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DEPARTAMENTO DE QUÍMICA Y FÍSICA

“Aplicación de nuevas técnicas de espectrometría de masas para la evaluación de plaguicidas en muestras vegetales y ambientales”

“Application of new techniques of mass spectrometry for the evaluation of pesticides in vegetable and environmental samples”

Memoria presentada para optar al grado de
Doctora en Química con Mención Internacional

Mar García Valverde
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UNIVERSIDAD DE ALMERÍA

Departamento de Química y Física

LOS DIRECTORES DE LA TESIS:

El Dr. Amadeo Rodríguez Fernández-Alba, Catedrático del Departamento de Química y Física de la Facultad de Ciencias Experimentales de la Universidad de Almería y la Dra. María Jesús Martínez Bueno, Profesora Titular de la Universidad de Almería, en calidad de director y codirectora, respectivamente,

CERTIFICAN:

Que la presente tesis doctoral, titulada: ¨ ***Aplicación de nuevas técnicas de espectrometría de masas para la evaluación de plaguicidas en muestras vegetales y ambientales*** ¨, presentada por Mar García Valverde graduada en Químicas, ha sido realizada dentro del programa de doctorado de Química Avanzada bajo nuestra supervisión, en el Área de Química Analítica del Departamento de Química y Física de la Universidad de Almería, y que a juicio de los directores, reúne los requisitos de originalidad, extensión e interés teórico y práctico necesarios para ser presentada como Memoria para optar al grado de Doctor en Química.

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Directores de la Tesis:

Dr. Amadeo Rodríguez Fernández-Alba

Dra. María Jesús Martínez Bueno

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RESUMEN

Debido al cambio climático, a la agricultura intensiva y aumento de la población, se está produciendo un incremento en la presión sobre los recursos hídricos. Esto tiene como consecuencia derivando en una escasez cada vez mayor de agua y deteriorando su calidad. Es por ello, que la utilización de aguas residuales tratadas o regeneradas para el riego agrícola está incrementándose a nivel mundial, a la vez se está convirtiendo en una alternativa con gran potencial para combatir la escasez de agua.

Recientemente, la Unión Europea ha publicado una nueva regulación (Regulación 2020/741) en relación con los requisitos mínimos de control y calidad para la reutilización de agua con fines agrarios. Su principal finalidad, se basa en que las aguas regeneradas sean seguras para el riego agrícola, además de promover la economía circular y asegurar la protección del medio ambiente y de la salud humana. En dicho documento, se recogen los parámetros mínimos de calidad del agua según sea su origen/categoría, así como el tipo de cultivo sobre el que se va a emplear. Además, en este reglamento se detallan los requisitos mínimos de control rutinario que deben ser realizados por los operadores de las estaciones regeneradoras de agua y controles de validación de estas, con requisitos rigurosos para el control de microorganismos.

Sin embargo, estas normas solo se centran en aspectos microbiológicos sin tener en cuenta otros contaminantes orgánicos de preocupación emergente, como productos farmacéuticos y plaguicidas. En las últimas décadas, los estudios sobre agua ambiental han llamado la atención debido a la presencia de una gran variedad de este tipo de contaminantes de origen antropogénico o agrícola. Todo ello evidencia que los procesos convencionales de las plantas de tratamiento de agua residuales no son completamente efectivos para eliminar completamente muchos de ellos. La mayoría son contaminantes orgánicos que se encuentran en concentraciones trazas de µg/L o ng/L, que, por su potencial bioacumulación, pueden causar efectos adversos en los ecosistemas y en la salud humana. Por tanto, el uso de aguas regeneradas para riego agrícola pueden ser el origen de estos contaminantes en la cadena alimentaria, originando peligros para la salud humana y para el medio ambiente.

Así, esta Tesis Doctoral se centra en el desarrollo y optimización de metodologías analíticas basadas en la cromatografía de líquidos acoplada a la espectrometría de masas (LC-MS/MS) con analizador triple cuadrupolo para la caracterización de plaguicidas y productos farmacéuticos y de cuidado personal tanto en productos de origen vegetal como origen ambiental (suelo y agua de riego). Además, se ha evaluado el potencial de absorción de varios contaminantes orgánicos en plantas, obteniéndose modelos estadísticos de acumulación para predecir aquellos que tienen mayor capacidad para ser absorbidos y translocados por la planta y cuales acumularse en el suelo. Por otro lado, se ha desarrollado y validado un método de extracción, basado en el método QuEChERS, con el fin de obtener un método analítico rápido y sensible que permita la determinación de una amplia gama de contaminantes de origen emergente en muestras de suelo agrícola. Por último, se ha estudiado el posible impacto a largo plazo del uso de aguas regeneradas para riego agrícola bajo condiciones agronómicas reales, considerando la ingesta de los frutos obtenidos en esta producción en el consumo humano

Las principales aportaciones de esta Tesis Doctoral, que condujeron a la publicación de artículos científicos son:

- Desarrollo y validación de un método analítico para la determinación en suelo agrícola de 30 de los contaminantes orgánicos de preocupación emergente y plaguicidas más detectados en agua regeneradas mediante el uso de un método QuEChERS modificado.
- Identificación de aquellos contaminantes que presentan una mayor capacidad de absorción por las plantas.
- Elaboración de modelos estadísticos de acumulación que permitan estimar niveles de contaminantes en suelo, así como en las diferentes partes de la planta.
- Evaluación del impacto del uso de agua regenerada en cultivos bajo condiciones agrícolas reales.

ABSTRACT

Due to climate change, intensive agriculture and population growth, there is increasing pressure on freshwater resources, leading to water scarcity and deteriorating water quality. Therefore, the use of treated or reclaimed wastewater for agricultural irrigation is increasing worldwide and is becoming an alternative with great potential to combat water scarcity.

Recently, the European Union has published a new regulation (Regulation 2020/741) regarding the minimum control and quality requirements for the reuse of water for agricultural purposes. The main purpose of this regulation is to ensure that reclaimed water is safe for agricultural irrigation, as well as to promote the circular economy and ensure protection of the environment and human health. In this document, we find the minimum water quality parameters according to the category of reclaimed water and the type of crop. In addition, this regulation details the minimum routine control requirements that must be carried out by operators of water reclamation stations and validation controls of reclaimed water with more stringent requirements where an evaluation of microorganisms is carried out.

However, the stipulated standards only focus on microbiological aspects without taking into account contaminants of emerging concern such as pesticides or pharmaceuticals. In recent decades, environmental water studies have drawn attention to the presence of a variety of contaminants of emerging concern (CECs), giving clear evidence that conventional wastewater treatment plant processes are poorly effective in removing many of these contaminants. Most are organic micropollutants found in concentrations of µg/L or ng/L, which can cause adverse effects on ecosystems and human health. Therefore, the use of reclaimed water for agricultural irrigation can introduce these contaminants into the food chain, causing hazards to human health and the environment.

Thus, this Doctoral Thesis focuses on the development and optimization of analytical methodologies based on liquid chromatography coupled to mass spectrometry (LC-MS/MS) with triple quadrupole analyzer for the characterization of pesticides and pharmaceutical and personal care products both in products of plant origin and environmental origin (soil and irrigation water). In addition, the contaminant absorption potential has been evaluated, obtaining statistical models of accumulation to predict which contaminants have a greater capacity to be absorbed and translocated by the plant. On the other hand, an extraction method has been developed and validated, based on the QuEChERS method, for the determination of a wide range of contaminants in agricultural soil samples, in order to obtain an optimal method to analyze soil irrigated with reclaimed water in the long term and prevent the accumulation of contaminants. Finally, the long-term impact of the use of reclaimed water for agricultural irrigation under real agronomic conditions has been studied. In addition to assessing the potential risk to human health from the consumption of the edible part of the plant irrigated with treated water, we have also studied the long-term impact of the use of reclaimed water for agricultural irrigation under real agronomic conditions.

The main contributions of this doctoral thesis, which led to the publication of scientific articles are:

- Development and validation of an analytical method for the determination in agricultural soil of 30 of the most detected contaminants in reclaimed water using the modified QuEChERS method.
- Determination of the compounds with the highest absorption capacity by the plant, obtaining statistical models of accumulation that allowed estimating the levels of contaminants in soil and different parts of the plant.
- Evaluation of the impact of the use of reclaimed water on crops under real long-term agricultural conditions.

HIPÓTESIS

HIPÓTESIS

Considerando la problemática mundial de la escasez de agua, el uso de aguas regeneradas desde plantas de tratamiento de agua residuales (EDAR) para riego agrícola está siendo una gran alternativa en zonas áridas y semiáridas para combatir dicho inconveniente. Aunque las plantas de tratamiento de agua realizan tratamiento de eliminación específicos de contaminantes, su eliminación no es completa. Consecuentemente, el uso de estas aguas para riego agrícola puede originar la translocación de estos contaminantes desde el suelo a las raíces, hojas y fruto pudiendo llegar a suponer un problema de salud pública.

El análisis de plaguicidas y fármacos en muestras de origen vegetal y ambiental presenta una serie de dificultades como consecuencia no solo por la complejidad de este tipo de matrices sino también por los bajos niveles de concentración a los que se pueden encontrar, especialmente sus productos de transformación. Todo ello, implica el desarrollo de procedimientos analíticos que posean una elevada sensibilidad y selectividad.

Este trabajo de investigación está dirigido, por una parte, al desarrollo y optimización de métodos analíticos que permitan el análisis simultáneo de una gran variedad de contaminantes orgánicos con propiedades físico-químicas diferentes en muestras vegetales y ambientales.

Por otro lado, el presente trabajo pretende también evaluar la capacidad de absorción por las plantas de algunos de los contaminantes orgánicos detectados más frecuentemente en las aguas tratadas, y establecer modelos estadísticos predictivos.

Por último, se evalúan los efectos a que puedan derivarse del uso de agua regenerada sobre los sistemas agrarios (suelo, cultivo), así como el riesgo potencial para el ser humano derivado del consumo de la parte comestible de estos productos.

OBJETIVOS

OBJETIVOS

El objetivo de este trabajo es desarrollar y optimizar nuevos procedimientos analíticos, siguiendo los criterios de validación establecidos por las guías oficiales europeas vigentes (SANTE/11312/2021), para la evaluación de residuos de plaguicidas, fármacos y algunos de sus principales productos de transformación en alimentos y muestras ambientales. Para ello, se evaluará el potencial analítico de técnicas avanzadas de espectrometría de masas en tándem (MS/MS) con el fin de:

- Desarrollar y optimizar metodologías analíticas basadas en el empleo de técnicas de espectrometría de masas de resolución unitaria para el estudio y la evaluación del destino y la propagación de plaguicidas y productos farmacéuticos desde diferentes tipos de aguas regeneradas a los cultivos por riego.

Tomando como base el objetivo principal, los objetivos específicos de la Tesis abordados en cada uno de los capítulos son:

Capítulo I:

- Desarrollo y validación de un método de extracción sensible, rápido, fácil y robusto para la determinación simultanea de 30 contaminantes orgánicos de origen emergente en suelo agrícola.
- Optimización del proceso de extracción de la muestra, comparando tres técnicas habitualmente usadas para la extracción de suelo como son ultrasonidos, extracción con líquido presurizado y QuEChERS modificado.
- Aplicación de la metodología desarrollada al análisis de muestras reales de suelo agrícola.

Capítulo II:

- Desarrollo y validación de un método analítico para la determinación de 30 contaminantes orgánicos en muestras vegetales y ambientales mediante cromatografía de líquidos acoplada a espectrometría de masas en tándem con analizador triple cuadrupolo.
- Aplicación de la metodología desarrollada al análisis de muestras reales tanto vegetales como ambientales.
- Identificación de aquellos compuestos que tienen una mayor capacidad de acumulación en la planta, y obtener modelos estadísticos predictivos que permitan estimar los niveles de contaminantes en el suelo, así como en las diferentes partes del cultivo (raíz, tallo, hojas y fruto).
- Evaluación del posible riesgo para las personas derivado del consumo de los frutos obtenidos desde plantas cultivadas con aguas regeneradas.

Capítulo III:

- Desarrollo y validación de un método analítico para el análisis de 293 contaminantes orgánicos en muestras vegetales y ambientales mediante cromatografía de líquidos acoplada a espectrometría de masas en tándem con analizador triple cuadrupolo.
- Evaluación del impacto a continuado del uso de aguas regeneradas en cultivos agrícolas.
- Identificación de aquellos contaminantes que poseen mayor riesgo para la salud humana.

INTRODUCCIÓN

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1. Problemática del estrés hídrico y su repercusión en la agricultura

El agua es un recurso imprescindible por ser fundamental para la existencia de toda vida, el crecimiento económico y para conservar el bienestar social. Los recursos hídricos, aunque son renovables tienen una serie de limitaciones. Existen depósitos (océanos, casquetes polares, aguas subterráneas, lagos, ríos y la atmósfera) que se reponen con mayor o menor rapidez. Entre estas reservas, las aguas subterráneas almacenan casi el total del agua dulce disponible para uso humano (99%), y también es el reservorio que se renueva más lentamente (Dalin, 2021). Esta renovación se origina a través del ciclo del agua (evaporación, condensación, precipitación y transpiración). Sin embargo, en los últimos años se ha ido observando que su demanda junto con el cambio climático, están haciendo que haya cada vez más discrepancia entre la demanda y el suministro disponible.

En muchas regiones en todo el mundo, el suministro de fuentes de agua dulce está en riesgo, sobre todo en las zonas áridas y semiáridas (Ofori et al., 2021; Rusănescu et al., 2022). Una cuarta parte de la población mundial se verá afectada por la escasez de agua en los próximos años. Además, si no se toman las medidas oportunas, urgentemente, para el año 2050 habrá un déficit del 40% del agua global (García-Valverde et al., 2023; López-Serrano et al., 2020). Se estima un incremento de las extracciones de aguas subterráneas de 1100 km^3 por año para 2050 en comparación con 800 km^3 por año de 2010 (Pradhan et al., 2023). Países como Israel, Egipto, o Arabia Saudí se encuentran con una extremada escasez de agua (con valores de pérdida superiores al 80% de los recursos renovables de agua). Mientras España se encuentra entre los países con moderado-alto estrés hídrico (20-40%) (Rusănescu et al., 2022).

Por tanto, los recursos de agua se están enfrentando a una degradación debido al gran crecimiento de la población, la expansión de actividades de producción como la agricultura y al cambio climático (López-Serrano et al., 2020). Si esto sigue agravándose, puede llegar a originarse la peor amenaza a nivel mundial, “la sequía”. Sin recursos hídricos se producirían alteraciones y cambios en el funcionamiento de los ecosistemas, sin embargo, lo peor se daría en el sector agrícola donde la producción de alimentos disminuiría y aumentarían los precios de estos.

2. Regulación del uso de aguas regeneradas/ tratadas

Es cierto que teniendo en cuenta el problema de la escasez de agua alrededor del mundo, el uso de agua regenerada, desde plantas de tratamiento de aguas residuales, para riego agrícola está siendo una gran alternativa en zonas áridas y semiáridas de Europa. La reutilización de aguas tratadas no solo promueve el uso sostenible del agua, sino que implica mejoras ambientales, humanas y económicas (Ait-Mouheb et al., 2018; García-Valverde et al., 2023; Martínez Bueno et al., 2022; Ofori et al., 2021). Sin embargo, cientos de publicaciones científicas han reportado la presencia de contaminantes de preocupación emergente (fármacos, pesticidas o productos de cuidado personal) en aguas regeneradas o agua de riego (Calderón-Preciado et al., 2011; Martínez Bueno et al., 2012, 2021; Picó et al., 2019; Quintana et al., 2019). Dando una clara evidencia que las plantas de tratamiento de aguas residuales convencionales no son capaces de eliminar de forma completa muchos de estos contaminantes.

La gestión de aguas residuales para uso agrícola está regulada por apropiadas políticas, legislaciones, marcos institucionales y regulaciones a niveles locales, nacionales e internacionales. La Directiva 91/271/EEC fue la primera directiva europea que consideraba la protección del medio ambiente. En ella se establecieron las medidas necesarias para que los Estados Miembros garantizasen un tratamiento adecuado de las aguas residuales urbanas antes de su vertido, además de sistemas de recogida y conducción de dichas aguas.

La siguiente Directiva Europea para la protección del medio ambiente fue la Directiva 96/61/CE (reemplazada por la Directiva 2008/1/CE) cuyo objetivo es la prevención y reducción de la contaminación procedente de actividades industriales muy contaminantes (industrias minerales, industrias químicas o gestión de residuos) en la atmosfera, el agua o el suelo.

A principio del siglo XIX fue adoptada la Directiva del Marco del Agua (Directiva 2000/60/CE), en la cual se establecía un marco para la protección de aguas superficiales, costeras, subterráneas y de transición. En ella se previene el deterioro de los ecosistemas acuáticos, se promueve un uso sostenible del agua, se reduce la contaminación del agua subterránea y se contribuye a paliar los efectos de las inundaciones y sequías.

La primera lista de sustancias prioritarias en el ámbito de la política de aguas fue aprobada en 2001 mediante la Decisión 2455/2001/CE. Dicha lista se añadió como anexo X a la Directiva 2000/60/CE formada por 33 sustancias prioritarias de diferentes clases como insecticidas, herbicidas, hidrocarburos aromáticos policíclicos, fungicidas, etc. En ella, las sustancias se clasificaron según persistencia, bioacumulación, toxicidad o alta producción que puedan causar riesgos al medio ambiente y a la salud humana.

Años más tarde, en 2008, mediante la Directiva 2008/105/CE, se establecieron normas de calidad ambiental (CNA) para las sustancias prioritarias en el ámbito de la política de aguas para obtener un estado químico óptimo de las aguas superficiales a través de niveles de concentraciones máximas en µg/L para estas sustancias.

Cinco años más tarde, la primera lista de sustancias prioritarias y las CNA fueron modificadas por la Directiva 2013/39/UE donde se ampliaron hasta 45 el número de sustancias prioritarias en el ámbito de la política de aguas y se actualizaron los niveles de concentración máxima (**Tabla 1**). Además, se incorporó el análisis de sedimentos y/o biota (peces) y se estableció un periodo de 20 años para el cese total del vertido y emisión de estas sustancias.

Tabla 1. Lista de sustancias prioritarias en el ámbito de la política de aguas con sus normas de calidad ambiental (Directiva 2013/39/UE).

MA: Media Anual
CMA: Concentración Máxima Admisible
Unidad: [µg/L] para columnas (4) a (7); [µg/Kg de peso húmedo] para columna (8)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Nº	**Nombre de la sustancia**	**N° CAS (1)**	**NCA-MA (2) Aguas superficiales continentales (3)**	**NCA-MA (2) Otras aguas superficiales**	**NCA-CMA (4) Aguas superficiales continentales (7)**	**NCA-CMA (4) Otras aguas superficiales**	**NCA Biota (12)**
1	Alacloro	15972-60-8	0.3	0.3	0.7	0.7	
2	Antraceno	120-12-7	0.1	0.1	0.1	0.1	
3	Atrazina	1912-24-9	0.6	0.6	2.0	2.0	
4	Benceno	71-43-2	10	8	50	50	
5	Difeniléteres bromados (5)	32534-81-9			0.014	0.014	0.0085
6	Cadmio y sus compuestos (en función de las clases de dureza del agua) (6)	7440-43-9	≤0.08 (clase 1) 0.08 (clase 2) 0.09 (clase 3) 0.15 (clase 4) 0.25 (clase 5)	0.2	≤0.45 (clase 1) 0.45 (clase 2) 0.6 (clase 3) 0.9 (clase 4) 1.5 (clase 5)	≤0.45 (clase 1) 0.45 (clase 2) 0.6 (clase 3) 0.9 (clase 4) 1.5 (clase 5)	
6a	Tetracloruro de carbono (7)	56-23-5	12	12	No aplicable	No aplicable	
7	Cloroalcanos C_{10-13} (8)	85535-84-8	0.4	0.4	1.4	1.4	
8	Clorfenvinfós	470-90-6	0.1	0.1	0.3	0.3	
9	Clorpirifós (Clorpirifós -etilo)	2921-88-2	0.03	0.03	0.1	0.1	
9a	Plaguicidas de tipo ciclodieno: Aldrina (7) Dieldrina (7) Endrina (7) Isodrina (7)	309-00-2; 60-57-1; 72-20-8; 465-73-6	Σ = 0.01	Σ = 0.005	No aplicable	No aplicable	
9b	DDT total (7) (9)	No aplicable	0.025	0.025	No aplicable	No aplicable	
	P,p´-DDT (7)	50-29-3	0.01	0.01			
10	1,2-Dicloroetano	107-06-2	10	10	No aplicable	No aplicable	
11	Diclorometano	75-09-2	20	20	No aplicable	No aplicable	
12	Ftalato de di(2-etilhexilo)	117-81-7	1.3	1.3	No aplicable	No aplicable	
13	Diurón	330-54-1	0.2	0.2	1.8	1.8	
14	Endosulfán	115-29-7	0.005	0.0005	0.01	0.004	
15	Fluoranteno	206-44-0	0.0063	0.0063	0.12	0.12	30

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Nº	**Nombre de la sustancia**	**N° CAS** (1)	**NCA-MA** (2) **Aguas superficiales continentales** (3)	**NCA-MA** (2) **Otras aguas superficiales**	**NCA-CMA** (4) **Aguas superficiales continentales** (7)	**NCA-CMA** (4) **Otras aguas superficiales**	**NCA Biota** (12)
16	Hexacloro-benceno	118-74-1			0.05	0.05	10
17	Hexacloro-butadieno	87-68-3			0.6	0.6	55
18	Hexacloro-ciclohexano	608-73-1	0.02	0.002	0.04	0.02	
19	Isoproturón	34123-59-6	0.3	0.3	1.0	1.0	
20	Plomo y sus compuestos	7439-92-1	1.2 (13)	1.3	14	14	
21	Mercurio y sus compuestos	7439-97-6			0.07	0.07	20
22	Naftaleno	91-20-3	2	2	130	130	
23	Níquel y sus compuestos	7440-02-0	4 (13)	8.6	34	34	
24	Nonilfenoles	84852-15-3	0.3	0.3	2.0	2.0	
25	Octifenoles	140-66-9	0.1	0.01	No aplicable	No aplicable	
26	Pentaclorobenceno	608-93-5	0.007	0.0007	No aplicable	No aplicable	
27	Pentaclorofenol	84-86-5	0.4	0.4	1	1	
28	Hidrocarburos aromáticos policíclicos (11)	No aplicable	No aplicable	No aplicable	No aplicable	No aplicable	
	Benzo(a)pireno	50-32-8	1.7x10^{-4}	1.7x10^{-4}	0.27	0.027	5
	Benzo(b)fluoranteno	205-99-2	Véase la nota 11	Véase la nota 11	0.017	0.017	Véase la nota 11
	Benzo(k)fluoranteno	207-08-9	Véase la nota 11	Véase la nota 11	0.017	0.017	Véase la nota 11
	Benzo(g,h,i)perileno	191-24-2	Véase la nota 11	Véase la nota 11	8.2x10^{-3}	8.2x10^{-4}	Véase la nota 11
	Indeno(1,2,3-cd)pireno	193-39-5	Véase la nota 11	Véase la nota 11	No aplicable	No aplicable	Véase la nota 11
29	Simazina	122-34-9	1	1	4	4	
29a	Tetracloroetileno (7)	127-18-4	10	10	No aplicable	No aplicable	
29b	Tricloroetileno (7)	79-01-6	10	10	No aplicable	No aplicable	
30	Compuestos de tributilestaño	36643-28-4	0.0002	0.0002	0.0015	0.0015	
31	Triclorobencenos	12002-48-1	0.4	0.4	No aplicable	No aplicable	
32	Triclorometano	67-66-3	2.5	2.5	No aplicable	No aplicable	
33	Trifluralina	1582-09-8	0.03	0.03	No aplicable	No aplicable	
34	Dicofol	115-32-2	1.3x10^{-4}	3.2x10^{-3}	No applicable	No aplicable	33
35	Ácido perflurorooctano-sulfónico y sus derivados	1763-23-1	6.5x10^{-4}	1.3x10^{-4}	36	7.2	9.1
36	Quinoxifeno	124495-18-7	0.15	0.015	2.7	0.54	

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Nº	**Nombre de la sustancia**	**N° CAS (1)**	**NCA-MA (2) Aguas superficiales continentales (3)**	**NCA-MA (2) Otras aguas superficiales**	**NCA-CMA (4) Aguas superficiales continentales (7)**	**NCA-CMA (4) Otras aguas superficiales**	**NCA Biota (12)**
37	Dioxinas y compuestos similares	Véase la nota 10, en el anexo X de la Directiva 2000/60/CE			No aplicable	No aplicable	Suma de PCDD + PCDF + PCB-DL 0.0065 µg/kg TEQ (14)
38	Aclonifeno	74070-46-5	0.12	0.012	0.12	0.012	
39	Bifenox	42576-02-3	0.012	0.0012	0.04	0.004	
40	Cibutrina	28159-98-0	0.0025	0.0025	0.016	0.016	
41	Cipermetrina	52315-07-8	$8x10^{-5}$	$8x10^{-6}$	$6x10^{-4}$	$6x10^{-5}$	
42	Diclorvós	62-73-7	$6x10^{-4}$	$6x10^{-5}$	$7x10^{-4}$	$7x10^{-5}$	
43	Hexabromo-ciclododecano	Véase la nota 12, en el anexo X de la Directiva 2000/60/CE	0.0016	0.0008	0.5	0.05	167
44	Heptacloro y epóxido de heptacloro	76-44-8/ 1024-57-3	$2x10^{-7}$	$1x10^{-8}$	$3x10^{-4}$	$3x10^{-5}$	$3.7x10^{-3}$
45	Terbutrina	886-50-0	0.065	0.0065	0.34	0.034	

(1) CAS: Servicio de resúmenes químicos (Chemical Abstracts Service)

(2) NCA expresada como valor medio anual (NCA-MA). Salvo que se especifique ora cosa, se aplica a la concentración total de todos los isómeros.

(3) Las aguas superficiales continentales incluyen los ríos y lagos y las masas de agua artificiales o muy modificadas conexas.

(4) Este parámetro es la NCA expresada como concentración máxima admisible (NCA-CMA). Cuando en la columna NCA-CMA se indica "No aplicable", se considera que los valores NCA-MA protegen contra los picos de contaminación a corto plazo en el caso de los vertidos continuos, ya que son significativamente inferiores a los valores calculados sobre la base de la toxicidad aguda.

(5) Por lo que respecta al grupo de sustancias prioritarias incluidas en los difeniléteres bromados (n o 5), las NCA se refieren a la suma de las concentraciones de los congéneres n o 28, 47, 99, 100, 153 y 154.

(6) Por lo que respecta al cadmio y sus compuestos (n o 6), los valores de las NCA varían en función de la dureza del agua con arreglo a cinco categorías (clase 1: < 40 mg $CaCO_3$ */l, clase 2: de 40 a < 50 mg* $CaCO_3$ */l, clase 3: de 50 a < 100 mg* $CaCO_3$ */l, clase 4: de 100 a < 200 mg* $CaCO_3$ */l, y clase 5: ≥ 200 mg* $CaCO_3$ */l).*

(7) Esta sustancia no es una sustancia prioritaria sino uno de los otros contaminantes para los cuales las NCA son idénticas a las establecidas en la legislación aplicable antes del 13 de enero de 2009.

(8) No se señala para este grupo de sustancias ningún parámetro indicativo. El parámetro o parámetros indicativos deberán definirse mediante el método analítico.

(9) El DDT total incluye la suma de los isómeros 1,1,1-tricloro-2,2-bis(p-clorofenil)-etano (n o CAS 50-29-3; n o UE 200-024-3); 1,1,1- tricloro-2-(o-clorofenil)-2-(p-clorofenil)-etano (n o CAS 789-02-6; n o UE 212-332-5); 1,1-dicloro-2,2-bis(p-clorofenil)-etileno (n o CAS 72-55-9; n o UE 200-784-6), y 1,1-dicloro2,2-bis(p-clorofenil)-etano (n o CAS 72-54-8; n o UE 200-783-0).

(10) No se dispone de suficiente información para establecer una NCA-CMA para estas sustancias.

(11) Por lo que respecta al grupo de sustancias prioritarias de hidrocarburos aromáticos policíclicos (HAP) (n o 28), las NCA de la biota y las correspondientes NCA-MA en el agua se refieren a la concentración de benzo(a)pireno, en cuya toxicidad se basan. El benzo(a)pireno puede considerarse como un marcador de los otros HAP, ya que solo tal s

ustancia debe ser objeto de seguimiento a efectos de comparación con las NCA de la biota o las correspondientes NCA-MA en el agua.

(12) *Salvo que se indique de otro modo, las NCA de la biota se refieren a los peces. Sustitutivamente podrá hacerse el seguimiento de otro taxón de la biota u otra matriz, siempre que las NCA aplicadas ofrezcan un nivel equivalente de protección. Para las sustancias con los números 15 (fluoranteno) y 28 (HAP), la NCA de la biota se refiere a crustáceos y moluscos. A efectos de evaluar el estado químico, no resulta adecuado el seguimiento del fluoranteno y de los HAP en los peces. Para la sustancia con el número 37 (dioxinas y compuestos similares), la NCA de la biota se refiere a los peces, los crustáceos y los moluscos en consonancia con el punto 5.3 del anexo del Reglamento (UE) n o 1259/2011 de la Comisión, de 2 de diciembre de 2011, por el que se modifica el Reglamento (CE) n o 1881/2006 en lo relativo a los contenidos máximos de dioxinas, PCB similares a las dioxinas y PCB no similares a las dioxinas en los productos alimenticios (DO L 320 de 3.12.2011, p. 18).*

(13) *Estas NCA se refieren a las concentraciones biodisponibles de las sustancias.*

(14) *PCDD: dibenzo-p-dioxinas policloradas; PCDF: dibenzofuranos policlorados; PCB-DL: policlorobifenilos similares a las dioxinas; TEQ: equivalentes tóxicos con arreglo a los Factores de Equivalencia Tóxica de 2005 de la Organización Mundial de la Salud.»*

Dos años después, se creó la primera lista de observación de 16 sustancias en el ámbito de la política de aguas (Decisión 2015/495). Los compuestos de la lista debían ser estudiados en el medio acuático para posteriormente ser incluidos o no en la lista de sustancias prioritarias.

En junio de 2018 fue actualizada dicha lista de observación, la cual, se redujo a 14 sustancias (Decisión 2018/840/EC). Entre ellas se encontraban hormonas, antibióticos como eritromicina o claritromicina y neonicotinoides como imidacloprid o acetamiprid. Para más información ver **tabla 2**.

Tabla 2. Lista de observación de sustancias prioritarias(Decisión 2018/840/EC).

Nombre de la sustancia	**N.º CAS (1)**	**N.º UE (2)**	**Método analítico indicativo (3) (4)**	**Límite máximo aceptable de detección del método (ng/L)**
17-alfa-etinilestradiol	57-63-6	200-342-2	SPE, LC-MS-MS en grandes volúmenes	0.035
17-beta-estradiol, estrona	50-28-2, 53-16-7	200-023-8	SPE, LC-MS-MS	0.4
Antibióticos macrólidos (5)			SPE, LC-MS-MS	19
Metiocarb	2032-65-7	217-991-2	SPE, LC-MS-MS o GC-MS-MS	2
Neonicotinoides (6)			SPE, LC-MS-MS	8.3
Metaflumizona	139968-49-3	604-167-6	LLE, LC-MS-MS o SPE, LC-MS-MS	65
Amoxicilina	26787-78-0	248-003-8	SPE, LC-MS-MS	78
Ciprofloxacina	85721-33-1	617-751-0	SPE, LC-MS-MS	89

(1) *Chemical Abstracts Service (Servicio de Resúmenes de Productos Químicos).*

(2) *Número de la Unión Europea. No disponible para todas las sustancias.*

(3) *Para garantizar la comparabilidad de los resultados de los diferentes Estados miembros, todas las sustancias serán objeto de seguimiento en toda la muestra de agua.*

(4) *Métodos de extracción: LLE:extracción líquido-líquido; SPE:extracción en fase sólida. Métodos analíticos: GC-MS:cromatografía de gases acoplada a espectrometría de masas; LC-MS-MS:cromatografía de líquidos acoplada a espectrometría de masas en tándem con triple cuadrupolo.*

(5) *Eritromicina (n.º CAS: 114-07-8; n.º UE: 204-040-1), claritromicina (n.º CAS: 81103-11-9), azitromicina (n.º CAS: 83905-01-5; n.º UE: 617-500-5).*

(6) Imidacloprid (n.º CAS: 105827-78-9/138261-41-3, n.º UE: 428-040-8), tiacloprid (n.º CAS: 111988-49-9), tiametoxam (n.º CAS: 153719-23-4; n.º UE: 428-650-4), clotianidina (n.º CAS: 210880-92-5; n.º UE: 433-460-1), acetamiprid (n.º CAS: 135410-20-7/160430-64-8).

La Unión Europea en los últimos años ha adoptado proyectos, informes y planes de acción para promover el uso de aguas residuales regeneradas ya que son una fuente efectiva para mitigar tanto la descarga continua de agua residual tratada al medioambiente como para hacer frente a la escasez de agua, además de tener un menor impacto ambiental.

Hace 11 años se comenzó a hablar de la reutilización de aguas para riego o fines industriales mediante la Comunicación del Plan para salvaguardar los recursos hídricos de Europa de la Comisión del Parlamento Europeo (Comisión Europea COM(2012) 673). En este plan se proponía realizar para 2015 un reglamento con normas europeas relativas a la reutilización de agua.

En 2016, la Comisión Europea puso en marcha la iniciativa de promover unos mínimos de calidad para la reutilización de agua con uso agrícola o recarga acuífera (European Commission, 2016).

Pero hasta 2018, la Comisión Europea no hizo una propuesta de reglamento sobre los requisitos mínimos para la reutilización de agua (Proposal 2018/0169/COD-337). Dos años más tarde, el 5 de Junio de 2020, se publicó una legislación resultante de esta propuesta por el parlamento Europeo, donde fueron publicados los requisitos mínimos de control y calidad para la reutilización de agua con fines agrícolas (Regulación 2020/741). La principal finalidad de esta, se basa en que las aguas regeneradas sean seguras para riego agrícola, además de promover la economía circular y asegurar protección al medio ambiente y a la salud humana. Entre los requisitos, que son detallados en el Anexo I de dicho documento, encontramos los parámetros mínimos de calidad del agua en primer lugar según la categoría de agua regenerada, clasificándose en 4 tipos: A, B, C y D (**tabla 3**)

Tabla 3. Requisitos mínimos de calidad de las aguas regeneradas para el uso agrícola (Regulación 2020/741).

Clase de calidad de las aguas regeneradas	**Tratamiento indicativo**	**Requisitos de calidad**				
		E. coli (número /100ml)	**DBO_5 (mg/l)**	**STS (mg/l)**	**Turbidez (UNT)**	**Otros**
A	Secundario, filtración y desinfección	≤ 10	≤ 10	≤ 10	≤ 5	Legionella spp.: < 1 000 UFC/l cuando exista un riesgo de aerosolización. Nematodos intestinales (huevos de helmintos): ≤ 1 huevo/l para el riego de pastos o forraje
B	Secundario y desinfección	≤ 100	De conformidad con la Directiva 91/271/CEE (anexo I, cuadro 1)	De conformidad con la Directiva 91/271/CEE (anexo I, cuadro 1)	-	
C	Secundario y desinfección	≤ 1000			-	
D	Secundario y desinfección	≤ 10000			-	

Y, en segundo lugar, los parámetros mínimos de calidad del agua según el tipo de cultivo (cultivos de consumo crudo, cultivos de alimento procesado y cultivos con alimento no destinado para el consumo humano) (**tabla 4**).

Tabla 4. Clase de calidad de agua regenerada según método de riego y cultivo agrícola (Regulación 2020/741).

Clase de calidad mínima de las aguas regeneradas	Categoría de cultivo (*)	Método de riego
A	Todos los cultivos de alimentos que se consumen crudos en los que la parte comestible está en contacto directo con las aguas regeneradas y los tubérculos que se consumen crudos	Todos los métodos de riego
B	Los cultivos de alimentos que se consumen crudos cuando la parte comestible se produce por encima del nivel del suelo y no está en contacto directo con las aguas regeneradas, los cultivos de alimentos transformados y los cultivos no alimenticios, incluidos los cultivos utilizados para alimentar a animales productores de carne o leche	Todos los métodos de riego
C		Riego por goteo (**) u otro método de riego que evite el contacto directo con la parte comestible del cultivo
D	Cultivos destinados a la industria y a la producción de energía y de semillas	Todos los métodos de riego (***)

(*) En caso de que un tipo determinado de cultivo regado corresponda a varias categorías del cuadro 1, se le aplicarán los requisitos de la categoría más estricta.

(**) El riego por goteo es un sistema de microrriego capaz de suministrar el agua en gotas o pequeños chorros a los vegetales y consiste en un goteo de agua sobre el suelo o directamente bajo la superficie en cantidades muy pequeñas (2-20 litros/hora) con un sistema de tubos de plástico de pequeño diámetro provistos de unos orificios denominados goteros de riego.

(***) En el caso de métodos de riego que imitan la lluvia, debe prestarse especial atención a la protección de la salud de los trabajadores o los transeúntes. A tal efecto, se aplicarán las medidas preventivas adecuadas.

En este reglamento se detallan también los requisitos mínimos de control rutinario que deben ser realizados por los operadores de las estaciones regeneradoras de agua **(tabla 5**). Por otro lado, aparecen controles de para garantizar la seguridad de la reutilización de las aguas regeneradas con requisitos más rigurosos donde se lleva a cabo una evaluación de los microorganismos (**tabla 6**). Estos controles deben ser aplicados cuando se ponga en funcionamiento una nueva estación regeneradora de agua o se cambien equipos y/o procesos, debiéndose cumplir los requisitos establecidos en la Norma ISO-17025 con el fin de garantizar un nivel de calidad.

Tabla 5. Control rutinario de las aguas regeneradas para riego agrícola (Regulación 2020/741).

Clase de calidad de las aguas regeneradas	**Frecuencia mínima de los controles**					
	E. coli	**DBO_5**	**STS**	**Turbidez**	***Legionella spp.* (cuando sea de aplicación)**	**Nematodos intestinales (cuando sea de aplicación)**
A	Una vez a la semana	Una vez a la semana	Una vez a la semana	Continuo	Dos veces al mes	Dos veces al mes o como determine el operador de la estación regeneradora de aguas en función del número de huevos en las aguas residuales que entran en la estación regeneradora de aguas
B	Una vez a la semana	De conformidad con la Directiva 91/271/CEE (anexo I, sección D)	De conformidad con la Directiva 91/271/CEE (anexo I, sección D)	-		
C	Dos veces al mes			-		
D	Dos veces al mes			-		

Tabla 6. Parámetros mínimos de control para garantizar la seguridad de las aguas regeneradas para uso agrícola (Regulación 2020/741).

Clase de calidad de las aguas regeneradas	**Microorganismos indicadores (*)**	**Objetivos de rendimiento de la cadena de tratamiento (reducción de $\log_{10}$)**
A	E. coli	≥ 5.0
	Colífagos totales/ colífagos F-específicos/ colífagos somáticos/ colífagos (**)	≥ 6.0
	Esporas de *Clostridium perfringens*/ bacterias formadoras de esporas reductoras de sulfato (***)	≥ 4.0 (en caso de esporas de *Clostridium perfringens)* ≥ 5.0 (en caso de bacterias formadoras de esporas reductoras de sulfato)

(*) Los patógenos de referencia Campylobacter, rotavirus y Cryptosporidium también podrán emplearse para el control de validación, en lugar de los microorganismos indicadores propuestos. En ese caso, se aplicarán los siguientes objetivos de rendimiento (reducción de log$_{10}$): Campylobacter (≥ 5,0), rotavirus (≥ 6,0) y Cryptosporidium (≥ 5,0).

(**) Se ha seleccionado colífagos totales como el indicador viral más adecuado. No obstante, si no es posible el análisis de los colífagos totales, se analizará al menos uno de ellos (colífagos F-específicos o somáticos).

(***) Se han seleccionado las esporas de Clostridium perfringens como el indicador de protozoos más adecuado. No obstante, las bacterias formadoras de esporas reductoras de sulfato son una alternativa si la concentración de esporas de Clostridium perfringens no permite validar la reducción de log$_{10}$ solicitada.

En este reglamento encontramos también un Anexo II, el cual se centra en un plan de gestión del riesgo del agua regenerada. Este se basa en la identificación y evaluación de los riesgos asociados al uso de aguas regeneradas para reducir el posible daño de los agentes peligrosos para el medio ambiente y la salud humana y/o animal. Entre los agentes peligrosos a evaluar se encuentran metales pesados, plaguicidas, fármacos, microcontaminantes y microplásticos. Dicha evaluación estará formada por:

A. Una confirmación de la naturaleza del agente peligroso (si es evaluación del riesgo para el medio ambiente se incluirá el nivel sin efecto, pero si es evaluación del riesgo para la salud se añadirá la relación dosis-repuesta).
B. Se evaluará el rango de exposición o dosis
C. Por último, se caracterizarán los riesgos.

3. Recurso alternativo de fuentes de agua: aguas residuales tratadas/ regeneradas para riego agrícola y su impacto medioambiental

El principal consumidor de agua en todo el mundo es el sector agrícola (FAO Aquastat, 2014; Lavrnić et al., 2017). Acorde con la **figura 1** obtenida por el Informe Mundial de las Naciones Unidas sobre el Desarrollo de los Recursos Hídricos, podemos observar cómo el 70 % del agua dulce se destinada a uso agrícola. Por ello, es crucial la gestión del agua en este sector, con el fin de combatir el aumento de la preocupación por la escasez mundial de agua dulce (FAO, 2013).

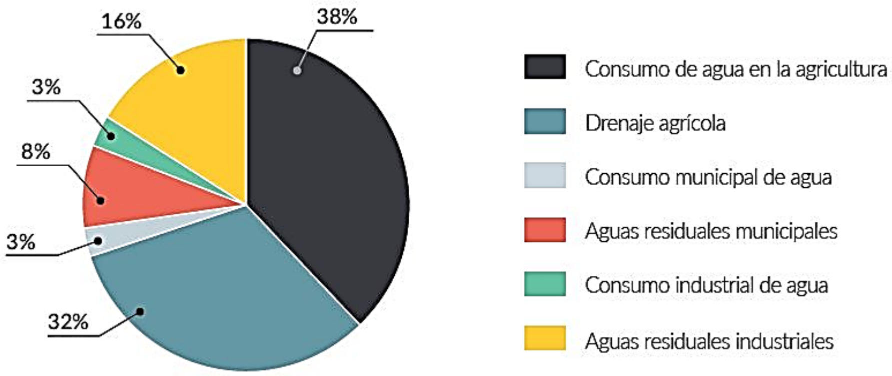

Figura 1. Destino del agua dulce a nivel mundial (fuente: Informe Mundial de las Naciones Unidas sobre el Desarrollo de los Recursos Hídricos). (Naciones Unidas, 2017)

Según las conclusiones tomadas por las Naciones Unidas se debe cambiar radicalmente la forma de utilizar, gestionar y compartir el agua, ya que el principal problema de la sociedad no es la falta de agua para satisfacer las necesidades, sino como la usamos y gestionamos (Naciones Unidas, 2017).

De acuerdo con el Instituto de Recursos Mundiales, países como Italia, Grecia o España estarán entre los países con peor escenario en los próximos años debido a la escasez de agua. (**figura 2**)

Los estados miembros de la Asamblea General de las Naciones Unidas establecieron en septiembre de 2015 la Agenda 2030 para el Desarrollo Sostenible, donde se incluían 17 objetivos (Naciones Unidas, 2017). En el sexto objetivo, donde se habla de los recursos hídricos, tenemos temas de especial importancia como:

- el agua potable y su saneamiento,
- una buena calidad del agua y aguas residuales,
- una mejora en la protección de ecosistemas y,
- un adecuado uso del agua para mitigar su escasez.

Una de las grandes alternativas para combatir esta escasez hídrica es la reutilización de agua residuales. En ella, en lugar de tratar el agua residual como un residuo, se convierte y se trata como un recurso hídrico. En las regiones áridas y semiáridas donde existe una mayor escasez de agua, la reutilización de agua es fundamental para gestionar los recursos de agua dulce (Ait-

Mouheb et al., 2018; Lavrnić et al., 2017; Morante-Carballo et al., 2022). En regiones áridas como Estados Unidos, Chile, Australia, Israel o Marruecos se está implantando desde hace años la reutilización de agua para uso agrícola (Jaramillo and Restrepo, 2017).

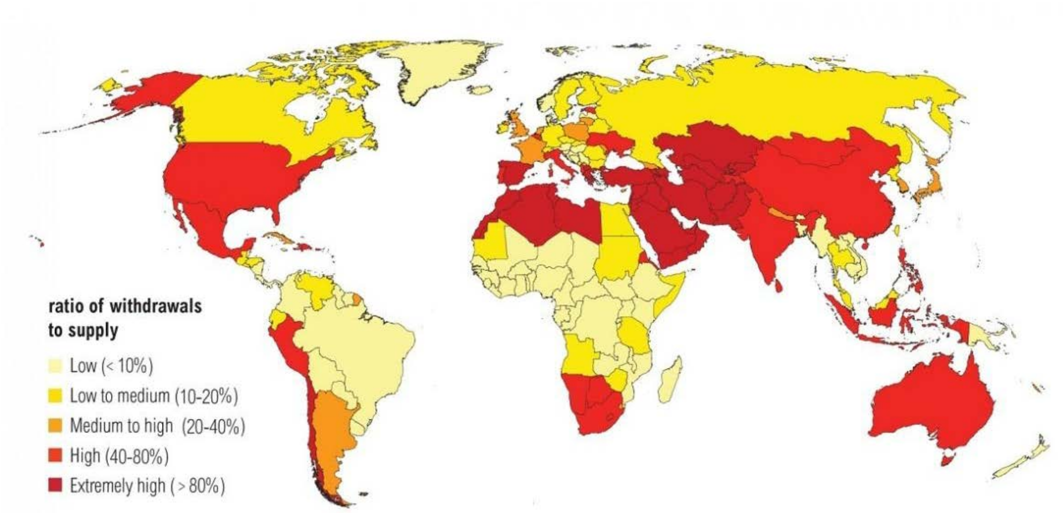

Figura 2. Estrés hídrico por países para el año 2040 según el Instituto de Recursos Mundiales.

La sociedad, de forma generalizada, cuando piensa en agua residual entiende que es agua de baja calidad afectada por influencias antropogénicas negativas (aguas usadas con fines domésticos, urbanos o industriales) (Naciones Unidas, 2017). Las aguas residuales son un recurso menospreciado a pesar de ofrecer oportunidades como sostenibilidad de agua, energía, nutrientes y materia orgánica. Sin embargo, actualmente alrededor del 80% las aguas residuales mundiales son liberadas sin ningún tipo de tratamiento, lo cual, se convertirá en una grave amenaza para el desarrollo sostenible porque potenciará la contaminación del agua en las próximas décadas (Boretti and Rosa, 2019).

Las aguas residuales tratadas o regeneradas son también denominadas aguas terciarias. Estas requieren de una planta de tratamiento de aguas que convierta dicha agua residual en un agua con condiciones óptimas para su destino final (ejemplo uso agrícola) (**Figura 3**). Aunque en las últimas décadas, el uso de agua regenerada se ha implementado cada vez más, hasta hace pocos años no ha sido más considerada. Esta importancia se ha originado por las ventajas que genera, como**:**

- disminuir las extracciones de agua fresca,
- contribuir positivamente en el suelo agrícola por contener nutrientes esenciales (lo cual actúa como fertilizante sin tener que hacer uso de ellos)
- impulsar la producción del cultivo.

Otro aspecto positivo de las aguas residuales tratadas para el riego agrícola es su contribución en la economía circular (EC), ya que el proceso de tratamiento de agua residuales potencia su cambio desde residuos a recursos, a través de reutilización y reciclado permite la sostenibilidad medioambiental y el ahorro de costes e ingresos de crecimiento para el agricultor (Naciones Unidas, 2020).

Ejemplos a seguir para alcanzar el concepto de economía circular en el sector del agua, serían, por un lago Singapur, donde el 40% de la demanda total de agua se cubre con agua regenerada producida en cuatro plantas del país y, por otro lado, Israel donde desde la década de los 80 han usado aguas residuales tratadas para cultivos y donde actualmente más del 50% del agua utilizada se dedica al sector de la producción agrícola (Avgar, 2018; Kog, 2020). Con relación a España, se ha previsto para 2025 una reutilización de aguas regeneradas de más de 1200 Mm^3/año (Beltrán et al., 2020).

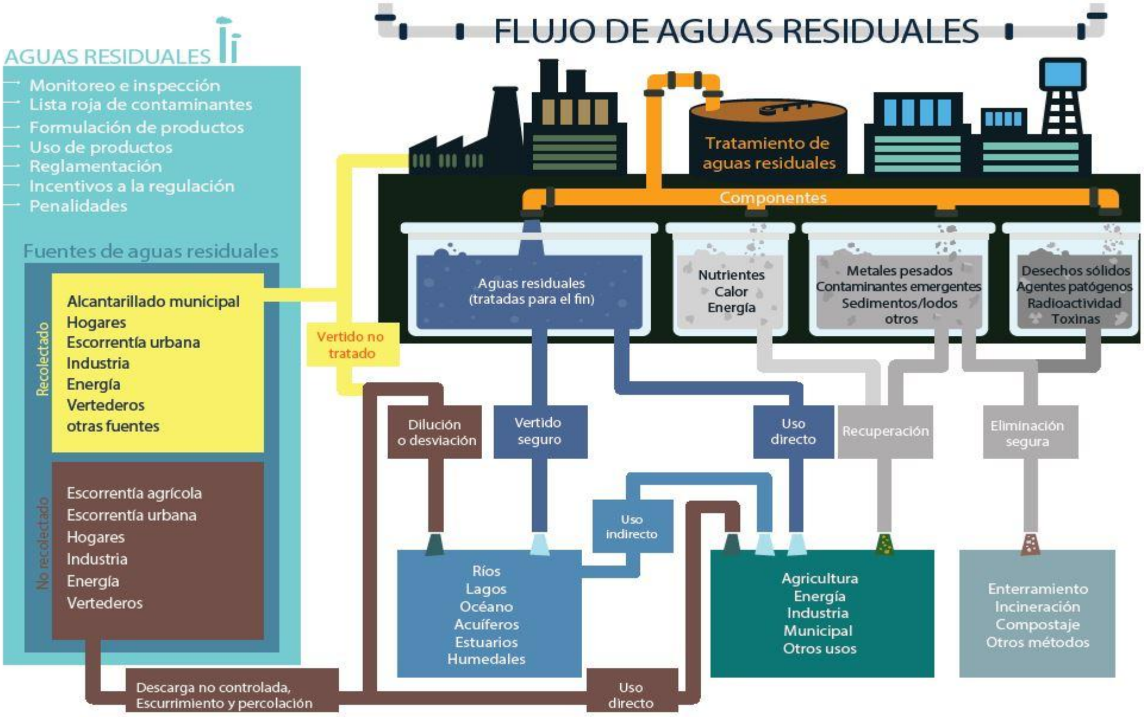

Figura 3. Flujo de aguas residuales en una planta de tratamiento de aguas residuales (fuente: Informe Mundial de las Naciones Unidas sobre el Desarrollo de los Recursos Hídricos)

Sin embargo, no hay que olvidarse de que las aguas residuales requieren de un adecuado tratamiento de limpieza para impedir serios problemas de contaminación que puedan producir problemas en la salud humana, en el medio ambiente y en el sistema económico (ej. aumento de número de personas enfermas por tener agua potable o agua de baño con menos calidad, degradación de los ecosistemas originando una reducción de la biodiversidad, incremento de la temperatura del agua, reducción de la producción tanto industrial como agrícola…) (Guven et al., 2023; Zahmatkesh et al., 2022).

Actualmente, existen numerosas publicaciones científicas que han demostrado la idoneidad del uso de las aguas residuales en el sector agrícola. Sin embargo, nos encontramos con pocas investigaciones sobre la aceptación de estas, en Europa, debido a que tradicionalmente no ha habido sequías, ni escasez de agua hasta los últimos años. La percepción del riesgo asociado a la salud (miedo a la contaminación) es el factor más significativo que determina la aceptación de la reutilización de aguas, por lo que hay que relacionar este riesgo con la confianza en las instituciones u organizaciones que supervisan la reutilización.

Una tendencia creciente y progresiva desde 2002 hasta 2022 se está originando respecto a la evolución de las investigaciones en torno al uso de aguas residuales en agricultura, obteniendo un total de 1261 publicaciones en estos 20 años (seleccionando las siguientes palabras en el buscador de Scopus: wastewater, reclaimed water, agricultura y crop). En la **figura 4** apreciamos como en los últimos años han aumentado significativamente la producción científica, siendo la categoría de Ciencias del Medioambiente la que posee mayor número de publicaciones (950) seguido de Ciencias Agrarias y Biológicas (379) e Ingeniería (179).

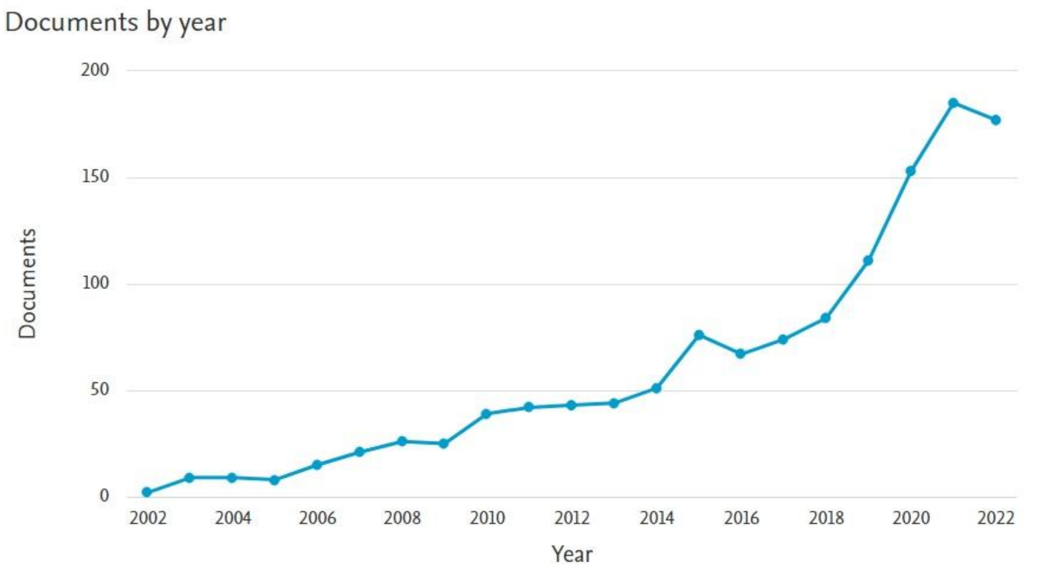

Figura 4. Artículos publicados en los últimos 20 años sobre uso sostenible de aguas residuales tratadas para riego agrícola sacado de la base de datos Scopus.

Los temas principales que aborda la categoría Ciencias del Medioambiente son el desarrollo de la sostenibilidad, el tratamiento de aguas residuales, la calidad y el suministro del agua. En cambio, los temas a tratar en Ciencias Agrarias y Biológicas son el regadío, la conservación del suelo, los problemas de los cultivos y la fertilización, mientras que para la categoría de Ingeniería son el tratamiento y reciclado de aguas residuales. Los países con mayor número de publicaciones en el ámbito del uso sostenible de las aguas residuales en agricultura son: en primer lugar, Estados Unidos con 280 (22%), España con 182 (14%) y China con un total de 148 (12%). Las dos principales líneas de investigación dentro de la temática de uso sostenible de aguas residuales son:

- líneas con perspectivas medioambientales donde encontramos términos como reutilización de aguas residuales, desarrollo de sostenibilidad, cambio climático o aguas subterráneas y,
- líneas de investigación relacionadas con la calidad del agua en términos de seguridad alimentaria y salud humana.

Respecto a la línea de investigación relacionada con la calidad del agua en términos de seguridad alimentaria y salud humana, en 2009 se iniciaron las primeras investigaciones, obteniendo un total de 157 publicaciones hasta 2022 (la búsqueda de publicaciones se realizó seleccionando las siguientes palabras en la base de datos, Scopus: emeging contaminants, wastewater, irrigation, agriculture, crop, plant uptake). En 2018 observamos un aumento significativo de la producción científica (ver **figura 5**). España fue el segundo país con mayor número de publicaciones (35) y la categoría más demandada fue Ciencias del Medioambiente con más de la mitad de la producción científica publicada en ella (53%).

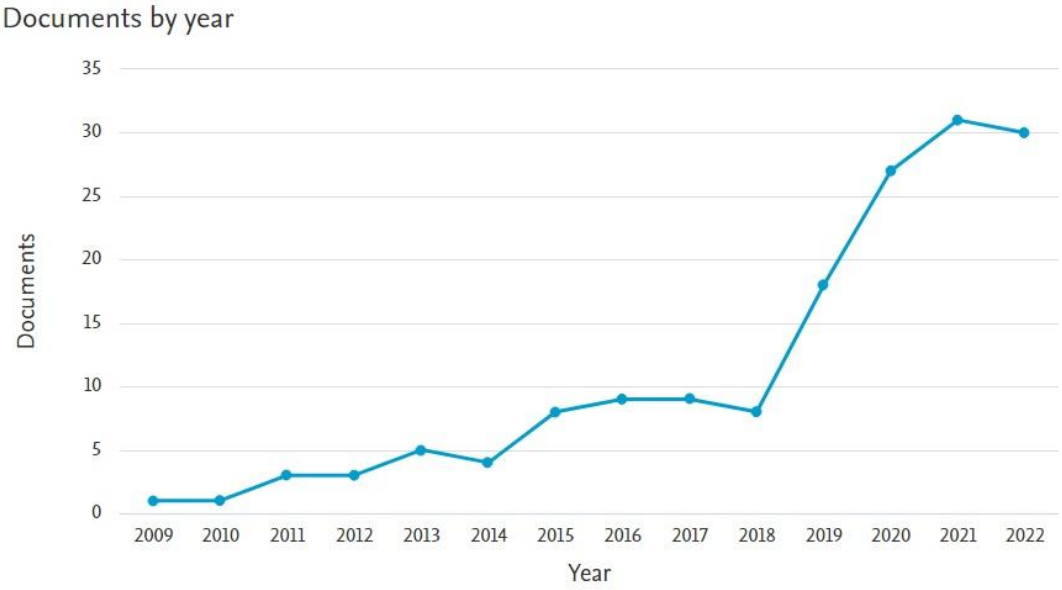

Figura 5. Artículos publicados en los últimos años sobre absorción de contaminantes emergentes provenientes de aguas regeneradas en cultivos agrícolas, sacado de la base de datos Scopus.

Aunque la reutilización de aguas tiene grandes beneficios, existen cuestiones sin responder, como, por ejemplo, consecuencias del uso de estas aguas a largo plazo. Las plantas de tratamiento de aguas no están diseñadas para eliminar completamente el gran número de contaminantes de preocupación emergente que entran en ellas, y es por ello por lo que algunos de ellos son detectados una vez han sido tratadas/regeneradas (Anagnostopoulou et al., 2022; Martínez Bueno et al., 2012; Rodríguez-Hernández et al., 2022). Además, las propiedades físico-químicas del contaminante pueden afectar a su eliminación en las plantas de tratamiento de aguas residuales. Por ejemplo, los compuestos con bajo coeficiente de absorción tienden a permanecer en la fase acuosa favoreciendo su movilidad por la planta y acabar en el agua receptora (Lopez et al., 2022).

Los contaminantes orgánicos de preocupación emergente son compuestos, especies químicas o elementos de origen sintético o natural que tiene efecto perjudicial sobre la biosfera. La mayoría son microcontaminantes orgánicos que se encuentran en concentraciones de µg/L o ng/L, que pueden causar efectos adversos en los ecosistemas y en la salud humana, además la mayoría no están actualmente regulados por administraciones públicas (Bustos Bustos et al., 2022; Rodríguez-Hernández et al., 2022). Lo cual puede ser que el uso de estas aguas a largo plazo genere problemas medioambientales y/o de salud humana.

Las propiedades físico-químicas de los estos contaminantes orgánicos determinan su destino y bioacumulación en el suelo, su persistencia en el medioambiente (Christou et al., 2019; Fu et al., 2019; Jaramillo and Restrepo, 2017), y su posterior translocación a través de los cultivos (Beltrán et al., 2020; García-Valverde et al., 2023; Picó et al., 2019; Wu et al., 2014). A pesar de que los artículos científicos publicados, muestran que los niveles de contaminantes encontrados no suponen repercusiones significativas para la salud humana (García-Valverde et al., 2023; Martínez Bueno et al., 2021), siguen existiendo lagunas de conocimiento para evaluar plenamente las consecuencias sobre la salud de la reutilización de aguas residuales regeneradas. Sobre todo, en relación con la presencia de una serie de compuestos susceptibles de translocarse a través de los cultivos y sus efectos sinérgicos (Rodriguez-Mozaz et al., 2021). Todavía es escaso el número de investigaciones sobre la evaluación de absorción en plantas de contaminantes emergentes en muestras bajo condiciones reales, puesto que la mayoría son en condiciones controladas en laboratorios (condiciones no agronómicas) (Carter et al., 2018, 2014; González García et al., 2018; Malchi et al., 2014; Wu et al., 2013).

Por tanto, son necesarios más estudios de investigación sobre este tema mediante el desarrollo y la aplicación de metodologías analíticas multiresiduo, así como bioensayos para comprender las implicaciones toxicológicas y las consecuencias de la reutilización en la agricultura con trascendencia para la salud humana y medioambiental.

3.1. Contaminantes orgánicos de preocupación emergente en aguas regeneradas

En las últimas décadas, los estudios sobre agua medioambiental han llamado la atención debido a la presencia de una gran variedad de contaminantes orgánicos de origen antropogénico (Agathokleous et al., 2023). Estos contaminantes son descargados en el medio ambiente a través de diferentes vías, aunque la principal fuente son las plantas de tratamiento de aguas residuales (Kumar et al., 2022; Martínez Bueno et al., 2012; Rodriguez-Mozaz et al., 2021) Las plantas de tratamiento de aguas residuales convencionales no son capaces de eliminar completamente muchos de estos contaminantes y sus productos de transformación, y como consecuencia muchos de ellos se vierten continuamente en las aguas naturales, lo que provoca la contaminación y/o el deterioro de estas (Ahmed et al., 2022; Guven et al., 2023).

Los tratamientos terciarios aplicados normalmente (radiación ultravioleta y sistema de cloración) se centran en cumplir las normas estipuladas en la normativa vigente (Regulación 2020/741), que sólo tiene en cuenta los aspectos microbiológicos. A pesar de que se han realizado mejoras en los tratamientos biológicos secundarios convencionales, es necesario aplicar procesos terciarios como, procesos de oxidación avanzada o de ultrafiltración, para alcanzar una eliminación más eficaz de estas sustancias químicas, cuando el objetivo es la recuperación y/o reutilización del agua, especialmente con fines agrícolas(Lopez et al., 2022; Rodríguez-Hernández et al., 2022; Vergine et al., 2020).

Cuando hablamos de contaminantes de preocupación emergentes nos referimos a fármacos, productos de cuidado personal, antibióticos, retardantes de fuego, aditivos de las comidas, metales, surfactantes o pesticidas (Ahmed et al., 2022; Fortunato et al., 2021; Mohapatra et al., 2023; Zahmatkesh et al., 2022). Además, los procesos degradativos naturales como la fotodegradación, originan la formación de especies químicas transformadas llamados productos de transformación que pueden presentar una mayor toxicidad que las moléculas originales (Kanan et al., 2022). Desde las últimas décadas, como resultado del desarrollo de estrategias analíticas, los controles a escala mundial de efluentes de aguas residuales, ríos y aguas subterráneas han revelado la presencia de estos compuestos en ng-µg/L (Farré et al., 2007; Gorga et al., 2013; Kosma et al., 2014; Kuster et al., 2008; Martínez Bueno et al., 2012, 2010; Nieto-Juárez et al., 2021; Ofrydopoulou et al., 2022; Petrović et al., 2003; Quintana et al., 2019). En la **tabla 7** podemos ver los productos farmacéuticos y plaguicidas detectados en agua de riego agrícola en los últimos años.

En años previos, los productos farmacéuticos han sido de gran interés ya que no se absorben ni se metabolizan completamente en humanos y animales (Bavumiragira et al., 2022; Feo et al., 2020; Martínez Bueno et al., 2012; Rodil et al., 2012). Se conoce que un alto porcentaje de la dosis ingerida se excreta de forma natural pocas horas después de la aplicación, ya sea como compuesto original o como metabolitos (Bustos Bustos et al., 2022; Christou et al., 2019). Debido a su presencia constante en las aguas ambientales y a sus efectos medioambientales adversos, algunos de estas sustancias se han incluido en la lista de vigilancia u observación (Decisión 2018/840/EC).

El extendido uso de los antibióticos para combatir microorganismos patógenos en animales y humanos ha producido un aumento del volumen descargado de estos fármacos en las aguas superficiales, originando resistencia microbiana. Debido a esto, está surgiendo en la comunidad científica uno de los problemas emergentes más serios en química ambiental. Los antibióticos pueden inducir la generación y propagación de bacterias resistentes a estos en las plantas de tratamiento de agua, en los suelos expuestos, en los cultivos y, por último, en el intestino del consumidor (Bustos Bustos et al., 2022; Christou et al., 2019; Garrido-Cardenas et al., 2020; Jaramillo and Restrepo, 2017; Lopez et al., 2022; Rodriguez-Mozaz et al., 2015; Wang et al., 2019). Esto es especialmente preocupante a nivel de salud pública si los procesos de desinfección para la recuperación del agua no son eficaces a la hora de inactivarlos; lo que significa su probable introducción en la cadena alimentaria a través del consumo de vegetales (Ben Mordechay et al., 2022; Christou et al., 2019; Fu et al., 2019). Entre los antibióticos incluidos en la lista de vigilancia encontramos los tres macrólidos azitromicina, claritromicina y eritromicina. El antibiótico azitromicina se utiliza para el tratamiento de infecciones respiratorias. En los últimos años ha estado en auge por su uso masivo para tratar el COVID-19, lo cual puede verse exacerbado por la pandemia actual (Bustos et al., 2022).

Los antiinflamatorios no esteroideos son los fármacos que se administran para tratar la fiebre, dolor y controlar la inflamación (Kalambate et al., 2021). Dentro de este grupo encontramos el ibuprofeno, naproxeno o diclofenaco, representando el 15% del total de fármacos detectados en muestras medio ambientales (Świacka et al., 2021). Diclofenaco tiene un consumo anual global de 1.443 tonelada, sin embargo, la tasa de eliminación de este analgésico en las EDAR es del 75% (Almeida et al., 2020; Martínez Bueno et al., 2012).

Los antidepresivos son otro grupo de compuestos orgánicos que se utilizan para tratar y controlar enfermedades mentales como depresión o insomnio. Este fármaco ha sido muy comercializado a nivel mundial en los últimos 20 años debido a que los trastornos depresivos afectan a más de 350 millones de personas (Murtada et al., 2019). Al igual que se ha producido un aumento en el consumo del antibiótico azitromicina, los antidepresivos también se han incrementado con los efectos en la salud mental que ha producido la pandemia en los recientes años debido al aislamiento social, desempleo o reducción de ejercicio físico (Melchor-Martínez et al., 2021). Por lo que se estima un aumento de este fármaco en las plantas de tratamiento de aguas y su eliminación tomará gran importancia próximamente.

Desde hace mucho tiempo, los pesticidas se han venido usando para combatir plagas no deseadas y mejorar el rendimiento de los cultivos. El problema principal es que son contaminantes poco estudiados en el medio acuático, debido a que se han considerado contaminantes de origen agrícola y no urbano (Paíga et al., 2021). Hasta el día de hoy, se conoce que el consumo mundial de pesticidas está en torno a 3,5 millones de toneladas al año (Huang et al., 2019). Además, aproximadamente el 70% de los fertilizantes aplicados en cultivos agrícolas pueden perderse en el medio ambiente debido a la lixiviación, descomposición y volatilización del amonio en el suelo, agua o aire (Duan et al., 2023). Es por ello, que se está generando una preocupación emergente por los posibles efectos a largo plazo que pueden causar a través del consumo de productos alimenticios (Christou et al., 2017; García-Valverde et al., 2023). En consecuencia, la Unión Europea estableció una lista de sustancias prioritarias en el ámbito de la política de aguas con sus normas de calidad ambiental (Directiva 2013/39/UE) donde aparecían plaguicidas junto con productos farmacéuticos, además de otra lista de vigilancia u observación (Decisión 2018/840/EC), la cual incluye cinco pesticidas neonicotinoides (tiacloprid, imidacloprid, tiametoxam, acetamiprid y clotianidina).

Tabla 7. Contaminantes orgánicos de preocupación emergente encontrados en aguas de riego agrícolas.

PAIS	COMPUESTOS	CONCENTRACIÓN (ng/L)	REFERENCIA
España, Madrid	Atenolol, carbamazepina, triclosán	15 – 491,8	*Beltrán et al., 2020*
España, Cádiz	Acetaminofeno, diclofenaco, ibuprofeno, ketoprofeno, naproxeno, bezafibrato, fenofibrato, gemfibrozilo, atenolol, propanolol, carbamazepina, cafeína, ciprofloxacino, ofloxacino, claritromicina, eritromicina, sulfametoxazol, trimetoprima, famotidina, ranitidina, furosemida, hidroclorotiazida	7 – 750	*Biel-Maeso et al., 2018*

PAIS	COMPUESTOS	CONCENTRACIÓN (ng/L)	REFERENCIA
España, Caldes de Montbui	Ibuprofeno, carbamazepina, naproxeno, diclofenaco, triclosán, tonalida, galaxólida, MCPA, simacina, atrazina, diazinón, alacloro, clorfenvinfos	< LOQ – 22,4 *	*Calderón-Preciado et al., 2013*
Chipre, Nicosia	Diclofenaco, sulfametoxazol, trimetoprima	22,1 – 55,2	*Christou et al., 2017*
España, Almería	Claritromicina, metronidazol, ofloxacino, sulfametoxazol, trimetoprima, diclofenaco, ketoprofeno, ácido mefenámico, naproxeno, tramadol, lidocaína, carbamazepina, lamotrigina, citalopram, venlafaxina, amisulprida, sulpirida, famotidina, furosemida, hidroclorotiazida, cafeína, ácido fenofíbrico, gemfibrozilo, atenolol, bisoprolol, propanolol, irbesartán, telmisartán, valsartán, azoxystrobin, carbendacima, propamocarb, tiabendazol, diurón, terbutrina, acetamiprid, DEET, imidacloprid	< LOQ – 130,7 *	*García-Valverde et al., 2023*
Israel, Rejovot	Bezafibrato, ácido clofíbrico, gemfibrozilo, diclofenaco, ibuprofeno, ketoprofeno, naproxeno, carbamazepina, lamotrigina, cafeína, metoprolol, sulfametoxazol, sulfapiridina	< 0,01 – 1,9 *	*Malchi et al., 2014*
España, Cataluña	Diclofenaco, ofloxacino, carbamazepina, naproxeno	454 - 1390	*Sunyer-Caldú et al., 2022*
España, Almería	4-AAA, 4-FAA, atenolol, azitromicina, bezafibrato, cafeína, carbamazepina, carbamazepina epóxido, cetirizina, citalopram, claritromicina, clomipramina, diazepam, eprosartán, ácido fenofíbrico, furosemida, gemfibrozilo, hidroclorotiazida, indometacina, irbesartán, ketoprofeno, lidocaína, lincomicina, naproxeno, , propanolol, ranitidina, salbutamol, sulfametoxazol, telmisartán, tramadol, trimetoprima, venlafaxina	15 – 14.424	*Martínez-Piernas et al., 2019*

PAIS	COMPUESTOS	CONCENTRACIÓN (ng/L)	REFERENCIA
Estados Unidos, California	Acetaminofén, atenolol, cafeína, carbamazepina, DEET, diclofenaco, dilatín, gemfibrozilo, ibuprofeno, naproxeno, primidona, sulfametoxazol, triclocarban, triclosán, trimetoprima, atorvastatina, diazepam, fluoxetina,	0,44 - 181	*Wu et al., 2014*
Israel, Rejovot	Benzotriazol, 4-aminoantipirina, acetaminofén, diclofenaco, ketoprofeno, atenolol, bisoprolol, digoxigenina, digoxina, diltiazem, doxazosina, metoprolol, sotalol, carbamazepina, carbamazepina epóxido, lamotrigina, difenhidramina, alprazolam, diazepam, fluoxetina, venlafaxina, crotamitón, tiabendazol, bezafibrato, ácido clofíbrico, codeína, cafeína, nicotina, azitromicina, ciprofloxacino, claritromicina, eritromicina, lincomicina, lomefloxacina, metilparabeno, miconazol, norfloxacino, ofloxacino, ormetoprim, ácido oxolínico, sulfadiazina, sulfadimetoxina, sulfamerazina, sulfametoxazol, triclocarban, trimetoprima, tilosina, virginiamicina M1, virginiamicina S1,	4 – 69.623	*Ben Mordechay et al., 2021*

* concentración expresada en µg/L

3.2. Impacto en el medio agrícola de la reutilización de agua

En los últimos años, el sector agrícola está buscando mejoras para mantener o incrementar la producción de los cultivos a la vez que reducir costes (Moreno-Teruel et al., 2022; Valera et al., 2017). Aunque se están intentando implantar prácticas agrícolas sostenibles como los empleados en la agricultura orgánica, es muy difícil cambiar la practica agrícola (Steingrímsdóttir et al., 2018). Debido a factores como el aumento de la población y/o el cambio climático, la utilización de aguas residuales tratadas para riego de cultivos, está siendo una alternativa en auge, ya que permite gastar menos cantidad de agua dulce además de reducir la aplicación de fertilizantes y por tanto costes Esta opción es acertada en regiones donde la demanda de agua es superior a la disponibilidad de esta (Morante-Carballo et al., 2022). Sin embargo, el uso de aguas residuales tratadas o regeneradas pude conducir a una contaminación asociada con la adsorción y/o acumulación de contaminantes de preocupación emergente en los suelos agrícolas, además de su posible posterior translocación desde los tejidos de la planta hasta el fruto o la parte comestible (García-Valverde et al., 2023; Martínez Bueno et al., 2021; Picó et al., 2019). En la **tabla 8** se muestra los contaminantes orgánicos detectados en frutos provenientes de cultivos regados con aguas tratadas o aguas fortificadas.

Tabla 8. Resumen de los contaminantes de preocupación emergente detectados en frutos de cultivos regados con aguas tratadas o aguas fortificadas en los últimos 12 años.

PAIS	FRUTO	COMPUESTOS	CONCENTRACIÓN (µg/Kg)	REFERENCIA
España, Madrid	Lechuga, maíz, rábano	Atenolol; Carbamazepina; Triclosán	0.5-2; 0-0.5; 0.5-1	*Beltrán et al., 2020*
España, Barcelona	Lechuga, zanahoria, judía verde	Ácido clofíbrico; Ibuprofeno; Carbamazepina; Naproxeno; Diclofenaco; Tonalida; Galaxolida; Simazina; Atrazina; Diazinon; Alachlor; Clorfenvinfos	< LOD – 18; < LOD – 30; < LOD – 60; < LOD – 113; < LOD – 19; < LOD – 124; < LOD – 270; < LOD; < LOD – 573; < LOD – 89; < LOD; < LOD	*Calderón-Preciado et al., 2013*
España, Almería	Tomate, pepino, pimiento	Carbamazepina; lidocaína; Cafeína	0.2-1.7; 0.1- 0.6; 0.4-1.7	*García-Valverde et al., 2023*
España, Murcia	Lechuga	Carbamazepina; Ibuprofeno; Ketoprofeno; Naproxeno	250 -1000; 5 -25; 25 – 80; 10 - 60	*González García et al., 2019*
Estados Unidos, Colorado	Lechuga,	Carbamazepina; Difenhidramina; Sulfametoxazol; Trimetoprima; TCEP	0.25 – 2; 0.05 – 0.4; 0.05 – 0.4; 0.1 – 0.7; 1 – 40;	*Hyland et al., 2015*
Israel, Rejovot	Zanahoria, batata	Carbamazepina; Lamotrigina, Cafeína; Sildenafilo; Ácido Clofíbrico; Bezafibrato; Sulfapiridina; Sulfametoxazol;	1 – 10; 0.5 – 10; 1 -5; 1 – 2.5; 1 – 3; 2 – 4; 0.25 – 0.5; 0.1 – 0.25;	*Malchi et al., 2014*
España, Barcelona	Lechuga	Dimetomorf; Surfynol 104; BPF; Carbamazepina; Carbendacima; Carbamazepina epóxido	0.59 – 19.5; 4.07 – 7.57; 1.61 – 62.0; 0.11 – 0.36; 2.2 – 19.9; 0.06 – 0.21	*Margenat et al., 2018*

PAIS	FRUTO	COMPUESTOS	CONCENTRACIÓN (µg/Kg)	REFERENCIA
España, Almería	Tomate	4-AAA; Cafeína; Carbamazepina; Carbamazepina epóxido; Hidroclorotiazida; Mepivacaína; Tramadol; Venlafaxina	<LOQ – 0.4; <LOQ – 1 <LOQ – 0.2; <LOQ – 0.07; <LOQ – 0.2; <LOQ – 0.1; 0.1 – 0.7; <LOQ – 0.1	*Martínez-Piernas et al., 2019*
Israel, Rejovot	Calabacín	Carbamazepina	6.4 – 25.6	*Shenker et al., 2011*
Israel, Rejovot	Aguacate, banana, zanahoria, naranja, judías verdes, patata, tomate	Acetaminofén; Aspartamo; Atenolol; Benzotriazol; Bisoprolol; Cafeína; Carbamazepina; Dihidroxi carbamazepina; Carbamazepina epóxido; Lamotrigina; Metoprolol; Nicotina; Tiabendazol; Venlafaxina	5; 7 – 10; 10; 50; 0.1 – 1; 10 – 50; 1 – 500; 1 – 10; 0.05 – 100; 1 – 10; 1; 1 – 10; 0.1 – 10; 0.1 – 5	*Ben Mordechay et al., 2021*
Arabia Saudita, Riad	Col, cebada, berenjena, judía verde, tomate, chili, calabacín	Atenolol; Atrazina desetil; Azoxystrobin; Bupirimato; Cafeína; Diazinón; Gemfibrozilo; Malatión; Pirimicarb	55 (col); 42 (berenjena); 892 (chili); 250 (calabacín); 48-125 (calabacín); 250 (tomate); 75 (col); 125 – 1000 (col); 98 (col)	*Picó et al., 2019*

En la actualidad, la evaluación de contaminantes de origen emergente en sistemas agroalimentarios carece de un enfoque armonizado. Para poder avanzar, haría falta realizar estudios de prevalencia para comprender lo que se sabe y lo que no, de los contaminantes en el entorno agrícola. El mayor reto para comprender la acumulación de estas sustancias en las plantas es el amplio número de contaminantes que existen, sus diferentes propiedades físico-químicas y sus productos de transformación. Por ello, se necesita crear un marco de trabajo para identificar aquellos contaminantes que presentan/suponen un mayor riesgo y daño potencial para la salud humana, según datos de absorción y acumulación en los alimentos. El movimiento de los contaminantes en el ciclo agua-suelo-planta es un proceso dinámico. Una mejor comprensión del flujo de agua en el suelo y los sistemas vegetales y, de los flujos químicos de estas sustancias, es esencial para dilucidar el transporte y la acumulación de estos.

3.2.1. Destino de los contaminantes en el suelo agrícola

El suelo es el receptor inicial de los contaminantes cuando los cultivos son regados con aguas residuales regeneradas. Diferentes aspectos deben ser considerados para evaluar el destino de los contaminantes emergentes en los sistemas agrícolas debido a su complejidad. En la **figura 6** se puede ver de forma general los factores que influyen para que una sustancia química sea absorbida por la planta y el suelo agrícola. Principalmente, existen 3 factores que van a determinar la adsorción de un compuesto orgánico en el suelo y, por tanto, su biodisponibilidad y potencial de absorción por las plantas. Estos son:

- Propiedades físico-químicas del contaminante (polaridad, pKa, solubilidad en agua e hidrofobicidad)
- Propiedades físico-química del suelo (contenido en arcilla, arena, en materia orgánica, comunidad microbiana del suelo)
- Química del poro de agua del suelo (pH, concentración mineral, capacidad de intercambio catiónico y materia orgánica disuelta)

La adsorción tiene un papel importante en la concentración de contaminante en el poro de agua del suelo y por consiguiente en la disponibilidad de este para su absorción por la planta. Si un contaminante es adsorbido por el suelo, se reduce su entrada en la planta. Esto es especialmente dado cuando el contaminante tiene alta hidrofobicidad (Peña et al., 2020). En cambio, los contaminantes con baja capacidad de adsorción al suelo, permanecer en la fase acuosa y son absorbidos fácilmente por las plantas, aunque pueden sufrir procesos de lixiviación o escorrentía. Por tanto, las propiedades químicas de los contaminantes químicos influyen en su destino medioambiental y entre las más importantes encontramos polaridad, hidrofobicidad y solubilidad en agua.

Las propiedades físico-química del suelo también van a determinar el destino de las sustancias/especies químicas. Cultivos que crecen en suelos agrícolas con bajo contenido de materia orgánica o suelos arenosos, tienen mayor probabilidad de adsorber contaminantes (Christou et al., 2019). Los contaminantes polares e ionizables, tienen mayor capacidad para interactuar con la materia orgánica del suelo. Por tanto, en suelo con pH > pKa del compuesto, tienen menos potencial para ser captadas por las plantas, debido a las fuerzas de repulsión ejercidas por la epidermis radicular (que está cargada negativamente). En cambio, los suelos con pH ácidos (pH < pKa del compuesto) pueden originar la presencia del contaminante en forma neutra facilitando la absorción por la planta.

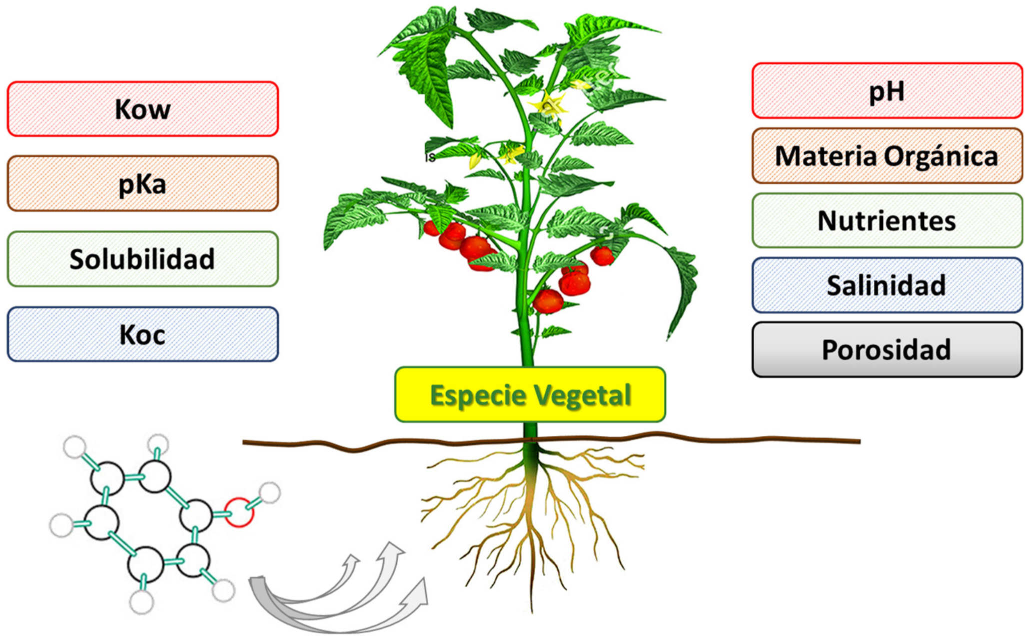

Figura 6. Esquema de los principales factores que influyen en la absorción de contaminantes orgánicos de preocupación emergente

La degradación abiótica y biótica también va a determinar la disponibilidad de los contaminantes para su absorción en la planta. La transformación mediante procesos bióticos puede ser a veces más rápida o lenta según las comunidades microbianas del suelo, el pH, la humedad o las propiedades físico-químicas del contaminante (Fu et al., 2019). Entre los procesos abióticos encontramos, la fotólisis por radiación solar, la hidrólisis o las reacciones de oxidación-reducción (Fortunato et al., 2021; Mohapatra et al., 2023). Además, la aplicación de aguas residuales tratadas tiene potencial para alterar las características bióticas y abióticas del suelo.

Hasta el día de hoy, los estudios para evaluar la adsorción de contaminantes en el suelo se han llevado a cabo con un número limitado de contaminantes, ignorando los efectos que pueden darse por mezclas químicas (Biel-Maeso et al., 2018; Caldas et al., 2011; Feng et al., 2015; Fernandes et al., 2013; García-Galán et al., 2013; Grossberger et al., 2014; Lee et al., 2017; Paz et al., 2016; Salvia et al., 2012).

3.2.2. Destino de los contaminantes en el cultivo agrícola

Las raíces son el punto de entrada de contaminantes desde el suelo a la planta a través de los poros de agua del suelo. Los compuestos entran en la raíz a través de la epidermis de las puntas radiculares en crecimiento y posteriormente atraviesan la corteza y la endodermis para llegar a los tejidos vasculares, donde pueden ser transportados por el xilema o floema a los tejidos aéreos (Fu et al., 2019).

En los últimos años, se ha propuesto una relación sigmoidal entre el factor de concentración de translocación y la hidrofobicidad (Log Kow) para una amplia gama de contaminantes con diferentes propiedades (Bagheri et al., 2019; Dettenmaier et al., 2009). Sin embargo, la translocación de los contaminantes orgánicos de origen emergente no depende solamente de la hidrofobicidad y se deben tener en cuenta otros parámetros como pKa, carga y tamaño molecular. Por tanto, la absorción de contaminantes por la raíz está determinada por:

- las propiedades físico-químicas del contaminante (como el tamaño molecular, carga e hidrofobicidad o lipofilia),
- la fracción biodisponible en el suelo y
- el tipo de cultivo (por ejemplo, cultivos con raíces con alto contenido lipídico origina menos translocación de contaminantes) (Carter et al., 2014; Christou et al., 2019; Malchi et al., 2014).

Los contaminantes orgánicos no iónicos (neutros) son capaces de atravesar fácilmente las membranas celulares y, por tanto, ser absorbidos por las raíces. Estos son transportados del xilema al floema y viceversa, en dirección a la corriente transpiración y, ser acumulados predominantemente en las hojas. En cambio, los contaminantes orgánicos ionizables (cationes y aniones) pueden disociarse en el poro de agua del suelo en función del pH y, según la hidrofobicidad del compuesto y ser más o menos absorbido y transportado en la planta. Los contaminantes iónicos (cationes) atrapados, se translocan en el floema más que por el xilema acumulándose en el fruto. En cambio, los aniónicos van a ser menos traslocados y serán más cumulados en las células de las raíces.

Los compuestos iónicos atraviesan las membranas celulares a una velocidad más lenta que los no iónicos, debido a atracciones y repulsiones que sufren con la superficie de la raíz cargada negativamente y los efectos de trampa de iones. En los últimos años se ha demostrado que carbamazepina o cafeína son fácilmente absorbidos por las raíces por ser compuestos no iónicos (Ben Mordechay et al., 2021; Carter et al., 2014; González García et al., 2018; Shenker et al., 2011).

En general, los contaminantes orgánicos de origen emergente neutros o catiónicos van a ser absorbidos por las raíces debido a 3 factores:

- Atracción eléctrica originada por la carga negativa de la superficie de la raíz y la carga positiva de los cationes.
- La acumulación en vacuolas debido a la trampa de iones.
- Adsorción en raíz cuando los compuestos tienen valores altos de Log Kow.

Previos estudios reportaron que los contaminantes orgánicos ácidos con pKa < 7 y log Kow < 3 permanecen en el floema debido a mecanismos de atrapamiento de iones y originan su acumulación en frutos. Mientras que los básicos con pKa > 7 y log Kow < 4 se desplazan por el xilema acumulándose en hojas (Miller et al., 2016).

La absorción y bioacumulación de contaminantes orgánicos de preocupación emergente en la parte comestible de los cultivos y su posterior entrada en la cadena alimentaria ha cobrado importancia en los últimos años. La mayoría de las investigaciones han sido realizadas bajo condiciones controladas donde se ha puesto de manifiesto que las plantas expuestas a estas sustancias a través del riego con agua residual tratada, agua fortificada o en suelos contaminados, absorben y acumulan dichos contaminantes en sus tejidos en concentraciones de µg/Kg (Beltrán et al., 2020; Ben Mordechay et al., 2021; Calderón-Preciado et al., 2013; Carter et al., 2018; García-Valverde et al., 2023; Hyland et al., 2015; Margenat et al., 2018; Martínez-Piernas et al., 2019; Montemurro et al., 2017; Picó et al., 2019). Se ha podido descubrir que la absorción depende de la transpiración y de las propiedades físico-químicas del contaminante (hidrofobicidad, estructura química, o carga) (González García et al., 2019).

Sin embargo, los mecanismos de transporte de los contaminantes orgánicos de origen emergente dentro del sistema vascular de la planta y su metabolización son complejos y existe todavía poca información. Por ello, hay que seguir investigando para entender las transformaciones y caminos que siguen estas sustancias químicas dentro del sistema vegetal.

4. Metodologías analíticas para la evaluación de contaminantes orgánicos en muestras medioambientales

La determinación de contaminantes de preocupación emergente en muestras medio ambientales sólidas requiere de ciertas etapas. La **figura 7** muestra de forma esquemática estas etapas a seguir.

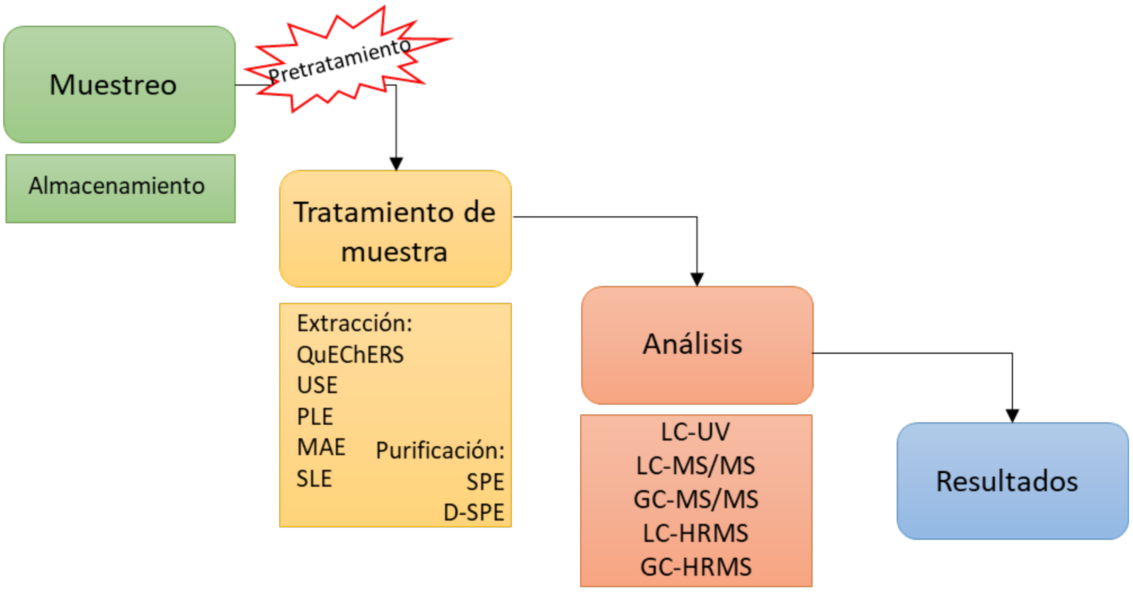

Figura 7. Esquema de las etapas a seguir en un análisis de contaminantes orgánicos de preocupación emergente

4.1. Muestreo

La primera etapa, de un proceso analítico, es el muestreo. Puede definirse como el proceso de selección de una pequeña porción de material, representativo del medio, la cual será transportada y manipulada posteriormente en el laboratorio. Esta etapa debe ser realizada con sumo cuidado ya que es crucial y determina la calidad de los resultados obtenidos. Los principales problemas que nos encontramos durante la toma de muestra es la falta de representatividad de muestra y la mala conservación de esta hasta su análisis (Wilga et al., 2008).

Por un lado, las muestras deben tomarse como muestras compuestas siguiendo un protocolo esquematizado para garantizar la representatividad de las muestras. Además, los materiales de almacenamiento son muy importantes y se deben utilizar recipientes de vidrio y con color ámbar para evitar posibles interacciones entre los analitos y el plástico o que se origine foto-degradación de los analitos. Por último, deben almacenarse las muestras a baja temperatura (-20ºC) para mantener la estabilidad y evitar la transformación de los analitos.

4.2. Tratamiento de la muestra

Después de la toma de muestra y antes de los procesos de extracción es necesaria, en algunos casos, la aplicación de etapas previas de pretratamiento. El pretratamiento es necesario para adaptar las muestras al método de extracción seleccionado y así asegurar reproducibilidad en el análisis. Por ejemplo, las muestras de suelo son deshidratadas a 30ºC durante 24 horas y tamizadas, mientras las muestras de fruto o planta son trituradas en pequeñas piezas usando un protocolo reproducible.

4.2.1. Extracción muestras agrícolas sólidas

En la etapa de extracción de muestra hay que considerar parámetros fundamentales relativos a la naturaleza de los compuestos a analizar, así como sus propiedades físico-químicas, la interacción matriz/contaminante y la complejidad de la matriz a estudiar (**tabla 9**) (Fernandes et al., 2013; Masiá et al., 2015). Uno de los desafíos, desde el punto de vista analítico durante la optimización de un método multi-analítico, son las etapas de extracción y purificación cuando los compuestos a analizar tienen diferentes propiedades, solubilidades en agua y constantes de disociación (pKa) (Vera et al., 2014).

- Polaridad: un parámetro que suele usarse para establecer la polaridad de un compuesto es el *coeficiente de partición de octanol-agua* (*Kow*). Actualmente, existen valores tabulados de este coeficiente o de su logaritmo decimal (*log Kow*) para muchos de los compuestos orgánicos. Según Fu et al., los compuestos con log Kow < 1 son polares, 1< log Kow < 3 tienen polaridad intermedia y los compuestos con log Kow > 3 son apolares.
- Acidez/Basicidad: pKa es el logaritmo negativo de la constante de acidez (Ka) y expresa el grado de acidez o basicidad de un compuesto en un disolvente determinado. A menor pKa mayor acidez. Sirve para establecer en que magnitud un compuesto estará disociado en disolución acuosa. El pKa coincide con el valor de pH al cual un compuesto está ionizado al 50%. Dependiendo del pKa el pH debe ser ajustado para obtener las condiciones óptimas para su extracción y extraer tanto compuestos como sea posible.
- Solubilidad: la solubilidad en agua de un compuesto depende de la temperatura y pH. Este nos puede ayudar a la hora de conocer la afinidad el compuesto con el adsorbente.

Un método de extracción optimo es aquel que proporciona la recuperación total de los compuestos y la menor cantidad posible de impurezas de matriz en los extractos (Santana-Mayor et al., 2019). Por otro lado, los métodos de extracción deben ser fáciles, rápidos, baratos y usar el mínimo volumen de disolventes orgánicos (Feng et al., 2015). El paso de purificación es otro aspecto clave ya que se extraen contaminantes orgánicos a niveles traza, por lo que, debe evitar la co-extracción de componentes de la matriz, reducir el efecto matriz y permitir bajos límites de cuantificación y detección (mediante el menor mantenimiento posible del sistema usado para el análisis de contaminantes de preocupación emergente).

Tabla 9. Propiedades físico-químicas de algunos contaminantes de preocupación emergente.

Compuesto	**Fórmula molecular**	**Peso Molecular (g/mol)**	**Solubilidad, agua (mg/L, 25ºC)**	**Log Kow**	**pKa**
PPCPs					
Antibióticos					
Ciprofloxacino	$C_{17}H_{18}FN_3O_3$	331.13	3x10^4	0.28	6.1
Claritromicina	$C_{38}H_{69}NO_{13}$	747.48	0.342	3.16	9.0
Eritromicina	$C_{37}H_{67}NO_{13}$	733.93	1.44	3.06	8.9
Metronidazol	$C_6H_9N_3O_3$	171.06	9500	-0.02	2.6; 15.4
Ofloxacino	$C_{18}H_{20}FN_3O_4$	361.4	1.08x10^4	-0.39	5.9; 9.3
Sulfametoxazol	$C_{10}H_{11}N_3O_3S$	253.28	610	0.89	6.2; 2
Trimetoprima	$C_{14}H_{18}N_4O_3$	290.14	400	0.91	7.1
NSAIDs					
Diclofenaco	$C_{14}H_{11}Cl_2NO_2$	296.15	2.37	4.51	4.2
Indometacina	$C_{19}H_{16}ClNO_4$	357.79	0.937	4.27	4.5
Ketoprofeno	$C_{16}H_{14}O_3$	254.28	51	3.12	4.5
Ácido Mefenámico	$C_{15}H_{15}NO_2$	241.28	41	5.12	4.2
Naproxeno	$C_{14}H_{14}O_3$	230.26	15.9	3.18	4.2
Analgésicos					
Acetaminofén	$C_8H_9NO_2$	151.16	1.4x10^4	0.46	9.4
Orfenadrina citrato	$C_{24}H_{31}NO_8$	461.20	30	3.77	8.9
Tramadol	$C_{16}H_{25}NO_2$	263.38	1151	3.00	9.4
Anestésicos					
Lidocaína	$C_{14}H_{22}N_2O$	234.34	4100	2.44	7.9
Ansiolíticos-Sedantes					
Clonazepam	$C_{15}H_{10}ClN_3O_3$	315.71	100	2.41	1.5; 10.5
Diazepam	$C_{16}H_{13}ClN_2O$	284.74	50	2.82	3.4
Anticonvulsivantes					
Carbamazepina	$C_{15}H_{12}N_2O$	236.09	17.7	2.45	13.9
Lamotrigina	$C_9H_7Cl_2N_5$	256.09	170	2.57	5.7
Antidepresivos					
Citalopram	$C_{20}H_{21}FN_2O$	324.16	5.9	3.50	9.8
Venlafaxina	$C_{17}H_{27}NO_2$	277.20	267	3.28	8.9
Antihistamínicos					
Cetirizina	$C_{21}H_{25}ClN_2O_3$	388.16	101	2.80	2.7; 3.6; 7.6
Antipsicótico					
Amisulprida	$C_{17}H_{27}N_3O_4S$	369.48	293	1.06	9.0
Sulpirida	$C_{15}H_{23}N_3O_4S$	341.40	537	0.6	9.12
Antiulceroso					
Famotidina	$C_8H_{15}N_7O_2S_3$	337.50	1000	-0.64	n.a.
Pantoprazol	$C_{16}H_{15}F_2N_3O_4S$	383.40	48	2.20	3.92; 8.19
Ranitidina	$C_{13}H_{22}N_4O_3S$	314.14	24700	0.27	8.2; 2.7
Anti-vértigo					
Betahistina	$C_8H_{14}Cl_2N_2$	209.11	1x10^6	0.68	10.1
UVA/UVB filtros					
BP-3	$C_{14}H_{12}O_3$	228.08	3.7	3.79	7.1
Agonista del receptor adrenérgico β2					
Salbutamol	$C_{13}H_{21}NO_3$	239.15	1.41x10^4	1.40	10.3
Diuréticos					
Furosemida	$C_{12}H_{11}ClN_2O_5S$	330.74	73.1	5.03	3.9
Hidroclorotiazida	$C_7H_8ClN_3O_4S_2$	297.7	722	-0.07	7.9; 9.2
Estimulante					
Cafeína	$C_8H_{10}N_4O_2$	194.08	2.16x10^4	-0.07	10.4
Fragancias sintéticas					
Galaxolida	$C_{18}H_{26}O$	258.40	1.75	5.90	n.a.
Tonalida	$C_{18}H_{26}O$	258.40	1.25	5.70	n.a.

Compuesto	Fórmula molecular	Peso Molecular (g/mol)	Solubilidad, agua (mg/L, 25°C)	Log Kow	pKa
Lípidos reguladores					
Bezafibrato	$C_{19}H_{20}ClNO_4$	361.11	1.55	4.00	3.8; -0.8
Ácido fenofíbrico	$C_{17}H_{15}ClO_4$	318.70	9.114	4.00	n.a.
Gemfibrozilo	$C_{15}H_{22}O_3$	250.33	11	4.77	4.5
Productos farmacéuticos para las vías urinarias					
Alfuzosín	$C_{19}H_{27}N_5O_4$	389.21	92	1.40	8.1
Beta-bloqueantes					
Atenolol	$C_{14}H_{22}N_2O_3$	266.16	13300	0.16	9.6
Bisoprolol	$C_{18}H_{31}NO_4$	325.23	2240	1.87	9.3; 14.1
Metoprolol	$C_{15}H_{25}NO_3$	267.18	16900	1.88	14.1
Propanolol	$C_{16}H_{21}NO_2$	259.16	61.7	3.48	9.4
Antihipertensivos					
Irbesartán	$C_{25}H_{28}N_6O$	428.23	0.059	6.00	4.1; 4.3
Telmisartán	$C_{33}H_{30}N_4O_2$	514.62	Insoluble	7.70	3.6; 6.1
Valsartán	$C_{24}H_{29}N_5O_3$	435.23	1406	5.80	3.6
Retardantes de llama organofosforados					
TBEP	$C_{18}H_{39}O_7P$	398.78	$1.2x10^{-11}$	3.75	n.a.
TCPP	$C_9H_{18}Cl_3O_4P$	327.57	n.a.	2.00	n.a.
PESTICIDAS					
Fungicida					
Azoxystrobin	$C_{22}H_{17}N_3O_5$	403.40	6	2.50	n.a.
Carbendazima	$C_9H_9N_3O_2$	191.19	29	1.52	n.a.
Fluxapyroxad	$C_{18}H_{12}F_5N_3O$	381.30	n.a.	3.10	n.a.
Myclobutanil	$C_{15}H_{17}ClN_4$	288.77	142	2.94	n.a.
Penconazol	$C_{13}H_{15}Cl_2N_3$	284.18	n.a.	3.70	n.a.
Propamocarb	$C_9H_{20}N_2O_2$	188.27	$9.0x10^5$	1.12	9.5
Tiabendazol	$C_{10}H_7N_3S$	201.25	50	2.47	4.6
Herbicida					
Atrazina	$C_8H_{14}ClN_5$	215.68	33	2.61	1.6
Diurón	$C_9H_{10}Cl_2N_2O$	233.09	42.0	2.68	n.a.
Isoproturón	$C_{12}H_{18}N_2O$	206.28	65	2.87	n.a.
Simazina	$C_7H_{12}ClN_5$	201.66	6.2	2.18	1.6
Terbutrín	$C_{10}H_{19}N_5S$	241.36	25	3.74	4.3
Insecticida					
Acetamiprid	$C_{10}H_{11}ClN_4$	222.67	$4.25x10^3$	0.80	0.7
Clorfenvinfos	$C_{12}H_{14}Cl_3O_4P$	359.60	124	3.81	n.a.
Clorpyrifos	$C_9H_{11}Cl_3NO_3PS$	350.60	1.4	4.96	n.a.
DEET	$C_{12}H_{17}NO$	191.27	912	2.02	n.a.
Diazinón	$C_{12}H_{21}N_2O_3PS$	304.35	40	3.60	2.6
Imidacloprid	$C_9H_{10}ClN_5O_2$	255.66	$6.1x10^2$	0.57	1.6; 11.1
Pymetrozine	$C_{10}H_{11}N_5O$	217.23	290	-0.18	n.a.
Tiacloprid	$C_{10}H_9ClN_4S$	252.72	185	1.26	n.a.
Tiamethoxam	$C_8H_{10}ClN_5O_3S$	291.72	$4.1x10^3$	-0.13	n.a.

*n.a.: no disponible

Los métodos multiresiduo están convirtiéndose en una importante herramienta para muestras medioambientales, ya que estos permiten analizar un gran número de posibles analitos presente en la matriz de manera simultánea. Sin embargo, es una de las tareas más laboriosas en el análisis ambiental, ya que se deben optimizar las condiciones de solubilidad de los compuestos, el disolvente de extracción, la temperatura y el pH del método, entre otras.

4.2.1.1. Suelo

Entre las técnicas más populares para la extracción de contaminantes de preocupación emergente en suelo agrícola que encontramos en bibliografía están extracción asistida por ultrasonidos (UAE), extracción con líquido presurizado (PLE), extracción asistida por microondas (MAE) y, desde hace recientemente poco, extracción mediante QuEChERS modificado (Acosta-Dacal et al., 2021; Beltrán et al., 2020; Caldas et al., 2011; Feng et al., 2015; Fernandes et al., 2013; García-Galán et al., 2013; García Valverde et al., 2021; Lee et al., 2017; Lesueur et al., 2008; Masiá et al., 2015; Pérez-Mayán et al., 2020). Estás técnicas de extracción han ido acopladas a una extracción en fase sólida (SPE) como etapa de limpieza o purificación del extracto.

a) Extracción asistida por ultrasonidos (UAE)

La extracción asistida por ultrasonidos (UAE) es la técnica más empleada para la extracción de suelos, debido a que suele ser más rápida que otras como la PLE, proporciona altas eficiencias de extracción con un consumo reducido de disolvente y rendimientos similares o mejores que otros métodos. Además, tiene aplicación de amplio alcance.

Este método de extracción genera una vibración adicional en las moléculas de la muestra, lo que aumenta la cavitación y la superficie de contacto entre la matriz de la muestra y el disolvente de extracción, mejorando las recuperaciones de los analitos durante un tiempo de extracción breve.

Las temperaturas y presiones extremadamente altas generadas durante el colapso de la burbuja (implosión) dan como resultado una mayor solubilidad y difusividad de los analitos, así como la penetración y el transporte en la interfase entre el disolvente de extracción y la matriz sólida. Aunque los baños ultrasónicos se han utilizado más ampliamente, las sondas ultrasónicas cilíndricas son más eficientes ya que enfocan la energía ultrasónica en la zona de la muestra, lo que da como resultado una mayor reproducibilidad y repetibilidad experimental (Gil García et al., 2018).

La extracción asistida por ultrasonidos ha sido aplicada para la extracción de una amplia variedad de contaminantes orgánicos de origen emergente en suelos agrícolas, como antibióticos, antiinflamatorios, lípidos reguladores, antiepilépticos o antidepresivos (Beltrán et al., 2020; Carter et al., 2014; García Valverde et al., 2021).

b) Extracción con líquido presurizado (PLE)

El segundo método de extracción más usado es la extracción con líquido presurizado (PLE), donde un disolvente a altas presiones y temperaturas por encima del punto de ebullición mantiene al disolvente de extracción en estado líquido. La eficiencia de extracción mejora al aumentar la temperatura lo que genera una menor viscosidad del disolvente aumentando así la capacidad de la matriz del suelo húmedo y la solubilidad de los compuestos. Aquí las muestras son colocadas dentro de un recipiente de acero inoxidable y son mezcladas con agentes dispersantes para evitar la agregación de partículas de muestras y reducir el volumen. El disolvente de extracción más usado es metanol, mezclas de este con agua o agua con otros disolventes orgánicos.

Entre las ventajas de este método encontramos un bajo consumo de disolventes y además se trata de un proceso fácil automatizado. Para lograr la mayor eficacia del método se optimiza tanto la temperatura como la presión. La presión en un proceso de extracción de contaminantes es habitualmente mantenida entre 500 y 1500 psi, mientras las temperaturas oscilan en torno a 100ºC. De esta forma, se evita la degradación de los analitos por elevadas temperaturas y encontrar una forma óptima de extraer cada compuesto lo máximo posible. Otros parámetros para tener en cuenta son el número de ciclos y el tiempo de extracción. Habitualmente el número de ciclos está entre 1 y 5 y el tiempo de extracción alrededor de 5 minutos. La extracción con líquido presurizado ha sido utilizada para la extracción de una amplia gama de productos farmacéuticos en suelos agrícolas. Un ejemplo, es el estudio llevado a cabo por Biel-Maeso et al., quien determinó 46 contaminantes emergentes en suelo regado con agua residual tratada de un municipio mediante PLE con agua caliente como disolvente de extracción. Otro ejemplo, es el trabajo realizado por García-Galán et al., quien mediante PLE analizó 22 antibióticos en suelo agrícola de Cataluña regado con agua residual tratada. En este estudio el disolvente utilizado fue una mezcla de metanol/agua (90:10, v/v) a 100ºC.

c) <u>Extracción asistida por microondas</u> (MAE)

Otro método de extracción usado para la determinación de contaminantes en matrices sólida es la extracción asistida por microondas, dónde se combinan técnicas de extracción tradicionales con microondas. Al igual que en PLE, la muestra es colocada en un recipiente cerrado donde el disolvente entra en contacto con esta. Mediante la aplicación de calor, a través de la energía de microonda, son extraídos los compuestos de la muestra. Entre las ventajas de esta técnica tenemos el rápido calentamiento de la mezcla muestra/disolvente, cortos tiempos de extracción o consumo reducido de disolventes de extracción. En cambio, entre las desventajas encontramos la optimización del disolvente de extracción porque solo disolventes con determinadas características como propiedades de absorción de microondas pueden ser usados. De la misma forma que en PLE, parámetros como el volumen de disolvente, tiempo de extracción, la presión o la temperatura tienen que ser evaluados y optimizados. La temperatura es otro punto crítico, ya que, si no está bien optimizada, los analitos pueden degradarse como en PLE.

d) <u>Extracción mediante QuEChERS modificado</u>

Otro método es la extracción sólido-líquido (SPLE), dónde destaca el método QuEChERS (acrónimo de Quick-rápido, Easy-fácil, Cheap-barato, Effective-eficaz, Rugged-robusto y Safe-seguro) (Santana-Mayor et al., 2019). Originalmente, fue creado en 2003 para la extracción de pesticidas en matrices de bajo contenido en grasa como frutas, verduras y cereales (Anastassiades et al., 2003). Sin embargo, en los últimos años, debido a su alta efectividad, se ha ido implementando en otras matrices y en otras áreas, como puede ser para el análisis multiresiduo de contaminantes emergentes en suelo agrícola (Acosta-Dacal et al., 2021; García Valverde et al., 2021; Picó et al., 2019).

El método QuEChERS tiene como procedimiento etapas analíticas simples, fáciles y rápidas de llevar a cabo. Básicamente, envuelve una extracción de muestra con un disolvente (habitualmente acetonitrilo) seguido de una etapa de limpieza (partición líquido-líquido) utilizando sales (NaCl) para eliminar interferentes polares y agentes desecantes ($MgSO_4$) para eliminar el contenido de agua de la muestra.

A partir del método QuEChERS se han aplicado modificaciones con la finalidad de mejorar el alcance del método. El método QuEChERS reduce el coste por muestra al 50%, el rendimiento por muestra es mayor a los métodos tradicionales y tiene una gran versatilidad que permite el análisis de un amplio rango de compuestos con diferentes propiedades físico-químicas.

En la **figura 8** se muestra el método QuEChERS original con las dos variantes más conocidas. Existen dos métodos estandarizados, cuya diferencia es el uso de distintos tampones mejorando la estabilidad y recuperación de los analitos sensibles al pH:

a) Método oficial de la AOAC 2007.01 (AOAC International, 2007) que emplea buffer de acetato y pH a 4.8.
b) Método estandarizado Europeo CEN-EN 15662 publicado por el Comité Europeo (European Commitee for Standarization, 2008) que utiliza buffer de citrato y pH desde 5 a 5.5.

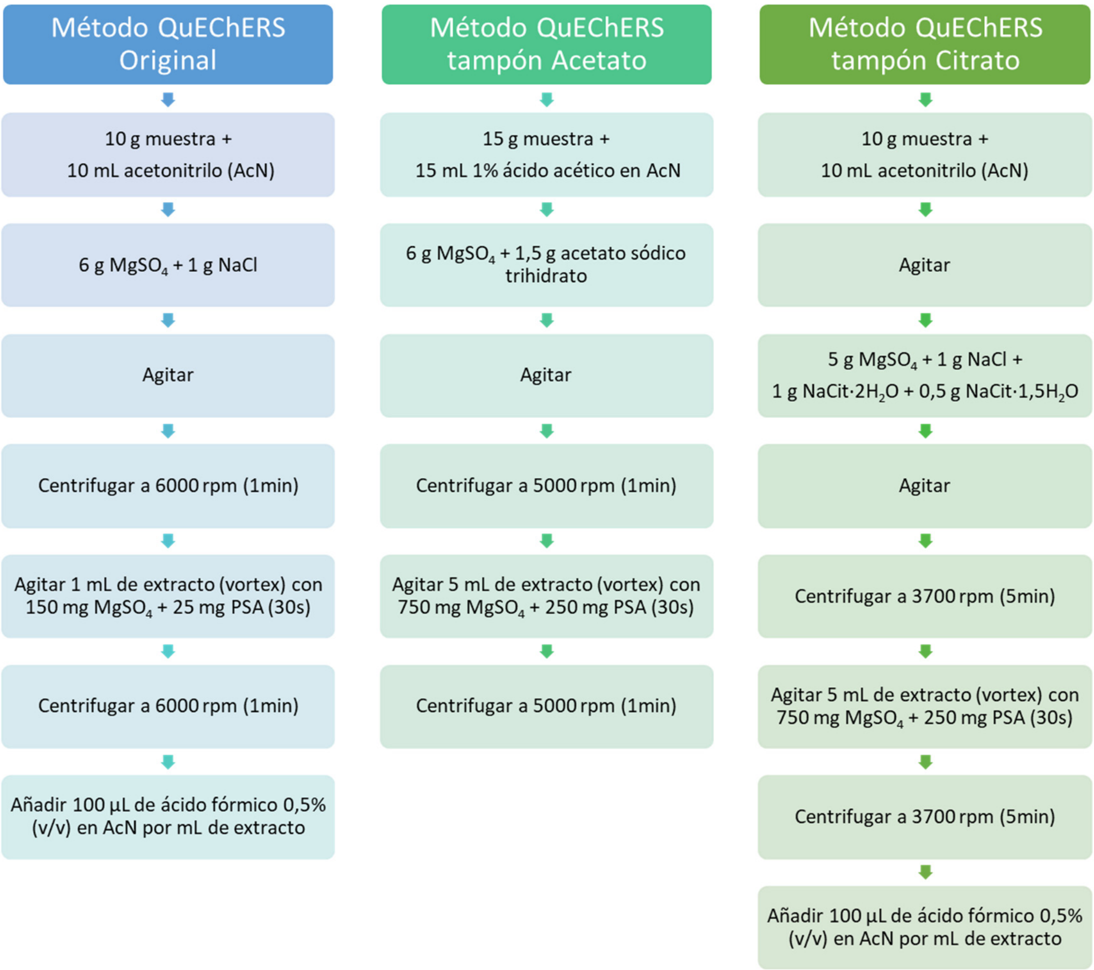

Figura 8. Diagrama de flujo de las etapas en los diferentes métodos QuEChERS

A pesar de su eficiencia, se extraen una gran cantidad de componentes de la matriz del suelo como ácidos húmicos, proteínas y lípidos que causan interferencias en el análisis de contaminantes orgánicos. Es por este motivo necesario una etapa de purificación para eliminarlos. La etapa de limpieza que se suele llevar a cabo es una extracción en fase sólida dispersiva (d-SPE), en la que los sorbentes se agitan junto a una fracción del extracto de la muestra. Entre los más comunes tenemos $MgSO_4$ actuando de agente desecante, C18 para eliminar interferentes apolares de la matriz y PSA para remover los ácidos orgánicos polares y pigmentos.

4.2.1.2. Planta

Los componentes de los tejidos de las plantas como pigmentos, clorofilas, azúcares o aceites hacen que estas matrices sean complejas pudiendo verse co-extraídos junto con los analitos de interés. y, por tanto, que la extracción de contaminantes sea todo un reto. Esta co-extracción origina presencia de interferentes y la disminución de sensibilidad durante el análisis de la muestra. Por tanto, se requiere el uso de método de extracción capaces de producir extractos limpios y de elevada eficacia y sensibilidad.

Los métodos más usados para la extracción de contaminantes orgánicos de origen emergente en matrices vegetales son prácticamente los mismos que los explicados anteriormente para las muestras de suelo agrícola, con excepción de ciertas modificaciones en la etapa de limpieza. Por ejemplo, Shenker et al., publicó la extracción de carbamazepina en hojas, tallo y fruto de calabacín usando la extracción asistida por ultrasonidos (UAE) , y Carter et al., extrajo benzodiacepinas como donazepam u oxazepam en hoja de remolacha y rábano con la misma técnica. Por otro lado, Calderón-Preciado et al., llevó a cabo la extracción de diferentes contaminantes emergentes en lechuga, zanahoria y judía verde usando la extracción con líquido presurizado (PLE). En cambio, Beltrán et al., realizó una extracción con el método QuEChERS modificado para extraer atenolol, carbamazepina y triclosán en lechuga, maíz y rábano.

4.2.2. Extracción de muestras acuosas ambientales. Inyección directa.

El análisis de contaminantes en muestras de agua es complicado debido a la complejidad de la matriz y a las bajas concentraciones en las que se encuentran los contaminantes en la muestra. Hasta ahora se han venido realizando procesos de extracción selectivos y eficaces para conseguir resultados óptimos en la extracción y concentración de los compuestos de interés.

Entre los métodos de extracción de contaminantes emergentes en muestras acuosas ambientales encontramos:

a) Extracción líquido-líquido (LLE)

Se basa en la partición de analitos entre la muestra acuosa y el disolvente orgánico. En un embudo de separación se introduce la muestra y posteriormente el disolvente inmiscible. Se agita y se abre la válvula varias veces para permitir la mezcla. Este método se suele aplicar para separar analitos apolares o semipolares y los disolventes más usados son n-hexano y ciclohexano. Sin embargo, cada vez está más en desuso debido a desventajas como alto consumo de disolventes, limitada eficacia para extraer analitos polares, baja reproducibilidad o selectividad entre otros.

b) Extracción en fase sólida (SPE)

Los principios en los que se basa la extracción en fase sólida son similares a los de extracción líquido-líquido. La distribución de los solutos o analitos entre dos fases es parecida. SPE no requiere de una mezcla de fases líquidas sino la dispersión del analito entre dos fases líquidas (medio de muestra y adsorbente). La muestra pasa a través de partículas adsorbentes por las que los analitos tienen una mayor afinidad que el líquido. Después, los analitos se extraen por elución con un disolvente apropiado. Este método de extracción se aplicó por primera vez en la década de 1940 (Liška, 2000). SPE es un método que simplifica el análisis por eliminar gran parte de los interferentes de la matriz y preconcentrar analitos de la muestra (Alexa et al., 2022). Existe un gran número de adsorbentes comerciales donde el mecanismo de retención del analito al adsorbente depende de las características de este. Las interacciones pueden ser dipolo/dipolo, ion/dipolo, hidrofóbicas, dispersión o intercambio iónico. Los adsorbentes genéricos son los más usados para la extracción de contaminantes emergentes en muestras acuosas. Entre ellos encontramos: absorbentes de carbón grafitizado, adsorbentes de sílica enlazada, adsorbentes de intercambio iónico y adsorbentes poliméricos. Estos últimos son los más usados por extraer una amplia gama de contaminantes orgánicos con diferente rango de polaridad y su gran estabilidad en toda la escala de pH. El adsorbente Oasis HLB® (Waters) es el más comúnmente usado en la actualidad (Rodriguez-Mozaz et al., 2015; Serra-Compte et al., 2021). Es un adsorbente con un equilibrio hidrofílico-lipofílico, originado por los monómeros de los que está formado (el divinilbenceno y la N-vinilpirrolidona), pudiendo secarse antes de la carga de muestra sin perder a capacidad de retención de analitos. Este adsorbente es útil para analitos ácidos, básicos y neutros, siendo posible realizar métodos multi-analito trabajando en pH neutro. Sin embargo, debido a que tiene limitada selectividad es probable extraer componentes de la matriz, como ácidos húmicos y fúlvicos, afectando a la separación cromatográfica.

c) Micro-extracción en fase sólida (SPME)

Este método fue introducido en 1990 como un método respetuoso con el medio ambiente ya que no utiliza disolventes orgánicos y reduce el tiempo de preparación de muestra (Arthur and Pawliszyn, 1990). El primer dispositivo de SPME se desarrolló rápido y fue por la incorporación de fibras revestidas en microjeringas. El mecanismo consiste en exponer una pequeña cantidad de fase de extracción (fibra de sílice fundida) recubierta con una capa delgada de polímero inmovilizado o un adsorbente sólido. La primera etapa es cuando los analitos se reparten entre la matriz y la fibra porque esta se expone a la muestra. La segunda etapa es cuando tiene lugar la separación y cuantificación de los analitos de la muestra, ya que la fibra con los analitos retenidos es transferida al instrumento analítico. Por tanto, la ventaja de este método es que permite la extracción y cuantificación de los analitos de una muestra.

Las metodologías convencionales (SPE, SPME o LLE) consumen mucho tiempo y se ven afectadas por una gran incertidumbre. Esto probablemente podría atribuirse a la introducción de errores humanos asociada con la manipulación de muestras. Por ello, en la actualidad se está implementando cada vez más la inyección directa de muestras ambientales acuosas (Fabregat-Safont et al., 2023; Ren et al., 2022; Restrepo-Vieira et al., 2022). Pero, en las metodologías convencionales simplemente por el hecho de necesitar una instrumentación más asequible y barata, están más disponible en todos los laboratorios analíticos. Sin embargo, la inyección directa reduce drásticamente el tiempo total de análisis, desde un día de trabajo a solo unos minutos, permitiendo el rendimiento analítico, es decir, poder procesar más muestras por día. Mediante estudios de validación donde se han comparado estos métodos, se ha visto que la efectividad y la veracidad del método, como las recuperaciones analíticas, arrojan peores resultados con la metodología convencional (de la Serna Calleja et al., 2023). Por lo que, la inyección directa implica un compromiso bueno que junta las ventajas de cada método. Es un método rápido y automático, confiando la mayor parte de la selectividad al detector de masas.

5. Técnicas de análisis

Tras la publicación del libro de Rachel Carson titulado "primavera silenciosa" en 1962 (Carson, 1962), se generó conciencia sobre los problemas ambientales causados por sustancias de origen antropogénico. La química analítica se ha convertido en una herramienta poderosa para lidiar con la contaminación medioambiental a través del desarrollo de técnicas de instrumentación especializadas y sensibles.

Años atrás, era todo un reto medir en una muestra de agua analitos en concentraciones en el orden de partes por millón (ppm). Así, uno de los objetivos de la química analítica ambiental ha consistido en aumentar el número de compuestos orgánicos de origen antropogénico que pueden ser detectados y determinados a concentraciones traza. Sin esta capacidad, no habría sido posible detectar muchos de los compuestos peligrosos en niveles a los cuales tienen efectos biológicos en el medio ambiente.

El análisis de contaminantes medioambientales, entre ellos los contaminantes emergentes, ha sido posible gracias al desarrollo de nuevas técnicas analíticas más sensibles y capaces de identificar y medir una gran variedad de este tipo de compuestos en matrices medioambientales complejas. Las mejoras analíticas van en dos direcciones:

1. Métodos más sensibles y selectivos que permitan límites de detección del orden de µg/L ng/g (ppb) o ng/L (ppt) para microcontaminantes orgánicos en el medio ambiente.
2. Versatilidad para analizar a la vez una amplia gama de compuestos con diferentes propiedades físico-químicas (log K_{ow}, solubilidad en agua, pK_a, peso molecular).

Con un gráfico de polaridad-volatilidad podemos apreciar la aplicabilidad de las dos técnicas de separación más usadas para analizar contaminantes orgánicos en muestras ambientales, como son la cromatografía de gases (GC) y la cromatografía de líquidos (LC) (**figura 9**).

Durante 40 años, la química analítica ha estado centrada en el análisis de contaminantes no polares volátiles y semivolátiles donde la técnica de separación GC ha jugado un papel importante para su determinación a nivel traza en el medo ambiente. Es por esto que, hasta mediados de los años 80, los métodos analíticos descritos para el análisis de fármacos, productos de cuidado personal y pesticidas estaban basados en GC (Ternes, 2001). En los años 90, el desarrollo de la cromatografía de líquidos acoplado a espectrometría de masas en tándem (LC-MS/MS) produjo la detección de muchos contaminantes de forma más efectiva y, en consecuencia, se convirtió en el método rutinario y robusto para el análisis de compuestos polares, metabolitos y productos de transformación, anteriormente difíciles o imposibles de analizar. En la actualidad, la mayoría de los trabajos de investigación analizan sus muestras medioambientales en equipos LC (Beltrán et al., 2020; Ben Mordechay et al., 2021; Carter et al., 2018; González García et al., 2019; Hyland et al., 2015; Malchi et al., 2014; Martínez-Piernas et al., 2019; Picó et al., 2019). Generalmente, la elección entre las técnicas cromatográficas GC y LC, está basada en las propiedades físico-químicas de los analitos de interés. Nos centraremos en explicar la cromatografía de líquidos puesto que es la técnica usada en esta tesis doctoral.

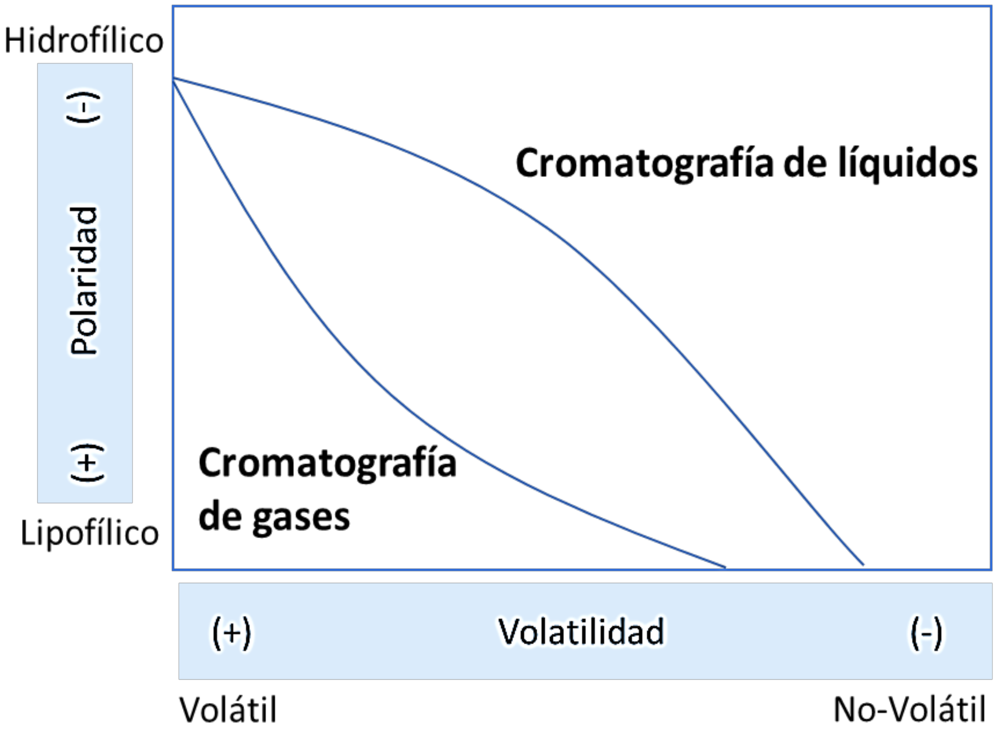

Figura 9. Aplicabilidad relativa de las técnicas de separación cromatográfica de gases y líquidos según polaridad y volatilidad de los compuestos

5.1. Cromatografía de líquidos (LC)

La cromatografía de líquidos es la técnica más apropiada para el análisis de sustancia polares o de polaridad intermedia. Actualmente, se está incrementando su uso, creando métodos que permiten el análisis simultaneo de diferentes clases de contaminantes emergentes, mejorando la separación cromatográfica de los analitos (Montemurro et al., 2017). Esta cromatografía se basa en la separación de sustancias en una fase liquida, utilizando una fase estacionaria sólida. La separación de las sustancias en una muestra depende de las interacciones físico-químicas entre los analitos y la fase estacionaria. Para el análisis de muestras medio ambientales, la cromatografía en fase reversa es la más usada desde hace décadas (Farré et al., 2007). En ella, la separación se da mediante columnas apolares y fases móviles polares. De modo que, los analitos polares atraviesan primero la comuna porque se retienen menos en la fase estacionaria. En cambio, los analitos polares, tienen mayor interacción y son más retenidos, eluyendo por la columna más tarde.

Columnas

Una buena separación cromatográfica es aconsejable para mejorar la detección y reducir el efecto matriz. Existen distintas columnas, según el analito problema u objetivo del análisis:

- Las columnas de grupos alquilos poseen grupos alquilo octadecil (C18) y octil (C8), siendo la columna C18 la más usada para el análisis de contaminantes emergentes. El desarrollo de columnas empacadas con partículas de menos de 2 µm permiten alcanzar una mayor eficiencia de separación, tiempos de retención menores, picos más estrechos y con mayor relación señal/ruido en el análisis de contaminantes orgánicos de origen emergente en muestras medio ambientales (Alygizakis et al., 2020; Asimakopoulos et al., 2017; Mayoudom et al., 2018). Por ello, en esta tesis se utilizó una columna Zorbax Eclipse Plus C8 con 1.8 µm de tamaño de partícula.
- Las columnas HILIC (interacción hidrofílica) utilizan fases estacionarias con grupos amida, ciclodextrina, ciano o amino y están siendo una gran alternativa a las C18 y C8. Son utilizadas para el análisis de sacáridos con abundantes grupos hidroxilo (compuestos polares e hidrofílicos) desde 1975 (Li et al., 2023).
- Las columnas quirales son útiles para la separación de enantiómeros. La separación se origina por la diferencia de afinidad por la fase estacionaria de uno de los enantiómeros en relación con el otro.

Fase móvil

La finalidad de la fase móvil es eluir los analitos de la columna y mejorar la ionización en la fuente. Entre las fases móviles orgánicas utilizadas para el análisis de contaminantes de preocupación emergente destacan el acetonitrilo y el metanol, aunque a veces se realizan mezclas de ambos disolventes (Ahmed et al., 2021; Beltrán et al., 2020; Serra-Compte et al., 2021). Para incrementar la mejora en la separación, se suele utilizar un gradiente donde inicialmente, este tiene un elevado porcentaje de contenido en agua que va disminuyendo al aumentar el porcentaje de disolvente orgánico con menor polaridad y mayor fuerza eluyente (Christou et al., 2017). Por otro lado, para conseguir una buena retención de los analitos en la columna y mejorar la sensibilidad en la retención cuando se trabaja con un espectrómetro de masas (MS), es aconsejable en la fase móvil acuosa añadir tampones y ácidos (modificadores). Entre los más comunes se encuentran acetato amónico, formiato amónico, ácido fórmico o ácido acético. A la hora de seleccionar el modificador y la cantidad debemos tener en cuenta el mínimo efecto de supresión en la ionización y la mejora de la separación.

En general, los contaminantes de preocupación emergente se clasifican en dos clases según su sensibilidad en ionización en modo positivo (PI) o en modo negativo (NI). Los contaminantes ácidos se determinan mejor en modo negativo utilizando disolventes orgánicos y agua ultrapura con un tampón de acetato amónico, mientras que los compuestos básicos o neutros se ionizan mejor en modo positivo utilizando tampones que modifiquen el pH de la fase móvil. Por ejemplo, el carácter ácido de los antinflamatorios hace que sea necesario para su determinación, tampones o ácidos en la fase móvil, como formiato amónico o ácido fórmico. El problema es que la adición de ácido puede disminuir la intensidad del anión molecular cuando se usa ESI en modo negativo, por lo que a veces se usa como alternativa acetato amónico.

5.1.1. Acoplamiento de la cromatografía líquida a espectrometría de masas- (LC-MS/MS)

Durante los años 70 la principal temática de investigación fue el acoplamiento de la cromatografía con la espectrometría de masas (MS). Después de la separación cromatográfica, los analitos llegan a un analizador, en el cual, se realiza la identificación. Debido a que el espectrómetro analiza iones en fase gaseosa se necesita una interfase que origine iones a partir de las moléculas neutras que salen del cromatógrafo.

Las interfases a presión atmosférica han sido la clave del desarrollo y son denominadas fuente de ionización. De este modo, la introducción de la técnica de ionización a presión atmosférica (API) han convertido la LC-MS en la técnica clave para el análisis de trazas de compuestos polares en el medio ambiente (Čelić et al., 2017; Farré et al., 2007). La tecnología API actúa a presión atmosférica y no a alto vacío como es normal en otras técnicas. En general, las interfases tienen varios problemas a los cuales tienen que hacer frente, entre ellos la evaporación de grandes volúmenes de disolvente, la incompatibilidad de los tampones y aditivos de pares iónicos no volátiles y la dificultad de la producción de iones en fase gas de los analitos no volátiles y termolábiles. La técnica de ionización a presión atmosférica (API) está formada por dos interfases: la electrospray (ESI) y la ionización química a presión atmosférica (APCI). En la **figura 10** podemos ver la aplicabilidad de las técnicas de ionización según el peso molecular del compuesto y su polaridad.

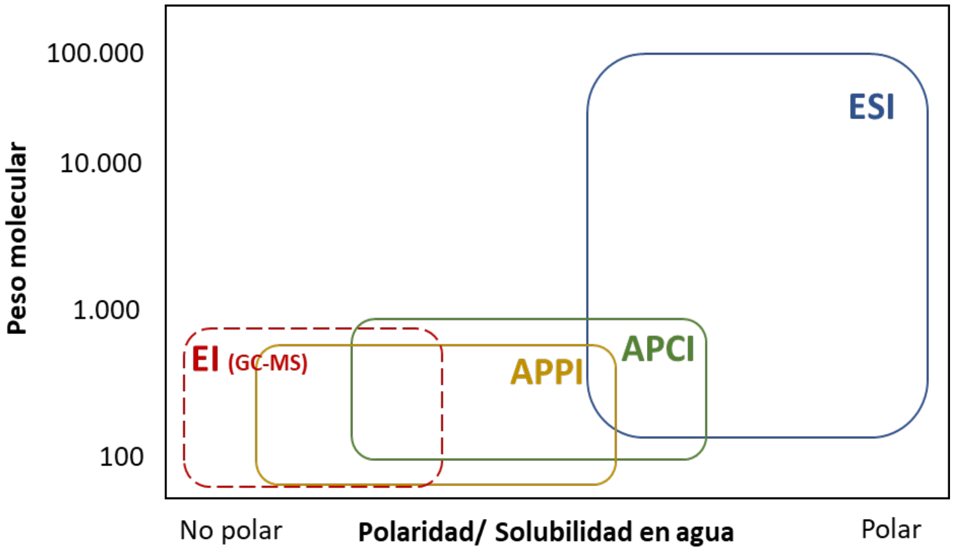

Figura 10. Aplicabilidad de las técnicas de ionización para LC-MS

Electrospray (ESI): se aplica un campo eléctrico de gran intensidad para favorecer el proceso de transformación de las gotas en iones. Los analitos disueltos en la fase móvil pasan al detector a través de un capilar de acero inoxidable a presión atmosférica sometido a un voltaje de entre 3 y 6 kV (ver **figura 11**). El líquido al fluir por el capilar se dispersa y las moléculas de los analitos y el disolvente forman un spray de gotas pequeñas cargadas en un proceso de nebulización. A continuación, las moléculas de disolvente se evaporan (desolvatación) al traspasar la región a presión atmosférica de la fuente del espectrómetro de masas. Conforme se evapora el disolvente, las gotas se hacen más pequeñas hasta que se originan iones en fase gaseosa. Estos iones entran al espectrómetro de masas. Su principal uso en las últimas décadas ha sido para analizar compuestos lábiles y con intermedia a alta polaridad (Gorga et al., 2013; Köck-Schulmeyer et al., 2019; Kuster et al., 2008). El efecto matriz se encuentra enlazado con la técnica de ionización de electrospray. Por ello, un aspecto crítico en el análisis cuantitativo es la aparición de efectos de matriz que provocan la supresión de iones debido a la presencia de componentes orgánicos e inorgánicos en la muestra que coeluyen con los analitos objetivo (Farré et al., 2007). Este fenómeno se debe a la cantidad de compuestos que compiten por la ionización en las microgotas que genera el electrospray. Por lo que, matrices muy complejas pueden afectar a la ionización de analitos de interés y consecuentemente influir en la sensibilidad. Para minimizar las interferencias de la matriz durante la ionización, se pueden cambiar la polaridad de la ionización de positiva a negativa o viceversa o se pueden disminuir el flujo dirigido a la interfaz ESI, lo que disminuye el tamaño de las gotas y la cantidad de moléculas que se ionizarán en un momento dado.

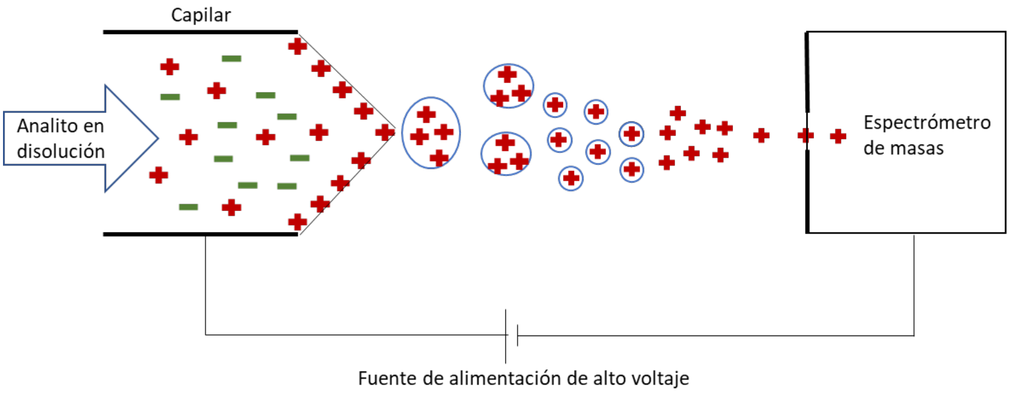

Figura 11. Esquema del sistema de ionización ESI

Ionización química a Presión atmosférica (APCI): las microgotas se generan con una aguja sometida a una diferencia de potencial, por el cual se rompen y generan iones. El mecanismo es un proceso de ionización química en el que las moléculas de fase móvil actúan como gas reactivo una vez ionizadas por una descarga de electrones. Así, el flujo del LC se nebuliza en un tubo vaporizador de sílice fundida caliente donde el disolvente se evapora casi al completo. La mezcla gas-vapor entra en la fuente a presión atmosférica donde se produce la ionización química a través de electrones que se generan en una aguja. Es válida para compuestos con baja a alta polaridad, requiere compuestos con cierta volatilidad y es una técnica con buena sensibilidad para compuestos de polaridad y peso molecular intermedio. Sin embargo, la sensibilidad es aproximadamente 10 veces menor en el modo APCI que en el modo ESI.

5.1.2. Analizadores en espectrometría de masas

Los iones producidos en las fuentes de ionización son guiados al espectrómetro de masa y separados según la relación masa/carga (*m/z*). El espectro de masas generado es una representación gráfica de la intensidad de la señal de los iones que llegan al detector en función de la relación *m/z*. El espectro se normaliza en función al pico base (pico con mayor intensidad). Del espectro se obtiene información estructural de los analitos además de su peso molecular.

La selección del analizador de masas está determinada por la sensibilidad y selectividad requerida, además, del objetivo general del análisis. Para incrementar la fragmentación en LC-MS existe como alternativa, el empleo de espectrometría en masas en tándem (MS/MS), usando analizadores de trampa de iones o sistemas híbridos como triple cuadrupolo. Dependiendo del objetivo del análisis encontramos dos tipos de estrategias:

- Análisis de compuestos objetivo: análisis que se basan en la determinación de compuestos seleccionados que se caracterizan mediante los correspondientes estándares. Se comparan tiempo de retención, fragmentos de masas característicos o transiciones y sus ratios.

- Análisis de compuestos no-objetivo o desconocidos: se presente determinar compuestos que no se han incluido en el análisis de rutina como metabolitos o productos de transformación.

Los 5 grupos de analizadores de masas más usados para el análisis de contaminantes orgánicos de origen emergente son: analizadores de cuadrupolo (Q), triple cuadrupolo (QqQ), trampa de iones, tiempo de vuelo (TOF) y orbitrap. Finalmente, se comentará en mayor profundidad el analizador triple cuadrupolo, ya que este ha sido el analizador usado en esta Tesis Doctoral.

Cuadrupolo sencillo (Q): Consiste en cuatro barras metálicas circulares paralelas entre ellas y dispuestas formando una circunferencia de tal forma que el haz de iones procedentes de la interfase coincide sobre el centro del dispositivo. Sobre las barras opuestas se aplica un voltaje constante y un potencial oscilante de radiofrecuencia superpuesto. Los iones se introducen en el campo del cuadrupolo, implicando una baja aceleración de su potencial, empezando a oscilar en un plano perpendicular longitudinal a las barras. Cuando las oscilaciones son estables, el ion es transmitido hasta el detector.

Trampa de iones: Se basa en el uso de una zona de confinamiento electromagnética. Está formado por tres electrodos con superficie hiperbólica; el electrodo central es anular y los electrodos superior e inferior forma el cierre de los extremos del anillo. En la cavidad que forman los tres electrodos se origina la ionización, fragmentación y el análisis de masas, a través del confinamiento electromagnético inducido por dos señales de radiofrecuencia

Tiempo de vuelo (TOF): en este analizador, los iones son acelerados mediante un potencial e introducidos en un tubo analizador de 50 cm o 1 m de longitud, que no está sometido a ningún campo eléctrico. Dentro del tubo, los iones tienen la misma energía cinética y con ello sus velocidades dentro de él variarán inversamente según su masa, por tanto, llegarán antes al detector los iones más ligeros. Mediante la relación entre el tiempo de vuelo y la relación m/z se obtiene la masa exacta del ion.

Orbitrap: este analizador está formado por un electrodo central con forma de huso combinado con dos electrodos aislados simétricos exteriores que complementa la forma del electrodo central. Estos electrodos establecen un campo de captura de electrones. Por otro lado, posee un dispositivo de acumulación de iones externo llamado C-trap, donde mientras se acumulan los iones, estos van perdiendo energía mediante suaves colisiones con el gas presente. Posteriormente, se aplica un voltaje en forma de pulso a los iones que son expulsados ortogonalmente al Orbitrap. Allí experimentan un campo de fuerza axial que genera la aceleración axial y un campo de fuerza radial que establece una órbita radial alrededor del electrodo central. La frecuencia de las oscilaciones es independiente de la velocidad del ion y es inversamente proporcional a la raíz cuadrada de la relación m/z del ion. La señal generada está formada por combinación de señales senoidales de las distintas m/z y las abundancias de cada ion.

Triple cuadrupolo (QqQ): Consiste en dos cuadrupolos sencillos con un segundo cuadrupolo entre ellos que actúa como celda de colisión (**figura 12**). Permitiéndose el análisis de masas en tándem. Los iones provenientes de la fuente de ionización van hacia el primer cuadrupolo donde se selecciona el ion precursor. A continuación, en la celda de colisión (segundo cuadrupolo), se fragmenta el ion precursor en iones fragmento mediante la generación de un potencial eléctrico y un gas inerte (He, N o Ar). Después, los iones pasan por el tercer cuadrupolo y por último llegan al detector fotomultiplicador.

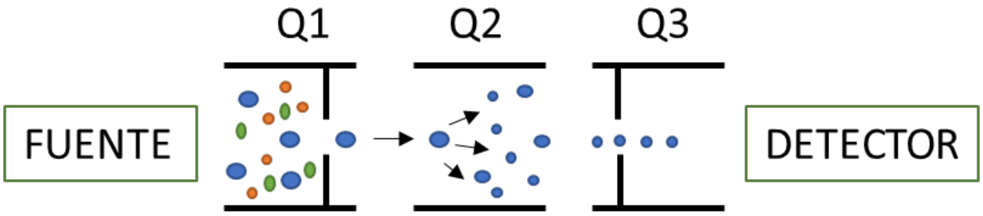

Figura 12. Esquema de un analizador triple cuadrupolo.

La alta sensibilidad de este tipo de analizadores convierte esta técnica en una de las más utilizada para el análisis de contaminantes orgánicos de preocupación emergente en muestras ambientales (Beltrán et al., 2020; Calderón-Preciado et al., 2013; López-Roldán et al., 2010; Martínez-Piernas et al., 2019).

Existen cuatro modos de trabajo: análisis del ion precursor (precursor ion scan), análisis de los iones producto (producto-ion scan), monitorización de reacciones seleccionadas (SRM-selected-reaction monitoring) o monitorización de reacciones múltiples (MRM) y pérdida neutra constante (neural loss scan). En la **tabla 10** podemos ver, en forma de resumen, las características de cada modo de trabajo.

Tabla 10. Características de los modos de trabajo de los instrumentos de triple cuadrupolo.

Modos de trabajo	**Características**
Precursor ion	Q1 realiza un barrido de iones precursores Q3 deja pasar al detector fragmentos comunes con una determinada relación m/z
Product ion	Q1 opera en modo SIM (seleccionando una m/z característica) Q2 (CE) se origina la fragmentación del precursor Q3 opera en modo scan generando el espectro de fragmentación del precursor
SRM/ MRM	Q1 selecciona el ion molecular CE origina la fragmentación Q3 selecciona y analiza un ion producto específico
Neutral loss	Q1 y Q3 analizan simultáneamente Se calculan las pérdidas de masa entre ambos cuadrupolos Útil para la detección de compuestos de misma familia y de compuestos con pérdidas comunes

El modo de trabajo más comúnmente empleado para el análisis cuantitativo es el SRM o MRM porque permite monitorizar una transición concreta aportando mayor sensibilidad y selectividad cuando se hacen análisis a niveles trazas (Acosta-Dacal et al., 2021; Alygizakis et al., 2020; Benedetti et al., 2020; Martínez Bueno et al., 2021; Tanoue et al., 2012; Wu et al., 2014). Por esos motivos en esta tesis se utilizó este modo de trabajo.

Primeramente, para trabajar en modo MRM, se necesita hacer una optimización de los voltajes utilizados en diferentes partes del equipo para obtener la señal lo más intensa posible. Durante esta optimización se debe trabajar en modo “scan” en el Q1 para obtener el ion precursor que suele coincidir con el ion molecular o un aducto de sodio o amonio. Seguidamente se filtra el ion precursor en Q1 haciéndolo pasar por la celda de colisión Q2. En el Q2 se colisiona el ion precursor con el gas inerte y se aplican diferentes voltajes para fragmentar dicho ion. Finalmente, se hace un barrido en el Q3 en un rango de masas inferiores al precursor para obtener los iones productos. De estos, se seleccionan los iones producto con los que se obtenga mayor selectividad (mayor *m/z*) y sensibilidad (iones más intensos). Una vez seleccionados los iones producto se escoge el voltaje óptimo para la celda de colisión con el fin de conseguir la mayor intensidad de cada uno.

Tras el paso de los iones por el analizador triple cuadrupolo, los iones alcanzan el detector fotomultiplicador. Los iones chocan con un dínodo (nombre que recibe cada uno de los electrodos de un fotomultiplicador) originando la emisión de electrones. A continuación, entran en contacto con una pantalla de fósforo produciendo una generación abrupta de protones. Posteriormente, los protones son introducidos en el multiplicador donde tiene lugar la cascada de amplificación de la señal.

6. Calidad de los resultados analíticos

El análisis de contaminantes de preocupación emergente en muestras ambientales requiere del desarrollo de métodos analíticos muy sensibles y selectivos debido a que estos compuestos se encuentran a bajas concentraciones, llegando a niveles traza (ng/kg o µg/kg y ng/L o µg/L).

En los resultados se pueden dar falsos positivos y falsos negativos, especialmente en los casos de análisis de matrices difíciles como suelo. Por lo que, para una correcta confirmación de los resultados analíticos es fundamental desarrollar métodos de análisis de alta selectividad que garantice la validez de los resultados. Para asegurar la calidad de los resultados, los métodos de análisis deben ser validados cumpliendo unos criterios de sensibilidad, selectividad, linealidad, precisión y exactitud. El análisis de blancos o muestras fortificadas (recuperaciones) son operaciones de control necesarios para garantizar que los resultados cumplen con los requisitos de calidad.

Un sistema de calidad riguroso y sistemático debe realizarse para garantizar estos dos puntos, donde este sistema afecte a todas las etapas de proceso analítico y disponga de controles que evalúen su correcto funcionamiento.

6.1. Validación de métodos analíticos

La validación es un proceso donde se demuestra experimentalmente que un método es apropiado para el propósito para el que fue desarrollado. Por tanto, garantiza un proceso analítico de calidad y resultados fiables. Diferentes organismos de carácter internacional como US EPA, EURACHEM, SANTE o IUPAC entre otros, han desarrollado directrices para la validación de métodos analíticos. Los criterios generales son similares entre las diferentes directrices y proporcionan la base para asegurar la fiabilidad de los métodos validados. Para el estudio de validación de un método de análisis debe incluir:

- Rango lineal
- Límite de detección y cuantificación del método
- Exactitud (Recuperación de los analitos)
- Precisión (en términos de repetitividad y reproducibilidad)
- Efecto matriz
- Empleo de blanco instrumentales
- Uso de estándares internos y estándares instrumentales

Rango lineal (linealidad): se define como la capacidad de mantener invariable la pendiente de la función de calibrado para el intervalo de concentraciones de trabajo. Los niveles de concentración más bajos deben coincidir con el LOQ (límite de cuantificación). Si se diese el caso de encontrarnos con un rango de trabajo mayor, tendríamos que realizar diluciones considerando la incertidumbre que generarían las mismas. Por otro lado, la linealidad debe ser comprobada usando 3 o más niveles de concentración usando una función linear, cuadrática y con o sin ponderación, siendo aceptada cuando su valor de coeficiente de correlación es ≥ 0.99. Además, la desviación de las de las concentraciones retro-calculadas de los estándares de calibración de las concentraciones reales, usando la curva de calibración en la región relevante, no debe exceder de ± 20 %

Límite de detección del método: es la menor concentración del analito a determinar que puede ser detectada. Según la IUPAC, se define el límite de detección como la concentración de analito, que resulta en un pico cromatográfico, con una relación señal/ruido ≥ 3.

Límite de cuantificación del método: es la menor concentración cuantificable con el método desarrollado en muestras reales y disoluciones de estándares. Aunque existen diferentes propuestas, la más extendida para su cálculo es la de la IUPAC que establece que este valor sea 10 veces superior a la señal de fondo (relación señal/ruido ≥ 10). Para evitar errores, es común considerar una señal ruido de 3 para la transición de confirmación (SRM2).

Exactitud (recuperación de los analitos): son estudios que se realizan para validar un método analítico usando muestras fortificadas. La recuperación debe realizarse a dos niveles de concentración, próximo al límite de cuantificación y 10 veces el LOQ, además de llevar a cabo 3 o 4 réplicas de cada uno. Los valores de recuperación son aceptables cuando el rango de este está entre 70-120%. La muestra fortificada a una concentración conocida y su respuesta es comparada con la respuesta obtenida de un extracto fortificado. Mediante la **ecuación 1** es calculado el valor de recuperación. En términos de precisión (reproducibilidad) las réplicas deber tener un RSD ≤ 20%.

$$\text{Rec (\%)} = \frac{respuesta\ de\ la\ muestra\ fortificada}{respueta\ del\ extracto\ fortificado} * 100 \quad \textbf{(ecuación 1)}$$

Precisión (en términos de repetitividad y reproducibilidad): cuantifican la variabilidad de los resultados analíticos en función del operador, las manipulaciones inherentes al método y el medio ambiente del laboratorio. Repetitividad: es una medida de dispersión interna (misma muestra analizada en el mismo laboratorio con el mismo equipo y operador dentro de un intervalo corto de tiempo) mientras la reproducibilidad incluye todas las posibles fuentes de variaciones aleatorias de datos, por lo que se evalúa mediante la obtención de resultados de ensayos independientes con el mismo método, aplicado a la muestra a analizar en diferentes condiciones como laboratorio, equipamiento, distintos operadores y diferentes días. La precisión es expresada en términos de RSD y su valor es aceptado cuando es ≤ 20%. Actualmente, los estudios de repetitividad y reproducibilidad son menos considerados para la validación del método porque mediante el estudio de recuperación que puede llevarse a cabo en el mismo o distinto día pueden obtenerse también valores de precisión

Efecto matriz: es el efecto que ejercen los constituyentes desconocidos presentes en la matriz en el análisis de los analitos individuales. Existen diferentes comportamientos, desde un desplazamiento en el tiempo de retención, una variación en la ionización, un cambio en el proceso de detección (aumento o supresión de la señal) o detección de falsos positivos debido a la presencia de compuestos co-extraidos con propiedades físico-químicas o relación masa/carga similar. El efecto matriz se estudia mediante la comparación de la pendiente de la recta de calibrado preparada en disolvente y en matriz, siguiendo la **ecuación 2**:

$$\text{ME (\%)} = \left(\frac{pendiente\ de\ la\ curva\ de\ calibrado\ en\ matriz}{pendiente\ de\ la\ curva\ de\ calibrado\ en\ disolvente} - 1\right) * 100 \quad \textbf{(ecuación 2)}$$

Es importante cuantificar el impacto de estos efectos en la medida de cada analito, para obtener resultados reproducibles y exactos. Por ello es aconsejable, el empleo de un blanco instrumental como disolvente orgánico, agua destilada o fase móvil en varias posiciones de la serie de medidas o detrás de un estándar de la concentración, para comprobar la contaminación que se haya podido quedar después del análisis en el instrumento, ya que como hemos dicho anteriormente, los contaminantes de preocupación emergente se encuentran en muestras ambientales a niveles traza, y cualquier pequeña contaminación puede alterar los resultados..

Con la validación de un método se proporciona una idea acerca de las capacidades y limitaciones del método y siempre que se implementa en rutina hay que comprobar que el método permanece bajo control. Para ello hay que seguir unos controles internos específicos realizados en el laboratorio para supervisar continuadamente las operaciones y resultados de medida con el fin de decidir si los resultados son fiables. Estos controles internos incluyen:

- Controles de recuperación en cada lote de muestras
- Estudio de reproducibilidad inter-días
- Aplicación de estándares internos en distintos puntos del método analítico que permitan controlar tanto la extracción de muestras como la cuantificación
- Bracketing

Con los controles de recuperación introducidos en cada lote de muestras se obtiene una medida del sesgo (recuperación) y de la precisión (desviación estándar relativa, RSD) obtenidos aplicando el mimo método dentro del mismo laboratorio en un período de tiempo acotado.

Estándares internos: son sustancias añadidas antes del proceso de extracción de la muestra (analitos marcados isotópicamente) para comprobar la eficacia del proceso. Se añaden a una concentración conocida y en una etapa determinada del análisis para verificar la correcta ejecución del método analítico. Durante la extracción la recuperación de dichos estándares debe estar en un rango de 60-140% respecto el valor de referencia. Entre los estándares más habituales para el análisis de plaguicidas están malathion-d6, carbendazim-d3, clorfenvinfos-d10, mientras que para el análisis de fármacos el más usado es caffeine13C.

Estándares instrumentales: es un compuesto añadido antes de la inyección del extracto en el equipo para comprobar la exactitud y fiabilidad del analizador, además de determinar pérdidas de analitos fortificados y estándar interno durante la preparación de la muestra. El estándar interno de inyección debe tener una señal constante sino se detectarían variaciones de volumen de inyección indicando fallos del instrumento analítico.

Bracketing: se inyectan los estándares de calibración al inicio y al final de la secuencia de muestras para comprobar que no ha sufrido el equipo ninguna derivación. La relación de la señal de los estándares del inicio con los del final de la secuencia no deben superar el 30%, sino las muestras deben ser nuevamente analizadas.

6.2. Confirmación de los resultados analíticos

La espectrometría de masa en el análisis de contaminantes orgánicos en muestras ambientales ha supuesto una mejora en la capacidad de confirmación, sin embargo, falsos negativos y falsos positivos pueden obtenerse principalmente debido a la supresión y aumento de la señal o insuficiente selectividad del método. Por tanto, para evitar estos resultados errores, es necesario establecer unos criterios de confirmación útiles considerando los distintos modos de operación e instrumentos utilizados.

En la Decisión 2002/657/EC están establecidos los criterios de identificación y confirmación para el análisis de contaminantes orgánicos en muestras alimentarias, pero se ha ido expandiendo a otras matrices incluyendo muestras ambientales. En ella, se describen una serie de criterios mínimos que debe cumplir el método para utilizarse en dos grupos de contaminantes:

Grupo A (compuestos prohibidos): donde encontramos estilbenos, derivados de estilbenos y sus sales y esteres, agentes antitiroideos, esteroides, lactonas de ácidos resorcílico…, es decir, sustancias con efectos anabolizantes.

Grupo B (compuestos con nivel de residuo establecido): sulfonamidas, quinolonas y otros fármacos veterinarios, antelmínticos, anticoccidiales como nitroimidazoles, carbamatos y piretroides, sedantes antinflamatorios no esteroideos, otra sustancias farmacológicamente activas y sustancias y contaminantes ambientales como compuestos organoclorados, compuestos organofosforados, elementos químicos, micotoxinas y colorantes.

La Decisión 2002/657/EC considera el uso de puntos de identificación (IP) obtenidos de acuerdo con los criterios de calidad para la identificación y confirmación espectrométrica (**tabla 11**). Para la confirmación de sustancias del grupo A se requieren de un mínimo de 4 IPs, mientras para sustancias del grupo B solo 3 IPs. Los IP son unos criterios estandarizados, establecidos internacionalmente, para la identificación y confirmación de analitos.

Tabla 11. Ejemplos de número de puntos de identificación (IPs) obtenidos para un rango de técnicas de espectrometría de masas (MS) y combinaciones de iones.

Técnica de MS	Ejemplo de iones	IPs obtenidos
GC-MS (Q)	1 ion (SIM)	1.0
GC-MS/MS (IT)	1 precursor, 2 productos MS^2	4.0
LC-MS (Q)	1 ion (SIM)	1.0
LC-MS/MS (QqQ)	1 precursor, 2 productos (SRM)	2.5
LC-MS/MS (IT)	1 precursor, 1 producto MS^2 y 2 MS^3	5.5
LC-TOF-MS	1 ion	2.0
LC-Q-TOF-MS	1 precursor, 1 producto (MS/MS)	4.5

Por tanto, de acuerdo con la **tabla 11,** para confirmar un compuesto usando la técnica LC-MS/MS en modo SRM, se necesitaría la presencia de un ion precursor y al menos dos transiciones SRM (obteniéndose así los 5 IPs). Con la aplicación precisa de los criterios de identificación y confirmación en términos de IPs, se asegura una correcta identificación del analito en muestras ambientales. Según las normas establecidas por la Comisión Europea en el documento Nº SANTE/11312/2021 las transiciones deben tener señales cromatográficas que cumplan los siguientes requisitos:

- solaparse completamente,
- tener una relación señal/ruido superior a 3

Otro criterio para confirmar la presencia de analitos es la relación entre las abundancias de las transiciones (ratio, SRM2/SRM1) y el tiempo de retención de cada compuesto, que es característico. Según la directiva, tiempo de retención no debe variar más de ± 0.1 min respecto al estándar analítico y la respuesta relativa no puede diferir en más de un 30% en términos relativos respecto a la correspondiente al promedio de los estándares de calibración del mismo.

En algunos casos, solo se puede usar una transición obteniéndose 2.5 IPs, de forma que se tendrían insuficientes datos para una correcta caracterización de un compuesto en la muestra. Así, la confirmación de compuestos se haría mediante la comparación del tiempo de retención con el obtenido en una disolución estándar.

7. Referencias

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CAPÍTULO I

Artículo Científico

“Determination study of contaminants of emerging concern at trace levels in agricultural soil. A pilot study”

M. García-Valverde, M. J. Martínez-Bueno, M. M. Gómez-Ramos, A. Aguilera,
M. D. Gil García, Amadeo R. Fernández-Alba

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Determination study of contaminants of emerging concern at trace levels in agricultural soil. A pilot study

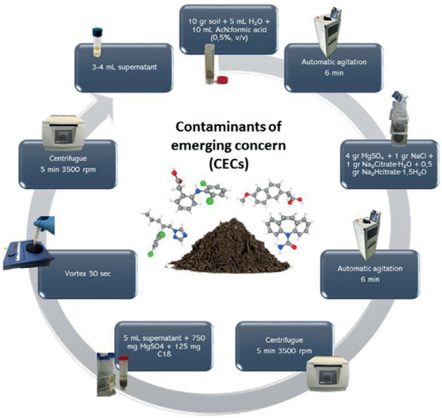
M. García Valverde, M.J. Martínez Bueno, M.M. Gómez-Ramos, A. Aguilera,
M.D. Gil García, A.R. Fernández-Alba *

University of Almería, Department of Chemistry and Physics, Agrifood Campus of International Excellence (ceiA3), Ctra. Sacramento s/n, La Cañada de San Urbano, 04120 Almería, Spain

HIGHLIGHTS

- Three different extraction methods were developed and compared.
- All they affordable for routinely control laboratories
- Sensitive quantification of 30 contaminants of emerging concern in agricultural soil
- A modified QuEChERS method showed the best results.
- A pilot study was carried out in real agronomic conditions in a greenhouse.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, we aimed to develop and validate a quick, easy, and robust extraction method for the simultaneous determination of 30 organic contaminants of emerging concern (CECs) including some transformation products in soil samples. Three different extraction methods based on an ultrasonic cylindrical probe (UAE), a pressurized liquid extraction (PLE), and a QuEChERS method were compared. Ultra-performance liquid chromatography coupled with electrospray tandem mass spectrometry (LC-MS/MS) was used for identification and quantification of the target analytes. A modified QuEChERS method showed the best results in terms of extractability and accuracy. The extraction procedure developed provided adequate extraction performances (70% of the target analytes were recovered within a 70–99% range), with good repeatability and reproducibility (variations below 20%) and great sensitivity (LOQ $<$ 0.1 ng/g in most cases). No matrix effects were observed for 70% of the compounds. Finally, the analytical methodology was applied in a pilot study where agricultural soil was irrigated with reclaimed water spiked with the contaminants under study. Of the 25 CECs added in irrigation water, a total of 13 pesticides and 5 pharmaceutical products were detected at concentration ranges from 0.1 to 1.2 ng/g (d.w) and from 0.1 to 2.0 ng/g (d.w), respectively.

1. Introduction

Water scarcity is one of the largest issues at a worldwide level. During recent decades, the use of reclaimed water for agricultural irrigation has steadily increased as an alternative to solve the lack of availability of

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* Corresponding author.
E-mail address: amadeo@ual.es (A.R. Fernández-Alba).

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freshwater (Lavrnić et al., 2017). It is estimated that approximately 32% of reclaimed water is used for agricultural irrigation, 20% for landscape irrigation and 19% for industrial processes (Helmecke et al., 2020). The drawback is that this water type contain many different organic contaminants of emerging concern (CECs) because as it has been displayed by different authors, the removal procedures of these chemicals in water treatment plants are deficient (Calderon-Preciado et al., 2011; Martinez-Bueno et al., 2012). Recently, the European Parliament and the Council has published a new Regulation with regarding the minimum requirements for water quality and control for the safe reuse of treated urban wastewater (Regulation (EU) 2020/741, 2020).

Nowadays, analytical methods are focused on the determination of CECs, such as pesticides, pharmaceuticals or personal care products, in reclaimed water and vegetable grown with this type of water (Beltran et al., 2020; Calderon-Preciado et al., 2013; Wu et al., 2014). Anti-inflammatory drugs (diclofenac, ibuprofen or naproxen), anti-hypertensives (atenolol or furosemide), antiepileptic (carbamazepine), analgesic (acetaminophen) and antibiotics (azithromycin, ciprofloxacin, clarithromycin, ofloxacin and oxytetracycline) have been the pharmaceuticals more analyzed to date (Carmona et al., 2017; Pico et al., 2019). Whereas neonicotinoid insecticides (acetamiprid, imidacloprid or thiacloprid) and fungicides (thiabendazole, azoxystrobin, penconazole or diazinon) have been the pesticides more reported in such matrices (Acosta-Dacal et al., 2021; Perez-Mayan et al., 2020; Pico et al., 2019). Nevertheless, there is a large gap of knowledge on the extent to which terrestrial ecosystems are affected by the use of reclaimed water for agricultural irrigation. CECs come into contact with agricultural soil and those with low hydrophobicity are accumulated in the soil through interactions with organic material (Beltran et al., 2020). Thus, the determination of CECs in agricultural soils irrigated with reclaimed water for assessing potential environmental and human health effects is necessary.

Several extraction techniques have been applied to the analysis of soil samples (Andreu and Picó, 2019; Santana-Mayor et al., 2019). Most of them are based on ultrasound-assisted extraction (UAE), pressurized liquid extraction (PLE) or supercritical fluid extraction (SFE). Sonication though the cylindrical probe is more efficient than an ultrasonic bath but this last is more common for its low cost (Lesueur et al., 2008). In PLE the solid sample is extracted with solvent at high pressures and high temperatures. This extraction method has good results in the efficiency of the extraction process because of the solvent with high temperature can penetrate better to solid sample (Perez-Mayan et al., 2020). SFE is often preferred because it is faster, requires less solvent and has a lower risk of sample contamination (Wilga et al., 2008). However, all they often require a further clean-up step to the purification of the extract. In the last years, an extraction procedure called QuEChERS, a method typically used to extract pesticides from food samples, it has been applied to extract organic compounds from soil. The procedure is based on a salting-out extraction with acidified acetonitrile, followed by a dispersive solid-phase extraction (d-SPE). It is rapid, simple, requires low solvent consumption and allows the extraction of a large number of compounds at the same time. This method applied to soil was first reported by Lesueur et al. (2008). The good recoveries obtained in soil samples are turning the QuEChERS method into an interesting alternative to extract CECs from soil (Acosta-Dacal et al., 2021; Benedetti et al., 2020; Masia et al., 2015).

Soil matrices extracts usually have a high content of organic components (such as humic acids), and lipids which increase the viscosity of the sample, and as consequence, also the matrix effects derived from the presence of interfering substances in the injection vial. According to recent reviews, analytical methods for the determination of CECs in soil are scarce and mainly developed for sediments and sewage sludge (Benedetti et al., 2020; Luque-Munoz et al., 2017; Malvar et al., 2020; Martin-Pozo et al., 2019; Ponce-Robles et al., 2017). Also, most of them are only to specific chemical families of contaminants, such as pesticides (Feng et al., 2015; Lesueur et al., 2008; Li et al., 2013) or pharmaceuticals, veterinary medicines and daily personal care products (Garcia-Galan et al., 2013; Lee et al., 2017; Salvia et al., 2012). Therefore, the development of a multi-residue extraction method that allows the simultaneous analysis of several classes of organic compounds with different physicochemical properties at trace levels in agricultural soil is necessary.

In this context, the main objective of this study was to develop and validate a quick, easy and robust extraction method for the simultaneous determination of 30 CECs (including pesticides, pharmaceutical products and some of the main transformation products) in agricultural soil. Three different extraction methods based on an UAE, a PLE without clean-up step, and a QuEChERS method were compared. To our knowledge, a multi-residue and inter-family extraction method for agricultural soil analysis has never been proposed.

2. Materials and methods

2.1. Chemicals and reagents

30 of most frequently reported as agricultural and urban organic environmental contaminants in the literature were selected in this study (Martinez-Bueno et al., 2012). They comprise 13 pesticides (acetamiprid, azoxystrobin, carbendazim, diazinon, diuron, fluxapyroxad, imidacloprid, myclobutanil, penconazole, pymetrozine, thiabendazole, thiacloprid, thiamethoxam), 12 pharmaceuticals (acetaminophen, atenolol, caffeine, carbamazepine, ciprofloxacin, codeine, diclofenac, furosemide, gemfibrozil, hydrochlorothiazide, naproxen, ofloxacin) and 5 transformation products (4-methylamino-antipyrine, 4-amino-antipyrine, 4-formylamino-antipyrine, 4,4-acetylamino-antipyrine and carbamazepine-10,11-epoxi). Table 1 shows some of their physicochemical properties. Caffeine-13C,

Table 1
Physicochemical properties of all target compounds selected in this study.

Family	Compound	Log Kow	pKa	Koc	Water solubility
Antibiotic	Ofloxacin	−0.4	5.9	44	28,300
Insecticide	Pymetrozine	−0.2	4.1	246–7875	270
Analgesic	4-AAA*	−0.1	12.4	n.a	40,226
Insecticide	Thiamethoxam	−0.1	0.4	32–237	4100
Stimulant	Caffeine	−0.1	14	741–7762	21,700
Diuretic	Hydrochlorothiazide	0.1	7.9	12	722
b-blocker	Atenolol	0.2	9.6	n.a	13,300
Analgesic	4-FAA*	0.2	12.7	n.a	101,289
Antibiotic	Ciprofloxacin	0.3	6.1	61,000	30,000
Analgesic	Acetaminophen	0.5	9.4	20,844	14,000
Analgesic	4-AA*	0.5	4.1	n.a	727,617
Analgesic	4-MAA*	0.6	n.a	n.a	28,897
Insecticide	Imidacloprid	0.6	11.1	156–800	610
Insecticide	Acetamiprid	0.8	0.7	132–267	4250
Analgesic	Codeine	1.2	8.2	700	<1
Insecticide	Thiacloprid	1.3	0.5	408–1584	185
Fungicide	Carbendazim	1.5	4.2	122–2805	8
Antiepileptic	Epoxide-CBZ*	1.6	n.a	n.a	1340
Diuretic	Furosemide	2.0	3.9	110	73
Herbicide	Diuron	2.3	13.6	55–962	37
Fungicide	Thiabendazole	2.4	4.7	2500–4680	30
Antiepileptic	Carbamazepine (CBZ)	2.4	13.9	510	18
Fungicide	Azoxystrobin	2.5	0.9	210–580	7
Fungicide	Fluxapyroxad	3.1	12.6	496–1424	3
Fungicide	Myclobutanil	3.2	2.3	950	115
Anti-inflammatory	Naproxen	3.2	4.1	330	16
Insecticide	Diazinon	3.3	2.6	191–1842	60
Fungicide	Penconazole	3.7	1.5	786–4120	73
Analgesic	Diclofenac	4.0	4.1	245	2
Lipid regulators	Gemfibrozil	4.8	4.5	430	11

Kow: octanol/water coefficient; pKa value: negative log of the acid dissociation constant; Koc (ml/g): organic carbon sorption constant; Water solubility at 25 °C (mg/L): solubility in water; *Metabolites (Epoxide-CBZ: carbamazepine-10,11Epoxi; 4-MAA: 4-methylamino-antipyrine; 4-AA: 4-amino-antipyrine; 4-FAA: 4-formylamino-antipyrine; 4-AAA: 4-4-acetylamino-antipyrine); n.a.: not available.

carbendazim-d3, malathion-d10 and dichlorvos-d6 were selected as internal standards to check the extraction efficiency. Dimethoate-d6 was used as injection standards for analytical efficiency. The reference standards with high purity (>98%) were acquired from Sigma-Aldrich (Steinheim, Germany), except codeine that was obtained by pill. Each pill contained 28.7 mg of codeine phosphate hemihydrate. Working solutions of pesticides and pharmaceuticals were prepared by dilution of the individual stock solutions at 10000 mg/L in AcN. Hydrochlorothiazide, acetaminophen and codeine were prepared in methanol at pH = 10 and a mixture water-methanol (50:50, v/v). These solutions were stored at −20 °C in amber screw-capped glass vials. For identification and quantification were daily prepared standard working solution at 1 mg/L.

For the optimization of the chromatographic and mass spectrometer conditions intermediate solutions at 200 μg/L were prepared. HPLC-grade acetonitrile was purchased from Merck (Darmstadt, Germany) and LC-MS optima grade water from Fisher Scientific (Fair Lawn, NJ, USA). Formic acid (purity 98%) was obtained from Fluka (Buchs, Germany). Magnesium sulphate anhydrous ($MgSO_4$), sodium chloride (NaCl), sodium hydrogenocitrate sesquihydrate ($Na_2HCitrate \cdot 1{,}5H_2O$) and sodium citrate tribasic dihydrate ($Na_3Citrate \cdot 2H_2O$) were purchased from Sigma-Aldrich (Steinheim, Germany). Primary-secondary amine (PSA) was obtained by Supelco (Bellefonte, PA, USA) and Bondesil-C18 sorbents from Agilent Technologies (Santa Clara, CA, USA).

2.2. Sample collection

A tomato (*Solanum lycopersicum* L.) crop was grown in a greenhouse located in Almeria (Southeast Spain), under controlled agronomic conditions and using reclaimed water to drip irrigation obtained from a wastewater treatment station with an ultrafiltration/chlorination process. The mixture had a nitrate content below 0,04 meq/L, 169 mg/L of chloride and 106 mg/L of sodium, and pH and electrical conductivity values of 8.0 and 536 μS/cm, respectively. The greenhouse's surface (540 m^2) was divided into two plots: one irrigated only with reclaimed water (control plot A) and the other with spiked reclaimed water (treatment plot B). No targeted organic contaminant residues were detected in the reclaimed water used to the study. The spiked reclaimed water used in the plot B was prepared to add t each one of the 25 selected CECs at 1 μg/L (considering the worst-case scenario, based on previous results of our research group) (Martinez-Bueno et al., 2012). No transformation products were added to the irrigation water to study the potential degradation of the precursor compounds (carbamazepine and metamizole). This solution was pumped through a dosing pump (3 L/h). The crop/soil was daily irrigated for 25 min. The total amount of spiked reclaimed water used in each plot was 2200 L. Irrigation water samples (100 mL) were taken once a week in clean polypropylene bottles from the exit of the drips at 10 cm depth, in each of the study plots. The agricultural soil contained 15% clay, 20% silt and 65% sand. It had 0.92% organic carbon, 100 ppm of total nitrogen, and pH and electrical conductivity values of 7.7 and 3240 μS/cm, respectively. Soil samples of the upper 10 cm layer were collected in polyethylene bags and transferred to the laboratory where they were sifted with a 2 mm diameter sieve and dehydrated in an oven at 30 °C for 24 h. Finally, samples were frozen and stored at −20 °C until their analysis.

2.3. Extraction method

Three extraction methods were assessed and compared in this work: (i) an ultrasonic extraction method with a cylindrical probe (UAE), (ii) a new pressurized liquid extraction method without clean-up step (PLE) and (iii) a QuEChERS method. In order to compare the results, solvent composition used in all they were the same. Prior to extraction step, 10 μL of a mix of internal extraction standards at 10 mg/L was added to check the extraction efficiency (caffeine-13C, carbendazim-d3, and dichlorvos-d6). Before injection, 100 μL of the final extract was evaporated to dryness under a nitrogen stream and reconstituted with 90 μL of AcN:water solution (1:9, v/v) and 10 μL of dimethoate-d6 in all methods.

2.3.1. Pressurized liquid extraction (PLE) method

PLE experiments were carried out with an automated extraction system (EDGE®, CEM Corporation, Charlotte, NC, USA). The experimental conditions used in this study were based on a previously reported method by our research group to pesticide residue analysis in dry food commodity samples (Diaz-Galiano et al., 2021). Briefly, samples were extracted with 10 mL of AcN (0.5% v/v, formic acid) at 40 °C and 25 psi. Soil samples (10 g d.w) were introduced into an aluminium Q-Cup sample holder. A thin 0.3 μm glass fibre filter (G1 Q-Discs®) and two cellulose filters (C9 Q-Disc®) were used to provide structural support and to filter the sample before analysis. Q-Cups and Q-Discs® (G1 and C9 varieties) were also provided by CEM Corporation. The Q-Cup was then placed into the EDGE instrument alongside a 50 mL PTFE falcon tube to collect the sample extract. Total time of extraction was 6 min for the sample. In comparison with other reported procedures based on PLE, no clean-up sorbents have been used in this study. A diagram of the procedure is presented in Fig. 1.

2.3.2. Ultrasonic accelerated extraction (UAE) method

UAE experiments were carried out with a Sonoplus HD 3100 ultrasonic system (Badelin Electronic GmbH & Co., KG, Germany). The apparatus was equipped with a GM3100 high-intensity generator (100 W), a UW 3100 ultrasonic converter, an SH 70G standard horn, and a 3 mm-diameter titanium MS73 probe for 2–50 mL volumes. Pre-treated soil samples (10 g d.w) were hydrated with 5 mL of distilled water (vortexed for 30 s and left for 10 min) and extracted with 10 mL of AcN (0.5% v/v, formic acid) by sonication at a 75% amplitude for 2 min with an ultrasonic probe (seven extraction cycles of 15 s each plus a 2 s pause during this 120 s). Set-up conditions used in this study were based on a previously reported method by our research group to pesticide residue analysis in dry food commodity (Gil-Garcia et al., 2018). After that, the tubes were centrifugated at 3500 rpm for 10 min. Then, 5 mL of supernatant was transferred to a 15 mL polyethylene tube with 750 mg of anhydrous $MgSO_4$, 120 mg of PSA and 120 mg of C18. Next, tubes were shaken with vortex for 30 s and centrifuged at 3500 rpm for 5 min. An operational scheme of the procedure is shown in Fig. 1.

2.3.3. Modified QuEChERS method

Sample extraction was based on a previously published method by our research group (García Valverde et al., 2021). Briefly, dried soil samples (10 g d.w) were rehydrated with 5 mL distilled water. Then the samples were vortexed for 30 s and left for 10 min. Subsequently, 10 mL of acidified AcN (0.5% v/v, formic acid) was added. The samples were shaken in an automatic axial extractor (AGITAX®, CirtaLab.S.L., Spain) for 6 min at 25 °C. Next, 4 g of anhydrous $MgSO_4$, 1 g of $Na_3Citrate \cdot 2H_2O$, 1 g of NaCl and 0.5 g of $Na_2HCitrate \cdot 1{,}5H_2O$ were added and shaken once more. The samples were centrifuged at 3500 rpm for 5 min. Then, 5 mL of supernatant was transferred to a 15 mL polyethylene tube with 750 mg of anhydrous $MgSO_4$, and 125 mg of C18. The tubes were vortexed for 30 s and centrifuged at 3500 rpm for 5 min. Fig. 1 shown a scheme of the QuEChERS procedure used for the soil sample extraction.

2.4. Liquid chromatography-tandem mass spectrometry analysis

A Sciex Exion HPLC coupled to a Sciex 6500+ TripleQuand-LC-MS/MS from Sciex was used for the analysis. Chromatographic separation was performed on a Zorbax Eclipse Plus C8 of 1.8 μm × 2.1 mm × 100 mm (Agilent). Mobile phases were 0.1% formic acid in water optima (solvent A) and AcN (solvent B) at a constant flow rate of 0.3 mL/min. The optimized gradient program was: 10% of B (initial conditions) for 0.5 min, after a linear gradient up to 100% of B in 11.5 min; kept at

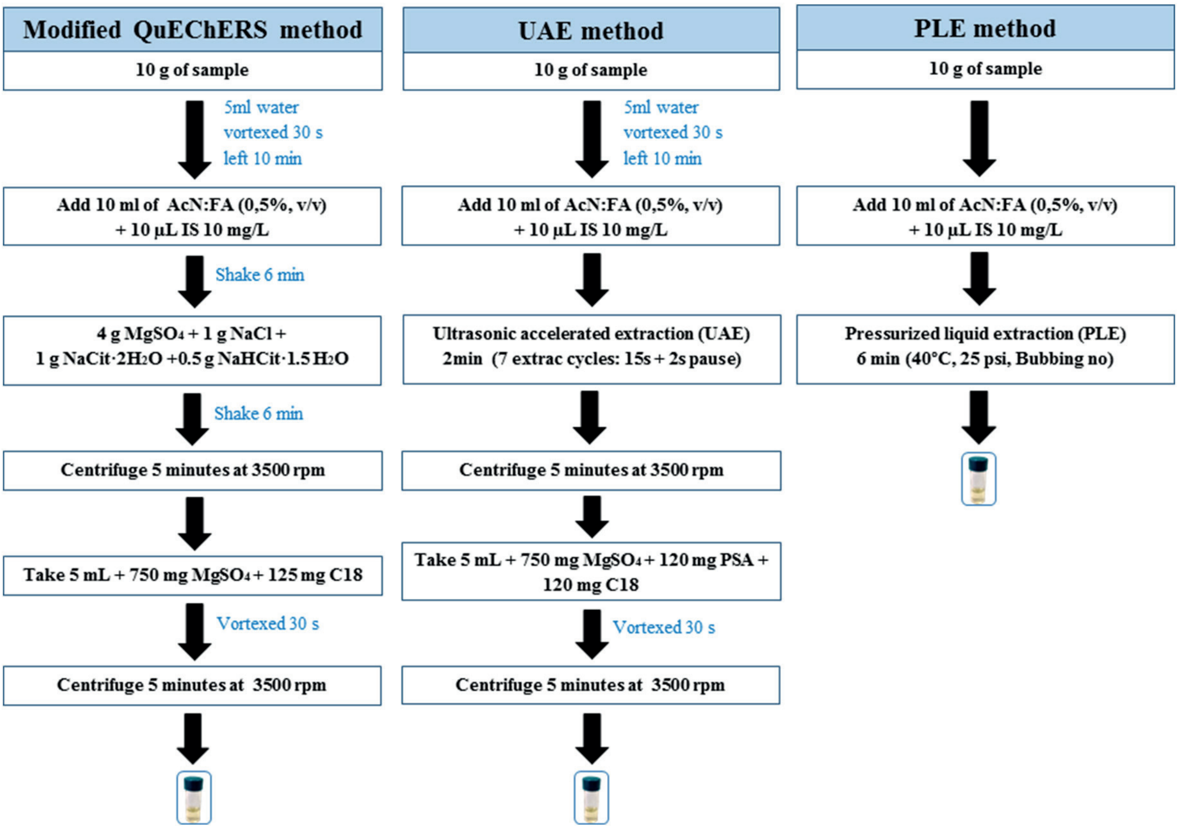

Fig. 1. Diagram of the three procedures used for the soil sample extraction.

100% of B for 4 min and finally, the mobile phase came back to the initial conditions (10% B). The total run time was 18 min and the injection volume was 5 μL. The HPLC was coupled to a QqQ-MS/MS with an ESI source (turbo spray iondrive), operating with positive and negative ionization modes. The ionization settings used were: curtain gas, 20 (arbitrary units); GS1, 50 psi; GS2, 40 psi; and temperature, 500 °C. The ionspray voltages were set at 5000 and −4500 V in positive and negative ionization mode. Nitrogen was used as the nebulizer gas and collision gas.

For the optimization of the chromatographic and mass spectrometer conditions individual pesticides and drugs standard solutions at 200 μg/L were used. The solutions were infused directly into the MS system in full-scan mode. The most intense ion was chosen as the precursor ion. Next, in product-ion mode the optimal collision energy (CEs) values for the two most intense transition were selected; the most intense ion was selected as the quantifier ion (SRM1) and the second ion as the qualifier ion (SRM2). The optimal mass spectrometric parameters for each target compound are presented in Table S1 as supplementary material. In the SCIEX OS acquisition and quantitative software (SCIEX) was applied schedule SRM with a retention time window of 0.4 min.

2.5. Analytical performance and quality control

The validation of the analytical approaches was performed according to the SANTE European Guideline 12682/2019 (SANTE/12682/2019, 2019). The analytical parameters evaluated were sensitivity, linearity, matrix effect, trueness (in terms of recovery), precision (in terms of method repeatability) and selectivity. The more demanding requirements regarding mass spectrometric confirmation currently set by EU regulations were taken into account for identification and confirmation of the target compounds (Directive 96/23/EC, 2002). These criteria were: The quantification transition (SRM1) with $s/n \geq 10$; the detection transition (SRM2) with $s/n \geq 3$; retention time ± 0.1 min with reference to standard and comparing of fragment ion area with precursor ion area (ion ratio) with a value ±30%.

In order to ensure quality measurements, each day before analysis a control standard mixture (2 ng/L) containing targeted analytes was injected to check the performance of the HPLC, analytical column, and QqQ-MS/MS system. Continuous monitoring of the quality of the analytical procedure was carried out through the inclusion of blanks (solvent) during the day-work sequence. No target analyte was detected in solvent blanks.

3. Results and discussion

3.1. Optimization of the extraction methods

Optimization of three proposed methodologies was carried out using blank soil sample (10 g d.w) spiked at 500 ng of each standard by triplicate. Soil samples acquired from control plot A were analyzed. No targeted compound residues were found in them. Therefore, they were selected as blank material. The spiked soil samples were stored 24 h at 25 °C until the total evaporation of the solvent and the correct balance between analytes/matrix before their extraction. The results of the recovery experiments were used to determine the effectivity of the extraction.

Firstly, PLE method optimisation was focused on the extraction solvent. The experimental conditions applied were based on a previously published method by our research group (Diaz-Galiano et al., 2021). In that work, satisfactory recoveries for the pesticide extraction from dried samples were obtained using AcN and a temperature of 40 °C. In this occasion, better results for pharmaceutical products were found when extraction organic solvent contained 0.5% of formic acid because a slightly acidic pH favours the extraction of basic compounds. The recoveries of diclofenac, furosemide, naproxen, gemfibrozil, and two transformation products of metamizole (4-AAA and 4-FAA) were 58%, 39%, 54%, 42%, 40%, and 33%, respectively, using AcN:0.5%FA. Values below 15% were obtained for all of them using only AcN as extraction solvent. Data included in Table S2 in supplementary material.

Secondly, UAE method was assessed. The parameters used to the extraction were previously optimized by our group (Gil-Garcia et al., 2018). According to results reported, we decided to select 75% amplitude by 120 s (seven extraction cycles of 15 s with a 2 s of pause) because recoveries are better when the number of cycles decreases and extraction time per cycles increases. Thus, in this work, UAE method optimisation was focused on the influence of clean-up sorbents. In the beginning, the UAE method was done without a clean-up step. The results were slightly higher for all target compounds when a mixture of clean-up sorbents (MgsO4, PSA and C18) was used. For example, the insecticide thiacloprid and the metabolite of the antiepileptic carbamazepine (carbamazepine-10,11-epoxide) were recovered a 68% without clean-up step. Instead, they were recovered a 74% using clean-up sorbents. Data included in Table S3 in supplementary material.

Finally, citrate QuEChERS method was evaluated in terms of the extraction organic solvent and clean-up sorbents. Two of the most commonly used extraction organic solvents were compared (AcN vs MeOH). No significant differences were observed in the results obtained among them. Taking into account that MeOH produces a greater matrix effect due to the co-extraction of a greater number of matrix interferences, AcN:0.5% FA was finally selected as the extraction solvent (Annesley, 2007). In relation to clean-up sorbents, we examined the use of PSA salt. As expected, the results showed that PSA had a high chelating effect, retaining fatty acids and other polar compounds from the organic extract (Caldas et al., 2011). For example, diclofenac, furosemide and naproxen showed recovery values below 22% using the conventional QuEChERS clean-up with PSA. However, their recoveries were 99%, 66% and 93%, respectively, when PSA was removed. Therefore, PSA sorbent was eliminated from the clean-up step. Data of each experiment can be seen in Table S4 in supplementary material. Last, the influence of an additional clean-up step was also tested. For that, the final extracts were filtered with a 0.45 μm syringe filter. No significant differences in the results were observed. Thus, the filtration step was not included in the final method.

3.2. Comparison of extraction methods

Fig. 1 shown a flow chart of the three proposed methodologies to quickly compare the operational parameters of each one of them. A summary of the main experimental conditions tested with each method is presented in Table 2. The composition and volume of organic solvent used for the extraction of analytes from the matrix (10 mL AcN:0.5%FA) as well as the amount of soil sample treated (10 g d.w) were similar for the three proposed methods.

Before UAE and QuEChERS extraction, dried soil samples were rehydrated with 5 mL of distilled water, in agreement with the DG-SANTE. The SANTE document recommends the addition of water to dry matrices before their extraction to improve the extraction efficiency (SANTE/12682/2019, 2019). This step makes sample pores more accessible to the extraction solvent improving the extraction efficiency of polar compounds. Nevertheless, the sample hydration was avoided in the PLE method since higher energy extraction conditions were applied in this approach (Diaz-Galiano et al., 2021).

PLE method was the fastest approach (6 min by sample), whereas UAE and QuEChERS methods required two steps, one for sample extraction (7 min and 17 min, respectively by sample) and other for a clean-up step (5.5 min). Nevertheless, the QuEChERS method allows the simultaneous and automatized extraction of up to 12 samples by run. PLE method also allows the automatized extraction but requires high-cost equipment. Our extraction method based on PLE was faster than ones reported by Perez-Mayan et al. (2020) and Masia et al. (2015) for the determination of pesticides in agricultural soils or by Malvar et al. (2020) for the determination of pharmaceuticals in sludge. Both methods required a total extraction time for 16 min. Perez-Mayan et al. (2020) required two extractions with two different solvents and temperatures whereas Masia et al. (2015) required an extraction for 7 min of heated. On the other hand, QuEChERS method proposed in this paper was less time consuming than that proposed by Salvia et al. (2012) and Malvar et al. (2020) to extract organic contaminants from soil and sludge, respectively. Salvia et al. (2012) reported an extraction in just 7 min but then, two clean-up steps by solid-phase extraction were required. In the approach published by Malvar et al. (2020) the agitation steps were done with a vortex, whereas we used an automatic agitation system.

Recoveries were calculated comparing the response of the analytes in spiked samples and the response in matrix extracts after the extraction process. Fig. 2 shows the recovery results obtained to selected CECs depending on the extraction method. As can be seen, (two transformation products of analgesic metamizole (4-AA and 4-MAA) could not be recovered with any approach. Only the proposed QuEChERS method allowed the recovery of the antibiotics ofloxacin, and ciprofloxacin, but below to 5% in both cases. The repeatability was similar for the three proposed method (RSD below 6%). Recoveries obtain by PLE had a range from 9 to 89% with a mean value of 46% (RSD 46%). Recovery values were lower than 50% for 12 of 26 recovered compounds using PLE. UAE method It presented recoveries ranged from 12 to 101% with a mean value of 62% (RSD 39%). The ultrasonic cylindrical probe allowed to enhance the efficiency of the extraction process and to duplicate the recovery values of three pharmaceuticals (acetaminophen, codeine, and carbamazepine-10,11-epoxide) and two pesticides (carbendazim and thiabendazole). The recoveries obtained by Malvar et al. (2020) to carbamazepine and its main metabolite carbamazepine-10,11-epoxide were 72% and 56%, respectively using an ultrasonic bath. In the present study, values of 87% to carbamazepine and 74% for its metabolite were obtained. Similar results were reported by Lesueur et al. (2008). They achieved recovery values of 50% to carbendazim and 60% to diuron using an ultrasonic cylindrical probe while their recoveries were 62%

Table 2
Experimental conditions evaluated with each extraction method.

Sample amount (g)	PLE		UAE		QuEChERS		
	10	10	10	10	10	10	10
Hydration (mL)	–	–	5	5	5	5	5
Extraction solvent	AcN	AcN 0.5%FA	AcN 0.5%FA	AcN 0.5%FA	AcN 0.5%FA	MeOH 0.5%FA	AcN 0.5%FA
Total volumen (mL)	10	10	10	10	10	10	10
Clean-up	–	–	–	MgSO4, PSA, C18	MgSO4, PSA, C18	MgSO4, PSA, C18	MgSO4, C18
Total extraction time (min, per sample)	6		2 + 5[a]		12 + 5[a]		
Total clean-up time (min, per sample)	–		–	0.5 + 5[a]	0.5 + 5[a]		
Total time of method (min, per sample)	6		7	12.5	22.5		
Total number of samples by run	12		1		12		
Total time of method (min, per 12 samples)	72		84	150	22.5		

[a] Centrifugation step.

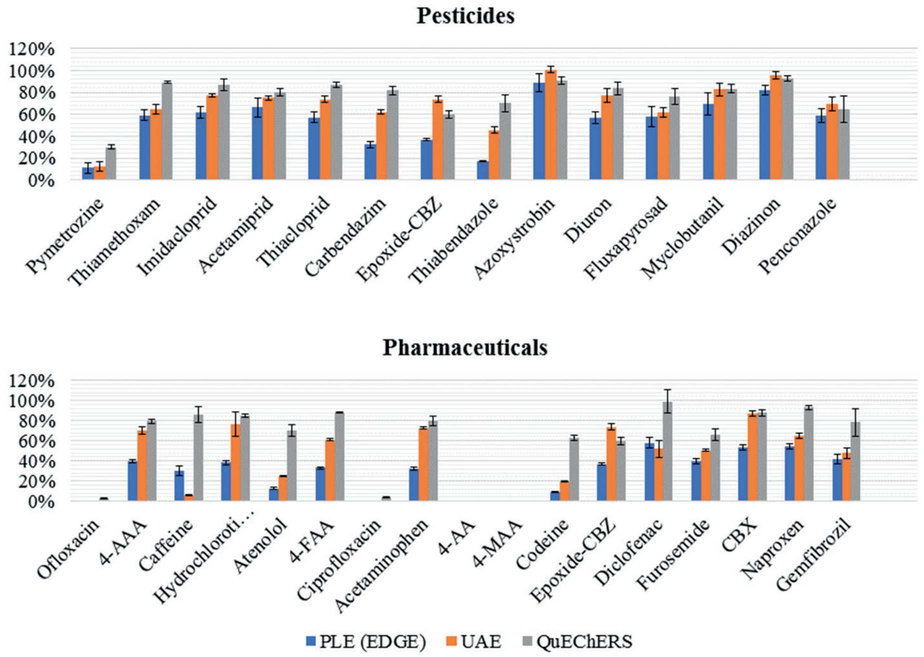

Fig. 2. Average recovery values (%) of the CECs at 50 ng/g from soil with the three extraction methods studied (error bars representing the standard deviation).

and 77%, respectively, with the proposed method in this work. This slight difference in the results can be explained by the diameter of the probe used in the extraction. Lesueur et al. (2008) used a probe with a diameter of 12.7 mm while in our study a smaller probe (3 mm) was used. That allows better penetration of the solvent into the matrix. The modified QuEChERS extraction method without PSA provided the highest extraction recoveries ranged from 24 to 120% (mean value 79%, RSD 33%). The recoveries were higher than 70% for all recovered analytes, except to pymetrozine (30%), codeine (63%), carbamazepine epoxide (60%), furosemide (66%), and penconazole (65%). These values were higher than other investigation previously published. According to the research of Carmona et al. (2017) diclofenac, gemfibrozil, naproxen and carbamazepine were recovered in values below 70% from soil samples. Malvar et al. (2020) reported recovery values below 55% for caffeine, carbamazepine and carbamazepine-10,11-epoxide. However, our extraction method also based on QuEChERS method recovered these compounds above 79%. The results obtained to some pesticides were also better than those reported by Fernandes et al. (2013). For example, azoxystrobin, diazinon or myclobutanil were recovered below 80% while values ranged from 82% to 93% were obtained with our method. In all proposed methods, the recoveries of the selected internal standards (caffeine-13C, carbendazim-d3, malathion-d10, dichlorvos-d6 and dimethoate-d6) were between 70 and 120%.

In summary, average recoveries obtained using UAE and QhEChERS were better than PLE, whose values were higher than 70% for many of the analytes studied. At last, the QuEChERS extraction method was selected based on the results obtained (higher recoveries) and taking into account the extraction times and tedious protocol associated with the UAE technique.

3.3. Method validation

Linearity, matrix effect, limit of quantification (LOQs), precision (repeatability) and trueness (recovery) were evaluated according to the European Union quality control guidance document (SANTE/12682/2019, 2019). A modified QuEChERS method using AcN:FA (0.5% v/v) without PSA was chosen as the best extraction method. Table 3 summarized the validation results obtained using the modified QuEChERS method proposed in this study.

The sensibility of the method was evaluated in terms of limit of quantification (LOQs). LOQs were estimated as the lowest concentration level in the matrix with a signal to noise ratio (S/N) of 10 to the first transition (quantitation transition) and 3 to the second transition (confirmation transition). As can be seen in Table 3, more than 75% of the target CECs (23/30) showed LOQ of 0.05 ng/g. The diuretic furosemide, the analgesics acetaminophen, the antibiotic ciprofloxacin and 4-FAA (a metabolite of metamizole) presented the highest LOQs (0.5 ng/g), followed by the anti-inflammatory naproxen, the antibiotic ofloxacin and the metabolite 4-AAA (0.1 ng/g). These values were lower than those published in the literature to soil samples. Acosta-Dacal et al. (2021) reported LOQs to diazinon, imidacloprid, myclobutanil, penconazole, thiacloprid and thiamethoxam between 0.5 and 2.5 ng/g, Lesueur et al. (2008) reported a LOQ of 0.08 ng/g for carbendazim and Carmona et al. (2017) obtained LOQ values between 15 and 20 ng/g to diclofenac, gemfibrozil, carbamazepine and codeine.

The linearity and matrix effect were evaluated using areas obtained of calibration curves prepared in matrix and solvent at seven concentration levels from 0.05 to 50 ng/g (range from LOQ to one hundred times more). Satisfactory results were observed because correlation coefficients (r2) were higher than 0.99 in all the cases (see Table 3). Matrix effect (ME) was studied comparing the slope of the calibration curve in the matrix with the slope of the calibration curve in the solvent. Results showed that 70% of the targeted CECs not presented matrix effect (≤20%), 26% shown intermediate matrix effect (between 20 and 50%) and only one, ofloxacin had strong effect matrix (62%). These results were better than other previously published in the literature (Acosta-Dacal et al., 2021; Fernandes et al., 2013; Masia et al., 2015).

Recovery studies were evaluated from spiked samples at 10 and 50 ng/g by triplicate. Recoveries were calculated comparing the response of the analyte in spiked samples and the response in matrix extracts after the extraction process at the concentration level previously mentioned. Two transformation products of analgesic metamizole (4-AA and 4-MAA) were not recovered, whereas two antibiotics (ofloxacin and ciprofloxacin) were extracted below 5%. An explanation for these low recoveries could be related to the fact of all them present very high water solubility values (≥25 mg/L), the pKa ranged from 4 to 6, and the Log Kow was below 1, suggesting a possibly high repartition in the water phase and as a consequence, a low concentration in the analyzed organic phase, which explains the low recoveries obtained (Vera

Table 3
Validations results using the modified QuEChERS method for soil samples.

Compound	Log Kow	LOQ (ng/g)	Linearity (r2)	ME (%)	Recovery (%)		Inter/intraday
					10 ng/g	50 ng/g	(%)
Ofloxacin	−0.4	0.1	0.999	−65%	–	3%	7/18
Pymetrozine	−0.2	0.05	1.000	−6%	30%	30%	1/12
4-AAA	−0.1	0.1	0.994	−7%	73%	79%	4/19
Thiamethoxam	−0.1	0.05	0.999	−9%	87%	89%	1/6
Caffeine	−0.1	0.05	1.000	−6%	72%	86%	1/16
Hydrochlorothiazide	0.1	0.05	1.000	1%	85%	85%	5/18
Atenolol	0.2	0.05	0.998	−25%	73%	75%	314
4-FAA	0.2	0.5	0.999	0%	81%	89%	3/4
Ciprofloxacin	0.3	0.5	0.999	−29%	–	4%	15/18
Acetaminophen	0.5	0.5	0.999	−5%	76%	80%	5/20
4-AA	0.5	0.05	0.999	−2%	–	–	4/10
Imidacloprid	0.6	0.05	1.000	−16%	86%	87%	2/15
4-MAA	0.6	0.05	1.000	−20%	–	–	3/19
Acetamiprid	0.8	0.05	0.998	−11%	83%	80%	8/10
Codeine	1.2	0.05	0.991	−7%	50%	63%	2/5
Thiacloprid	1.3	0.05	0.998	−20%	82%	87%	3/6
Carbendazim	1.5	0.05	0.998	−17%	70%	82%	4/4
Epoxide-CBZ	1.6	0.05	0.995	−13%	68%	60%	3/10
Furosemide	2.0	0.5	1.000	2%	63%	66%	15/7
Diuron	2.3	0.05	0.998	−11%	79%	83%	2/6
Thiabendazole	2.4	0.05	0.992	−15%	62%	70%	7/19
CBZ	2.4	0.05	1.000	−26%	79%	88%	6/3
Azoxystrobin	2.5	0.05	0.992	−33%	71%	90%	5/6
Fluxapyroxad	3.1	0.05	0.999	−21%	73%	76%	4/3
Myclobutanil	3.2	0.05	0.999	−17%	81%	83%	15/15
Naproxen	3.2	0.1	1.000	−17%	82%	93%	3/9
Diazinon	3.3	0.05	1.000	−34%	84%	93%	4/6
Penconazole	3.7	0.05	0.999	−30%	73%	65%	7/19
Diclofenac	4.0	0.05	0.997	−18%	86%	99%	15/10
Gemfibrozil	4.8	0.05	0.999	−33%	76%	79%	9/13

LOQs: Limits Of Quantification; Linearity expressed by the correlation coefficient; ME: Matrix effect; Inter/Intra repeatability expressed as relative standard deviation (RSD, in parenthesis).

et al., 2013). Considering only the analytes that were recovered above 5%, more than 80% of targeted CECs were recovered above 70%, four were between 60% and 66%, and only one was extracted below 50% (see Table 3). The results were compared with those from previous research. Masia et al. (2015) reported recoveries lower than 80% to carbendazim, diazinon, diuron, imidacloprid and thiabendazole, whereas we obtained values above 83% to all them. Malvar et al. (2020) obtained average recoveries to caffeine (25%), carbamazepine (53%), or carbamazepine 10,11-epoxide (48%) lower than our results, which were higher than 70% for the above-named.

Intra and inter-day precision (repeatability/reproducibility) were calculated for each analyte from results obtained of the recovery study in terms of relative standard deviations (RSD, %). The repeatability was acceptable with values ranged from 0% to 19%. Finally, the specificity/selectivity of the analytical methodology was assessed through the analysis of three blank samples extracted by the proposed method. No other significant peaks (S/N > 3) were found at the specific retention times of the target pesticides.

3.4. Application of the method to real samples

The developed analytical method was applied to agricultural soil samples obtained from a pilot study carried out under controlled real conditions in an experimental farm located in Almería (Spain) for sixteen weeks in 2020. Some examples of chromatograms of the identified compounds in the agricultural soil samples analyzed are shown in the Fig. 3. The recoveries of each internal standard in all samples (extraction and injection) were between 75 and 100%.

The distribution and average concentration of the CECs detected in the irrigation water samples (16 samples) and in the agricultural soil samples (3 samples) irrigate with contaminated reclaimed water can be seen in Fig. 4. The measured concentrations of CECs in contaminated reclaimed water were generally between 0.9 μg/L and 1.1 μg/L, except to the diuretics furosemide (0.2 μg/L) and hydrochlorothiazide (0.4 μg/L), and the insecticide diazinon (0.4 μg/L). These low concentrations can be related to losing in the irrigation system and/or the reservoir tank used for storing the contaminated recycled water solution in the field, due to degradation processes. As it has been found in literature, both furosemide, hydrochlorothiazide, and diazinon can be degraded by exposure to strong UV light (Cies et al., 2015; Mansour et al., 1997). The results could suggest that furosemide was highly sensitive to UV exposure with more than 30% of the compound degraded after 3 h of treatment, whereas diazinon was 50% degraded after 5 days of exposure to sunlight.

Out of the 25 CECs added compounds in irrigation water, a total of 13 pesticides and 5 pharmaceutical products were detected in soil samples irrigated with contaminated reclaimed water. None of the selected transformation products was found in the agricultural soil samples analyzed. The pesticides concentration levels ranged from 0.1 to 1.2 ng/g (d.w.), whereas pharmaceuticals were found between 0.1 and 2.0 ng/g (d.w.). As can be seen in Fig. 4, in general, CECs with low and medium polarity (Log Kow ≥ 2.5) shown a greater accumulation rate in soil. The fungicides penconazole and myclobutanil were the target compounds detected at the highest concentrations (2.0 ng/g d.w). Considering the amount of pesticide added of each and the irrigation time, their accumulation rates were 62%, and 49%, respectively. Their high octanol-water partition coefficient (Kow > 3.0) as well as their soil sorption coefficient (Koc > 500) implying a strong binding to soil and therefore low mobility in soil (see Table 1). Similar concentrations to both substances were found by Acosta-Dacal et al. (2021) in different types of agricultural soil samples (up to 3.9 ng/g to penconazole and 2.4 ng/g to myclobutanil). Pymetrozine also showed a high accumulation rate (38%). Despite its low Kow (−0.2), it presents a high Koc, which explains that this compound can firmly be fixed in the organic matter of the soil and tends to accumulate in it. Acetamiprid, diuron and diazinon were the pesticides found at the lowest concentration levels in the soil samples, 0.1, 0.4 and 0.4 ng/g d.w, respectively.

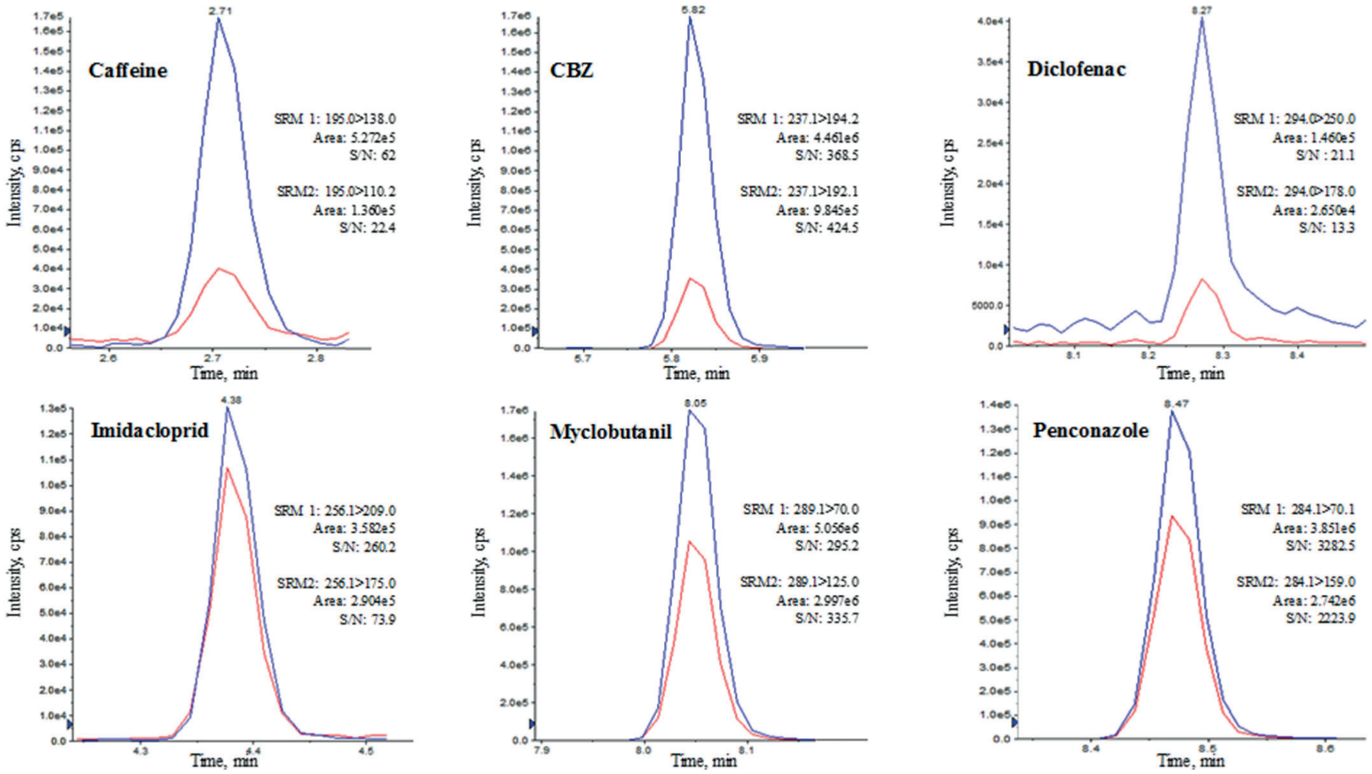

Fig. 3. Selected Ion Chromatogram (XIC) of some of the CECs detected in agricultural soil samples irrigated with reclaimed water.

Regarding pharmaceutical products, the antiepileptic carbamazepine was the compound detected at the highest levels in the soil samples, up to 1.2 ng/g d.w, whereas atenolol was found at lowest concentrations (0.1 ng/g d.w). Similar data were found by Beltran et al. (2020). They explained the low concentrations found to atenolol based on its high solubility in water (30,000 mg/L) and its low Kow (0.2). Caffeine was detected at a concentration of 0.9 ng/g which suppose an accumulation percent in the soil of 21%. Caffeine has been one of the pharmaceutical most detected in soil (Pico et al., 2019). Diclofenac should be the most accumulated compound in the soil due to its low solubility in water and its high Kow value (4.0), however, it showed an accumulation rate of 10%. Carter et al.

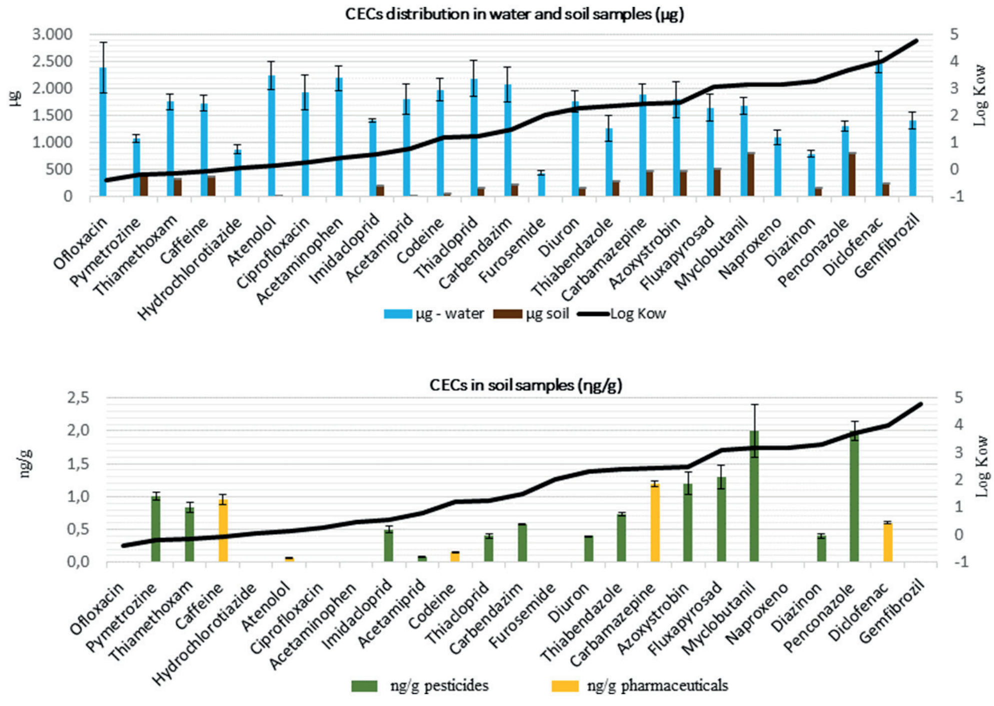

Fig. 4. Average concentration of the CECs detected in water and agricultural soil samples irrigate with spiked reclaimed water at 1 μg/L of each selected compound (error bars representing the standard deviation).

(2014) reported that diclofenac was easily biodegraded, which can explain its low value.

4. Conclusions

The modified QuEChERS method developed in this study presented the highest recoveries (between 29 and 99%, average 79%), followed by the UAE (between 12 and 101%, average 62%) whereas the PLE showed the lowest recoveries (between 9 and 89%, average 46%) of the three proposed methods at a 50 ng/g fortification level. In none of the tested methods, 4-AA, 4-MAA, ciprofloxacin and ofloxacin, compounds with a water solubility higher than 25×10^3 mg/L, moderate acidity ($4 \leq pKa \geq 6$), and very low polarity ($\text{Log Kow} \leq 1$) could be extracted properly. However, the modified QuEChERS method was the most efficient method with around 80% of the extracted target CECs with recoveries range from 70 to 99%. Codeine and pymetrozine presented low recoveries in all the methods. The proposed QuEChERS method demonstrated to be a useful tool for the simultaneous extraction of multiclass organic contaminants with different physicochemical properties from soil samples. It enabled to obtain higher recoveries than those found in the literature to date, for most compounds included in this study. Therefore, this method can be an alternative to techniques more used recently to analyze soil, sludge and sediments such as PLE or UAE.

Finally, the validated method was applied to agricultural soil samples obtained from an experimental farm of UAL-ANECOOP located in Almería (Spain). The total amount of pesticides released during the irrigation was 41×10^3 μg and the CECs total load detected in the soil samples was 5872 ng/g (d.w). The accumulation rates of pesticides in the soil samples ranged from 2% to 62%, whereas pharmaceuticals ranged from 1% to 26%. It means a final concentration that ranged from 0.1 to 1.2 ng/g (d.w) in pesticides and from 0.1 to 2.0 ng/g (d.w) in pharmaceutical products. The simulated reclaimed water was spiked at similar levels than those typically found in reclaimed water for most of the CECs selected in this study. The results obtained under greenhouse conditions highlight the importance to carry out analysis of soil irrigated with reclaimed water to evaluate the long-term accumulation process of these substances in agricultural soil. Future research about the application of the analytical methodology proposed in this study to agricultural soil samples with different content of organic matter is needed.

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CRediT authorship contribution statement

1. **guarantor of integrity of the entire study**: MMG; ARF
2. **study concepts and design**: MJM; MMG; AA; ARF
3. **literature research**: MGV; MJM; AA; ARF
4. **laboratory work**: MGV; MJM;
5. **data analysis**: MGV; MJM; MDG
6. **statistical analysis**: MGV; MJM; MDG; ARF
7. **manuscript preparation**: MGV; MJM; AA; MGD; ARF
8. **manuscript editing**: MJM; ARF

Declaration of competing interest

The authors declare no conflict of interest. This is an independent research. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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CAPÍTULO II

Artículo Científico

“Fate, modeling, and human health risk of organic contaminants present in tomato plants irrigated with reclaimed water under real-world field conditions”

M. J. Martínez-Bueno, M. García-Valverde, M. M. Gómez-Ramos,
J. A. Salinas Andújar, D. Barceló, Amadeo R. Fernández-Alba

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Fate, modeling, and human health risk of organic contaminants present in tomato plants irrigated with reclaimed water under real-world field conditions

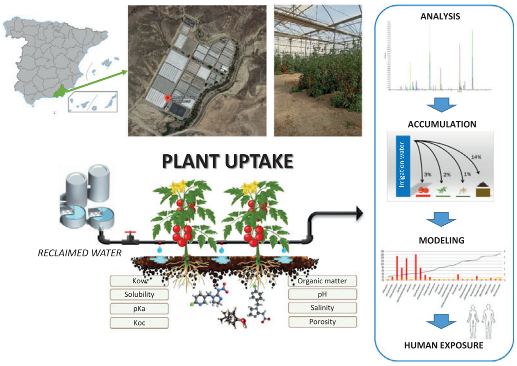
M.J. Martínez Bueno [a], M. García Valverde [a], M.M. Gómez-Ramos [a], J.A. Salinas Andújar [b], D. Barceló [c,d], A.R. Fernández-Alba [a,*]

[a] *University of Almería, Department of Physics and Chemistry, Agrifood Campus of International Excellence (ceiA3), La Cañada de San Urbano, 04120, Almería, Spain*
[b] *University of Almería, Department of Engineering, Agrifood Campus of International Excellence (ceiA3), La Cañada de San Urbano 04120, Almería, Spain*
[c] *Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Spain*
[d] *Catalan Institute for Water Research (ICRA)-CERCA, Girona, Spain*

HIGHLIGHTS

- Soil samples exhibited the highest content of CECs followed by fruit ≥ leaf ≥ roots.
- We identify CECs with the highest potential for plant uptake and soil accumulation.
- We propose mathematical models to estimate the CECs uptake in soil and plants.
- A correlation was found between CECs uptake and log*Kow*.
- Daily human intake was estimated considering the worst-case scenario.

GRAPHICAL ABSTRACT



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ABSTRACT

Using reclaimed water to irrigate crops can be an important route for organic contaminants of emerging concern (CECs) to be introduced into agricultural production and thus find their way into the food chain. This work aims to establish accumulation models for the different parts of a crop (fruit/leaves/roots) and the soil of some of the most commonly detected CECs in reclaimed water, through field trials in greenhouses. For this, tomato plants were permanently irrigated under realistic agricultural conditions with a mixture of the selected compounds at approx. 1 μg/L. A total of 30 contaminants were analyzed belonging to different compound categories. A modified QuEChERS extraction method followed by liquid chromatography coupled to tandem mass spectrometry was the procedure used. The study revealed the presence of 21 target contaminants in the tomatoes, and 18 CECs in the leaves, roots, and soil. The average total concentration of pesticides detected in the tomatoes was 3 μg/kg f.w., whereas the average total load of pharmaceuticals was 5.8 μg/kg f.w. after three months, at the time of crop harvesting. The levels of pharmaceutical products and pesticides in the non-edible tissues were up to 3.5 and 2.1 μg/kg f.w., respectively, in the leaves and up to 89.3 and 31.3 μg/kg f.w., respectively, in the roots. In the case of the soil samples, the pesticide concentration found after crop harvesting was below 11.4 μg/kg d.w., and less than 3.0 μg/kg d.w. for pharmaceuticals. Overall, the concentration levels of CECs detected in the tomatoes, which were permanently irrigated with contaminated reclaimed water, do not pose a risk to human health via dietary intake.

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* Corresponding author.
E-mail address: amadeo@ual.es (A.R. Fernández-Alba).

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1. Introduction

Climate change, intensive agriculture and the increasing population are significantly contributing to the great pressure on water resources, leading to water scarcity and deterioration in water quality. The use of reclaimed wastewater for agricultural irrigation is increasing around the world and is a potential alternative for combating water scarcity. Water reuse has much less impact on the water cycle compared, for example, with water transfers from rivers; it also offers important environmental benefits (extending the life cycle of the water and zero discharge) as well as economic and social benefits (Ait-Mouheb et al., 2018). In this sense, the use of treated water constitutes a strategic resource that can reduce the structural water deficit suffered by many regions, such as countries in southern Europe. According to the data from Eurostat Statistics, Spain accounted for about a third of the total volume of EU water reuse (347 Mm^3/year) in 2019, followed by Italy (233 Mm^3/year) and Germany (42 Mm^3/year). In Spain, approx. 71% of reclaimed water is used for agricultural irrigation, 17% for landscape irrigation, 11% for recreational/urban use and only 0.3% for industrial processes (Eurostat Statistics Explained, 2019).

The European Union has recently published a new Regulation (EU) 741/2020, concerning the minimum requirements for water quality and control to ensure the safe use of treated urban wastewater (The European Parliament and the Council, 2020). This Regulation aims to guarantee that reclaimed water is safe to use and to provide a high level of human, animal, and environmental health protection, in addition to tackling water scarcity.

To date, hundreds of scientific papers have reported the presence of organic contaminants of emerging concern (CECs), such as pharmaceuticals, personal care products, or pesticides, in treated water or irrigation water (Calderón-Preciado et al., 2011; Martínez Bueno et al., 2012; Quintana et al., 2019; Renau-Pruñonosa et al., 2020). These studies provide clear evidence that conventional wastewater treatment plants (WWTPs) are poorly effective to comprehensively remove of most CECs and advanced treatment steps are needed to effectively remove CECs (Krzeminskia et al., 2019). Therefore, the use of reclaimed water in crop irrigation can be an important route by which emerging pollutants are introduced into agricultural production and subsequently enter the food chain; this could be hazardous to human health and to the environment. For example, Calderón-Preciado et al. (2011) detected a total of 26 chemical contaminants in reclaimed water used for agricultural crop irrigation. The average concentration for pesticides ranged between 0.05 and 0.1 μg/L, while pharmaceutical products were found at concentrations between 0.03 and 0.7 μg/L.

Contaminant uptake in plants can be influenced by a wide variety of factors, both biotic and abiotic. The main biotic factors affecting adsorption are the plant itself (the species, variety, and physiological state) and soil microorganisms, which are the principal cause of contaminant biodegradation/biotransformation in the soil. Climatic conditions (such as temperature, UV radiation, salinity and wind speed), the contaminants' physicochemical properties (hydrophobicity, polarity and solubility in water) and the structure and composition of the soil constitute the main abiotic factors influencing the plants' uptake potential (Christou et al., 2019). In spite of some scientific works related to the study of the mechanism by which plants absorb organic pollutants have recently been published, it is still relatively unknown. Most of these works have been focused on evaluating the accumulation of pharmaceutical and personal care products (PPCPs) in crops irrigated with treated wastewater. For example, Wu et al. (2014) investigated the accumulation of 19 PPCPs in eight types of vegetables irrigated with treated wastewater under field conditions. The total concentration of PPCPs detected in the edible tissues was in the 0.01–3.87 μg/kg range. In a recent study, Picó et al. (2019) evaluated the potential for plant uptake in crops irrigated with treated wastewater under uncontrolled environmental conditions. The results reported the presence of 7 pharmaceutical products at concentrations ranging from 25 to 96 μg/kg in the soil samples, and from 35 to125 μg/kg in the plants, whereas 7 pesticides were detected at levels between 25 and 366 μg/kg in the soil and between 35 and 5650 μg/kg in the vegetable samples. To date, most of this research has been carried out in the laboratory (unrealistic agricultural conditions), under uncontrolled environmental conditions or in field trials at concentration levels higher than those expected in reclaimed water (Wu et al., 2013; Malchi et al., 2014; Paz et al., 2016; Madikizela et al., 2018; Picó et al., 2018; Ju et al., 2019). Moreover, the mathematical models reported in the literature on plant uptake are limited to only a few substances or require compound-specific parameters to run (Collins and Finnegan, 2010; González García et al., 2019; Prosser et al., 2014).

Therefore, the general objective of this work is to measure the uptake of some of the most commonly detected CECs in treated water. The measurements were taken from the soil, plant tissues and fruit grown using reclaimed water under realistic agricultural conditions. This study determines the compounds that have a higher capacity in reaching the plant; these are then used as chemical markers to develop accumulation statistical models which allow us to estimate the levels of organic contaminants in the soil as well as in the different parts of the plants. Finally, the potential human risks from consuming the edible part of plants are assessed.

2. Materials and methods

2.1. Reagents and materials

A total of 30 CECs including 13 pesticides, 12 pharmaceutical products, and 5 transformation products were investigated. The target analytes' selection was based on their environmental relevance and the authors' previous experience (Martínez Bueno et al., 2012). The physicochemical properties of all the selected analytes have been included in Table 1. All reference standards were purchased from Sigma-Aldrich (Steinheim, Germany) at a high purity grade (>98%), except codeine which was obtained in pill form. Stock standard solutions were prepared individually in acetonitrile at concentrations ranging from 1000 to 2000 mg/L and stored in amber glass vials with screw caps in the dark at −40 °C.

Acetonitrile (AcN), methanol (MeOH), and formic acid were of LC-MS grade and supplied by Fluka Analytical (Steinheim, Germany), whereas the ultrapurewater was supplied by Fisher Scientific (Fair Lawn, NJ, USA). Anhydrous magnesium sulphate $(MgSO_4)_{anh}$, sodium chloride (NaCl), sodium hydrogenocitrate sesquihydrate ($Na_2HCitrate \cdot 1{,}5H_2O$), and sodium citrate tribasic dihydrate ($Na_3Citrate \cdot 2H_2O$) were obtained from Sigma–Aldrich (Steinheim, Germany). C-18 sorbent was purchased from Supelco (Bellefonte, PA, USA) and ChloroFiltr® dispersive centrifuge tubes containing 900 mg $MgSO_4$ and 150 mg ChloroFiltr® were acquired from United Chemical Technologies (UCT, Ref. ECMSGG15CT, Bristol).

2.2. Field experiments and sampling strategy

Tomatoes are commonly used in salads, and people often consume them raw. They are the second most important vegetable crop in the world after potatoes. The EU's production of tomatoes was 16.5 million tonnes in 2019, according to data released by the European Statistical Office (Eurostat). Almost two thirds of the EU-27s tomato production in 2019 came from Italy (5.3 million tonnes) and Spain (5.0 million tonnes) (Eurostat Statistics Explained, 2020). Based on these data, a tomato (*Solanum lycopersicum L.*) crop was grown in a greenhouse located in Almería (Spain) under controlled agronomic conditions and using reclaimed water applied by drip irrigation. The reclaimed water was spiked with a solution containing a mixture of the CECs selected for this study, each one at a concentration of approx. 1 μg/L, considering the worst-case scenario according to previous results from our research group (Martínez Bueno et al., 2012). In order to assess the degradation potential of carbamazepine and metamizole, none of their respective

Table 1
Physicochemical properties of all CECs selected in this study.

Compound	Family	Log*Kow*	pKa	Water solubility	Koc	DT$_{50}$ soil	Soil/environmental fate
Ofloxacin	Antibiotic	−0.4	5.9	28,300	44	952–1820	Immobile in soil. Non-volatile.
Pymetrozine	Insecticide	−0.2	4.1	290	246–7875	707	Very Persistent. Low mobility in soil. Rapidly degraded in soil.
4-AAA	Analgesic	−0.1	12.4	40,226	240.7	n.d.	n.d.
Thiamethoxam	Insecticide	−0.1	0.4	4100	56	50	Moderately persistent. High mobile in soil. Photodegraded.
Caffeine	Stimulant	−0.1	14	21,700	741–7762	10–34	Low mobility in soil.
Hydrochlorothiazide	Diuretic	−0.1	8.0	722	12	9–11	High mobility in soil. Photodegraded. Non-biodegradable.
Atenolol	b-blocker	0.2	9.6	13,300	148.1	n.d.	High mobility in soil.
4-FAA	Analgesic	0.2	12.7	101,289	17	n.d.	n.d.
Ciprofloxacin	Antibiotic	0.3	6.1	30,000	61,000	1155–3466	Immobile in soil. Non-volatile.
Acetaminophen	Analgesic	0.5	9.4	14,000	20,844	30	High mobility in soil. Photodegraded and readily biodegradable.
4-AA	Analgesic	0.5	4.1	727,617	282.9	n.d.	n.d.
Imidacloprid	Insecticide	0.6	11.1	33	478	191	Persistent. Medium mobility in soil. Photodegraded.
4-MAA	Analgesic	0.6	n.d.	28,897	410.7	n.d.	n.d.
Acetamiprid	Insecticide	0.8	0.7	2950	200	1.6	Non-persistent. High mobility in soil. Biodegradable.
Codeine	Analgesic	1.2	8.2	<1	700	120	Low mobility in soil. Photodegraded.
Thiacloprid	Insecticide	1.3	1.6	184	1100	1–4	Non-persistent. Low mobility in soil. Biodegradable.
Carbendazim	Fungicide	1.5	4.2	8	122–2805	40	Moderately persistent. Medium mobility in soil.
Epoxide-CBZ	Antiepileptic	1.6	n.d.	1340	388.5	n.d.	Medium mobility in soil.
Furosemide	Diuretic	2.0	3.9	73	110	120	High mobility in soil. Photodegraded. Biodegradable.
Diuron	Herbicide	2.3	13.2	35	680	146	Persistent. Low mobility in soil. Non-biodegradable.
Thiabendazole	Fungicide	2.4	4.7	30	3983	500	Very persistent. Low mobility in soil.
Carbamazepine (CBZ)	Antiepileptic	2.4	13.9	18	510	462–533	Very persistent. Medium mobility in soil. Non-biodegradable.
Azoxystrobin	Fungicide	2.5	0.9	7	589	78	Moderately persistent. Medium mobility in soil.
Fluxapyroxad	Fungicide	3.1	12.6	3	1907	183	Persistent. Low mobility in soil.
Myclobutanil	Fungicide	3.2	2.3	132	950	560	Very persistent. Low mobility in soil.
Naproxen	Anti-inflammatory	3.2	4.1	16	330	17–69	Medium mobility in soil. Non-biodegradable.
Diazinon	Insecticide	3.4	2.6	60	609	9.1	Non-persistent. Low mobility in soil.
Penconazole	Fungicide	3.7	1.5	73	786–4120	117	Persistent. Low mobility in soil.
Diclofenac	Analgesic	4.0	4.1	2	245	3–20	Medium mobility in soil. Biodegradable.
Gemfibrozil	Lipid regulators	4.8	4.5	11	430	224–231	Medium mobility in soil. Non-biodegradable.

https://sitem.herts.ac.uk/aeru/ppdb/en/atoz.htm#; http://www.chemspider.com/; https://pubchem.ncbi.nlm.nih.gov/; *Kow*: octanol/water coefficient; pKa: negative log of the acid dissociation constant; Water solubility at 20 °C (mg/L); Koc: average coefficient of sorption (mL/g); DT$_{50}$: time required for the concentration to decline to half of the initial value (days); Kf: Freundlich constant; n.d.: Not data. Epoxide-CBZ (carbamazepine-10,11Epoxi); 4-MAA (4-methylamino-antipyrine); 4-AA (4-amino-antipyrine); 4-FAA (4-formylamino-antipyrine); 4-AAA (4-acetylamino-antipyrine).

metabolites were added to the irrigation water. The total amount of contaminated reclaimed water used was 2200 L. A detailed description of the field-plots location as well as the sampling strategy is given in the Supplementary material section.

2.3. Sample extraction

Vegetable and soil samples were extracted with a modified QuEChERS method, which was based on a method (with some small modifications) that our research group had recently published (García Valverde et al., 2021). Briefly, 10 g of plant tissue samples were weighed in a 50-mL PTFE centrifuge tube, and a surrogate standard mixture was added. Subsequently, the samples were shaken in an automatic axial extractor (AGYTAX®, Cirta Lab. S.L., Spain) for 4 min at 25 °C after the addition of 10 mL of acidified AcN (0.5% v/v, FA). For the soil samples, 5 mL of Milli-Q water was also added before the extraction solvent and left to stand for 5 min. Afterwards, 4 g MgSO4, 1 g NaCl, 1 g $Na_3Citrate \cdot 2H_2O$ and 0.5 g $Na_2HCitrate \cdot 1{,}5H_2O$ were added and centrifuged at 3500 rpm for 5 min. Then, 3 mL of the extract was transferred to a 15-mL PTFE centrifuge tube containing 750 mg of anhydrous $MgSO_4$ and 125 mg C-18, vortexed for 30 s and centrifuged at 3500 rpm for 5 min. In the case of the leaf/root samples, 900 mg $MgSO_4$ and 150 mg ChloroFiltr® were added to eliminate possible pigment interferences during the analysis. Finally, 100 μL of each extract was transferred to vials with screw caps, evaporated to dryness and reconstituted with 100 μL of AcN:water solution (1:9, v/v) containing dimethoate-d6. Fig. 1 shows a diagram of the extraction methods used for each of the studied matrices (soil, leaves/roots, fruit).

The water samples were filtered using a 0.45-mm PTFE syringe filter (Millipore, USA) to remove suspended solids and particulate matter, and then spiked with the selected labeled standard (dimethoate-d6) before analysis.

2.4. Sample analysis and quality control

The high-performance liquid chromatography analyses were performed in a Sciex Exion HPLC system connected to a Sciex 6500+ TripleQuad-LC-MS/MS. The chromatographic and acquisition parameters for the analyses are described elsewhere (García Valverde et al., 2021). The retention times, transitions and collision energies for the analyzed compounds are included as Supplementary material in Table S1. The data analysis was performed with the Sciex Analyst 1.7.1 software for the data acquisition/processing and MultiQuant 3.0.1 software for the data quantification. The criteria for the mass spectrometric confirmation and quantification of the target compounds were in line with current EU regulations (Commission Decision 2002/657/EC, 2002). The trueness, precision, selectivity, sensitivity, range, ruggedness, and limit of quantification of the developed analytical methodology were evaluated according to the EU quality control procedures (European Commission DG-SANTE, 2019).

To ensure the quality of the measurements, continuous monitoring of the analytical procedure was carried out. To check the correct performance of the analytical procedure, several labeled standards were used. Caffeine-13C, carbendazim-d3, dichlorvos-d6, and malathion-d10 were selected as surrogate standards to check the extraction efficiency. Dimethoate-d6 was used as the injection standard. A standard mixture (2 μg/L) containing all the targeted analytes was injected each day before the analysis in order to check the functioning of the analytical column and the mass spectrometer. Blank samples (solvent) were also included during the daily work sequence.

2.5. Bioconcentration factor (BCF) and human exposure

The bioconcentration factor (BCF) was employed to estimate the plant uptake of the selected CECs, similarly to previous published

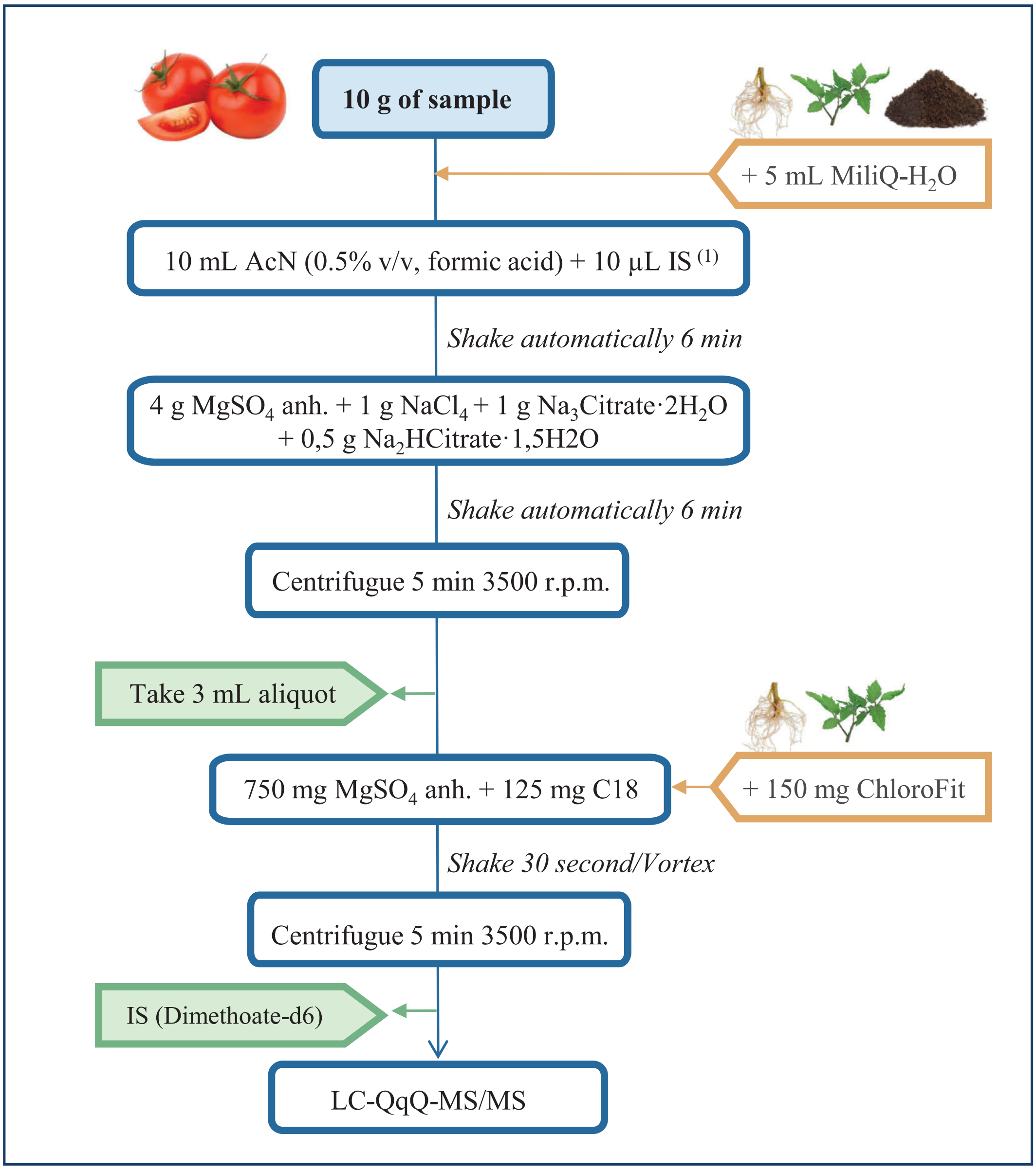

Fig. 1. Scheme of the extraction methods used to extract CECs in each of the matrices studied (fruit, leaf, root, and soil).

works (González García et al., 2019) It was calculated from the concentration of each individual contaminant measured in the tomato against the contaminant concentration applied to the crop via the irrigation water. That is because, the potential efficiency of drip irrigation systems is greater than 90% (Hedley et al., 2014). Thus, the difference between the amount of water that the plant absorbs and that we supply is less than 10%.

$$BCF\ (L/kg) = \frac{concentration\ in\ edible\ part\ of\ plant\ (\mu g/kg)}{concentration\ in\ irrigation\ water\ (\mu g/L)}$$

The daily human intake of each selected CEC was estimated by multiplying the concentration measured in the edible part of the crop (ng/g in f.w.) and the daily consumption per capita of fresh vegetables (g f.w./day). According to the latest reported data from Blázquez (2021), tomato was the most consumed vegetable in Spain in 2019. In fact, its consumption volume amounted to 613 million kilograms in that year, which is 13.3 kg per person/year (36.4 g/day).

3. Results

3.1. Validation of analytical methods

Table 2 summarizes the validation data obtained for the selected target compounds of each compound/matrix combination. The method sensitivity was calculated in terms of the limit of quantitation (LOQ). It was estimated as the lowest spiked level meeting the identification and method performance criteria for recovery and precision (European Commission DG-SANTE, 2019). The values were experimentally evaluated for each analyte/matrix combination. All the compounds showed LOQ values ranging from 0.05 to 0.1 ppb (µg/L or µg/kg) in all the matrices, except to ciprofloxacin and acetaminophen, which presented values of 0.5 ppb in the tomato matrix. Only 20% and 13% of the analytes showed LOQs higher than 0.1 ppb in the non-edible vegetable tissue and the soil matrix, respectively.

The linearity of the analytical response was evaluated based on the linear regression and squared correlation coefficient (r^2). Matrix-matched calibration curves were prepared by fortifying

Table 2
Validation data for target compounds in the reclaimed water, fruit, leaf, root, and soil matrices.

Compound	Water				Tomato					Leaf/root					Soil				
	LOQ	r2	ME	Inter/intraday	LOQ	r2	ME	Rec.	Inter/intraday	LOQ	r2	ME	Rec.	Inter/intraday	LOQ	r2	ME	Rec.	Inter/intraday
Ofloxacin	0.10	0.996	9	2/11	0.10	0.996	9	70	2/19	0.50	0.997	−78	52	3/20	0.10	0.999	−65	n.r	7/18
Pymetrozine	0.05	1.000	0	0/8	0.05	1.000	0	40	3/18	0.10	0.999	−15	47	1/19	0.05	1.000	−6	30	1/12
4-AAA	0.10	0.996	9	1/4	0.10	0.996	9	75	2/10	0.10	1.000	−15	83	2/15	0.10	0.994	−7	73	4/19
Thiamethoxam	0.05	1.000	2	1/7	0.05	1.000	2	97	2/11	0.10	1.000	−29	98	3/10	0.05	0.999	−9	87	1/6
Caffeine	0.05	1.000	2	1/4	0.05	1.000	2	90	3/12	0.05	0.996	−12	92	2/15	0.05	1.000	−6	72	1/16
Hydrochlorothiazide	0.05	1.000	−4	5/3	0.05	1.000	−4	94	3/20	0.10	1.000	17	86	2/9	0.05	1.000	1	85	5/18
Atenolol	0.05	1.000	13	1/5	0.05	1.000	13	116	1/15	0.10	0.998	−64	100	1/6	0.05	0.998	−25	73	314
4-FAA	0.10	0.999	4	2/6	0.10	0.999	4	96	0/6	0.10	1.000	−3	85	1/6	0.50	0.999	0	81	3/4
Ciprofloxacin	0.50	0.997	5	3/7	0.50	0.997	5	80	4/4	0.50	0.998	−68	64	11/13	0.50	0.999	−29	n.r	15/18
Acetaminophen	0.50	1.000	9	1/2	0.50	1.000	9	89	3/15	0.50	1.000	−31	92	3/16	0.50	0.999	−5	76	5/20
4-AA	0.05	0.999	0	1/3	0.05	0.999	0	50	3/20	0.10	0.998	5	74	1/5	0.05	0.999	−2	n.r	4/10
Imidacloprid	0.05	1.000	4	1/2	0.05	1.000	4	91	2/10	0.05	0.999	−40	98	1/11	0.05	1.000	−16	86	2/15
4-MAA	0.05	0.998	−2	1/3	0.05	0.998	−2	52	2/10	0.10	0.998	−19	51	2/13	0.05	1.000	−20	n.r	3/19
Acetamiprid	0.05	1.000	3	1/6	0.05	1.000	3	90	1/8	0.05	0.999	−40	97	1/6	0.05	0.998	−11	83	8/10
Codeine	0.05	0.991	13	2/7	0.05	0.991	13	82	6/7	0.10	1.000	−50	81	1/9	0.05	0.991	−7	50	2/5
Thiacloprid	0.05	0.999	0	3/3	0.05	0.999	0	90	1/9	0.05	1.000	−52	96	1/8	0.05	0.998	−20	82	3/6
Carbendazim	0.05	0.998	6	2/3	0.05	0.998	6	80	2/7	0.10	1.000	−45	99	1/6	0.05	0.998	−17	70	4/4
Epoxide-CBZ	0.05	0.998	5	1/5	0.05	0.998	5	86	5/12	0.05	0.998	−30	91	2/11	0.05	0.995	−13	68	3/10
Furosemide	0.10	1.000	6	4/6	0.10	1.000	6	94	2/9	0.50	1.000	−5	95	1/4	0.50	1.000	2	63	15/7
Diuron	0.05	1.000	1	2/6	0.05	1.000	1	80	2/6	0.10	1.000	−36	82	1/15	0.05	0.998	−11	79	2/6
Thiabendazole	0.05	0.998	8	1/1	0.05	0.998	8	82	1/6	0.05	0.999	−50	80	1/5	0.05	0.992	−15	62	7/19
CBZ	0.05	0.998	4	2/8	0.05	0.998	4	82	2/9	0.05	0.999	−31	89	1/12	0.05	1.000	−26	79	6/3
Azoxystrobin	0.05	0.997	3	1/4	0.05	0.997	3	90	1/9	0.05	0.995	−30	117	1/9	0.05	0.992	−33	71	5/6
Fluxapyroxad	0.05	1.000	−1	1/5	0.05	1.000	−1	83	2/11	0.05	0.998	−46	70	3/14	0.05	0.999	−21	73	4/3
Myclobutanil	0.05	1.000	−2	1/2	0.05	1.000	−2	87	1/10	0.05	0.997	−35	56	1/8	0.05	0.999	−17	81	15/15
Naproxen	0.10	1.000	7	1/9	0.10	1.000	7	86	1/12	0.50	0.999	−54	74	9/14	0.10	1.000	−17	82	3/9
Diazinon	0.05	0.998	1	1/1	0.05	0.998	1	84	0/16	0.05	1.000	−50	60	2/15	0.05	1.000	−34	84	4/6
Penconazole	0.05	1.000	−1	2/2	0.05	1.000	−1	84	1/18	0.10	0.997	−40	28	1/3	0.05	0.999	−30	73	7/19
Diclofenac	0.10	1.000	2	2/3	0.10	1.000	2	77	3/7	0.10	1.000	−20	10	2/5	0.10	0.997	−18	86	15/10
Gemfibrozil	0.05	1.000	0	1/8	0.10	1.000	0	87	3/19	0.50	0.992	−16	28	3/17	0.10	0.999	−33	76	9/13

LOQ: Limits of quantification (μg/L; μg/kg); Linearity expressed by the correlation coefficient (r2); ME: Matrix effect (%); Rec: Average recoveries (n = 5, %, 1 μg/kg to tomato, 10 μg/kg to leaf/soil); Inter/Intra repeatability expressed as relative standard deviation (RSD, %), n.r: Not recovered; Epoxide-CBZ (carbamazepine-10,11Epoxi); CBZ: Carbamazepine; 4-MAA (4-methylamino-antipyrine); 4-AA (4-amino-antipyrine); 4-FAA (4-formylamino-antipyrine); 4-AAA (4-4-acetylamino-antipyrine).

blank extracts of each matrix at five concentration levels (from 0.05 to 5 μg/L for the water and from 0.05 to 50 μg/kg for the soil and vegetable tissue). These were used to minimize matrix interference and to avoid any under/over estimation during the quantification step. All the selected compounds presented a very good response of three orders of magnitude, with correlation coefficients above 0.992 in all cases. Likewise, the matrix effects were studied comparing the calibration curve slopes in the matrix and the solvent. According to our results, no matrix effect (≤20%) was observed in the water or the tomato matrices (see Table 2). In the leaf matrix, only 5 of the 30 studied compounds presented a matrix effect over 50% (strong). In the case of the soil matrix, 70% of the targeted CECs presented no matrix effect, 27% showed an intermediate matrix effect (between 20 and 50%) and only one, ofloxacin, had a strong matrix effect (>50%).

The recovery studies evaluated per quintuplicate ($n = 5$) using spiked samples with each of the compounds selected in this study at 1 ng/g for the tomato, and 10 ng/g for the leaf and soil matrices. All the targeted CECs showed recovery values above 70% in the tomato matrix, except for two metabolites of the analgesic metamizole (4-AA and 4-MAA) and the insecticide pymetrozine (see Table 2). Regarding the leaf/root and soil matrices, 21 compounds were recovered above 70%. Only three compounds (penconazole, diclofenac and gemfibrozil) presented values below 30% in the non-edible tissue whereas four pharmaceuticals (4-AA, 4-MAA, ofloxacin and ciprofloxacin) were not recovered from the soil samples.

The repeatability and reproducibility expressed as the relative standard deviation (RSD, %) ranged between 0% and 20% in both cases. These results demonstrate the method's analytical precision and, therefore, its effectiveness for quantitative purposes. Finally, three blank samples of each matrix studied (water, fruit, leaf, and soil), extracted by the proposed method, were analyzed to assess the method's specificity and selectivity. At the specific retention times for the target compounds, no other significant peaks were detected.

3.2. Presence of CECs in the vegetable and environmental samples

Tomatoes grown in an experimental greenhouse with drip irrigation, consumed a total of 2200 L of contaminated reclaimed water during the three months of the crop cycle. The results obtained showed that the tomato crop in a greenhouse consumed a total of approx. 8 L of water by kg of plant, and therefore an average of 90 mL of water per day by plant. The data showed that 2 m^3 of water, that is to say, 2000 l, produced 150 kg of drip-irrigated greenhouse tomatoes. The analytical approach developed was applied to agricultural samples obtained from a pilot study under real-world field conditions. Three independent extractions of each sample type were analyzed. The internal standards (extraction and injection) were recovered between 70 and 120% in all cases. No targeted CEC residues were detected in the control samples (natural water, plant, and soil) at concentration levels higher than the LOQ values. The concentration ranges and average levels of the pesticides and pharmaceutical products found in each part of the crop irrigated with contaminated reclaimed water are summarized in Table 3. In accordance with the International System of Units, the data concentrations for the vegetable tissue were presented as fresh weight (f.w.) whereas for the soil, they were presented as dry weight (d.w.).

Table 3
Concentration levels of CECs detected in the plant and environmental samples ($n = 3$).

Compound	Reclaimed water[a]			Tomatoes			Leaf			Root					Soil
	Range μg/L	Average μg/L	μg	Range μg/kg	Average μg/kg	μg	Range μg/kg	Average μg/kg	μg	Range μg/kg	Average μg/kg	μg	Range μg/kg	Average μg/kg	μg
Pharmaceutical															
Ofloxacin	0.9–1.2	1.1	2395	–	–	–	–	–	–	–	–	–	–	–	–
4-AAA	n.a	n.a	n.a	–	–	–	–	–	–	–	–	–	–	–	–
Caffeine	0.7–0.9	0.8	1737	0.7–1.2	0.9	135	0.3–0.4	0.4	48	2.9–3.4	3.3	16	0.8–1.0	0.9	372
Hydrochlorotiazide	0.3–0.4	0.4	882	0.4–1.1	0.8	116	0.1	0.1	12	0.2–0.3	0.3	1	–	–	–
Atenolol	0.9–1.0	1.0	2243	–	–	–	–	–	–	2.0–2.5	2.4	12	0.1	0.1	28
4-FAA	n.a	n.a	n.a	1.5–2.3	2.1	308	–	–	–	–	–	–	–	–	–
Ciprofloxacin	0.7–1.2	0.9	1938	–	–	–	–	–	–	–	–	–	–	–	–
Acetaminophen	0.8–1.1	1.0	2205	–	–	–	–	–	–	–	–	–	–	–	–
4-AA	n.a	n.a	n.a	–	–	–	–	–	–	–	–	–	–	–	–
4-MAA	n.a	n.a	n.a	–	–	–	–	–	–	–	–	–	–	–	–
Codeine	0.7–1.0	0.9	1985	–	–	–	0.1	0.1	14	–	–	–	0.1–0.2	0.2	62
Epoxide-CBZ	n.a	n.a	n.a	0.1–0.2	0.2	23	0.4–0.6	0.5	63	–	–	–	–	–	–
Furosemide	0.1–0.3	0.2	441	0.1	0.1	21	–	–	–	–	–	–	–	–	–
Carbamazepine	0.8–1.0	0.9	1898	0.1	0.1	9	0.2–0.4	0.3	33	9.5–13.0	11.0	53	1.0–1.3	1.2	492
Naproxen	0.5–0.6	0.5	1103	≤LOQ	≤LOQ	≤LOQ	–	–	–	–	–	–	–	–	–
Diclofenac	1.0–1.2	1.1	2505	1.4–1.9	1.7	260	0.6–0.7	0.7	84	12.1–15.1	14.3	69	0.5–0.7	0.6	246
Gemfibrozil	0.6–0.7	0.6	1420	–	–	–	–	–	–	–	–	–	–	–	–
Total		9.4	20.751		5.8	871		2.1	254		31.3	150		3.0	1.200
Pesticide															
Pymetrozine	0.5–0.6	0.5	1078	0.1–0.2	0.2	26	–	–	–	2.0–2.7	2.5	12	1.0–1.1	1.0	410
Thiamethoxam	0.7–0.8	0.8	1764	1.5–1.9	1.8	267	0.3–0.4	0.4	47	1.8–2.1	2.0	10	0.8	0.8	328
Imidacloprid	0.5–0.7	0.6	1421	0.1–0.2	0.2	30	0.3–0.5	0.4	48	4.3–5.0	4.8	23	0.7–0.9	0.5	205
Acetamiprid	0.7–0.8	0.8	1807	0.1	0.1	15	0.1	0.1	10	2.8–3.6	3.5	17	0.1	0.1	34
Thiacloprid	0.9–1.0	1.0	2191	0.1	0.1	12	0.1–0.2	0.2	18	1.0–1.3	1.2	6	0.3–0.4	0.4	164
Carbendazim	0.8–1.0	0.9	2080	0.1	0.1	11	0.1	0.1	17	1.0–2.0	1.9	9	0.6	0.6	238
Diuron	0.7–0.9	0.8	1764	0.1	0.1	9	0.4–0.5	0.5	60	2.0–3.0	2.3	11	0.3–0.4	0.4	165
Thiabendazole	0.5–0.6	0.6	1260	0.1	0.1	12	0.1	0.1	11	6.8–8.6	8.0	38	0.7	0.7	300
Azoxystrobin	0.7–0.9	0.8	1804	0.1–0.2	0.2	32	0.1–0.2	0.2	25	9.0–11.2	10.0	48	1.0–1.3	1.2	492
Fluxapyroxad	0.7–0.8	0.8	1654	0.1	0.1	10	0.4–0.6	0.5	60	14.2–16.7	15.8	76	1.2–1.4	1.3	533
Myclobutanil	0.7–0.9	0.8	1684	0.1	0.1	9	0.5–0.6	0.6	72	15.2–16.8	16.0	77	2.0–2.2	2.0	820
Diazinon	0.3–0.4	0.4	794	0.1	0.1	20	0.1	0.1	7	3.0–4.5	4.0	20	0.3–0.4	0.4	164
Penconazole	0.5–0.7	0.6	1315	≤LOQ	≤LOQ	≤LOQ	0.1–0.2	0.2	20	16.0–18.0	17.3	83	1.5–3.0	2.0	820
Total		9.3	20.616		3.0	452		3.5	396		89.3	429		11.4	4.673

[a] Reclaimed water spiked at 1 μg/L of each selected CECs; LOQ: Limits of quantification; n.a: not add; R.S·D: Relative standard deviation (%).

3.2.1. CECs in the irrigation water

The total amount of CECs released over the three months of the crop cycle was 41,367 μg, which correspond to a total of 18.7 μg/L in the water. The analysis of the water samples showed that, generally, all the compounds were presented at levels between 0.9 μg/L and 1.1 μg/L, except for the insecticide diazinon (0.4 μg/L), and the diuretics furosemide (0.2 μg/L) and hydrochlorothiazide (0.4 μg/L). These lower concentrations (with respect to the added concentrations) may be due to degradation processes. Other authors have reported that these compounds are highly sensitive to UV light exposure (Mansour et al., 1997; Cies et al., 2015), with degradation rates of 30% and 50% for furosemide and diazinon, respectively. No degradation products of the anti-epileptic carbamazepine (carbamazepine-10,11-epoxide) or the analgesic metamizole (4-methylamino-antipyrine; 4-amino-antipyrine, 4-formylamino-antipyrine and 4-4-acetylamino-antipyrine) were detected in the irrigation water.

3.2.2. CECs in the tomatoes

Out of the 30 compounds selected in this study, 12 pesticide residues and 7 pharmaceutical products were identified in the tomato samples irrigated with contaminated reclaimed water (see Table 3). Of the pesticides, the insecticide thiamethoxam was found at the highest concentration level (1.8 μg/kg f.w.). The rest of the pesticides included in this study were also detected in the tomato at concentrations ranging from 0.1 to 0.2 μg/kg f.w. Only the fungicide penconazole was found at levels below its LOQ (<0.05 μg/kg). The analgesic diclofenac, the stimulant caffeine, and the diuretic hydrochlorothiazide were the pharmaceuticals detected at the highest levels - 1.7, 0.9 and 0.8 μg/kg f.w., respectively. The anti-epileptic carbamazepine (CBZ) and the diuretic furosemide were also found in the edible part of the plant, but at lower concentrations (up to 0.1 μg/kg f.w.). These results are in agreement with previous works. Wu et al. (2014) detected CBZ levels ranging from 0.19 ± 0.32 ng/g in tomatoes grown using fortified water in irrigated plots at approx. 300 ng/L. Neither of the antibiotics evaluated in this study (ofloxacin and ciprofloxacin) were detected. In contrast, two transformation products that had not initially been added to the irrigation water were determined in tomato samples: carbamazepine-10,11-epoxide (epoxy-CBZ) and 4-formylamino-antipyrine (4-FAA). The first of these, epoxy-CBZ, was measured at higher levels than the parent product (CBZ), 0.2 μg/kg f.w., while the second, the metabolite of the analgesic metamizole, was the drug found at the highest levels in the fruit, up to 2.1 μg/kg f.w. Previous works have reported the occurrence of transformation products in crops irrigated with treated municipal wastewater; however, all were taken up from the water by the plants (Margenat et al., 2018; Picó et al., 2019). For example, Margenat et al. (2018) reported similar levels of epoxy-CBZ in lettuce grown in a peri-urban area of northern Spain using furrow-irrigated water in open air channels from industrial, urban and agricultural activities. Nevertheless, the results from this study suggest that CBZ and metamizole can also be metabolized in the soil or synthesized in the plant since neither were detected in the irrigation water samples analyzed at the dripper outlets. This hypothesis is supported by other authors. Paz et al. (2016) suggest that CBZ is metabolized in the soil and, therefore, its main metabolite, 10,11-epoxy-CBZ, is available for uptake. In another work, Malchi et al. (2014) reported that the metabolite is synthesized in the plant through the metabolization of its precursor (CBZ) by CYP450 enzymes.

The presence of the pharmaceuticals (caffeine, hydrochlorothiazide, 4-FAA, and CBZ) and the pesticides (imidacloprid, diuron, and fluxapyroxad) can be explained based on their pKa values (>8.0, see Table 1). These non-ionic compounds can pass through the plants' cell membranes, entering via the roots and translocating to other parts of the plant through transpiration. Nonetheless, diclofenac, despite being an ionic compound (with a pKa of 4.1), was detected in the tomato samples. According to its properties, diclofenac has a negative charge in the soil environment (pH 7.7) so it should be repulsed by the cells in the roots – in our case, however, it was detected in the fruit. This is in line with the results reported by Christou et al. (2017), in which diclofenac was detected in tomato samples when the crop was irrigated for long periods with wastewater. The authors reported concentration levels ranging from 1.3 to 11.63 μg/kg f.w. in the tomatoes. Picó et al. (2019) explained the presence of ionic compounds in the plants as being due to differing pH levels, depending on the plant organs. Therefore, the pH could reach values as low as 4 in some tissues, meaning that these compounds would be primarily neutral. In fact, the pH value for a ripe tomato is about 4.6. This theory explains the high diclofenac levels found in the tomatoes in the present work.

In summary, the total amounts of pharmaceutical products and pesticide residues found in the tomato samples were 871 μg and 452 μg, respectively, which correspond to a total of 8.8 μg/kg f.w. The results suggest that all these CECs have the potential to accumulate in the fruits. However, the total load of CECs is not solely due to the contribution of contaminants present in the irrigation water, but the metabolization/degradation processes produced in the plant or in the soil are also another source of products, such as transformation products.

3.2.3. CECs in the leaves/roots

To determine the plant translocation rate, the roots and leaves (including stems) were separated and individually analyzed. In the case of the leaves, 6 pharmaceutical products and 12 pesticides residues were detected. Diclofenac and the CBZ metabolite (epoxide-CBZ) were the drugs measured at the highest concentrations, 0.7 and 0.5 μg/kg, respectively. The highest average concentration values were measured as follows: diclofenac (0.7 μg/kg f.w.), epoxide-CBZ (0.5 μg/kg f.w.), caffeine (0.4 μg/kg f.w.), and CBZ (0.3 μg/kg f.w.). In contrast, the lowest concentrations were found for hydrochlorothiazide and codeine (0.1 μg/kg f.w.). Regarding the pesticide residues, myclobutanil, diuron, fluxapyroxad, thiamethoxam and imidacloprid were the substances detected at the highest levels in the leaves, ranging from 0.5 to 0.7 μg/kg f.w. Similar results were observed in the roots, where 5 pharmaceutical products and 13 pesticides were found. CBZ and diclofenac were the drugs measured at higher concentrations, 11 and 14.3 μg/kg f.w., respectively. Thiabendazole, azoxystrobin, fluxapyroxad, myclobutanil and penconazole were the pesticides found in the roots at levels above 8 μg/kg. The other pesticides studied in this work were also detected at concentrations ranging from 0.1 to 0.2 μg/kg f.w. in the leaves and from 1.2 to 4.8 μg/kg f.w. in the roots (see Table 3). In general, the CEC concentrations detected in the roots were up to 10-times higher than those found in the leaves. These findings accord well with other studies, which suggest that CEC accumulation generally decreases in the order of root > leaf/stem. Wu et al. (2013) carried out a study to compare the translocation of pharmaceutical and personal care products (PPCPs) by common vegetables. The authors found higher concentrations of diclofenac in the roots than in the leaves in four different vegetable species (lettuce, spinach, cucumber and pepper). Ju et al. (2019) suggested that hydrophobic compounds with low water solubility accumulate more in the roots than in the leaves because the translocation is restricted. Curiously, the CECs detected at higher levels in the roots have moderate hydrophobicity (a logK_{ow} between 2.5 and 3.7) and low water solubility (<132 mg/L), which coincides with that discussed above.

Overall, the total amounts of pharmaceutical products and pesticide residues found in the leaves were 254 μg and 396 μg, respectively, whereas in the roots, the amounts were 150 μg and 429 μg, respectively. These measurements correspond to a total load of 5.6 μg/kg f.w. in the leaves and 120.6 μg/kg f.w. in the roots. The main CBZ metabolite was only detected in the leaves, thus supporting the hypothesis that it is synthesized in the plant.

3.2.4. CECs in the soil

All the targeted pesticides (13) and pharmaceuticals (5) were detected at concentrations above their LOQs in the agricultural soil samples irrigated with contaminated reclaimed water. The CECs total concentration was measured by kg of soil dry weight. The pesticide residues were found at levels between 0.1 and 2.0 μg/kg d.w. The fungicides myclobutanil and penconazole were the compounds found at the highest concentrations (2.0 μg/kg d.w.) whereas the insecticide acetamiprid was detected at the lowest level (0.1 μg/kg d.w). The physicochemical properties of these compounds support these data. As we can see in Table 1, in general, persistent pesticides (DT$_{50}$ ≥ 75 days) were detected at higher concentrations than those with high mobility and low persistence in the soil. Similar results have recently been reported in different types of agricultural soil samples for penconazole (3.9 μg/kg d.w.) and myclobutanil (2.4 μg/kg d.w.) (Acosta-Dacal et al., 2021). Concerning the pharmaceutical products, the average concentrations ranged from 0.1 to 1.2 μg/kg d.w. Carbamazepine was the drug measured at the highest levels in the soil samples analyzed, followed by caffeine, with average concentrations of 1.2 and 0.9 μg/kg d.w., respectively. Conversely, codeine and atenolol were quantified at the lowest concentrations, 0.2 and 0.1 μg/kg d.w., respectively. Caffeine was the second pharmaceutical detected at high levels, with an average concentration of 0.9 μg/kg d.w. Previous studies agree with our result. Beltrán et al. (2020) found CBZ concentrations higher than atenolol in three different crops. Conversely, caffeine levels 10-times higher than those found in this study were reported in soil samples irrigated with reclaimed water in Saudi Arabia (Picó et al., 2019). Despite their high persistence (DT$_{50}$ ≥ 1000 days) and low mobility in soil, neither of the antibiotics selected in this study (ofloxacin and ciprofloxacin) were detected in any of the soil samples analyzed. This can be explained because these compounds were not recovered from the soil samples using the developed extraction method. Two diuretics (furosemide and hydrochlorothiazide), and the analgesic acetaminophen, were not detected in any of the soil samples analyzed in this study. This can be explained because all are bio/photodegradable compounds, which is in line with the bibliographic data (see Table 1).

In summary, the total amounts of pesticides and pharmaceuticals measured in the agricultural soil samples were 4673 μg and 1200 μg, respectively, which corresponds to a total of 14.4 μg/L d.w. None of the selected degradation products were detected in the soil samples analyzed.

3.3. Accumulation and modeling of the CECs in a tomato crop

The accumulation rates were calculated from the concentrations of individual contaminants measured in each part of the crop against the contaminant concentration applied to the crop via the irrigation water. The results from the pilot study conducted in a greenhouse under real field conditions showed that the average total load measurements were as follows: 41368 μg in the irrigation water, 5873 μg in the soil, 1323 μg in the tomatoes, 650 μg in the leaves, and 579 μg in the roots. Considering these values, the accumulation rates in each part of the crop were: 14% in the soil, 3% in the edible part (fruit), 2% in the leaves, and 1% in the roots (see Fig. 2).

3.3.1. Accumulation in the fruit

The CECs' accumulation percentages in the tomatoes ranged from 0.5% to 15%, both for the pesticides and for the pharmaceutical products. Thiamethoxam (15%), 4-FAA (15%), hydrochlorothiazide (13%), diclofenac (10%), and caffeine (8%) were the compounds detected at the highest accumulation rates. All these substances have a high polarity (−0.1 ≤ logKow ≤ 0.2), expect diclofenac, which has a log*Kow* = 4. The concentrations of the detected compounds were compared with the log*Kow* values. As can be seen in Fig. 3, a trend in the outcomes was observed. The experimental data obtained under agronomic field conditions were used to determine a mathematical equation. Most of the compounds studied fitted well to a second-order quadratic equation ($y = 0.0005x^2 - 0.0167x + 0.1705$), the correlation coefficient (r^2) being acceptable at 0.8857. Subsequently, the theoretical accumulation values for each compound were calculated using the equation obtained

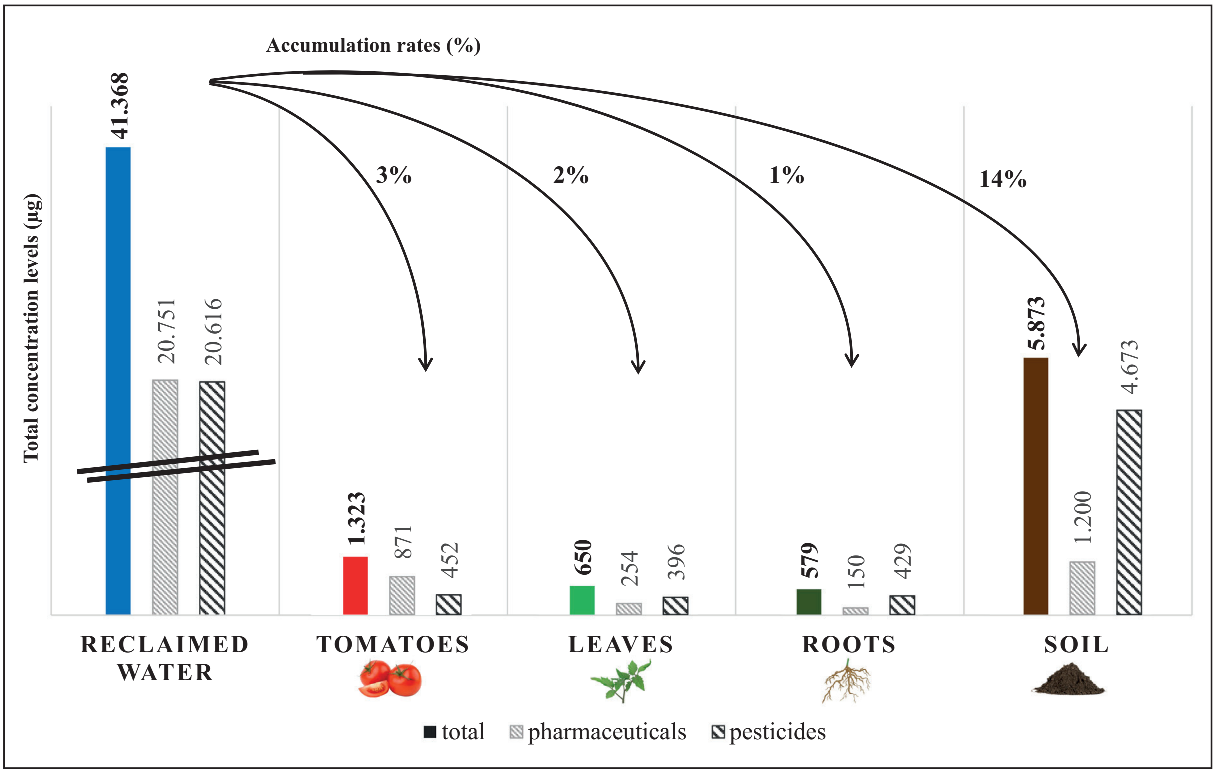

Fig. 2. Distribution of the total concentration levels (μg) and the accumulation rates (%) of the pharmaceuticals and pesticides detected in each part of the crop (reclaimed water, fruit, leaf, root, and soil).

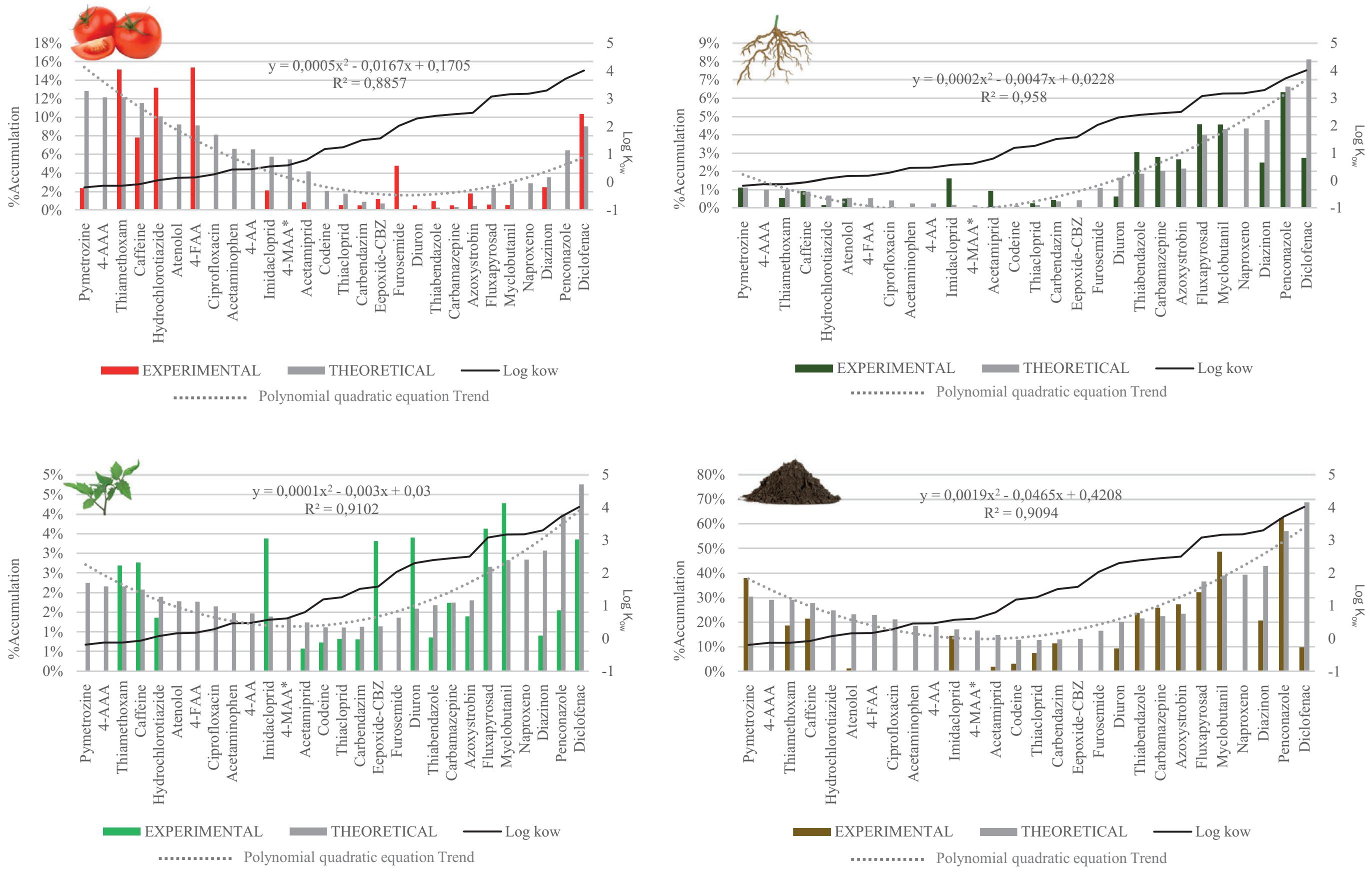

Fig. 3. CEC accumulation (%) detected in each part of the crop (tomato, leaf, root, and soil).

from the experimental data. As can be seen in Fig. 3, the detected compounds generally presented experimental values higher than the theoretical values. The variations between the experimental and theoretical results were lower than 70% in all the detected cases, except for pymetrozine, acetamiprid, furosemide, azoxystrobin, fluxapyroxad and myclobutanil. These data are supported by the low half-life values for acetamiprid ($DT_{50} = 1.6$ days), or the low mobility in the soil of pymetrozine, fluxapyroxad and myclobutanil. Acetaminophen and penconazole were not detected in any of the tomato samples analyzed in this study. This can be explained because acetaminophen is a photodegraded compound whereas penconazole is a persistent compound in the soil (see Table 1).

3.3.2. Accumulation in leaves/roots

The accumulation percentages of the pesticides and the pharmaceutical products in the non-edible parts of the plants were similar. In the leaves, they ranged from 0.9% to 4% for the pesticides and from 1% to 3% for the drugs, whereas in the roots, they ranged from 0.3% to 6% and from 0.2% to 3%, respectively. The highest accumulation rates in the leaves were presented by the fungicides fluxapyroxad and myclobutanil (4%) followed by thiamethoxam, caffeine, imidacloprid, epoxy-CBZ, diuron, and diclofenac (3%). In the roots, the fungicide penconazole presented the most elevated accumulation rates (6%) followed by the fungicides fluxapyroxad and myclobutanil (5%), and by the CECs azoxystrobin, CBZ, thiabendazole, and diclofenac (3%). Again, a correlation between the log*Kow* and the CECs' accumulation in the plant tissue (the non-edible parts) was observed. Two different polynomial equations having adequate r^2 were obtained both for the leaves and for the roots. Fig. 3 summarizes the variations between the experimental and the theoretical results. For the leaves, the following mathematical equation, $y = 0.0001x^2 - 0.003x + 0.03$, was obtained with an $r^2 = 0.912$. The variations between the experimental and the theoretical results were below 70% for all the detected compounds, except for imidacloprid, epoxide-CBZ, and diuron. Most of them presented experimental values lower than the theoretical values. With respect to the roots, another polynomial quadratic equation was obtained ($y = 0.0002x^2 - 0.0047x + 0.0228$) with a satisfactory coefficient ($r^2 = 0.958$). As can be seen in Fig. 3, the experimental results were similar to the theoretical results. Only three compounds (hydrochlorothiazide, imidacloprid, and acetamiprid) presented differences higher than 70%.

3.3.3. Accumulation in the soil

The accumulation percentages of the pesticides and the pharmaceutical products in the soil ranged from 2% to 62% and from 1% to 26%, respectively. The pesticides found at the highest accumulation rates were penconazole (62%), myclobutanil (49%), pymetrozine (38%), and fluxapyroxad (32%). Thiabendazole, azoxystrobin and diazinon were the other pesticides detected at high percentages, between 21% and 27%. Acetamiprid was the pesticide measured at the lowest accumulation percentage (2%) in the agricultural soil samples. The biodegradation data supported this low value (DT_{50} in soil $= 1.6$ days). The pharmaceuticals CBZ (26%), caffeine (21%), and diclofenac (10%) were the compounds with the highest accumulation percentages in the agricultural soil. Atenolol and codeine were the pharmaceuticals found at the lowest accumulation percentages in the agricultural soil samples, at 1% and 3%, respectively. Looking at the physicochemical properties (see Table 1), both compounds are highly hydrophilic in character with a log*Kow* ≤ 1.2, implying a light binding to the soil; however, codeine is also a photodegradable compound whereas atenolol has a high mobility in soil (solubility in water = 13,300 mg/L). Hence, the experimental results under controlled conditions suggested that the CECs' accumulation in the soil was strongly influenced by the lipophilic

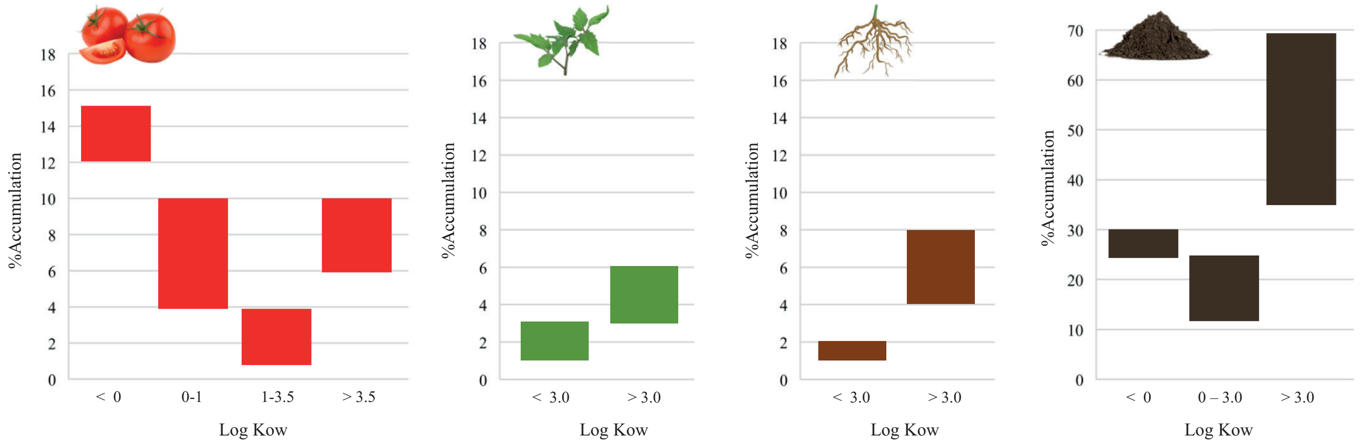

Fig. 4. CEC uptake model in each part of the crop based on the log*Kow* parameter.

character of the compounds. Overall, CECs with moderate and high polarities (log*Kow* ≥ 2.5) presented greater accumulation rates in the soil (see Fig. 3). Pymetrozine and caffeine also presented high accumulation rates, 38% and 21%, respectively. Despite their low log*Kow* values (−0.2 and −0.1, respectively), both compounds have high sorption coefficients in soil (Koc between 246 and 7875 mL/g and between 741 and 7762 mL/g, respectively), which explains how they can be firmly fixed to the organic matter in the soil and accumulate in it. A linear curve derived from plotting the concentration of each of the contaminants measured in the agricultural soil against their log*Kow* values was obtained. Again, the majority of the detected compounds fitted well to a second-order quadratic equation ($y = 0.0019x^2 - 0.0465x + 0.4208$), with a suitable correlation coefficient ($r^2 = 0.9094$). The differences between the experimental/theoretical results observed in the soil samples ranged from 9% to 53%, the exceptions being atenolol (95%), imidacloprid (87%), codeine (76%), and diclofenac (86%). Diclofenac presented an accumulation rate of 10%. However, given its high log*Kow* value (4.0) and its low solubility in water (2 mg/L), higher percentages should be found. As reported in previous works, diclofenac is easily degraded in the environment (Carter et al., 2014), which support this low result.

Overall, some small differences between the experimental/theoretical accumulation data were observed in all the matrices analyzed. In general, the mathematical equations found in this study allowed a suitable estimation of the accumulation percentages in each part of the tomato crop (the soil, leaves, roots and fruit) for many different classes of CECs, based on the hydrophobic/lipophilic character of the compounds. However, the accumulation rates were also influenced by other parameters such as persistence, mobility, and solubility.

Finally, the data obtained were used to establish an uptake model of the contaminants in each part of the crop, based on the log*Kow* parameter (see Table 1). It is expected that compounds with a log*Kow* below 0 will present accumulation rates between 12% and 15%, while compounds with a log*Kow* greater than 3.5 will have an uptake between 6% and 10% in the tomatoes. In the soil, CECs with a log*Kow* greater than 3 will present an accumulation rate between 40% and 70%, while those with a log*Kow* lower than 3 will accumulate at between 10% and 30%. In the leaves and roots, only compounds with a log*Kow* greater than 3 will reach accumulation levels between 4% and 8%.

3.4. Human exposure

This work has shown that tomato plants, when irrigated with reclaimed water containing CECs, are capable of selectively accumulating contaminants in their edible parts. In all cases, the values for the detected CECs complied with the levels established for tomato by the latest Regulation, (EC) No 155/2021, applicable from 02/09/2021, concerning maximum residue levels (MRLs) for pesticides in food (European Commission, 2021). As can be seen in Table 4, these were between 100 and 10,000-times lower than the levels established by the European Commission. According to our experimental data, the pesticides detected in the fruit posed no risk to human health.

Three pharmaceutical products and one pesticide were the compounds with the highest bioconcentration factor values (BCF). Caffeine, diclofenac, and hydrochlorothiazide had BCF values of 1.1, 2.0 and 1.5 (L/kg), respectively, indicating that they tend to accumulate a lot in the fruit. Thiamethoxam was the only pesticide with a high BCF value (2.3 L/kg); of all the selected CECs, this compound had the greatest tendency to accumulate in the fruit.

Consumption of these contaminated tomatoes could potentially pose a risk to humans via their dietary intake. Some scientific papers have reported on studies regarding the human health risks that arise from consuming vegetables irrigated with treated water containing contaminants, especially pharmaceutical and personal care products (PPCPs). Most of these works have been carried out under hydroponic conditions (Wu et al., 2013), under unrealistic agricultural conditions (Malchi et al., 2014), or in field trials at concentration levels higher than those expected in reclaimed water (González García et al., 2019). In our study, we estimated the daily human intake values for each detected CEC (pesticides and pharmaceutical products) based on experimental data obtained from the pilot study carried out under agronomic conditions and considering the latest data reported on per capita consumption of fresh tomato (Blázquez, 2021). As can be seen in Table 4, the highest daily human exposure from consuming contaminated tomatoes in a conventional diet came from the metamizole metabolite (4-FAA, 0.075 μg/day), followed by diclofenac (0.062 μg/day), caffeine (0.033 μg/day), and hydrochlorotiazide (0.029 μg/day), while thiamethoxam was the only pesticide with a high daily exposure value (0.066 μg/day). On the other hand, it has been estimated that these values will be as much as 3-times higher in a vegetarian diet than in a conventional diet, ranging from 0.225 μg/day (4-FAA) to 0.09 μg/day (hydrochlorotiazide). These results are in agreement with another work, in which Wu et al. (2014) reported annual CBZ exposure levels (0.08 μg/year) in tomato grown using treated water fortified at 0.2 μg/L (0.9 μg/L in our case). The total daily values for exposure to pesticides and pharmaceutical products were 0.11 μg/day and 0.21 μg/day, respectively, in a conventional diet; whereas the values rose to 0.33 μg/day and 0.64 μg/day, respectively, in a vegetarian diet. These amounts were more than 3 orders of magnitude less than are present in a single medical dose of these pharmaceutical products (typically between 10 and 200 mg), both for the conventional and vegetarian diets.

The definition of ADI (the acceptable daily intake) is established as "an estimate of the amount of a residue, expressed on a body-weight basis, that can be ingested daily over a lifetime without appreciable

Table 4
Estimated per capita daily exposure values to CECs (μg) from the intake of vegetables.[a]

Compound	Water	Tomato			Daily human intake (μg/day)		
	Average concentration (μg/kg)	Average concentration (μg/kg)	MRL (μg/kg)	BCF (L/kg)	Conventional diet	Vegetarian diet	ADI (mg/day)
Pharmaceutical							
Caffeine	0.8	0.9	–	1.1	0.033	0.10	1000
Hydrochlorotiazide	0.4	0.8	–	2.0	0.029	0.09	3500
4-FAA	Not add	2.1	–	–	0.075	0.22	–
Epoxide-CBZ	Not add	0.2	–	–	0.005	0.02	–
Furosemide	0.2	0.1	–	0.7	0.005	0.02	1500
CBZ	0.9	0.1	–	0.1	0.004	0.01	1200
Diclofenac	1.1	1.7	–	1.5	0.062	0.19	150
Pesticide							
Pymetrozine	0.5	0.2	20	0.4	0.007	0.02	2.1
Thiamethoxam	0.8	1.8	200	2.3	0.066	0.20	1.8
Imidacloprid	0.6	0.2	500	0.3	0.007	0.02	4.2
Acetamiprid	0.8	0.1	500	0.1	0.004	0.01	1.7
Thiacloprid	1.0	0.1	500	0.1	0.003	0.01	0.7
Carbendazim	0.9	0.1	300	0.1	0.003	0.01	1.4
Diuron	0.8	0.1	10	0.1	0.002	0.01	0.5
Thiabendazole	0.6	0.1	10	0.1	0.003	0.01	70
Azoxystrobin	0.8	0.2	3000	0.2	0.007	0.02	14
Fluxapyroxad	0.8	0.1	600	0.1	0.002	0.01	1.4
Myclobutanil	0.8	0.1	600	0.1	0.002	0.01	1.7
Diazinon	0.4	0.1	10	0.4	0.005	0.01	0.01
Total					0.323	0.968	

[a] Data calculated from a tomato crop irrigated with contaminated reclaimed water at 1 μg/L under controlled field conditions; MRL: maximum residue levels of pesticides in tomato (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN); BCF: bioconcentration factor; Consumption data: 13.3 kg per person/year in a conventional diet (https://www.statista.com/statistics/745474/fresh-vegetables-consumption-per-person-in-spain-2015-by-product/#statisticContainer), in a vegetarian diet is estimated 3 times more (approx. 40 kg per person/year); ADI: Maximum acceptable daily intake without appreciable health risk, for pesticides estimated values considering an average weight of 70 kg per individual (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN; https://www.vademecum.es).

health risk" (World Health Organization, 1987). These values are summarized in Table 4, taking 70 kg as the average weight of an individual. In all cases, the estimated daily human intake values were more than 3 orders of magnitude less than the acceptable limits, except in the case of diazinon. This pesticide was found at similar levels to the maximum daily intake value for a vegetarian diet (0.01 μg/day) at which there is no health risk. However, given that fortified irrigation water (in the worst-case scenario, this would be about 1 μg/L) was used to obtain these preliminary experimental data, the human exposure from consuming vegetables irrigated with reclaimed water is expected to be even lower than the above results. A more realistic view of the risk to human health that the consumption of these products entails would be the evaluation of the synergistic effect of exposure to the mixture of detected CECs in them. However, according to the best of authors' knowledge, the current models to consider a mix of effects are not established.

4. Conclusion

From the total amount of CECs released during the crop irrigation (41×10^3 μg), the soil samples exhibited the highest CEC content (5873 μg), followed by the fruit (1323 μg), leaves (650 μg), and roots (579 μg). These values represent accumulation rates from the total load of 14%, 3%, 2%, and 1%, respectively. Overall, the edible part of the plant presented a total CEC amount between 40 and 50% more than the non-edible parts. Some CECs (e.g., ofloxacin, ciprofloxacin, acetaminophen, and gemfibrozil) were not detected in any plant tissue, indicating that these compounds have a limited potential for plant uptake under the field conditions studied here. The results obtained in our study have highlighted that the physicochemical properties of the contaminants (mainly the pKa, log*Kow*, and log*K*oc) play a crucial role in the uptake and translocation through the plants. A negative correlation was observed between the environmental fate with the distribution of CECs within the plants and the log*Kow*. As was expected, hydrophilic compounds tended to translocate from roots to leaves and finally to the fruit, whereas hydrophobic contaminants tended to remain in the roots and soil. Overall, it was noted that the CEC accumulation percentages in the soil were approx. 5-times higher than those found in the plant.

Further studies on different crops are needed to acquire better theoretical accumulation models to allow us to estimate the CEC levels in relation to different physicochemical properties in the soil as well as in the different parts of plants that are irrigated with reclaimed water over a long period. It is strongly recommended that future works also monitor the three macrolide antibiotics included in Commission Implementing Decision (EU) 2018/840 (azithromycin, used in the treatment of Covid, clarithromycin, and erythromycin), as well as other ubiquitous and highly concentrated compounds, such as venlafaxine, due to their incessant discharge into WWTPs. Additionally, the analgesic diclofenac presented the highest bioconcentration factor values, indicating that this compound tends to be highly accumulated in the fruit, while also showing high accumulation rates in the soil. Although this compound has been removed by the new EU 2018/840 legislation and has not been included in the latest European Commission watch list decision, the results obtained in this study highlight the importance of its continued monitoring.

Regarding the human risk derived from consuming tomatoes that are permanently irrigated with contaminated reclaimed water, the obtained results suggest that an adult would need to consume a few hundred kilograms of contaminated tomatoes daily to reach the acceptable intake limit. However, even though the present study might be considered a worst-case scenario, it only encompasses 30 substances in a tomato crop. Therefore, the total daily exposure values may be higher when using more generalized reclaimed water and when screening for other chemicals. It is also recommended that specific soil cleaning treatments are undertaken between crops to facilitate the soil's continuous reuse.

CRediT authorship contribution statement

1. Guarantor of integrity of the entire study: MMG; ARF.
2. Study concepts and design: MJM; MMG; JASA; DB; ARF.
3. Literature research: MJM; MGV.
4. Laboratory work: MGV; MJM; MMG.

5. Data analysis: MGV; MJM; JASA; DB; ARF.
6. Statistical analysis: MJM; ARF.
7. Manuscript preparation: MJM; MGV; ARF.
8. Manuscript editing: MJM; DB; ARF.

Declaration of competing interest

The authors declare no conflict of interest. This is an independent research. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Appendix A. Supplementary data

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CAPÍTULO III

Artículo Científico

“Long-term effects on the agroecosystem of using reclaimed water on commercial crops”

M. García-Valverde, A. M. Aragonés, J. A. Salinas Andújar, M. D. Gil García,
M. J. Martínez-Bueno, Amadeo R. Fernández-Alba

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Long-term effects on the agroecosystem of using reclaimed water on commercial crops

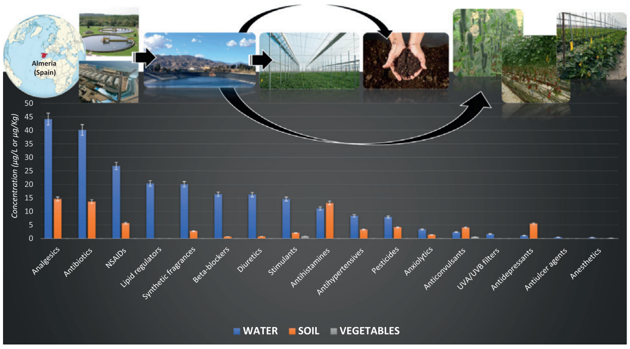
M. García-Valverde [a], A.M. Aragonés [a], J.A. Salinas Andújar [b], M.D. Gil García [a],
M.J. Martínez-Bueno [a,*], A.R. Fernández-Alba [a]

[a] *University of Almería, Department of Physics and Chemistry, Agrifood Campus of International Excellence (ceiA3), Ctra. Sacramento s/n, La Cañada de San Urbano, 04120 Almería, Spain*
[b] *University of Almería, Department of Engineering, Agrifood Campus of International Excellence (ceiA3), La Cañada de San Urbano, 04120 Almería, Spain*

HIGHLIGHTS

- Data from 5 greenhouses irrigated with reclaimed water are presented.
- Irrigation water exhibited the highest levels of CECs followed by soils ≥ vegetables.
- Carbamazepine and caffeine were the only CECs in all water-soil-plant continuum.
- Lidocaine exhibited the greatest bioaccumulation factor in all crops.
- None of vegetables represented a risk to human health.

GRAPHICAL ABSTRACT



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ABSTRACT

The use of reclaimed water for crop irrigation has been proposed as a suitable alternative for farmers in the coastal areas of Mediterranean countries, which suffer from greater water scarcity. In this work we study the impact on the water-soil-plant continuum of using reclaimed water for commercial crops irrigated over a long period, as well as the human risks associated with consuming the vegetables produced. Forty-four CECs were identified in the reclaimed water used for crop irrigation. Of these, twenty-four CECs were identified in the irrigated soil samples analysed. Tramadol, ofloxacin, tonalide, gemfibrozil, atenolol, caffeine, and cetirizine were the pharmaceuticals detected at the highest levels in the water samples (between 11 and 44 μg/L). The CECs with the highest average soil concentrations were tramadol (14.6 μg/kg), followed by cetirizine (13.2 μg/kg) and clarithromycin (12.7 μg/kg). In the irrigated vegetable samples analysed over the study period, carbamazepine, lidocaine, and caffeine were only detected at levels from 0.1 to 1.7 μg/kg. The CEC accumulation rate detected in the edible parts of the vegetables permanently irrigated with reclaimed water was very low (~1 %), whereas it was 33 % in the soils. The results revealed that consuming fruits harvested from plants irrigated for a long period with reclaimed water does not represent a risk to human health, opening the door to a circular economy of water. Nevertheless, for crop irrigation, future studies need to be conducted over longer periods and in other matrices to provide more scientific data on the safety of using reclaimed water.

1. Introduction

The availability of suitable quality water is essential for the growth of those economic sectors that depend on it, and for society in general. However, according to the latest data from UNESCO, it is estimated that, by

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* Corresponding author.
E-mail address: mjbueno@ual.es (M.J. Martínez-Bueno).

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2050, 40 % of the global population will be living under conditions of severe water scarcity (UNESCO, 2020). Using reclaimed water is a very important water supply alternative, offering significant environmental (extending the water life cycle), economic and social benefits (Delli Compagni et al., 2020). Therefore, this practice is being incorporated as an integral part of water resource management plans in many regions around the world (Singh, 2021).

In Europe, water reuse is a priority area within the Strategic Implementation Plan of the European Innovation Partnership on Water. At present, about 1 billion m^3 of treated urban wastewater is reused annually, accounting for approximately 2.4 % of overall treated urban wastewater effluents, yet this is <0.5 % of annual EU freshwater withdrawals. It is estimated that, by 2025, the wastewater reuse volume will reach 3222 Mm^3/year in Europe (Alcalde Sanza and Gawlik, 2014). Spain is the European leader in wastewater reuse, the annual volume of which is 347 hm^3/year, the main application being in agriculture, as is the case worldwide. Specifically, 71 % of the regenerated water volume is used for crop irrigation, 17 % for environmental use, 7 % for recreational use, 4 % for urban use, while only 0.3 % is used in industries (Jodar-Abellan et al., 2019).

One of the advantages of using reclaimed wastewater is that less fertilizer has to be applied; this is because reclaimed wastewater contains nitrogen, phosphorus and potassium as well as micronutrients (Singh, 2021). However, this practice can affect soil salinity, potentially inducing a water absorption deficit in the plant (Shakir et al., 2017). Furthermore, as has been reported by several authors, the treated waters contain a wide range of organic contaminants known as contaminants of emerging concern (CECs), including pharmaceutical and personal care products (PPCPs) and pesticides (Martínez Bueno et al., 2012; Ofrydopoulou et al., 2022; Renau-Pruñonosa et al., 2020). The bio-physico-chemical properties of these molecules determine their fate in the water-soil-plant continuum. In this regard, over recent years, several studies have reported that using reclaimed water for crop irrigation might be an important pathway for organic contaminants to be introduced into agricultural production, and then subsequently enter the food chain, potentially posing a risk to health and to the environment (Christou et al., 2019; Picó et al., 2019). Nonetheless, most of these works were carried out in field trials at concentration levels higher than those expected in reclaimed water (under controlled greenhouse conditions) or on a specific group of organic contaminants (pharmaceutical products) (Beltrán et al., 2020; Christou et al., 2017; González García et al., 2019; Martínez-Piernas et al., 2019). To date, scarce scientific data are available regarding CEC concentrations in commercially grown crops. In a recent publication, Ben Mordechay et al. (2021) reported data on CECs in crops collected from 445 commercial fields irrigated with reclaimed wastewater in Israel.

Until 2020, water use in the European Union was regulated by Directive 2013/39 & Commission Implementation Decision (EU) 2018/840, and by Directive 91/271/EE. However, these frameworks did not sufficiently specify the conditions and parameters for using reclaimed water for crop irrigation. Consequently, a new regulation has recently been established by the European Commission regarding the minimum water reuse requirements for agricultural irrigation (Regulation 2020/741/EU, 2020). Nevertheless, this only regulated the physicochemical and microbiological parameters; again, CECs were not considered, creating commercial difficulties for agricultural products cultivated with reclaimed water.

Considering all of the above, the goal of the current work was to evaluate the impact of the long-term use of reclaimed water for crop irrigation under real agronomic conditions. The specific objectives were: (1) to develop, validate and apply an analytical approach based on a multi-residue analysis of environmental samples (water and soil) and food samples; and (2) to identify those contaminants that, due to their physicochemical properties, may pose a greater risk to health from being consumed in the edible part of the plant and/or from their environmental impact. For that, a multiresidue method based on QuEChERs (Quick, Easy, Cheap, Effective and Rugged) extraction coupled to liquid chromatography tandem mass spectrometry (LC–MS/MS) was the procedure used. The risk to human health was estimated based on the daily human intake values found of each detected target compound and compared with reported acceptable daily intake values (ADI), both in the conventional as well vegetarian diet. To the best of the authors' knowledge, this is the first time that such a large panel of target organic pollutants have been investigated (a total of 293 substances: 49 pharmaceutical products and 244 pesticides) on commercial crops permanently irrigated with reclaimed water in greenhouses in Spain. Therefore, the current work aspires to meet one of the main challenges facing European society (The Strategic Implementation Plan of the European Innovation Partnership on Water), providing improved scientific-technical knowledge regarding the long-term effects on the water-soil-plant continuum of using reclaimed water for crop irrigation.

2. Materials and methods

2.1. Reagents and materials

Ultrapure water was obtained from Fisher Scientific (Fair Lawn, NJ, USA). Methanol, HPLC-grade acetonitrile, and LC-MS-grade acetonitrile were purchased from Honeywell (Charlotte, North Carolina). Formic acid was supplied by Fluka Analytical (Steinheim, Germany). QuEChERS salts (anhydrous magnesium sulphate, sodium chloride, sodium hydrogen citrate sesquihydrate and sodium citrate tribasic dihydrate) were obtained from Sigma-Aldrich (Steinheim, Germany) while C-18 sorbent was supplied from Supelco (Bellefonte, PA, USA). An automatic axial extractor (AGITAX®) was purchased from CirtaLAb, S.L., Spain. The centrifuge was supplied by Ortoalresa (Daganza, Madrid, Spain). Carbendazim-d^3, dichlorvos-d^6, malathion-d^{10} and caffeine-^{13}C were used to check the extraction efficiency while dimethoate-d^6 was selected to check the analytical efficiency.

The analytes were acquired from Sigma-Aldrich (Steinheim, Germany) at analytical grade (>98 %), except for hydrochlorothiazide, betahistine, sulpiride, famotidine, pantoprazole, clonazepam, and diazepam, which were obtained in pill form. In the Supplementary Material section (Table S1), detailed information is given on the physicochemical properties of the compounds selected in this study. Individual stock solutions of each organic emerging contaminant were prepared at 2000–10,000 mg/L in MeOH, placed in amber screw-capped glass vials, and stored in the dark at −40 °C. Standard working solutions were prepared daily in ACN at 1000 mg/L by diluting the stock solution; this working solution was then used for identification and quantification purposes.

2.2. Sampling site and sample collection

The province of Almería is situated in the southeast of Spain (the Western Mediterranean area). Almería is the main production area of horticultural products for Spain and Europe (Caparrós-Martínez et al., 2020). It produces >3.5 MT of fruit/vegetables per year, mostly grown under plastic (35,000 ha), of which 2.7 MT are exported (76 %). Among the typical crops cultivated in Almería are tomato (*Solanum lycopersicum*), cucumber (*Cucumis sativus*), pepper (*Capsicum annuun*), melon (*Cucumis melo*), eggplant (*Solanum melongena*) and zucchini (*Cucurbita pepo*). According to data published by the Government of Andalusia, 48 % of Almerian farmers use reclaimed water as the only supply source, and the remaining 52 % mix it with conventional water from local water sources and wells (Segura and Fernández, 2014).

Irrigation water samples were directly collected from the water treatment plant of the General Community of Water Users of Almería (CGUAL). Reclaimed wastewater effluents and desalinated seawater are mixed and treated using an ultrafiltration and sodium hypochlorite disinfection process prior to distribution. The reclaimed water complies with the main European Directives on water reuse requirements (Directive 2013/39/EU, 2013; Directive 91/271/EEC, 1991; Regulation 2020/741/EU, 2020). Vegetable and soil samples were obtained from greenhouses managed by CGUAL farmers over two agricultural seasons (from September 2021 to May 2022). A total of 22 irrigation water samples were collected weekly in glass bottles (2 L). Four different crops (cucumber, tomato, pepper, and zucchini) were analysed. The vegetable samples were collected in plastic bags and transferred to the laboratory, where they were triturated. The irrigated soil samples were taken from the top 10 cm layer. They were collected in glass bottles and transferred to the laboratory. There, they were sifted through a 0.5 mm diameter sieve and dehydrated in an oven at 30 °C for 24 h. All the samples were frozen and stored at −20 °C prior to analysis.

2.3. Sample extraction

The water samples were analysed by direct injection after a centrifugation step at 3500 rpm for 5 min to remove suspended solid particles. The vegetable and soil samples were extracted using two procedures that had been previously reported and validated by our research group (García Valverde et al., 2021; Martínez Bueno et al., 2022). Each sample was extracted in triplicate. Briefly, 10 g of sample was weighed in a 50-mL PTFE centrifuge tube. To rehydrate the soil samples, 5 mL of distilled water was added. After this, a deuterated standard mixture was added to check the extraction procedure (caffeine-^{13}C, carbendazim-d^3, dichlorvos-d^6 and malathion-d^{10}). Next, 10 mL of acidified ACN (0.5 % v/v, formic acid) was added to the samples to improve the extraction. The samples were shaken for 6 min in an automatic axial extractor at room temperature (AGITAX®, CirtaLAb, S.L., Spain). After that, 4 g of anhydrous $MgSO_4$, 1 g of $Na_3Citrate{\cdot}2H_2O$, 1 g of NaCl and 0.5 g of $Na_2HCitrate{\cdot}1.5H_2O$ were added. The samples were shaken again and then centrifuged at 3500 rpm for 5 min. Subsequently, 5 mL of the supernatant was transferred to a 15 mL polyethene tube, to which 750 mg of anhydrous $MgSO_4$ and 125 mg of C18 were added. After this, the tubes were vortexed for 30 s. The last step was to centrifuge the tubes once more. Prior to injection, 100 μL of each extract was evaporated and reconstituted with 100 μL of ACN:water solution (10:90, v/v), which contained dimethoate-d^6 as the surrogate injection standard.

2.4. Sample analysis

For target analysis purposes, a wide-scope method harmonized with DG SANTE 11312/2021 guidelines was employed. Samples were analysed on a Sciex high-performance liquid chromatography system (Exion HPLC 6500+) connected to a mass spectrometer equipped with a turbo spray ion-drive source (LC-ESI-TripleQuad-MS/MS, Sciex) operating in positive and negative mode. Chromatographic separation was performed on a Zorbax Eclipse Plus C8 of 1.8 μm × 2.1 mm × 100 mm (Agilent). The mobile phases were 0.1 % formic acid in ultrapure water (solvent A) and ACN (solvent B) at a constant flow rate of 0.3 mL/min. The gradient programme was 10 % of B and 90 % of A for 0.5 min (the initial conditions) after a linear gradient up to 100 % of B in 11.5 min; B was kept at 100 % for 4 min and, finally, the mobile phase returned to 10 % B and 90 % A. The total run time was 18 min, and the injection volume was 5 μL. The ionization settings used were: ion spray voltage, 5000 V and −4500 V (for the positive and negative ionization modes, respectively); GS1, 50 psi; GS2, 40 psi; curtain gas, 20 (arbitrary units) and temperature, 500 °C. Nitrogen was used as the nebulizer gas and collision gas.

To optimize the target compounds, an individual standard solution at 200 μg/L was used. The chromatographic and mass spectrometer conditions for each compound were obtained with these individual solutions. In full-scan mode, the solutions were infused directly into the MS system and the precursor ion was selected by choosing the most intense ion. The analyses were performed in multiple reaction monitoring (MRM) mode. Then, the optimal collision energies (CEs) were selected using the two most intense transitions in product-ion mode. Consequently, the quantifier ion (SRM1) was the ion with the most intense and the qualifier ion (SRM 2) was the ion with the second most intensity. The optimal mass spectrometric parameters for each target compound using LC-MS/MS are shown in Table S2 in the Supplementary Material section.

Data analysis was performed with Sciex Analyst version 1.7.3 software for data acquisition/processing and SCIEX OS version 2.0.0.45330 software for data quantification. In addition, an SRM schedule with a retention time window of 0.4 min was applied during the acquisition and quantification.

2.5. Target compounds

The analytes chosen in the present study were selected based on previously published data on the presence of CECs in treated water (Martínez Bueno et al., 2012; Ofrydopoulou et al., 2022). A total of 293 target compounds were selected from the following categories: 49 pharmaceutical products belonging to 19 different therapeutic classes and 244 pesticides belonging to 5 different pesticide types (acaricides, biocides, herbicides, insecticides, and nematicides). More information about the target compounds can be found in the Supplementary Material section (Table S1).

2.6. Analytical performance and quality control

To confirm and quantify the target compounds, the requirements on mass spectrometric confirmation set by EU regulations (Commission Decision 2002/657/EC, 2002) were considered. The four criteria were: SRM1 with an $s/n \geq 10$; SRM2 with an $s/n \geq 3$; a retention time ± 0.1 min with reference to the standard; and a value of ±30 % when comparing the fragment ion area with the precursor ion area (the ion ratio).

The validation of the analytical approaches was performed according to EU quality control procedures, DG-SANTE/11312/2021 (European Commission DG-SANTE, 2021). The linearity, matrix effect, sensitivity, trueness (in terms of recovery), precision (in terms of method repeatability and reproducibility) and selectivity were evaluated for each matrix studied (water, fruit, and soil).

A control was injected prior to analysis to check the performance of the HPLC, the analytical column, and the QqQ-MS/MS system. This control contained a selection of analytes at 2 μg/L. The analytical procedure was checked at two different stages of the process. During the extraction, caffeine-^{13}C, carbendazim-d^3, malathion-d^{10} and dichlorvos-d^6 were added to check the extraction efficiency. To check the analytical stage, dimethoate-d^6 was added while the injection vials were being prepared. To evaluate the selectivity and specificity of the method, different blank samples of water, fruit and soil were extracted. No other peaks caused by matrix co-eluting interferences were detected for the target analytes in a time range of ±0.2 min.

2.7. Bioconcentration factor (BCF) and human exposure

The plant uptake was estimated using the bioconcentration factor (BCF) (González García et al., 2019; Martínez Bueno et al., 2022). The BCF values were calculated through the average concentration of each target compound detected in the edible part of the plant (cucumber, pepper, tomato, and zucchini) compared to the average concentration of each target compound in the irrigation water.

In addition, human exposure was estimated to gauge the daily human intake of each target compound. This value was obtained by multiplying the average concentration of each contaminant in the edible part of the plant (μg/kg) and the volume of fresh vegetables consumed per capita in Spain in 2021 (kg/day). According to the most recent data reported, the annual consumption of fresh vegetables in 2020 in Spain was around 2.9 billion kg (Trenda, 2022). Tomato, pepper, zucchini, and cucumber were the most consumed vegetables with consumption volumes per capita of 13.3, 4.8, 4 and 2 kg/person/year, respectively (36.4, 13.2, 11 and 5.5 g/day). Human exposure was calculated as follows:

$$\text{Human exposure} = C \times D \times T$$

with C standing for the concentration of CECs in the vegetables (μg/kg in fresh weight, fw), D being the amount of vegetables consumed daily per capita (kg/day) and T being the exposure time of these contaminated vegetables (days).

3. Results and discussion

3.1. Method validation

A validation study was carried out for each matrix studied (water, fruit and soil). Due to the difficulty in obtaining a "blank" of the reclaimed water (without CECs or below their LOQ levels), the water sample was previously analysed and the signal of the target analytes was subtracted to calculate the validation data.

To simplify the validation step, the guidance from the European Union (European Commission DG-SANTE, 2021) was used to classify the plant commodities according to their physicochemical properties. Cucumber, tomato, pepper, and zucchini are commodities with similar water contents. Therefore, the tomato matrix was selected to perform the validation study in vegetables. The analytical performance data for each compound/matrix combination are summarized in Table S3. The LOQ values were evaluated for each analyte/matrix combination. Overall, the LOQ values were below 0.5 μg/kg for >80 % of the analytes studied in the tomato and soil matrices. In the reclaimed water, a total of 209 compounds out of 293 (71 %) presented a LOQ ≤ 0.5 μg/L. Less than 2 % of the target compounds presented LOQ values at 10 μg/L in the matrices under study. All of these were pesticides, except for the analgesic acetaminophen in the water matrix and the antiulcer agent pantoprazole in the tomato matrix. Specifically, four pesticides presented LOQ values at 10 μg/L in the water matrix (acephate, cyazofamid, cyhalophos-butyl and MCPB), two in the tomato matrix (cyhalophos-butyl and procymidone), and five in soil matrix (acephate, cyhalophos-butyl, fluopyram, propiconazole and tolfenpyrad).

The linearity of the method was evaluated based on the linear regression and correlation coefficient (r^2). Matrix-matched calibration curves at 7 levels (from 0.1 to 100 μg/L or μg/kg) were prepared to study the linearity in each matrix. All the analytes presented a good response with correlation coefficients higher than 0.99 in all cases.

The calibration curves in the matrix and solvent were compared to evaluate the matrix effect (ME). Signal suppression was the most common effect found in the soil and vegetable matrices (>95 %), while the enhancement/suppression effect was similar for the reclaimed water (see Table S3). A ME ≤ 20 % was considered a weak matrix effect, between 20 % and 50 % a moderate matrix effect, and >50 % a strong effect. According to our results, no matrix effect was observed for >90 % and 70 % of the target analytes in the reclaimed water and the soil matrix, respectively. However, the number of compounds that presented matrix effects was greater in the tomato matrix — >75 % of the contaminants presented a moderate or strong matrix effect. Some authors have explained this issue as being caused by several enzymes/sugars present in the plant (Picó et al., 2019).

Recovery studies were carried out per quintuplet (n = 5) using spiked samples at different levels (1, 5, 10 and 50 μg/kg). The response of each contaminant in the spiked matrix extract was compared with the response detected in the spiked samples. Recoveries were considered acceptable when consistent results were obtained within the 70 to 120 % range. The recoveries obtained were satisfactory considering the wide range of contaminants being studied and their different properties. More than 75 % of the target compounds presented satisfactory recovery values within the 70–120 % range in the soil and tomato samples spiked at 1 μg/kg. The percentage of compounds was higher than 90 % when the soil and tomato samples were spiked at concentration levels above 10 μg/kg. The results demonstrate the method's good performance at low concentration levels. Only 13 compounds out of 293 presented poor recoveries in the soil matrix at any concentration (<37 %) — 4 pharmaceutical products (ciprofloxacin, famotidine, ofloxacin and ranitidine) and 9 pesticides (alfuzosin, cyromazine, formetanate hydrochloride, matrine, matrine-n-oxide, phosmet, prothioconazole, pyridalyl and pyridate). In tomato, 3 pharmaceuticals (ciprofloxacin, erythromycin, and ranitidine) and 7 pesticides (2,4-D, dodine, fluazifop-p, haloxyfop, matrine, matrine-n-oxide and propiconazole) presented the lowest recovery values (<39 %). For more details, see Table S3 in the Supplementary Material section.

Repeatability and reproducibility (intra and inter-day precision) were calculated for each analyte/matrix from the results obtained from five injections of a blank sample spiked at two levels: 1 and 10 μg/L, covering the different concentrations of the average linearity range of the target compounds. The results were acceptable with %RSD values between 1 % and 20 % for all the matrices.

3.2. Irrigation water analysis

All the field-collected irrigation water samples contained CECs. A total of forty-four compounds were detected in the irrigation water samples analysed. Table 1 shows the detection frequencies (%), concentration ranges, and average concentrations (μg/L) of all the CECs detected in the irrigation water samples analysed. Tramadol, ofloxacin, tonalide, gemfibrozil, atenolol, caffeine, and cetirizine were the pharmaceutical products quantified at the highest concentrations, with mean values between 44.1 and 11.1 μg/L. On the other hand, the most frequently detected pesticides were the fungicide carbendazim and the insecticides acetamiprid and imidacloprid, with detection frequencies higher than 75 % and concentration levels up to 1.5, 35.7 and 9.2 μg/L, respectively. The herbicides, terbutryn and diuron were the least detected pesticides (<25 %). As can be seen in Table 1, a total of 14 contaminants were detected in all the water samples analysed. Among them were the antibiotic ofloxacin (a mean of 33.5 μg/L), the NSAIDs ketoprofen and mefenamic acid (means of 10 and 0.3 μg/L, respectively), the β-blocker propranolol (a mean of 0.6 μg/L), the anticonvulsant lamotrigine (a mean of 1 μg/L), the antihypertensives valsartan and irbesartan (means of 4.8 and 2.0 μg/L, respectively), the lipid regulator gemfibrozil (a mean of 16.9 μg/L), the diuretic hydrochlorothiazide (a mean of 5.8 μg/L), the antipsychotic drugs amisulpride and sulpiride (means of 0.3 and 3.1 μg/L, respectively), the antihistamine cetirizine (a mean of 11.1 μg/L), the anaesthetic lidocaine (a mean of 0.4 μg/L), and the insecticide imidacloprid (a mean of 1.8 μg/L). Other CECs with high detection frequencies were atenolol (95 %), galaxolide (95 %), telmisartan (91 %), venlafaxine (91 %), acetamiprid (91 %) and bezafibrate (86 %). Fig. 1 presents the data collected. In general, pharmaceutical products were detected at higher concentration levels than pesticides, with average total loads of 227.8 μg/L and 8.1 μg/L, respectively. The average total loads according to the different groups were: 44.1 μg/L for analgesics, 40.1 μg/L for antibiotics, 26.8 μg/L for NSAIDs, 20.4 μg/L for lipid regulators, 20.1 μg/L for synthetic fragrances, 16.4 μg/L for β-blockers, 16.2 μg/L for diuretics, 14.6 μg/L for stimulants, 11.1 μg/L for antihistamines, 8.4 μg/L for antihypertensives and 8.0 μg/L for pesticides (see Fig. 1). With regard to pesticides, none of the compounds included in the list of priority substances were detected at concentrations above their LOQs, except for diuron and terbutryn (Directive 2013/39/EU, 2013). However, neither exceeded the maximum limits permitted 1.8 μg/L and 0.34 μg/L, respectively. The most relevant CECs detected in the irrigation water samples analysed are discussed below.

Antibiotics are a group of drugs widely prescribed to treat bacterial infections. They can be expelled into the environment via effluents from hospitals, pharmaceutical industries, and wastewater treatment plants (WWTPs). Moreover, it is known that WWTPs provide only a low removal efficiency (Martínez Bueno et al., 2012). In a study carried out on Spain's surface water, the authors reported ofloxacin concentration levels of up to 0.4 μg/L (Martínez Bueno et al., 2010). In our study, six antibiotics were detected. Ofloxacin was detected in all the analysed samples at concentrations ranging from 5.6 to 130.7 μg/L, an average concentration of 33.5 μg/L. In contrast, clarithromycin, metronidazole, trimethoprim and sulfamethoxazole were detected at concentrations that were an order of magnitude lower than ofloxacin, with averages of 3.4, 1.4, 1.1 and 0.7 μg/L, respectively. A previously published study by Rodriguez-Mozaz et al. (2015) reported concentration levels similar to our results for clarithromycin, sulfamethoxazole, and trimethoprim (0.92, 0.64 and 0.97 μg/L, respectively).

Around 10 % of patients worldwide are diagnosed with chronic pain each year (Ahmed et al., 2021). Analgesics are used to provide pain relief. Tramadol is a typical analgesic although paracetamol is the most used. To date, there have been few studies evaluating tramadol. In our study, tramadol was detected at a detection frequency of 73 % and its average

Table 1
CECs in irrigation water (μg/L) and irrigated soil samples (μg/kg). Soil data per each crop are presented in Tables S4.

Compound	Irrigation water			Irrigated soils		
	Detection frequency (%)	Concentration range (μg/L)	Average concentration (μg/L)	Detection frequency (%)	Concentration range (μg/kg)	Average concentration (μg/kg)
PPCPs						
Antibiotics						
Clarithromycin	82	<LOQ–7.6	3.4	75	<LOQ–17.0	12.7
Metronidazole	64	<LOQ–4.6	1.4	–	–	–
Ofloxacin	100	5.6–130.7	33.5	–	–	–
Sulfamethoxazole	23	<LOQ–0.9	0.7	–	–	–
Trimethoprim	82	<LOQ–1.9	1.1	75	<LOQ–1.9	1.0
NSAIDs						
Diclofenac	18	6.7–15.0	9.5	–	–	–
Ketoprofen	100	<LOQ–21.3	10.0	–	–	–
Mefenamic acid	100	0.1–0.6	0.3	100	0.1–0.4	0.2
Naproxen	18	<LOQ–7.7	6.9	25	<LOQ–5.7	5.5
Analgesics						
Tramadol	73	15.6–81.0	44.1	75	<LOQ–24.5	14.6
Anesthetics						
Lidocaine	100	0.1–0.7	0.4	–	–	–
Anticonvulsants						
Carbamazepine	73	0.7–2.2	1.4	100	1.1–7.6	3.5
Lamotrigine	100	0.4–2.0	1.0	75	<LOQ–0.9	0.6
Antidepressants						
Citalopram	27	<LOQ–0.6	0.5	100	0.9–7.1	3.7
Venlafaxine	91	<LOQ–1.2	0.6	100	0.6–3.3	1.8
Antihistamines						
Cetirizine	100	2.5–29.7	11.1	75	<LOQ −20.0	13.2
Antipsychotic drugs						
Amisulpride	100	0.1–0.6	0.3	100	0.2–0.4	0.3
Sulpiride	100	0.9–5.8	3.1	100	0.2–2.4	1.2
Antiulcer agents						
Famotidine	27	<LOQ–0.9	0.5	–	–	–
UVA/UVB filters						
BP-3	14	1.6–1.9	1.7	–	–	–
Diuretics						
Furosemide	14	<LOQ–20.2	10.4	–	–	–
Hydrochlorothiazide	100	2.0–12.0	5.8	37	<LOQ-0.9	0.8
Stimulant						
Caffeine	73	<LOQ–66.5	14.6	100	0.8–4.9	2.1
Synthetic fragrances						
Galaxolide	95	<LOQ–3.0	1.2	100	0.3–7.4	2.8
Tonalide	50	6.9–27.7	18.9	–	–	–
Lipid regulators						
Bezafibrate	86	<LOQ–2.7	1.5	–	–	–
Fenofibric acid	50	0.7–3.8	2.0	–	–	–
Gemfibrozil	100	7.1–45.2	16.9	–	–	–
Beta-blockers						
Atenolol	95	5.9–23.8	15.0	–	–	–
Bisoprolol	27	<LOQ–0.9	0.7	–	–	–
Propanolol	100	0.2–1.5	0.6	37	<LOQ–0.9	0.7
Antihypertensives						
Irbesartan	100	0.6–3.5	2.0	75	<LOQ–1.0	0.5
Telmisartan	91	<LOQ–3.0	1.6	100	0.5–8.2	2.9
Valsartan	100	0.8–12.8	4.8	–	–	–
Total load of PPCPs			227.8			67.3
Pesticides						
Fungicides						
Azoxystrobin	50	<LOQ	<LOQ	75	<LOQ–1.2	0.9
Carbendazim	77	<LOQ–1.5	0.5	25	<LOQ–2.4	2.3
Propamocarb	32	<LOQ–0.9	0.5	100	0.2–0.4	0.3
Thiabendazole	23	0.3–0.4	0.3	100	0.1–0.2	0.1
Herbicides						
Diuron	23	<LOQ–1.7	1.5	100	0.2–0.3	0.2
Terbutryn	18	<LOQ–0.3	0.2	75	<LOQ–0.5	0.4
Insecticides						
Acetamiprid	91	0.1–35.7	2.9	–	–	–
DEET	59	0.3–0.7	0.4	–	–	–
Imidacloprid	100	0.2–9.2	1.8	–	–	–
Total load of pesticides			8.1			4.2

<LOQ: limits of quantification (μg/L).

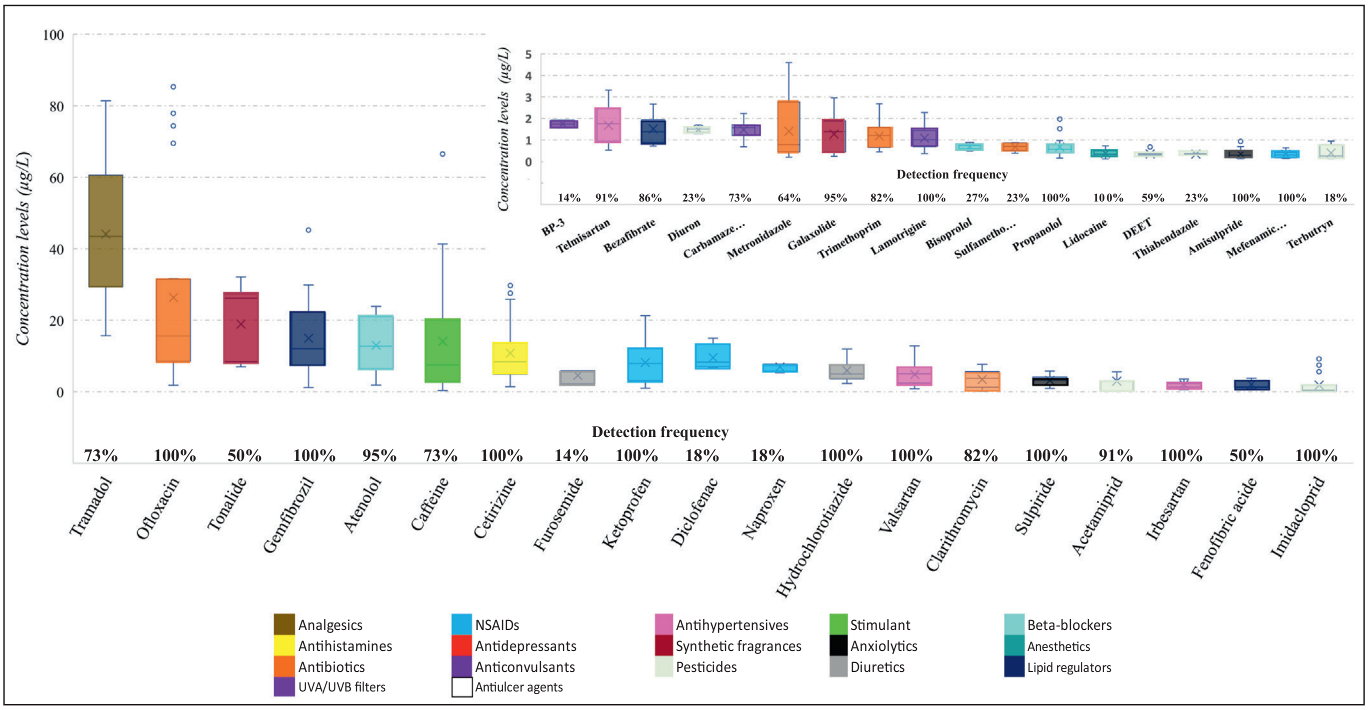

Fig. 1. CECs detected in the irrigation water samples analysed. Data <LOQ are not presented. The median is presented as boxplots. Detection frequencies are listed for each compound. The vertical lines represent the maximum and minimum concentrations detected for each compound in the samples analysed. Circles represent outlier measurements.

concentration was 44.1 µg/L in the irrigation water samples analysed. Ahmed et al. (2021) reported concentration levels of tramadol between 0.2 and 0.5 µg/L in different treated waters from Australia. Similar results were found by Nieto-Juárez et al. (2021) in treated wastewater from South America.

Fragrance substances have wide applications such as in cleaning, detergent and cosmetic products. In the EU alone, around 1000 tons per year are produced (Chen et al., 2007; Klaschka et al., 2013; Tasselli et al., 2021). The synthetic fragrances tonalide and galaxolide were detected in the irrigation water samples. Galaxolide was found in almost all the samples (95 %) at an average concentration of 1.2 µg/L, while tonalide was only detected in half of the analysed samples (50 %), although at higher concentrations (18.9 µg/L). These results are in line with the data reported by other authors. Chen et al. (2007) found similar concentration levels for tonalide in treated wastewater from a cosmetic plant (5.4 µg/L) whereas galaxolide was quantified at concentrations up to 32.1 µg/L.

Lipid regulators, such as gemfibrozil, fenofibric acid or bezafibrate, are used to treat abnormal lipid levels in the blood. Gemfibrozil was the lipid regulator detected at the highest average concentration (16.9 µg/L) in treated wastewater samples from Spain (Gros et al., 2012) and Costa Rica (Ramírez-Morales et al., 2020). These works reported gemfibrozil levels 20-times lower than in our results, while bezafibrate and fenofibric acid were detected at concentrations one order of magnitude lower (between 1.5 and 2.0 µg/L). To the best of our knowledge, this is the first time that fenofibric acid has been found in agricultural irrigation water.

Atenolol, propranolol and bisoprolol are β-blockers that are usually used to treat hypertension or cardiac arrhythmias. Nowadays, they are found in treated water because of their persistence (Biel-Maeso et al., 2018; Gabet-Giraud et al., 2010; Gros et al., 2012; Marothu et al., 2019; Martínez-Piernas et al., 2019; Martínez Bueno et al., 2012; Picó et al., 2019). Picó et al. (2019) detected atenolol in treated wastewater used for crop irrigation in an area of Saudi Arabia at concentrations ranging from 0.1 to 0.9 µg/L. In another study carried out in southern Spain, atenolol and propranolol were found in irrigation water samples at mean concentrations of 1.1 µg/L and 0.1 µg/L, respectively (Biel-Maeso et al., 2018). In our study, atenolol was detected at higher levels than those found in the literature, at concentrations ranging from 5.9 to 23.8 µg/L.

Caffeine is the main stimulant used by humans due to the consumption of coffee and carbonated drinks. As indicated in the previous work by Martínez Bueno et al. (2012), caffeine is a drug that is removed very efficiency in WWTPs but its high consumption still leads to significant detection rates in the treated water. In this study, caffeine was detected at a detection frequency of 73 % and an average concentration level of 14.6 µg/L. Numerous studies have reported the presence of caffeine in treated water. Kosma et al. (2014) detected caffeine at concentrations between 0.05 and 4.1 µg/L. In another recent study carried out in Israel, Ben Mordechay et al. (2021) found caffeine in 71 % of the irrigation water samples analysed at concentration levels up to 3.9 µg/L.

Additionally, the irrigation water samples were subjected to chemical analysis where the metals, anions, physicochemical and agronomic parameters were evaluated. The physicochemical parameters evaluated were conductivity, water hardness, phosphorus, the degree of acidity or basicity and the dissolved solids, the values of which were 2510 µS/cm, 25.4 °F, 2.4 mg/L, 7.5 U. pH, and 1547 mg/L, respectively. In terms of anions, the chlorides, nitrates, and sulphates were analysed. The concentration values measured were 491.0 mg/L, <0.5 mg/L and 145.5 mg/L, respectively. A total of 9 metals were detected at levels above their respective LOQs in the irrigation water samples analysed. The mean concentrations were 93.4 mg/L for calcium, 5.0 mg/L for magnesium, 32.2 mg/L for potassium, 1.4 µg/L for arsenic, 1.3 µg/L for copper, 123.1 µg/L for iron, 25.2 µg/L for manganese, 19.3 µg/L for nickel, and 15.4 µg/L for zinc. Regarding the agronomic parameters, residual sodium carbonate, bicarbonates, carbonates, the sodium percentage, and the sodium and calcium ratio were analysed, the values of which were 4.4 meq/L, 579.4 mg/L, < 2.0 µg/L, 73.9 %, 313.9 mg/L and 0.25, respectively. The COD (chemical oxygen demand) and BOD (biochemical oxygen demand) were also evaluated. The COD is the amount of oxygen needed to oxidize organic matter by chemical means and convert it into carbon dioxide and water. The BOD is the amount of oxygen that microorganisms, especially bacteria, fungi, and plankton, consume during the degradation of organic substances. The COD and

BOD obtained from the samples were 32.4 mg O_2/L and 5.4 mg O_2/L, respectively.

3.3. Irrigated soil analysis

None of the agricultural soil samples analysed were free of CECs. A total of 24 CECs out of 293 were detected at concentrations above their LOQs in the samples from the soil irrigated with reclaimed water over a long period. All of them presented detection frequencies between 75 % and 100 %, except for the β-blocker propranolol and the diuretic hydrochlorothiazide (both 37 %), the pesticide carbendazim and the anti-inflammatory naproxen (both 25 %). Table 1 shows the detection frequencies, the average, and the ranges of concentration, as well as the average total load detected in the agricultural soil samples analysed. As one can see, the CEC concentrations in the reclaimed wastewater/irrigated soils ranged from the low μg/kg range to tens of μg/kg. Overall, 75 % of the CECs detected in the irrigated soil samples were pharmaceutical and personal care products (PPCPs) while the remaining 25 % were pesticides.

As can be observed in Fig. 2, analgesics, antibiotics, and antihistamines were again the groups quantified at the highest average total load in the samples under study (14.6, 13.7, 13.2 μg/kg, respectively). This finding highlights the stability of these organic contaminants in the irrigation water-soil continuum. Similar to the reclaimed water samples, the analgesic tramadol was the pharmaceutical detected at the highest concentrations. Its average total load was 14.6 μg/kg. Garduño-Jiménez et al. (2022) reported that tramadol can be retained in soils that have a high organic content and/or are acidic (pH < 6.5). The antihistamine cetirizine was the pharmaceutical product detected at the second highest average concentration level (13.2 μg/kg). Only two of the seven antibiotics studied were detected in the agricultural soil samples. Clarithromycin was found at an average total load of 12.7 μg/kg while trimethoprim was detected at 1.0 μg/kg. According to the scientific literature, data on clarithromycin, trimethoprim and cetirizine have not yet been reported in soils irrigated with treated water. The stimulant caffeine was also detected at high concentrations ranging from 0.8 to 4.9 μg/kg (an average of 2.1 μg/kg). The levels of caffeine found by Biel-Maeso et al. (2018) in soil irrigated with treated water were of the same order of magnitude as those found in this study (1.3 μg/kg). With regard to NSAIDs, naproxen was detected at a higher average concentration level than mefenamic, 5.5 μg/kg and 0.2 μg/kg, respectively. Nevertheless, naproxen was only detected in two samples at levels above its LOQ while mefenamic was found in all the samples analysed. Other relevant pharmaceuticals that also exhibited high detection frequencies and relatively high mean concentrations (above 1 μg/kg) were the antidepressant citalopram (3.7 μg/kg), the anticonvulsant carbamazepine (3.5 μg/kg), the antihypertensive telmisartan (2.9 μg/kg), the synthetic fragrance galaxolide (2.8 μg/kg), the anxiolytic venlafaxine (1.8 μg/kg), and the anxiolytic sulpiride (1.2 μg/kg). The anticonvulsants carbamazepine and lamotrigine, and the antidepressant agent venlafaxine were previously reported in a similar concentration range in soils irrigated with reclaimed wastewater (Ben Mordechay et al., 2021). Furthermore, the antidepressant citalopram was detected in 27 % of the irrigation water samples and in 100 % of the irrigated soil samples analysed. These results, along with those reported in previous scientific works, emphasize the persistency of these compounds in the agricultural environment (Martínez Bueno et al., 2022; Paz et al., 2016; Picó et al., 2019). Thus, the compounds presenting the highest accumulation rates were citalopram (86 %), clarithromycin (73 %), venlafaxine (66 %), carbamazepine (59 %) and galaxolide (55 %). This fact is related to the physicochemical properties of the molecules and the high sorption capacity of hydrophobic and non-ionic pharmaceuticals. All of them presented Log K_{ow} values > 3, except for carbamazepine, which presented a Log K_{ow} value of 2.4; this is in agreement with previous results published by our group (Martínez Bueno et al., 2021, 2022). Other compounds, such as the antibiotic ofloxacin, the anti-inflammatory ketoprofen and the β-blocker gemfibrozil were not detected in the irrigated soil samples analysed even though they were found at high concentrations (>10 μg/L) in all the irrigation water samples. This finding has been supported by other authors and is due to the relatively low stability of these types of compounds in the water distribution system (Ben Mordechay et al., 2021) or because of the low recovery percentage from the soil matrix (<25 % in the case of ofloxacin).

Regarding the pesticides, four fungicides (azoxystrobin, carbendazim, penconazole and thiabendazole) and two insecticides (imidacloprid and

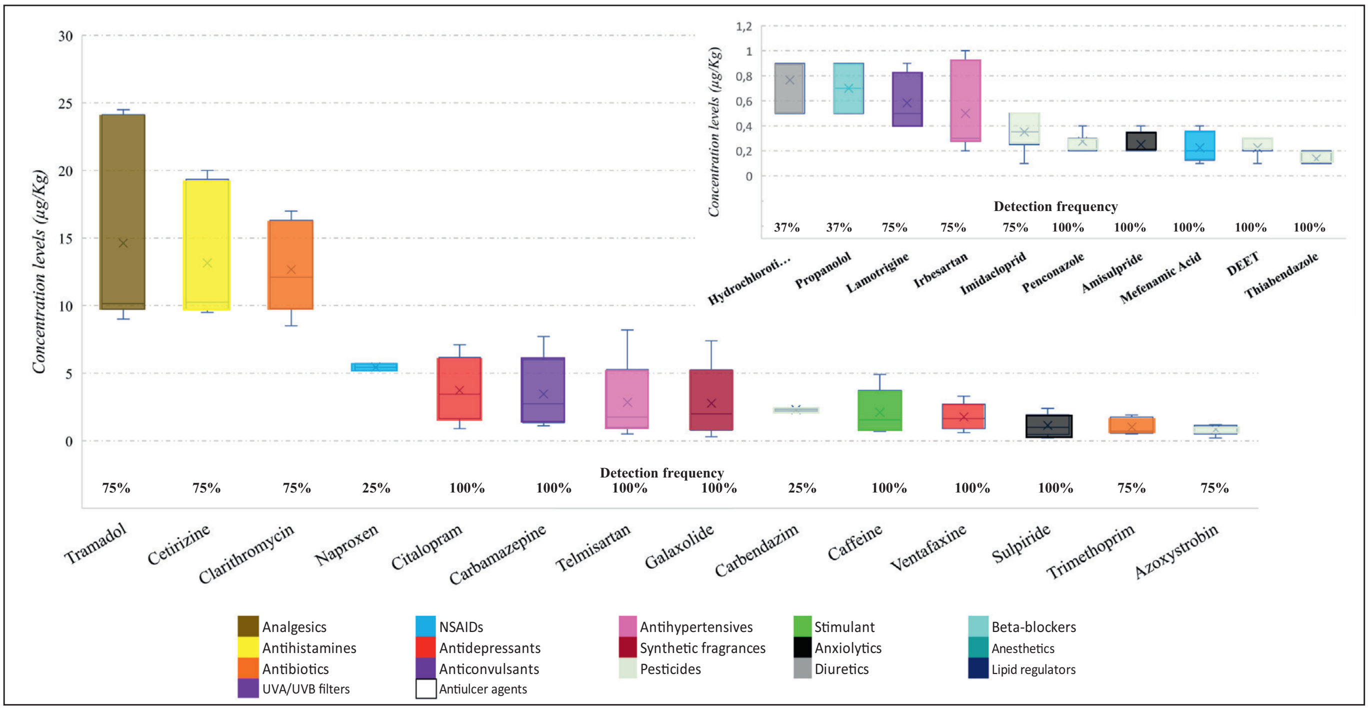

Fig. 2. CECs detected in the irrigated soil analysed. Data <LOQ are not presented. The median is presented as boxplots. Detection frequencies are listed for each compound. The vertical lines represent the maximum and minimum concentrations detected for each compound in the samples analysed.

DEET) were detected in the agricultural soil samples analysed. The average concentrations of the fungicides were 0.8, 2.2, 0.3 and 0.1 μg/kg, respectively. In contrast, the average total concentrations of the insecticides were 0.4 and 0.3 μg/kg, respectively. Picó et al. (2019) evaluated the accumulation of CECs in agricultural soil samples irrigated with treated water. The work reported levels of imidacloprid that were 250-times higher than our results (108 μg/kg) in cabbage soil samples. The fungicides penconazole and thiabendazole, and the insecticide DEET, were found in all the samples while carbendazim was only detected in one sample but at a higher concentration (2.2 μg/kg).

In summary, the concentration and fate of CECs in agricultural soils permanently irrigated with reclaimed water vary depending on the physicochemical properties of the CECs and the soil characteristics (Christou et al., 2019). In the Supplementary Material section (Table S4), additional information is given on the CEC concentration levels found in each soil sample irrigated with reclaimed water (μg/kg) according to the kind of crop. As can be observed, no significant differences were found in the CEC concentration levels detected in the analysed soil samples within the same crop. However, due to the differences in the soil characteristics, some small variations were found in the CEC concentrations between different crops. The highest average total load of CECs was detected in the cherry tomato soil samples (a mean of 101.2 μg/kg), followed by pepper (a mean of 47.6 μg/kg), cocktail tomato (a mean of 47.3 μg/kg), and cucumber soils (a mean of 43 μg/kg). The zucchini soil samples presented the lowest total load of CECs (a mean of 23.4 μg/kg).

3.4. Irrigated vegetable analysis

Of the 293 CECs selected in this study, only three pharmaceutical products were detected in the irrigated vegetable samples analysed over the study period at concentration levels above their LOQ (no pesticide was detected in those samples). These compounds were carbamazepine, lidocaine, and caffeine, with a detection frequency of 100 %. All were found in the irrigation water samples, whereas only carbamazepine and caffeine were detected in the irrigated soils. The carbamazepine and caffeine concentration levels quantified in the vegetable samples were as much as 5-times lower than in the soil samples. Table 2 shows the CEC concentration levels detected in each vegetable. The average CEC concentrations found in the fruits were 0.9 μg/kg for caffeine, 0.6 μg/kg for carbamazepine and 0.2 μg/kg for lidocaine. Two factors might explain the uptake of these compounds into the plant — the Log Kow and pKa. Compounds with Log Kow values lower than 2.5 have lower hydrophobicity and higher solubility in water. Therefore, these types of CECs can be taken up by the plant instead of accumulating in the soil (Christou et al., 2019; Martínez Bueno et al., 2022). Such was the case for the anaesthetic lidocaine, which was detected in 100 % of the irrigation water and vegetable samples analysed but not in the irrigated soil samples, thus indicating its translocation from the roots to the fruit. Furthermore, the plant uptake of carbamazepine and caffeine might be explained by the pKa of these compounds. Carbamazepine (pKa 3.8) and caffeine (pKa 10.4) are neutral compounds in the irrigation water (at pH 7.8). Therefore, they can cross the cell membranes, enter through the roots, and translocate to different parts of the plant by transpiration. Previous publications have reported that caffeine and carbamazepine are taken up in the edible parts of the plant (Beltrán et al., 2020; Ben Mordechay et al., 2022; González García et al., 2019; Gworek et al., 2021; Hyland et al., 2015; Martínez Bueno et al., 2022; Picó et al., 2019; Wu et al., 2014). As can be seen in Table 2, caffeine was detected at higher concentration levels than carbamazepine and lidocaine in all the commercial produce, except in the case of zucchini. In this crop, the anticonvulsant carbamazepine was the compound detected at the highest concentrations (a mean of 1.6 μg/kg). As in the irrigated soil samples, no significant differences were found in the CEC concentration levels detected within the same crop over the study period. Only small variations were found between vegetable types due to the differences in the matrix and the soil characteristics. The highest average total load of CECs was found in the zucchini samples (a mean of 2.9 μg/kg), followed by cherry tomato (a mean of 2.0 μg/kg) and cucumber (a mean of 1.9 μg/kg). The pepper and cocktail tomato samples presented the lowest total CEC load (a mean of 0.9 μg/kg). Our results are in line with previously reported works in the literature. For example, Martínez-Piernas et al. (2019) quantified caffeine and carbamazepine in tomato fruit at average concentrations of 0.3–1.0 μg/kg and 0.01–0.2 μg/kg, respectively, at concentrations similar to our results. On the other hand, Picó et al. (2019) reported caffeine at concentrations ranging from 48 to 125 μg/kg in crops irrigated with treated water under real-world environmental conditions. However, Picó et al. (2019) did not detect carbamazepine in the fruit even though it was found in the irrigation water and the soil. Regarding the anaesthetic lidocaine, this was found in all the vegetable samples analysed at concentrations ranging from 0.1 to 0.6 μg/kg. To the best of our knowledge, this is the first time that lidocaine has been detected in crops irrigated with treated water under real agricultural conditions.

Finally, Fig. 3 summarizes the average total load of CECs detected in the irrigation water samples (μg/L), the irrigated soil samples and the vegetable samples analysed (μg/L) as well as the accumulation rates in the soil and crops. The accumulation rates were calculated from the average total concentrations of CECs measured in the irrigation water samples against the concentrations found in the irrigated soils and vegetables. The results reveal that the accumulation rates were 31 % in the soils and only 1 % in the vegetables that were permanently irrigated with reclaimed water.

3.5. Human exposure

The different plants irrigated with reclaimed water took up contaminants into their edible parts (zucchini, cucumber, tomato and pepper). Table 3 presents the bioconcentration factor (BCF) values and the per capita daily exposure data on the three CECs detected in the irrigated vegetables. Using the average concentration of CECs in the edible part of the plant (μg/kg) and the average concentration in the irrigation water samples (μg/L), the BCF values were calculated for the three CECs detected in each vegetable. The BCF values ranged from 0.1 to 1.4 L/kg. The average BCF values found in this study were 0.1 L/kg for caffeine, 0.4 L/kg for carbamazepine and 0.6 L/kg for lidocaine. Of all the CECs detected, the anaesthetic lidocaine had the greatest tendency to accumulate in the edible part of the plant in all the crops studied. In all cases, the BCF values were below 1 L/kg, except for carbamazepine and lidocaine in the zucchini samples.

Table 2
Detection frequency (%), concentration range (μg/kg), average concentration (μg/kg), and total load (μg/kg) of the CECs detected in the analysed vegetable samples.

	Cucumber 12/21	Pepper 01/22	Tomato-cocktail 04/22	Tomato-cocktail 05/22	Tomato-cherry 04/22	Tomato-cherry 05/22	Zucchini 04/22	Zucchini 05/22	Detection frequency (%)	Concentration range (μg/kg)	Average concentration (μg/kg)
Anticonvulsants											
Carbamazepine	0.5	0.3	0.2	0.3	0.2	0.3	1.7	1.5	100	0.2–1.7	0.6
Anesthetics											
Lidocaine	0.1	0.2	0.1	0.1	0.1	0.1	0.6	0.5	100	0.1–0.6	0.2
Stimulant											
Caffeine	1.3	0.4	0.6	0.5	1.7	1.6	1.0	0.4	100	0.4–1.7	0.9
Total load (μg/kg)	1.9	1.0	0.9	0.9	2.0	2.0	3.3	2.4			

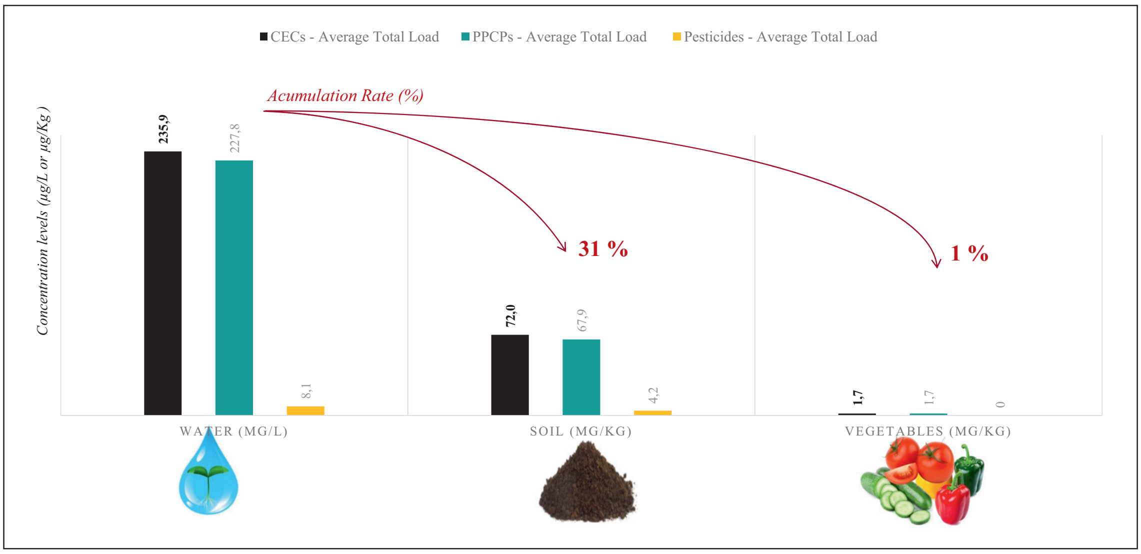

Fig. 3. Impact of reclaimed water reuse on an agroecosystem. Average total load of CECs detected in the irrigation water samples (μg/L), irrigated soil samples, and vegetable samples analysed (μg/L). Accumulation rate of CECs in the irrigated soils and vegetables.

These were found at levels of 1.1 L/kg and 1.4 L/kg, respectively, indicating that these CECs accumulate in the edible part of this plant.

According to various publications, consuming vegetables that contain contaminants, especially PPCPs, can pose a risk to human health. However, in all the previously published papers, the estimated levels were several orders of magnitude below the maximum permitted for daily human intake (Beltrán et al., 2020; González García et al., 2019; Hyland et al., 2015; Martínez Bueno et al., 2022; Wu et al., 2014). As can be seen in Table 3, in a conventional diet, the highest daily human exposure came from consuming irrigated cocktail tomatoes, which contained a total contaminant load of 0.073 μg/day (caffeine 0.06 μg/day, carbamazepine 0.009 μg/day, and lidocaine 0.004 μg/day). In contrast, cucumbers irrigated with reclaimed water were the commercial produce showing the lowest daily human exposure, with a total contaminant load of 0.011 μg/day. These values could be as much as 3-times higher in a vegetarian diet than in a conventional diet (0.219 μg/day for cocktail tomatoes and 0.032 μg/day for cucumbers). The total daily CEC exposure values ranged from 0.09 to 0.13 μg/day and from 0.26 to 0.38 μg/day in a conventional and vegetarian diet, respectively.

No regulation exists regarding the concentration levels of PPCPs in food. Regardless, all of them presented concentrations lower than the levels established by the latest regulation — (EC) No 155/2021, concerning the maximum residue limits (MRLs) for most of the pesticides found in food (10 μg/kg) (European Commission, 2021). In any case, the levels found in the commercial vegetables were as much as 3 orders of magnitude lower than the typical medical dose of these pharmaceutical products.

According to the World Health Organization (1987), the acceptable daily intake (ADI) is the maximum amount of a residue that can be ingested daily over a lifetime without posing an appreciable health risk. The ADI values for caffeine, carbamazepine, and lidocaine are 1.0×10^6, 1.2×10^6 and 1.4×10^6 μg/day, respectively (https://www.vademecum.es). In all cases, the daily human intake was as much as 5 orders of magnitude lower than the ADI, both in the conventional diet and the vegetarian diet, meaning that none of them pose a risk to human health. The obtained

Table 3
Estimated per capita daily exposure values to the three CECs detected (taken up) in the irrigated vegetables.

	Compound	Water	Vegetable		Daily human intake (μg/day)	
		Average concentration (μg/L)	Average concentration (μg/kg)	BCF (L/kg)	Conventional diet	Vegetarian diet
Cucumber	Caffeine	14.6	1.3	0.1	0.007	0.022
	CBZ	1.4	0.5	0.3	0.003	0.008
	Lidocaine	0.4	0.1	0.3	0.001	0.002
Pepper	Caffeine	14.6	0.4	0.1	0.006	0.018
	CBZ	1.4	0.3	0.2	0.004	0.012
	Lidocaine	0.4	0.2	0.5	0.003	0.008
Tomato-cocktail	Caffeine	14.6	0.6	0.1	0.020	0.060
	CBZ	1.4	0.3	0.2	0.009	0.027
	Lidocaine	0.4	0.1	0.3	0.004	0.011
Tomato-cherry	Caffeine	14.6	1.7	0.1	0.060	0.180
	CBZ	1.4	0.3	0.2	0.009	0.027
	Lidocaine	0.4	0.1	0.3	0.004	0.011
Zucchini	Caffeine	14.6	0.7	0.1	0.008	0.023
	CBZ	1.4	1.6	1.1	0.018	0.053
	Lidocaine	0.4	0.6	1.4	0.006	0.018
				Total	0.09[a]–0.13[b]	0.26[a]–0.38[b]

CBZ: carbamazepine; BCF: bioconcentration factor; Consumption data: 13.3 kg/person/year to tomato, 4.8 kg/person/year to pepper, 4 kg/person/year to zucchini and 2 kg/person/year to cucumber in a conventional diet (https://www.statista.com/statistics/745474/fresh-vegetables-consumption-per-person-in-spain-2015-by-product/#statisticContainer). Daily human exposure to a conventional diet (in the case of vegetarian diet it was established 3 times more).

[a] Total load (μg/day) considering the intake of zucchini, cucumber, pepper and cocktail tomato.

[b] Total load (μg/day) considering the intake of zucchini, cucumber, pepper and cherry tomato.

results suggest that an adult would have to consume around 100,000 kg of irrigated vegetables a day to reach the intake limit.

4. Conclusion

One of the three macrolide antibiotics included in the Commission Implementing Decision (EU) 2018/840 (clarithromycin) and two of the pesticides (diuron and terbutryn) included in the list of priority substances covered by the Water Framework Directive (Directive 2013/39/EU) were detected in the irrigation water samples and irrigated soil samples. However, none of them were detected in the vegetables permanently irrigated with that water. Carbamazepine and caffeine were the only compounds detected across the entire water-soil-plant continuum. The anaesthetic lidocaine was detected in all the irrigation water samples and vegetables samples analysed, but not in the irrigated soil samples. This finding emphasizes the great potential of this CEC to translocate through the plant.

The results obtained in this work support the reuse of water for agricultural irrigation since the concentration levels of the CECs detected in all the commercial produce analysed were very low compared to their therapeutic doses. Thus, their intake does not pose a risk to human health. This enables a circular economy to be established, improving confidence among consumers towards agricultural products grown with this type of water, and especially those produced in greenhouses, as these are very important to the EU market. Nonetheless, the study highlights the importance of carrying out a long-term control strategy on agricultural soil that is permanently irrigated with reclaimed water to avoid high accumulation rates among certain organic contaminants that could migrate over further crop seasons. The compounds that presented soil accumulation rates above 50 % were citalopram, clarithromycin, venlafaxine, carbamazepine and galaxolide. Undertaking specific soil cleaning treatments, such as washing and/or solarization processes, between crop rotations could be a useful agronomic strategy for improving and extending the reuse of the soil.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare no conflict of interest. This is an independent research. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Appendix A. Supplementary data

A detailed description of the physicochemical properties of all the pesticides selected in this study (Table S1) shows the optimal mass spectrometric parameters for each target compound using LC-MS/MS (Table S2). Detailed information on the validation data (Table S3) and on the CECs concentration levels in the irrigated soil samples for each crop (Table S4) are presented in this section. Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.160462.

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CONCLUSIONES

CONCLUSIONES

La primera conclusión general que se puede extraer de la presente Tesis Doctoral es que los métodos analíticos desarrollados han proporcionado excelentes resultados en términos de sensibilidad, repetitividad, recuperaciones y efecto matriz para el análisis de un elevado número de contaminantes de forma simultánea, rápida y fiable, en muestras vegetales y ambientales.

Por otro lado, se pueden considerar las siguientes conclusiones más concretas relacionadas con los tres capítulos en los que está dividida la Tesis Doctoral.

Capítulo I:

- La metodología desarrollada ha permitido el análisis de 30 contaminantes orgánicos con diferentes propiedades físico-químicas en muestras vegetales y ambientales utilizando un sistema de cromatografía de líquidos acoplado a espectrometría de masas en tándem con analizador triple cuadrupolo.
- El método optimizado y validado se aplicó a muestras reales procedentes de la finca experimental UAL-ANECOOP. La mayor tasa de acumulación se detectó en las muestras de suelo analizadas (14%), seguido por el fruto (3%), las hojas (2%) y las raíces (1%).
- En los tejidos de la planta, no se detectaron contaminantes como el ofloxacino, ciprofloxacino, acetaminofén y gemfibrozilo, por su limitado potencial para ser absorbidos/acumulados por la planta.
- Con los resultados obtenidos en este estudio se confirma, que propiedades fisicoquímicas de los contaminantes orgánicos (principalmente el pKa, logKow y logKoc) desempeñan un papel crucial en la absorción y translocación a través de las plantas. Los compuestos hidrofílicos tienden a translocarse desde las raíces a las hojas, acabando en el fruto, mientras que los contaminantes hidrofóbicos son retenidos en el suelo y la raíz.
- En cuanto al riesgo humano derivado del consumo de tomates regados de forma continua con aguas regeneradas contaminadas, se demuestra en base a los datos obtenidos que un adulto necesitaría consumir cientos de kilogramos de tomates contaminados al día para superar el límite de ingesta de riesgo sobre la salud.

Capítulo II:

- Se ha desarrollado un método analítico para el análisis cuantitativo de 30 residuos de plaguicidas y productos farmacéuticos con una amplia variedad de estructuras y propiedades físico-químicas en una matriz compleja como es el suelo agrícola. El método se basa en la utilización de un sistema LC-MS/MS, con analizador triple cuadrupolo en modo scheduled MRM.
- El método QuEchERS desarrollado ha proporcionado las mejores recuperaciones (entre 29%-99%), seguido por el método de extracción UAE (12%-101%). Contaminantes con una alta solubilidad en agua, moderada acidez y baja polaridad

solo se extrajeron ligeramente desde la matriz (ej. ciprofloxacino u ofloxacino). Con el método QuEChERS modificado desarrollado se consiguió extraer el 80% de los contaminantes estudiados con recuperaciones entre 70-99%.

- El método de extracción desarrollado y validado reduce/elimina las interferencias analíticas mejorando la sensibilidad del método, llegando a ser una herramienta muy útil para la determinación simultanea de una amplia gama de contaminantes orgánicos.
- El método validado fue aplicado a muestras reales de la finca experimental UAL-ANECOOP, donde se tomaron muestras de suelo perteneciente a cultivos regados con agua tratada. Se alcanzaron tasas de acumulación en el sistema agrícola suelo-planta-fruto entre el 2%-62% para los pesticidas, mientras que para los productos farmacéuticos fue entre 1% y 26%.
- Con los resultados obtenidos en este trabajo en condiciones agronómicas controladas en invernadero se ha podido comprobar la importancia de realizar análisis del suelo agrícola regado permanentemente con agua regenerada. Esta evaluación prevendrá el proceso de acumulación a largo plazo de estas sustancias en el suelo agrícola y evitará una alta acumulación de contaminantes orgánicos que podrían migrar/translocarse durante las siguientes campañas agrícolas.

Capítulo III:

- Se desarrolló y validó una nueva metodología para el análisis de 293 contaminantes orgánicos de origen emergente en muestras vegetales y ambientales, obteniéndose excelentes resultados en términos de sensibilidad, recuperación, efecto matriz, repetitividad, reproducibilidad o límites de cuantificación. El método se aplicó a muestras reales procedentes de invernaderos gestionados por agricultores de la Comunidad de Regantes de Las Cuatro Vegas de Almería (CGUAL).
- En las muestras de agua de riego regeneradas fueron detectados 44 contaminantes químicos de los 293 optimizados en el método analítico. Tramadol, gemfibrozilo, cafeína, atenolol o cetirizina se detectaron a niveles más elevados en dichas aguas (11-44 µg/L).
- Se alcanzó un porcentaje de acumulación en el suelo del 33% en comparación a la media detectada en el agua de riego, mientras que en las muestras vegetales fue tan solo del 1%, donde únicamente se detectaron carbamazepina, lidocaína y cafeína.
- La técnica LC-MS/MS utilizada para el análisis de compuestos “objetivo”, ha proporcionado información analítica de alta calidad tanto desde el punto de vista cualitativo como cuantitativo.
- Los resultados obtenidos en este trabajo apoyan la reutilización del agua para riego agrícola ya que los niveles de concentración de los productos farmacéuticos detectados en todos los productos comerciales analizados fueron muy bajos a niveles de 2 µg/kg. Por tanto, se puede concluir que su ingesta no supone un riesgo para la salud humana.

- Finalmente, se propone como una posible estrategia agronómica útil para mejorar y prolongar la reutilización del suelo, la realización de tratamientos específicos de limpieza del suelo, como puede ser el lavado y/o solarización entre rotaciones de cultivos

CONCLUSIONS

The first general conclusion that can be drawn from this Doctoral Thesis is that the analytical methods developed provide excellent results in terms of sensitivity, repeatability, recoveries, and matrix effect for the analysis of CECs in plant and environmental samples.

On the other hand, more specific conclusions related to the three chapters presented in this Thesis can be considered.

Chapter I:

- The methodology developed has allowed the analysis of 30 organic contaminants with different physicochemical properties in plant and environmental samples using a liquid chromatography system coupled to tandem mass spectrometry with a triple quadrupole analyzer.
- The optimized and validated method was applied to real samples from the UAL-ANECOOP experimental farm. The highest accumulation rate was detected in the soil samples analyzed (14%), followed by the fruit (3%), leaves (2%) and roots (1%). Between 40 and 50% more emerging contaminants were found in the edible part of the plant than in the non-edible part.
- Contaminants such as ofloxacin, ciprofloxacin, acetaminophen and gemfibrozil were not detected in plant tissues, possibly because of their limited potential to be absorbed/accumulated by the plant.
- The results obtained in this study confirm that the physicochemical properties of organic contaminants (mainly pKa, logKow and logKoc) play a crucial role in their uptake and translocation through plants. Hydrophilic compounds tend to translocate from the roots to the leaves, ending up in the fruit, while hydrophobic contaminants are retained in the soil and root.
- Regarding the human risk derived from the consumption of tomatoes permanently irrigated with contaminated reclaimed water, it is demonstrated on the basis of the data obtained that an adult would need to consume hundreds of kilograms of contaminated tomatoes per day to exceed the limit of intake that a person of 70 kg can consume daily throughout life without appreciating a health risk.

Chapter II:

- An analytical method has been developed for the quantitative analysis of 30 pesticide and pharmaceutical residues with a wide variety of structures and physicochemical properties in a complex matrix such as agricultural soil. The method is based on the use of a LC-MS/MS system, with a triple quadrupole analyzer in scheduled MRM mode.
- The QuEchERS method developed has provided the best recoveries (between 29%-99%), followed by the UAE extraction method (12%-101%). Contaminants with high water solubility, moderate acidity and low polarity were only slightly

extracted from the matrix (e.g. ciprofloxacin or ofloxacin). With the modified QuEChERS method developed, 80% of the contaminants studied were extracted with recoveries between 70-99%.

- The extraction method developed and validated has reduced/eliminated interferences improving the sensitivity of the method, becoming a very useful tool for the simultaneous determination of a wide range of organic contaminants with different physicochemical properties.
- The validated method was applied to real samples from the UAL-ANECOOP experimental farm, where soil samples were taken from crops irrigated with treated water. Accumulation rates in the soil-plant-fruit agricultural system were achieved between 2%-62% for pesticides, while for pharmaceuticals it was between 1% and 26%.
- With the results obtained in this work under controlled agronomic conditions in a greenhouse, it has been possible to prove the importance of carrying out analyses of agricultural soil permanently irrigated with reclaimed water to evaluate the long-term accumulation process of these substances in agricultural soil in order to avoid a high accumulation of organic pollutants that could migrate/translocate during the next agricultural campaigns.

Chapter III:

- A new methodology was developed and validated for the analysis of 293 organic contaminants of emerging origin in plant and environmental samples, obtaining excellent results in terms of sensitivity, recovery, matrix effect, repeatability, reproducibility, or quantification limits. The method was applied to real samples from greenhouses managed by farmers of the Comunidad de Regantes de Las Cuatro Vegas de Almería (CGUAL).
- In the reclaimed irrigation water samples, 44 chemical contaminants were detected out of the 293 optimized in the analytical method. Tramadol, gemfibrozil, caffeine, atenolol, or cetirizine were detected at higher levels in these waters (11-44 µg/L).
- An accumulation rate of 33% was reached in the soil, while in the plant samples it was only 1%, where only carbamazepine, lidocaine and caffeine were detected.
- The LC-MS/MS technique used for the analysis of "target" compounds has provided high quality analytical information both qualitatively and quantitatively.
- The results obtained in this work support the reuse of water for agricultural irrigation since the concentration levels of the pharmaceuticals detected in all the commercial products analyzed were very low compared to their therapeutic doses. Therefore, it can be concluded that their ingestion does not pose a risk to human health.
- Finally, specific soil cleaning treatments, such as washing and/or solarization between crop rotations, are proposed as a possible agronomic strategy to improve and prolong soil reuse.

ANEXO

ANEXO I. ÍNDICE DE ACRÓNIMOS

AOAC	Asociación de Químicos Analíticos Oficiales
APCI	Ionización química a presión atmosférica
API	Ionización a presión atmosférica
CNA	Normas de calidad ambiental
DG-SANTE	Dirección General de Salud y Seguridad Alimentaria
d-SPE	Extracción en fase sólida dispersiva
EC	Economía circular
EDAR	Estación Depuradora de Aguas Residuales
EI	Ionización Electrónica
ESI	Ionización por Electrospray
GC-HRMS	Cromatografía de Gases acoplada a espectrometría de masas de alta resolución
GC-MS/MS	Cromatografía de gases acoplada a espectrometría de masas en tándem
IP	Puntos de Identificación
IUPAC	Unión Internacional de Química Pura y Aplicada
Ka	Constante de disociación
Kow	Coeficiente de partición de octanol-agua
LC-HRMS	Cromatografía de líquidos acoplada a espectrometría de masas de alta resolución
LC-MS/MS	Cromatografía de líquidos acoplada a espectrometría de masas en tándem
LC-UV	Cromatografía de líquidos acoplada a ultravioleta
LLE	Extracción líquido-líquido
LOQ	Límite de cuantificación
MAE	Extracción Asistida por Microondas
ME	Efecto Matriz

MRM	Método Multirresiduo
MS/MS	Espectrometría de masas en tándem
NI	Ionización en modo Negativo
pH	Medida de acidez o alcalinidad
PI	Ionización en modo Positivo
pKa	Logaritmo negativo de la constante de disociación de un ácido débil
PLE	Extracción con Líquido Presurizado
Q	Analizador de Cuadrupolo sencillo
QqQ	Analizador triple Cuadrupolo
QuEChERS	Quick, Easy, Cheap, Effective, Rugged & Safe (Met. Extracción)
RSD	Desviación Estándar Relativa
SLE	Extracción Sólido-Líquido
SPE	Extracción en Fase Sólida
SPLE	Extracción en Fase Sólida dispersiva
SPME	Micro-extracción en Fase Sólida
SIM	Monitoreo del ión seleccionado
SRM	Seguimiento de la Reacción Seleccionada
TOF	Analizador de tiempo de vuelo
US EPA	Agencia de Protección Ambiental de los Estados Unidos
UAE	Extracción Asistida por Ultrasonidos

ANEXO II. PUBLICACIONES CIENTÍFICAS NO INCLUIDAS EN LA TESIS

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2. Cutillas, V., García-Valverde, M., Gómez-Ramos, M. del M., Díaz-Galiano, F.J., Ferrer, C., Fernández-Alba, A.R., 2020. Supercritical fluid chromatography separation of chiral pesticides: Unique capabilities to study cyhalothrin and metalaxyl as examples. J. Chromatogr. A 1620. https://doi.org/10.1016/j.chroma.2020.461007

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4. Molina-Aiz, F.D., Valera, D.L., López, A., Moreno, M.A., Marı, P., Garcıá-Valverde, M., 2020. Effects of the increase of ventilation surface area on the microclimate and yield of a tomato crop in Mediterranean greenhouses. Acta Hortic. 1296, 177–184. https://doi.org/10.17660/ActaHortic.2020.1296.23

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