectro de los iones producto dependiente de la información (LC-MS-QqTOF en IDA-MS/MS) automatizada para el análisis cualitativo y cuantitativo permitió la determinación simultánea de 43 plaguicidas y la identificación de un gran número de otros plaguicidas y compuestos farmacéuticamente activos no seleccionados en el análisis dirigido en muestras de aguas residuales y de río. El método es una alternativa simple,

rápida y viable para el control rutinario de contaminantes orgánicos en aguas, útil para la detección de un número ilimitado de contaminantes sin necesidad de realizar diversos análisis.

3. La preparación de la muestra mediante extracción en fase sólida es conveniente, simple y rápida y cubre una gran variedad de sustancias con diferente polaridad. Todas las muestras analizadas contenían plaguicidas, algunos de ellos en concentración superior a 0,5 µg L^{-1}. Estos resultados ponen de manifiesto la coherencia, buena sensibilidad y el alto poder de identificación del método desarrollado.

4. El acoplamiento de microextracción en fase sólida "en tubo" (IT-SPME) con cromatografía líquida espectrometría de masas en tándem (UHPLC-MS/MS) para la identificación y cuantificación de contaminantes orgánicos en muestras de agua proporcionó buena precisión y reproducibilidad (RSD<20%), alto factor de enriquecimiento (ca. 15) y respuesta lineal (r>0.99). El sistema permite el enriquecimiento en-línea de los analitos en el rango de ppt (ng L^{-1}), ya que aumenta la sensibilidad aproximadamente 100 veces en comparación con la inyección directa en el cromatógrafo. Sin embargo, dado que el acoplamiento de la extracción y la determinación se debe realizar manualmente, es menos robusto que la extracción en fase sólida cuando se quiere aplicar a la determinación de un amplio rango de plaguicidas (> 20).

5. La comparación de la extracción con QuEChERS y con líquidos presurizados (PLE) para la extracción simultánea de 50 plaguicidas en suelos, sedimentos y lodos demostró que la eficiencia y la eficacia del método QuEChERS optimizado para sedimentos es superior a la de PLE, permitiendo reducir el tiempo y el coste del análisis, así como aumentar el rendimiento.

6. El estudio de plaguicidas en influentes, efluentes y lodos deshidratados en las plantas de tratamiento de aguas residuales de las principales cuencas hidrográficas españolas pone de manifiesto la presencia de plaguicidas y la baja eficacia en su eliminación. Debido a estas bajas eficiencias de eliminación, las EDARs se pueden considerar un foco de contaminación puntual de las aguas superficiales a causa de estos compuestos.

7. Se monitorizó la presencia de plaguicidas en muestras de aguas, sedimentos y biota en las cuencas hidrográficas del Guadalquivir y el Llobregat durante dos años consecutivos (2010 y 2011). En ambas, los plaguicidas se detectaron principalmente en agua, donde aparecieron casi continuamente. Su presencia en sedimentos fue mucho más intermitente y en biota más bien escasa. Los insecticidas organofosforados y los herbicidas triazínicos fueron los plaguicidas predominantes en ambas cuencas.

8. El estudio realizado en el Guadalquivir revela que organofosforados (malatión, clorpirifós, diazinon), carbamatos (metiocarb) y triazinas (terbutilazina, deisopropilatrazina), fueron las familias detectadas con mayor frecuencia y concentración en ambos períodos. Los productos de transformación de la atrazina y la terbutilazina se encontraron en concentraciones más altas que los compuestos originales, como resultado de su largo proceso de degradación. La distribución espacial de los plaguicidas mostró que los puntos de muestreo localizados en Jaén y Córdoba están dominados por los insecticidas y herbicidas utilizados en el cultivo del olivo mientras que los de las otras provincias contenían una mezcla compleja de todos los plaguicidas estudiados, que reflejan las diferencias en los patrones de cultivo.

9. Los plaguicidas predominantes en el Llobregat (en términos de frecuencia y concentración) eran bencimidazoles (carbendazima), organofosforados (malatión, clorpirifós), triazinas (terbutilazina-2-hidroxi, simazina), neonicotinoides (imidacloprid)

y triazoles (tebuconazole). Los niveles son mayores en la confluencia de los ríos Anoia y Llobregat. Esta zona es eminentemente agrícola y dedicada al cultivo de la vid.

10. La comparación de los resultados de este estudio con datos históricos confirman la existencia de una contaminación de fondo en la zona del río Llobregat en los últimos 20 años y demuestran que el seguimiento periódico del estado de la calidad ambiental de los ríos ayudará al control de la contaminación y determinar el efecto de diferentes parámetros físico-químicos.

11. El estudio de la distribución temporal durante los dos años del seguimiento indica que podría estar relacionada con el caudal del río. El flujo medio-bajo comparado con un flujo alto produce un aumento de la concentración en el agua y los sedimentos. En ambas cuencas hidrográficas varios plaguicidas excedían el límite de 0,1 µg L^{-1} para los compuestos individuales. Los puntos de muestreo GUA-3 y ANO-3, localizados en los ríos Guadalquivir y Llobregat respectivamente, excedieron el límite de 0,5 µg L^{-1} para la suma de los plaguicidas en régimen de caudal medio-bajo.

12. Los diferentes patrones de contaminación por plaguicidas en diferentes condiciones de flujo revelaron que puede afectar a la toxicidad para la biota. Aunque los valores del cociente de riesgo (RQ) mostraron un bajo riesgo de toxicidad por la presencia de plaguicidas en el agua, la bioacumulación de clorpirifós y diazinon, plaguicidas lipófilos (Kow>3) en peces, destaca la necesidad de enfoques alternativos y complementarios a los previstos por el simple cumplimiento de las normas de calidad ambiental (NCA).

13. Se realizó un estudio en el río Turia sobre la posible infiltración de las triazinas y ureas de las aguas superficiales a las aguas subterráneas. Estos datos se confrontaron con los mapas de vulnerabilidad obtenidos por el Sistema de Información

Geográfica (GIS) en la zona para estos mismos herbicidas. Los pozos analizados se caracterizan por tener un nivel de vulnerabilidad diferente a lo establecido mediante la aplicación de modelos GIS. Los resultados obtenidos apuntan a la existencia de factores adicionales al nivel de vulnerabilidad de los suelos. Estos resultados se compararon con los obtenidos en diversas zonas de Italia sobre la vulnerabilidad de los acuíferos subterráneos al glifosato (herbicida que de acuerdo con los modelos de GIS no debería lixiviar). Ambos estudios confirman la importancia de la integración entre los datos de la vigilancia y el modelo para subrayar los casos en los que el comportamiento no se ajusta al predicho por el modelo y los problemas específicos vinculados a malas prácticas agrícolas y a las especificidades territoriales.

Referencias bibliográficas:

[1] La ciencia que protege a los consumidores desde el campo hasta la mesa. http://www.efsa.europa.eu/en/corporate/doc/efsacorporatebrochure_es.pdf.

[2] J. Cooper, H. Dobson, Crop Prot. 26 (2007) 1337.

[3] Los plaguicidas, como contaminantes del agua. http://www.fao.org/docrep/w2598s/w2598s06.htm.

[4] Promoción de la Agricultura ecológica: su producción y consumo, de la Red Carrefour de Información y Animación Rural de la Comisión Europea. http://www.crie.uji.es/agric/santa.htm.

[5] Comisión Europea, Directiva 2000/60/CE del Parlamento Europeo y del Consejo de 23 de octubre de 2000 por la cual se establece un marco comunitario de actuación en el ámbito de la política de aguas (Directiva Marco del Agua), D. Of. Comun. Eur. L327 (2000).

[6] Decisión No 2455/2001/CE del Parlamento Europeo y del Consejo de 20 de noviembre de 2001 por la cual se aprueba la lista de sustancias prioritarias en el ámbito de la política de aguas, y por la cual se modifica la Directiva 2000/60/CE, D. Of. Comun. Eur. L 331 (2001).

[7] Comisión Europea, Directiva 2008/105/CE del Parlamento Europeo y del Consejo de 16 de diciembre de 2008 relativa a las normas de calidad ambiental en el ámbito de la política de aguas, por la cual se modifican y derogan ulteriormente las Directivas 82/176/CEE, 83/513/CEE,

84/156/CEE,84/491/CEE y 86/280/CEE del Consejo, y por la cual se modifica la Directiva 2000/60/CE, D. Of. Comun. Eur. L348 (2008).

[8] Directiva 98/83/CE del Consejo de 3 de noviembre de 1998 relativa a la calidad de las aguas destinadas al consumo humano, D. Of. Comun. Eur. L330 (1998).

[9] Scarce 2014. http://www.scarceconsolider.es/publica/P000Main.php.

[10] A. Masiá, C. Blasco, Y. Picó, TrAC 2 (2014) 11.

[11] M.A. Fernández, C. Alonso M.J. Gonzalez, L.M. Hernández, Chemosphere 38 (1999) 33.

[12] P. Palma, M. Kock-Schulmeyer, P. Alvarenga, L. Ledó, I.R. Barbosa, M. Lopez de Alda, D. Barceló, Sci. Total Environ. 488-489 (2014) 208.

[13] C. Flores, F. Ventura, J. Martin-Alonso, J. Caixach, Sci. Total Environ 461 (2013) 618.

[14] V. Matamoros, V.Salvado, J. Environ Manage 117 (2013) 96.

[15] J.C. Lopez-Doval, N. De Castro-Català, I. Andrés-Domenech, J. Blasco, A. Ginebreda, I. Muñoz, Sci. Total Environ 440 (2012) 194.

[16] C. Planas, J. Caixach, F.J. Santos, J. Rivera, Chemosphere 34 (1999) 2393.

[17] A. Navarro-Ortega, V. Acuña, R.J. Batalla, J. Blasco, C. Conde, F.J. Elorza, A. Elosegi,F. Francés, F. La-Roca, I. Mu˜noz, M. Petrovic, Y. Picó, S. Sabater, X. Sanchez-Vila,M. Schuhmacher, D. Barceló, Environ. Sci. Pollut. Res. 19 (2012) 918.

[18] A. Belmonte Vega, A. Garrido Frenich, J.L. Martínez Vidal, Anal. Chim. Acta 538(2005) 117.

[19] A. Lazartigues, C. Fratta, R. Baudot, L. Wiest, C. Feidt, M. Thomas, C. Cren-Olive, Talanta 85 (2011) 1500.

[20] Y.B. Li, F.S. Dong, X.G. Liu, J. Xu, J. Li, Z.Q. Kong, X. Chen, W.C. Song, Y.H. Wang, Y.Q. Zheng, J. Chromatogr. A 1218 (2011) 6667.

[21] M.P. Ensminger, R. Budd, K.C. Kelley, K.S. Goh, Environ. Monit. Assess. 185(2013) 3697.

[22] K.L. Smalling, T.J. Reilly, M.W. Sandstrom, K.M. Kuivila, Sci. Total Environ. 447(2013) 179.

[23] L.H. Nowell, P.W. Moran, R.J. Gilliom, D.L. Calhoun, C.G. Ingersoll, N.E. Kemble,K.M. Kuivila, P.J. Phillips, Arch. Environ. Contam. Toxicol. 64 (2013) 32.

[24] M.L. Hladik, J.L. Domagalski, K.M. Kuivila, Sci. Total Environ. 408 (2009) 356.

[25] L.A. Knight, M.K. Christenson, A.J. Trease, P.H. Davis, A.S. Kolok, Environ. Toxicol. Chem. 32 (2013) 1544.

[26] N.E. Kemble, D.K. Hardesty, C.G. Ingersoll, J.L. Kunz, P.K. Sibley, D.L. Calhoun, R.J. Gilliom, K.M. Kuivila, L.H. Nowell, P.W. Moran, Arch. Environ. Contam. Toxicol.64 (2013) 52.

[27] S.J. Kalkhoff, A.V. Vecchia, P.D. Capel, M.T. Meyer, J. Environ. Qual. 41 (2012)1566.

[28] A.V. Vecchia, R.J. Gilliom, D.J. Sullivan, D.L. Lorenz, J.D. Martin, Environ. Sci.Technol. 43 (2009) 9096.

[29] A. Masiá, K. Vásquez, J. Campo, Y. Picó, J. Chromatogr. A. (2014) enviat.

[30] Método de validación y control de calidad de los procedimientos de análisis de residus de plaguicidas en alimentos y piensos. Comisión Europea Documento No. SANCO/12495/2011, Bruselas, 1 de enero, 2012.

SUMMARY IN ENGLISH

Continued population growth in the last century has created the need to increase food production. However, pests and diseases destroy about a third of crops during production, transport and storage. In these circumstances the use of pesticides that control the action of pests is essential. [1]

The use of pesticides has provided indisputable economic benefits because they increase agricultural productivity and reduce labour force as well as food costs. Along with the benefits of agricultural use, the use of pesticides have also benefited the field of health, where they have contributed to the control and even eradication in some areas, vector-borne diseases [2].

All of that, coupled to their synthesis is economical, their excellent chemical stability and toxicological risk to human health and the environment has become evident relatively late, it has led to its widespread and indiscriminate use. [3]

Pesticides are not selective to the pest that seek to control and affect not only the vectors associated with this, but also to other species, causing imbalances in ecosystems. According to some studies carried out in the USA, among the 500 million kilos of pesticides used annually, only 1% of the products reach the harmful organisms which they are intended. The other 99% remains in ecosystems, they are transferred to the atmosphere by volatilization, to the soil and to the groundwater [4]. While PPPs increase food production, the negative effects on the environment are undeniable. From surface water, pesticides enter the lower levels of the food chain at low concentrations, they bioaccumulate in each trophic level and they biomagnify successively up to the top of the food chain (raptors, fish or mammalian predators), where they reach concentrations between 10 and 100 times higher than the originals.

This bioaccumulation affects biodiversity causing a decline in their populations, since all members of the food chain are exposed to sublethal concentrations causing effects on individuals that can be undetected or severe damage affecting their reproduction and survival.

Figure 1 shows how pollutants reach the aquatic ecosystems. Chemicals found in pesticides penetrate into waterways due to the diffusion of the spray. The degree and extent of diffusion depends on external climatic factors such as ambient temperature and rainfall and winds.

The rain mobilizes pesticides from the place where they were applied of surface runoff or infiltration phenomena through the soil into groundwater. As rain, irrigation increases the leaching degree, decreasing the amount of pesticide that it is volatilized from the ground. Excessive irrigation can leach pesticides directly into the aquifer. Part of pesticides evaporate passing air or joining soil particles, such as dust or steam. They can be transported long distances and be deposited again on the ground or surface water through rainfall.

In recent decades, there has been growing concern about the conservation of natural resources from a sustainable perspective and the availability of water has become a major issue of concern to all governments.

This widespread contamination of aquatic ecosystems and soil has led to the adoption of restrictive legislative measures by the European Union:

- The Water Framework Directive (WFD) (Directive 2000/60 / EC) provides the basis for regulating water resources in order to preserve, protect and improve its quality and sustainable use [5].

• Decision 2455/2001/EC, establish a list of 33 priority substances to be controlled, a third part of which are pesticides [6].

• Directive 2008/105 / EC defines environmental quality standards (EQS), the annual average (MA) and the maximum allowable concentrations (MAC) in surface waters to the list of priority substances mentioned above. Although many pesticides are currently included in this list in the regulations of the European Union (EU), many others are not still regulated [7].

• Directive 98/83/EC sets limits for pesticides in water intended for human consumption (100 ng/L for individual pesticides and 500 ng/L for the sum of all pesticides) [8].

The Mediterranean watersheds are subjected mainly to pressures from the expansion and human development in its various expressions (agricultural, industrial, urban ...). The climate change models conclude that the Mediterranean region will be the most impacted in next years and the consequences of global change will affect both the availability and the quality of water. Although these contaminants are usually found at trace levels in aquatic environments, it is important to know their distribution and evolution in the environment to control and evolution, since chronic exposure to these low doses may suppose a threat to the aquatic biota and human health [9].

Above mentioned shows that the analysis of pesticide residues is a crucial instrument for human and environmental protection. This situation creates the need for greater efforts in the development of rapid and versatile methodologies for the detection of emerging contaminants and their potential metabolites in environmental matrices [10].

Studies on the impact of emerging contaminants in the Iberian Peninsula are scarce [11-16]. In general, in Europe there are few studies that determine the occurrence

of currently used pesticides in environmental compartments other than water and most of them are erratic sampling performed to demonstrate the reliability of an analytical method, but not systematic studies evaluating pesticide occurrence and levels in a River Basin [17-20]. These studies, without being many, are more prevalent in the United States [21-28].

The low number of publications related to the presence of pesticides in sediments and biota, in part is due to the lack of NCA for organic contaminants in these matrices, including pesticides and the difficulty of extraction. Sediment and fish are complex and variable matrices, due to the strong interactions between analytes and the different sample constituents (organic matter and clay sediment, fats and proteins in fish), turning both matrices into a deposit of these compounds that difficult their extraction [29].

According to the existing environmental requirements, the overall objective of this thesis was defined, based on providing an overview of the water quality from representative Mediterranean River Basins from the Iberian Peninsula, as well as toxicological risk assessment of pesticides on biota (algae and bacteria, macroinvertebrates and fish). As a result, this thesis aims to contribute to the knowledge of the Mediterranean basin functionality, and its resilience to the impact of human activities, as well as, be useful as a solid foundation for the development and planning strategies and more suitable for the recovery and protection of Mediterranean ecosystems distributed across the earth, taking as a study case the presence of pesticide residues methodologies.

Therefore, the methodology used in the preparation of this thesis was:

1 To develop and validate analytical methodologies based on solid phase extraction (SPE), QuEChERS (acronym for Quick, Easy, Cheap,

Effective, Rugged, Safe) and pressurized liquid extraction (PLE) followed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) using a triple quadrupole analyzer and quadrupole time of flight for the determination of pesticides in water, sediments and fish samples.

2 To study concentrations, distribution and fate of these contaminants in Mediterranean river ecosystems from the Iberian Peninsula (Guadalquivir, Llobregat and Turia) trying to determine the source and pattern of distribution of these compounds.

3 To assess the hazard derived from the presence of these pollutants for the aquatic fauna, based on the available toxicological data of pesticides. The target organisms were those belonging to the three levels of the food chain to obtain a whole picture of the potential impact of these substances to the aquatic environment.

For this, publications that make up this thesis have been divided into 9 chapters that can be grouped into three blocks:

• Chapters 1-5, focuses on the development of analytical methods that solve the challenges in pesticide residues analysis.

• Chapters 6-8 bases on the application of the methodology previously developed on several Mediterranean riverine ecosystems from the Iberian Peninsula, through a survey in which more than 40 pesticides were analyzed during 2010 and 2011 campaigns.

To evaluate the main stressors in these basins a network of representative sampling points covering the most affected areas from the point of view of the chemical, hydrological, morphological and ecological was

established, as well as, reference sites in which higher quality was expected.

• Chapter 9 focuses on the study of models on the behavior of pesticides in the Mediterranean basins.

The purpose of each chapter is detailed below:

Chapter 1 is a general introduction in which different aspects are addressed. Firstly, a critical overview of the current workflow within pesticide residue analysis by LC–MS because the many reviews that treat this topic are more general and focus not only on LC–MS but on a sort of techniques. Special attention is paid to provide comprehensive coverage of ultimate innovations in the field. Finally, possible future trends and developments in this area are briefly discussed.

Chapters 2 to 9 presents the experimental work undertaken in this PhD, compiled in the form of publications, which was planned and designed in order to achieve the proposal objectives.

In chapter 2, 60 samples of surface and wastewater are analyzed by LC-triple quadrupole (QqQ) MS/MS and LC-quadrupole time of flight (QqTOF)-MS/(MS) after conventional SPE to investigate whether the combined use of both systems is useful in the routine performance of the systematic determination of pesticide residues.

Chapter 3 This study goes an step forward into the analytical strategies developed for quantitative and non-quantitative non-target screening of contaminants using a last generation LC–QTOF-MS (ABSciex TripleTOF™ 5600) by ion extraction in front of a database of more than 2000 compounds with accurate mass information and, if available, retention times. An information dependent acquisition (IDA) methods allow to obtain automatically MS/MS spectra of the most intense precursor ions (without previous

selection) to additionally confirm the identity of the detected compounds by MS/MS library searching. Furthermore, for the first time and in order to identify relevant unexpected contaminants for the water systems and define changes in the water fingerprints, statistical data analysis using principal component analysis (PCA) and principal components variable grouping (PCVG) was combined with empirical formula calculation, online database (chemspider or other internet databases) searching, and MS/MS fragment ion interpretation to successfully detect unknown contaminants as an insight within environmental forensics. Last but not least, the quantitative capabilities of the system were explored for 42 currently used pesticides, the determination of which was validated according to the European guidelines [30]. The possibility to limit the analysis to one instrument will provide advantages in terms of saving time and cost of the determination.

In Chapter 4, an analytical method is developed for in-tube solid phase microextraction (IT-SPME) coupled to ultra-high pressure-chromatography electrospray - tandem mass spectrometry (UHPLC-QqQ-MS/MS) for the multi-residue analysis of nine priority substances covered by Directive 2008/105/EC in inland surface waters. This procedure has been chosen to reach selectivity and sensitivity necessary to determine organic pollutants in water samples by coupling a technique that needs relatively low pressure, and UHPLC that operates to relatively high pressure. The new coupling is compared with the previously established IT-SPME and Capillary Liquid Chromatography (CapLC).

Chapter 5 describes, evaluates and compares two extraction methods −PLE and QuEChERS− to establish the most suitable technique for the extraction of 50 pesticides in soils, sediments and sludges. The determination was carried out using liquid chromatography-triple quadrupole-mass spectrometry (LC-MS/MS).The QuEChERS

and LC–MS/MS were advantageous and was further applied for the first time to determine the pesticide residues in soil, sediment and sludges collected during a monitoring campaign in Turia River Basin.

In Chapter 6, pesticide concentrations have been analysed in the influent, effluent and dehydrated sludge from main STPs located along the rivers Ebro, Guadalquivir, Jucar and Llobregat. With these data, removal efficiencies of such STPs have been calculated and reported. The final objective of this study is to improve the knowledge about the causes of aquatic environments contamination considering the STPs as point sources of contaminants such as pesticides.

In Chapter 7, 50 currently used pesticides are monitored in two consecutive years (2010 and 2011) in water, sediment and biota (only 2010) in the Guadalquivir River Basin. This is the first extensive pilot study undertaken in this Spanish River Basin and it intends to improve the knowledge of these pesticides occurrence in the aquatic environment. The concentrations of currently-used pesticides associated with sediments and biota can also ascertain which pesticides are more likely to partition to the suspended sediment phase, or to bioaccumulate in the aquatic trophic chain, and this information will be useful for other watersheds where these compounds are applied.

In chapter 8, an extensive monitoring (including 50 pesticides) has been developed in two consecutive campaigns (2010–2011) in surface water, wastewater effluents, sediment, and biota samples along the entire course of the Llobregat River and its tributaries, with the purpose of establishing pesticide occurrence and distribution. The results obtained by this monitoring were compared with historical data gathered during other monitoring programs in this river basin to describe trends in water quality status and determine potential risks for human health.

This is the first time that a so extensive number of pesticides (i.e. 50 active ingredients) is determined in the basin; (ii) three environmental compartments (i.e. water/sediment/biota) were studied for the first time, because most of the previous studies were restricted to water, and (iii) the contamination levels of the Llobregat River were reviewed only once, dealing with the presence and biological effects of any type of emerging contaminants. Thus, it is also the first time that data related to pesticide occurrence in this river have been assembled and compared in a detailed way. Furthermore, the results of this study can be widely applicable to other basins that follow the hydrological pattern of the Mediterranean rivers and suffer the increasing effect of climate change.

Finally, in Chapter 9 the driving forces of the processes involved in the movement of pesticides in some Italian and Spanish regions are identified. In addition, tools are being developed to simulate the behavior of pesticides at different scales in order to define pesticide use permission (or restrictions) at a regional level, planning monitoring programs, optimizing the study budget by focusing sampling in the areas where higher pesticide concentrations are likely to be found.

The results of this thesis have contributed to the development of more sensitive and reliable analytical methods, as well as the advancement of knowledge about the distribution of these toxic compounds in the environment.

According to the objectives of this thesis, the research carried out and the results described in the previous chapters, the following conclusions can be outlined:

1. The combination of liquid chromatography tandem mass spectrometry using a triple quadrupole (LC-QqQ-MS / MS) and quadrupole time of flight (QTOF UHPLC-MS) proved to be a feasible and efficient way the systematic pesticide residue

determination. Analyzer Triple Quadrupole (QqQ) is able to detect more pesticides because of its higher sensitivity, while analyzer time of flight (QTOF MS) can identify other contaminants also present in the samples, such as pharmaceuticals (Valsartan and Irbesartan) not selected a priori.

2. Liquid chromatography coupled with a quadrupole time-of-flight spectrum acquisition dependent information product ions (LC-MS-QqTOF on IDA-MS / MS) automated for the qualitative and quantitative analysis allowed the simultaneous determination of 43 pesticides and identification of a large number of non-target pesticides and pharmaceutically active compounds in wastewater and river water samples. The method has been demonstrated to be a very simple, fast, and viable alternative for routine monitoring of organic contaminants in waters in one run.

3. The sample preparation by solid phase extraction is suitable, simple and fast, and covers a large variety of substances with different polarity. All samples contained pesticides, some of them exceeding 0.5 mg L -1 concentration. These results highlight the consistent and good sensitivity and the high identification power that can be achieved using the developed method.

4. The coupling of solid phase microextraction "tube" (IT-SPME) with liquid chromatography tandem mass spectrometry (UHPLC-MS / MS) for identification and quantification of organic pollutants in water samples provided good accuracy and reproducibility (RSD <20%), high enrichment factor (ca. 15) and linear response (r> 0.99). The system allows the on-line enrichment of the analytes in the range of low parts per billion as it increases sensitivity 100 times approximately compared with direct injection 5 μL water sample in chromatograph. However, it is less robust than the solid phase extraction to determine a wide range of pesticides (> 20), because extraction and determination coupling must be done manually.

5. Comparison of extraction Quechers and pressurized liquid (PLE) for simultaneous extraction of 50 pesticides in soils, sediments and sludges showed that the efficiency and efficacy of optimized Quechers for sediment demonstrated to be superior to PLE, reducing time consuming and increasing throughput.

6. The study of pesticides in influent, effluent and dehydrated sludges in wastewater treatment plants from main Spanish River Basins reveals the presence of pesticides and low efficiency in removal. Accordingly, sewage treatment plants can be considered a focal point of contamination to surface waters.

7. The presence of pesticides was monitored in samples of water, sediments and biota in the Guadalquivir and the Llobregat River Basin for two consecutive years (2010 and 2011). In both, pesticides were detected mainly in water, where they appeared almost continuously. Their presence in sediments was much more intermittent and rather scarce in biota. Organophosphorus insecticides and triazine herbicides were predominant in both river basins.

8. The study reveals that the Guadalquivir organophosphorus (malathion, chlorpyrifos, diazinon), carbamates (methiocarb) and triazines (terbuthylazine, deisopropilatrazina) families were detected with highest frequency and concentration in both periods. The transformation products of atrazine and terbuthylazine were found at higher concentration than the parent compounds, as a result of their degradation process. The spatial distribution of pesticides showed that the sampling sites located in Jaén and Córdoba are dominated by insecticides and herbicides used in olive cultivation while other provinces contain a complex mixture of all pesticides studied, which reflect differences in crop patterns.

9. The predominant pesticides in the Llobregat (in terms of frequency and concentration) were benzimidazoles (carbendazim), organophosphorus (malathion, chlorpyrifos), triazines (2-hydroxy-terbuthylazine, simazine), neonicotinoids (imidacloprid) and triazoles (tebuconazole). The levels are higher at the confluence of the rivers Llobregat and Anoia. This is an agricultural area, mainly with vineyards.

10. Comparison of the results of this study with historical data confirm the existence of background contamination in the river Llobregat in the last 20 years and periodic monitoring on the environmental quality status of the river in combination with previous data will assist in controlling the contamination of the river basin and ascertain the effect of different physicochemical parameters.

11. The temporal distribution during both sampling campaigns could be related to the river flow. Low-medium flow compared to high one produced an increasing of the concentration in water and sediments. In both River Basins several pesticides exceeded the limit of 0.1 μg L^{-1} for individual compounds. Sampling sites GUA-3 and ANO-3, located in Guadalquivir and Llobregat rivers respectively, exceeded the limit of 0.5 μg L^{-1} for the sum of pesticides under a medium-low flow.

12. Different patterns of pesticide contamination in different flow conditions were revealed that can affect toxicity to biota. Although the RQ values showed low risk of toxicity from the presence of pesticides in water, the bioaccumulation of chlorpyrifos and diazinon, lipophilic pesticides (Kow> 3) in fish, highlight the need of alternative and complementary approaches to that provided by the simple compliance with the environmental quality standards (EQS).

13. A study was conducted in the Turia River on the possible infiltration of triazines and ureas of surface water to groundwater. These data were compared with

vulnerability maps obtained by the Geographic Information System (GIS) in the area for these herbicides. The studied wells are characterized by a level of vulnerability different to the GIS models predicted. The results suggest the existence of additional factors to the level of vulnerability of soils. These results were compared with those obtained in various regions from Italy on groundwater aquifer vulnerability to glyphosate (according to GIS models, this herbicide should not leach). Both studies confirm the importance of the integration between monitoring data and modeling in order to highlight cases in which the behavior does not conform to that predicted by the model and specific problems related to poor agricultural practices and territory specificities.

References:

[1] The science that protects consumers from field to table. http://www.efsa.europa.eu/en/corporate/doc/efsacorporatebrochure_es.pdf.

[2] J. Cooper, H. Dobson, Crop Prot. 26 (2007) 1337.

[3] Pesticides as water pollutants. http://www.fao.org/docrep/w2598s/w2598s06.htm.

[4] Promotion of Organic farming: production and consumption, Carrefour Information Network and Rural Life of the European Commission. http://www.crie.uji.es/agric/santa.htm.

[5] European Commission, Directive 2000/60 / EC of the European Parliament and of the Council of 23 October 2000 for which a framework for Community action in the field of water policy (Water Framework Directive), as D. Of. Common. Eur. L327 (2000).

[6] Decision No 2455/2001 / EC of the European Parliament and of the Council of 20 November 2001 by which the list of priority substances in the field of water policy is approved, and why 2000 amending Directive / 60 / EC, D. Of. Common. Eur. L 331 (2001).

[7] European Commission, Directive 2008/105 / EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, whereby amending and subsequently repealing Directives 82/176 / EEC, 83/513 / EEC, 84/156/84/491 / EEC and 86/280 / EEC and why amending Directive 2000/60 / EC, D. Of. Common. Eur. L348 (2008).

[8] Directive 98/83 / EC of 3 November 1998 on the quality of water intended for human consumption, D. Of. Common. Eur. L330 (1998).

[9] Scarce 2014 http://www.scarceconsolider.es/publica/P000Main.php.

[10] A. Masiá, C. Blasco, Y. Picó, Trac 2 (2014) 11.

[11] MA Fernandez, C. Alonso MJ Gonzalez, LM Hernandez, Chemosphere 38 (1999) 33.

[12] P. Palma, M. Kock-Schulmeyer, P. Alvarenga, L. Ledo, IR Barbosa, M. Lopez de Alda, D. Barceló, Sci. Total Environ. 488-489 (2014) 208.

[13] C. Flores, F. Ventura, J. Martin-Alonso, J. Caixach, Sci. Total Environ 461 (2013) 618.

[14] V. Matamoros, V.Salvado, J. Environ Manage 117 (2013) 96.

[15] JC Lopez-Doval, N. De Castro-Catala, I. Andrés-Domenech, J. Blasco, A. Ginebreda, I. Muñoz, Sci. Total Environ 440 (2012) 194.

[16] C. Planas, J. Caixach, FJ Santos, J. Rivera, Chemosphere 34 (1999) 2393.

[17] A. Navarro-Ortega, V. Acuña, RJ Batalla, J. Blasco, C. Conde, FJ Elorza, A. Elosegi, F. French, F. La-Roca, I. Muñoz, M. Petrovic, Y. Pico, S. Sabater, X. Sanchez-Vila, M. Schuhmacher, D. Barceló, Environ. Sci. Pollut. Res. 19 (2012) 918.

[18] A. Belmonte Vega, A. Garrido Frenich, JL Martínez Vidal, Anal. Chim. Acta 538 (2005) 117.

[19] A. Lazartigues, C. Fratta, R. Baudot, L. Wiest, C. Feidt, M. Thomas, C. Cren-Olive, Talanta 85 (2011) 1500.

[20] YB Li, Dong FS, XG Liu, J. Xu, J. Li, ZQ Kong, X. Chen, WC Song, YH Wang, YQ Zheng, J. Chromatogr. A 1218 (2011) 6667.

[21] MP Ensminger, R. Budd, KC Kelley, KS Goh, Environ. Monit. Assess. 185 (2013) 3697.

[22] KL Smalling, TJ Reilly, MW Sandstrom, KM Kuivila, Sci. Total Environ. 447 (2013) 179.

[23] LH Nowell, PW Moran, RJ Gilliom, DL Calhoun, CG Ingersoll, NE Kemble, KM Kuivila, PJ Phillips, Arch. Environ. Contam. Toxicol. 64 (2013) 32.

[24] ML Hladik, JL Domagalski, KM Kuivila, Sci. Total Environ. 408 (2009) 356.

[25] LA Knight, MK Christenson, AJ Trease, PH Davis, AS Kolok, Environ. Toxicol. Chem. 32 (2013) 1544.

[26] NE Kemble, DK Hardesty, CG Ingersoll, JL Kunz, PK Sibley, DL Calhoun, RJ Gilliom, KM Kuivila, LH Nowell, PW Moran, Arch. Environ. Contam. Toxicol.64 (2013) 52.

[27] SJ Kalkhoff, AV Vecchia, PD Capel, MT Meyer, J. Environ. Qual. 41 (2012) 1566.

[28] AV Vecchia, RJ Gilliom, DJ Sullivan, DL Lorenz, JD Martin, Environ. Sci.Technol. 43 (2009) 9096.

[29] A. Masiá, K. Vasquez, J. Campo, Y. Picó, J. Chromatogr. A. (2014) Enviat.

[30] Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed. European Commission Document No. SANCO/12495/2011, Brussels, January 1, 2012.

Annexos

^{3D}IT: Trampa Iònica Tridimensional (*Tridimensional Ion Trap*)

ACE: Acetona (*Acetone*)

AcN: Acetonitril (*Acetonitrile*)

AEPLA: Associació Empresarial per a la Protecció de les Plantes

AF: Factor d'Avaluació (*Assessment Factor*)

AOAC: Associació de Comunitats Analítiques (*Association of Analytical Communities*)

BOD_5: Demanda Bioquímica d'Oxigen (5 dies) (*Biochemical Oxygen Demand (five days)*)

C18: Octadecil Silica (*Octadecyl Silica*)

CapLC: Cromatografia Líquida Capil·lar (*Capillary Liquid Chromatography*)

CAS Number: Nombre d'identificació numèrica única per a compostos químics (*Chemical Abstracts Service registry number*)

CCα: Límit de Decisió (*Decision limit*)

CCβ: Capacitat de Detecció (*Detection capability*)

CE: Energia de Col·lisió (C*ollision Energy*)

CEC: Capacitat d'intercanvi de cations (*Cation- Exchange Capacity*)

Cef: Concentració a l'efluent (*Effluent Concentration*)

CEN: Comitè Europeu de Normalització (*European Committee for Standardization*)

Cin: Concentració a l'influent (*Influent Concentration*)

CMA-NQA/MAC-EQS: Concentracions Màximes Admissibles (*Maximum Allowable Concentration*)

CUR: Gas Cortina (*Curtain Gas*)

DAD: Detector de files de diodes (*Photodiode Array Detector*)

Dehp: Di (2-dietilhexil) ftalat (*Di (2-diethylhexyl) phthalate*)

DLLME: Microextracció Líquid-Líquid Dispersiva (*Dispersive Liquid–Liquid Microextraction*)

DMA/WFD: Directiva Marc de l'Aigua (*Water Framework Directive*)

DMC: Diclorometà (*Dichloromethane*)

DP: Potencial de Desagrupació (*Declustering Potential*)

dSPE: Extracció en Fase Sòlida Dispersiva (*Dispersive Solid-Phase Extraction*)

DWTP: Planta de Tractament d'Aigua Potable (*Drinking Water Treatment Plant*)

EC_{50}: Concentració efectiva que provoca la mort o un altre efecte en el 50 % dels individus exposats (*Effective concentration*)

EDAR/WWTP/STP: Estació Depuradora d'Aigües Residuals (*Wastewater Treatment Plants*)

EMS scan: Espectre de Masses ampliat (*Enhanced MS*)

EPI: Ió Producte ampliat (*Enhanced product ion scan*)

ESI: Ionització per Electropolvorització (*Electrospray Ionization*)

EtAc: Acetat d'Etil (*Ethyl Acetate*)

EU: Unió Europea

EUA/US: Estats Units d'Amèrica

FAO: Organització per a l'Alimentació i l'Agricultura (*Food and Agriculture Organization*)

FLD: Detector de Fluorescència (*Fluorescence Detector*)

FRAG: Fragmentador (*Fragmentor*)

FT: Transformada de Fourier (*Fourier Transform*)

FWHM: Amplària Completa a la Meitat del Pic (*Full Width Half Maximum*)

GBC: Carboni Negre Grafititzat (*Graphitized Black Carbon*)

GC: Cromatografia de Gasos (*Gas Chromatography*)

GIS: Sistema d'Informació Geogràfica (*Geographical Information Systems*)

GS: Gas de la Font d'ionització (*Ion Source Gas*)

GUS Index: Puntuació de la ubiqüitat de les aigües subterrànies (*Ground-water Ubiquity Score index*)

HE: Funció d'Alta Energia (*High Energy Function*)

HPLC: Cromatografia de Líquids d'Alta Resolució (*High Performance Liquid Chromatography*)

HRT: Temps de Retenció Hidràulica (*Hydraulic Retention Time*)

HTpSPE: SPE planar d'Alt Rendiment (*High Throughput planar SPE*)

I.S.: Estàndard Intern (*Internal Standard*)

ICMAN: Institut de Ciències Marines d'Andalusia

ICRA: Institut Català de Recerca de l'Aigua

IDA: Informació d'Adquisició Depenent (*Information-Dependent Acquisition*)

IDAEA: Institut de Diagnòstic Ambiental i Estudis de l'Aigua

IN: Influent (Influent)

IT-SPME: Microextracció en Fase Sòlida "en-tub" (*In-tube Solid Phase Microextraction*)

k_{ow}: Coeficient de Partició Octanol-Aigua (*Octanol-Water Partition Coefficient*)

LC: Cromatografia Líquida (*Liquid Chromatography*)

LCL: Nivell de calibratge més baix (*Lowest Calibration Level*)

LC–MS: Cromatografia Líquida - Espectrometria de Masses (*Liquid Chromatography–Mass Spectrometry*)

LC-MS/MS: Cromatografia Líquida acoblada a Espectrometria de Masses en tándem (*Liquid Chromatography–tándem Mass Spectrometry*)

LDMHLLE: Extracció Líquid - Líquid de Baixa Densitat Homogènia i Miniaturitzada (*Low Density Miniaturized Homogenous Liquid–Liquid Extraction*)

LDTD: Desorció Tèrmica amb Díodes de Làser (*Laser Diode Thermal Desorption*)

LE: Funció de Baixa Energía (*Low Energy Function*)

LLE: Extracció Liquid–Liquid (*Liquid–Liquid Extraction*)

LLME: Microextracció Liquid–Liquid (*Liquid–Liquid Microextraction*)

LOD/MLD: Límit de Detecció (*Limit of Detection*)

LOQ/MLQ: Límit de Quantificació (*Limit of Quantification*)

LVI: Inyecció de gran Volum *(Large Volume Injection)*

MA-NQA/AA-EQS: Mitjana Anual (*Annual Average value*)

MAE: Extracció Assistida per Microones (*Microwave-Assisted Extraction*)

MAGRAMA: Ministeri d'Agricultura, Alimentació i Mediambient

MASE: Extracció amb Dissolvent Assistida per Membrana (*Membrane-Assisted Solvent Extraction*)

MCP: Placa de Micro-Canals (*Micro-Channel Plate*)

ME: Efecte Matriu (*Matrix Effect*)

MeOH: Metanol (*Methanol*)

MF: Factors de la Matriu (*Matrix Factors*)

MRL: Nivells Màxims de Residus (*Maximum Residue Levels*)

MRM: Monitorització de Múltiples Reaccions Seleccionades (*Multiple Reaction Monitoring*)

MS/MS: Espectrometria de Masses en Tándem (*Tandem Mass Spectrometry*)

MS: Espectrometria de Masses (*Mass Spectrometry*)

MSPD: Dispersió de Matriu en Fase Sòlida (*Matrix Solid Phase Dispersion*)

MTBE: Metil Ter-Butil Èter (*Methyl Tertiary Butyl Ether*)

n.a.: No s'analitzen (*not analysed*)

n.d: No detectat (*not detected*)

NI: Mode d'Ionització Negatiu (*Negative Ionization Mode*)

NOEC: Concentració Sense Efectes Observats (*No Observed Effect Concentration*)

NQA/EQS: Normes de Qualitat Ambiental (*Environmental Quality Standards*)

OUT: Efluent (*Efluent*)

p.s. /d.w.: Pes Sec (D*ry Weight*)

PCA: Anàlisi de Components Principals (*Principal Component Analysis*)

PCPG: Components Principals d'Agrupació de Variables (*Principal Components Variable Grouping*)

PDMS: Polidimetilsiloxà (*Polydimethylsiloxane*)

PI: Mode d'Ionització Positiu (*Positive Ionization Mode*)

PLE: Extracció amb Líquids Pressuritzats (*Pressurized Liquid Extraction*)

PNEC: Concentració Prevista Sense Efecte (*Predicted No-Effect Concentration*)

PSA: Amina Primària-Secundària (*Primary-Secondary Amine*)

Q: Analitzador de tipus Quadrupol (*Single Quadrupole*)

QC: Control de Qualitat (*Quality Control*)

QqLIT: Analitzador híbrid Quadrupol - Trampa Lineal d'ions (*Linear ion trap*)

QqQ: Analitzador triple Quadrupol (*Triple Quadrupol*)

QqTOF: Analitzador híbrid Quadrupol - Temps de Vol (*Quadrupol Time of flight*)

QuEChERS: Extracció Ràpida, Fàcil, Barata, Efectiva, Robusta i Segura (*Quick, Easy, Cheap Effective Rugged and Safe*)

RD: Reial decret (*Royal Decree*)

RP (RP-LC): Fase Reversa (*Reversed-phase*)

RQ: Quocient de Risc (*Risk Quotient*)

RSD: Desviació Estàndard Relativa (*Relative Standard Deviation*)

RSDE: Extracció sobre Discos Rotatoris (*Rotating Disk Sorptive Extraction*)

RT: Temps de Retenció (*Retention Time*)

S/N: Relació Senyal-Soroll (*Signal-to-Noise Ratio*)

SAX: Intercanvi Aniònic Fort (*Strong Anion Exchange*)

SBSE: Extracció per Sorció sobre Barres Magnètiques (*Stir Bar Sorptive Extraction*)

SE: Extracción amb dissolvents (Solvent Extraction)

SFE: Extracció amb Fluids Supercrítics (*Supercritical Fluid Extraction*)

SPE: Extracció en Fase Sòlida (*Solid-Phase Extraction*)

SPME: Microextracció en Fase Sòlida (*Solid-Phase Microextraction*)

SRM: Monitorització de reacciones seleccionades (*Selected Reaction Monitoring*)

SRM_1: Transició Quantitiva (*Quantification Transition*)

SRM_2: Transició Qualitativa (*Qualified Transition*)

SRT: Temps de Retenció dels Sòlids (*Solid Retention Time*)

SS: Sediments en Suspensió (*Suspended Sediments*)

SUSME: Microextracció basada en Dissolvents Supramoleculars (*Supramolecular Solvent-based Microextraction*)

TD: Desorció Tèrmica (*Thermal Desorption*)

TIC: Cromatograma Total dels Ions (*Total Ion Chromatogram*)

TOF: Analitzador Temps de Vol (*Time of Flight*)

TOTAD: Transferència d'Adsorció-Desorció a través del Forn (*Through Oven Transfer Adsorption Desorption*)

TPs: Productes de Transformació (*Transformation Products*)

UAE: Extracció Assistida per Ultrasons (*Ultrasonic Assisted Extraction*)

UAME: Extracció amb Microones Assistida per Ultrasons (*Ultrasound Assisted Microwave Extraction*)

UHPLC: Cromatografia d'Ultra Alta Pressió (*Ultra-High Performance Liquid Chromatography*)

USAEME: Microextracció-Emulsificació Assistida per Ultrasons (*Utrasound-Assisted Emulsification–Microextraction*)

UTM: Sistema de coordenades Universal Transversal de Mercator (*Universal Transverse Mercator*)

WW: Aigua Residual (*Waste Water*)

XIC: Cromatograma de l'ió Extret (*Extracted Ion Chromatogram*)

Evolució del consum del fitosanitaris

Capítol 1: Antecedents bibliogràfics

Capítol 2: Screening sistemàtic de plaguicides per Cromatografia Líquida-Espectrometria de masses

Capítol 3: Cromatografia Líquida d'Ultra-alta Pressió acoblada a un quadrupol temps de vol per a identificar contaminants en aigües. Una introducció a la forensia mediambiental

Capítol 4: Microextracció en fase sòlida-en tub acoblada a Cromatografia Líquida d'Ultra-alta Pressió-Espectrometria de Masses

Capítol 5: Avaluació de dos mètodes d'extracció per a la determinació de plaguicides en sòls, sediments i fangs

Capítol 6: Incidència i eficiència de l'eliminació de plaguicides en Plantes de Tractament d'Aigües Residuals (EDARs) en quatre conques mediterrànies

Capítol 7: Screening dels plaguicides utilitzats en l'actualitat en aigües, sediments i biota de la conca del Guadalquivir (Espanya)

Capítol 8: Seguiment de plaguicides en la conca del Llobregat (Catalunya, Espanya) i comparació amb dades històriques

Capítol 9: Integració de diferents models predictius per a calcular la concentració de plaguicides i la informació derivada de les campanyes de seguiment

Introducció-Objectiu

Evolució del consum de fitosanitaris

Capítol 1: Antecedents bibliogràfics

Capítol 2: Screening sistemàtic de plaguicides per Cromatografia Líquida-Espectrometria de masses

Capítol 3: Cromatografia Líquida d'Ultra-alta Pressió acoblada a un quadrupol temps de vol per a identificar contaminants en aigües. Una introducció a la forensia mediambiental

Capítol 4: Microextracció en fase sòlida-en tub acoblada a Cromatografia Líquida d'Ultra-alta Pressió-Espectrometria de Masses

Capítol 5: Avaluació de dos mètodes d'extracció per a la determinació de plaguicides en sòls, sediments i fangs

Capítol 6: Incidència i eficiència de l'eliminació de plaguicides en Plantes de Tractament d'Aigües Residuals (EDARs) en quatre conques mediterrànies

Capítol 7: Screening dels plaguicides utilitzats en l'actualitat en aigües, sediments i biota de la conca del Guadalquivir (Espanya)

Capítol 8: Seguiment de plaguicides en la conca del Llobregat (Catalunya, Espanya) i comparació amb dades històriques

Capítol 9: Integració de diferents models predictius per a calcular la concentració de plaguicides i la informació derivada de les campanyes de seguiment

Resultats i discussió

Capítol 3: Cromatografia Líquida d'Ultra-alta Pressió acoblada a un quadrupol temps de vol per a identificar contaminants en aigües. Una introducció a la forensia mediambiental

Capítol 4: Microextracció en fase sòlida-en tub acoblada a Cromatografia Líquida d'Ultra-alta Pressió-Espectrometria de Masses

Capítol 5: Avaluació de dos mètodes d'extracció per a la determinació de plaguicides en sòls, sediments i fangs

Capítol 6: Incidència i eficiència de l'eliminació de plaguicides en Plantes de Tractament d'Aigües Residuals (EDARs) en quatre conques mediterrànies

Capítol 7: Screening dels plaguicides utilitzats en l'actualitat en aigües, sediments i biota de la conca del Guadalquivir (Espanya)

Capítol 8: Seguiment de plaguicides en la conca del Llobregat (Catalunya, Espanya) i comparació amb dades històriques

Capítol 3: Cromatografia Líquida d'Ultra-alta Pressió acoblada a un quadrupol temps de vol per a identificar contaminants en aigües. Una introducció a la forensia mediambiental

Capítol 4: Microextracció en fase sòlida-en tub acoblada a Cromatografia Líquida d'Ultra-alta Pressió-Espectrometria de Masses

Capítol 5: Avaluació de dos mètodes d'extracció per a la determinació de plaguicides en sòls, sediments i fangs

Capítol 6: Incidència i eficiència de l'eliminació de plaguicides en Plantes de Tractament d'Aigües Residuals (EDARs) en quatre conques mediterrànies

Capítol 7: Screening dels plaguicides utilitzats en l'actualitat en aigües, sediments i biota de la conca del Guadalquivir (Espanya)

Capítol 8: Seguiment de plaguicides en la conca del Llobregat (Catalunya, Espanya) i comparació amb dades històriques