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Determinación de la eficiencia de degradación de un sistema de biopurificación de plaguicidas mediante análisis multirresidual

Tesis presentada al Tribunal Examinador del Programa de Maestría Académica en Manejo de Recursos Naturales de la Escuela de Ciencias Exactas y Naturales para optar por el grado de *Magister Scientiae* con énfasis en gestión ambiental

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Dedicatoria

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INDICE

Determinación de la eficiencia de degradación de un sistema de biopurificación de plaguicidas mediante análisis multirresidual

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RESUMEN GENERAL

Los sistemas de biopurificación (SBP) son una herramienta que ayuda a tener un mejor manejo de los residuos que se generan durante la aplicación de productos químicos en el campo, siendo por lo tanto una forma de mitigación de la contaminación puntual en el ambiente. Uno de los componentes de un SBP es la biomezcla. En este estudio se trabajó con una biomezcla optimizada compuesta por fibra de coco, compost y suelo. Con la biomezcla preparada, se realizaron cuatro experimentos correlacionados entre sí. Primero se optimizó y validó una metodología analítica para la determinación de residuos de 43 moléculas de plaguicidas con triazinas (10), triazoles (13) y organofosforados (20), en una biomezcla por la técnica de LC-MS/MS. Se verificó el cumplimiento de los parámetros de mérito como selectividad, especificidad, límite de detección, límite de cuantificación, precisión, veracidad, efecto matriz, robustez, ámbito lineal y ámbito de trabajo. Se obtuvieron resultados satisfactorios para 41 de ellas. Ciromazina y diclorvós fueron las dos moléculas que presentaron un mayor número de incumplimientos de los criterios de aceptación de los parámetros de mérito. Después de validar la metodología de análisis, se evaluó el efecto de degradación de la biomezcla para cada grupo de plaguicidas en estudio, con concentraciones iniciales entre 4 y 8 mg/kg, por un periodo de 128 días, con condiciones de humedad y temperatura controlada. Se observó una eliminación para las triazinas y organosoforados de 59.3% y 68.5% respectivamente; los triazoles no presentaron una eliminación significativa. Simultáneamente se realizaron dos experimentos de degradación en dos ~~SPB~~ SBP a escala piloto. El primer sistema trabajó con aguas residuales de laboratorio, con una mezcla de residuos de plaguicidas (38) que contenía triazinas, triazoles, organosforados y carbamatos, entre otros, con concentraciones iniciales variadas (0.0036-0,25 mg/kg en biomezcla), por un periodo de 281 días. El sistema eliminó organofosforados (90%), triazoles (73.4%), carbamatos (71.3%) y triazinas (54.3%) en diferentes porcentajes. En este sistema se

observó una desintoxicación completa de la biomezcla según los exámenes ecotoxicológicos con *Daphnia magna* y de forma parcial para los que se hicieron con la germinación de *Lactuca sativa*. En el otro SBP se evaluó la degradación de 8 moléculas de residuos de formulaciones comerciales de plaguicidas, con concentraciones iniciales entre 3.9 – 51.1 mg/kg por un periodo de 53 días. En este sistema se registró la misma tendencia de los ejercicios de degradación con una degradación rápida de triazinas y organofosofrados y una degradación lenta para los triazoles. Estos resultados demuestran que la metodología de trabajo cumple con los criterios de aceptación para las moléculas de estudio y que la biomezcla es efectiva para la eliminación de triazinas y organofosforados, no tanto así para los triazoles. Se recomienda el análisis de las moléculas de forma separada por los grupos de familia química, para obtener mejores resultados de degradación, complementando cada análisis con los estudios ecotoxicológicos.

ABSTRACT

Biopurification systems (BPS) are a tool that helps to have a better management of the residues that are generated during the application of chemical products in the field, being therefore a way of mitigating the point pollution in the environment. One of the components of BPS is biomixture. In this study we worked with an optimized biomixture composed of coconut fiber, compost and soil. With the prepared biomixture, four experiments were correlated with each other. First, an analytical methodology was optimized and validated for the determination of 43 pesticide residue molecules, triazines (10), triazoles (13) and organophosphates (20), in a biomixture by the LC-MS/MS technique. Compliance with the merit parameters was verified such as selectivity, specificity, limit of detection, limit of quantification, precision, trueness, matrix effect, robustness, linear scope and scope of work. Satisfactory results were obtained for 41 of them. Ciromazine and dichlorvos were the two molecules that presented a greater number of breaches of the criteria of acceptance of the merit parameters. After the analysis methodology validation, the degradation effect of the biomixture for each study group of pesticides was evaluated with initial concentrations between 4 and 8 mg/kg, for a period of 128 days, with controlled conditions of humidity and temperature. An elimination was observed for triazines and organophosphates of 59.3% and 68.5% respectively; the triazoles did not present a significant elimination. Simultaneously, two degradation experiments were conducted in two BPSs at pilot scale. The first system worked with laboratory wastewater, with a mixture of pesticide residues (38) containing triazines, triazoles, organosphosphates and carbamates, among others, with varying initial concentrations (0.0036-

0.25 mg/kg in biomixture), for a period of 281 days. The system eliminated organophosphates (90%), triazoles (73.4%), carbamates (71.3%) and triazines (54.3%) in different percentages. In this system, a complete detoxification of the biomixture was observed according to the ecotoxicological tests with *Daphnia magna* and partially for those that were made with the germination of *Lactuca sativa*. In the other BPS, the degradation of 8 residue molecules of commercial pesticide formulations was evaluated, with initial concentrations between 3.9 - 51.1 mg/kg for a period of 53 days. In this system the same trend of degradation exercises was recorded with a rapid degradation of triazines and organophosphonates and a slow degradation for triazoles. These results demonstrate that the working methodology meets the acceptance criteria for the study molecules and the biomixture is effective for the elimination of triazines and organophosphates, not so much for the triazoles. The analysis of the molecules is recommended separately by chemical family group to obtain better degradation results and it is necessary to complement each analysis with ecotoxicological studies.

INTRODUCCIÓN GENERAL

Los estudios en la agricultura han permitido el desarrollo de cultivos más extensos, con productos de mejor calidad, tanto de la planta como del fruto (Adou et. al., 2001). Como parte del avance en la agricultura, surgieron agentes biológicos, sustancias o mezclas de sustancias, de naturaleza química sintética o biológica que se destinaron a combatir, controlar, prevenir, atenuar, atraer, repeler o regular la acción de diferentes plagas. Todos estos compuestos son conocidos de forma general como plaguicidas. (Bernal et. al., 1995; Esteve, 2007; Ferrer, 2003; Huete-Soto et al., 2017).

La agroindustrialización y la necesidad de tener mayor control sobre las plagas que afectan los cultivos, favoreció la producción de una mayor cantidad de plaguicidas y como consecuencia un incremento en el consumo de estos en los campos agrícolas (Ramírez & Lacasaña, 2001). El aumento en la fabricación de nuevos formulados de plaguicidas y el uso irracional, ha causado efectos indeseables en el ser humano y sobre el medio ambiente. Estos efectos son variables ya que dependen de diferentes niveles de toxicidad, tipo de plaguicida, vías de contacto y tiempo de exposición, afectando también a otras especies (Kim et al., 2017; Ramírez & Lacasaña, 2001; Van der Werf, 1996).

La intoxicación en seres humanos por lo general se da por una entrada al organismo a través cutánea, vías respiratorias o por ingesta alimentaria, presentando en los casos agudos síntomas de descomposición o hasta la muerte, además de la aparición en enfermedades graves como cáncer, leucemia y enfermedades hepáticas. (Esteve, 2007; Kim et al., 2017; Van der Werf, 1996).

En Costa Rica se han reportado más de 1500 casos de intoxicación por plaguicidas desde el año 2002 siendo la mayoría clasificados como accidentes laborales. Las zonas del país con mayor incidencia fueron las regiones Brunca, Huetar Atlántico y Pacífico Central, regiones cuya actividad económica predominante es de carácter agrícola (MS, 2013; OPS, 2003).

La presencia de plaguicidas en el ambiente es preocupante debido a su estabilidad, persistencia y toxicidad. El mal manejo de los plaguicidas puede causar impacto en aves, animales acuáticos, suelo, insectos polinizadores y la calidad de producción de las granjas (Masiá et al., 2015; Reichenberger, S. et al., 2007; Ying-Dong, 2006). Las zonas agrícolas son las que presentan mayor contacto con los plaguicidas, ya que se aplican de forma aérea o terrestre, con lo cual se identifican fuentes de contaminación difusas o puntuales (Castillo el al., 2008; De Wilde et al., 2007; Ramírez & Lacasaña, 2001).

En la contaminación difusa, el contacto puede deberse a factores climatológicos como lluvia, viento, temperatura, y por medio procesos y propiedades físicas y químicas de los plaguicidas como solubilidad, fotodegradación y volatilización (Castillo et al., 2008; De Wilde et al., 2007; Ramírez & Lacasaña, 2001; Reichenberger et al., 2007). Estos factores se observan en eventos como lixiviación, escorrentía, erosión, deriva, filtración del agua con arrastre de contaminantes, lo cual implica que este tipo de contaminación es difícil de controlar y de determinar la fuente (Castillo et al., 2008; De Wilde et al., 2007; Ramírez & Lacasaña, 2001; Reichenberger et al., 2007).

La contaminación puntual permite identificar las fuentes de contaminación, ya sea por la forma de preparación del formulado de aplicación, fugas de los equipos de aplicación, indumentaria de los encargados del manejo de los plaguicidas, descarte inadecuado de residuos de aplicación, lugares inapropiados de lavados de equipo o mal almacenaje de los productos (Castillo et al., 2008; De Wilde et al., 2007; Reichenberger et al., 2007). Al ser fuentes puntuales, es factible ejercer un control sobre el residuo contaminante y por lo tanto minimizar su impacto. Existen varios procesos que se utilizan para tratar este tipo de contaminación: procesos de quemado, deshidratación, degradación con agentes químicos, fotocatalíticos o de tipo biológico; sin embargo, la mayoría son costosos (De Wilde et al., 2007).

Otro tipo de tratamiento de muy bajo costo, es la biorremediación, en donde los microorganismos o sus enzimas degradan el desecho orgánico a un estado inocuo o a concentraciones menores (De Wilde et al., 2007). Dentro de estos sistemas destacan los sistemas de biopurificación como el biofiltro, el Phytobac® y el biobed, los cuales se comportan de forma similar ya que poseen una matriz biológicamente activa que retiene al contaminante en la materia orgánica y se da una rápida degradación de este por la acción microbiana, siendo el biobed, quizás, el sistema de mitigación más atractivo por su bajo costo y buen funcionamiento (Antoniuos, 2012; Castillo et al., 2008; Chin-Pampillo et al., 2015a; De Wilde et al., 2007; Karanasios et al. 2012; Vischetti, Capri, Trevisan, Casucci & Perucci, 2004).

El biobed es un sistema de mitigación efectivo, diseñado para el descarte de aguas residuales agrícolas contaminadas con plaguicidas. En la biomezcla, centro biológicamente activo del sistema de biopurificación (SBP) y conformado por suelo de la zona, turba o compost y un material lignocelulósico, se da la eliminación de los plaguicidas, teóricamente con vidas medias más cortas que en el ambiente (Antoniuos, 2012; Castillo et al., 2008; Tortella et al., 2013). Esto hace que el SBP actúe como una estrategia de biorremediación de aguas residuales que permite contener los efectos adversos de fuentes de contaminación puntual con plaguicidas en la industria agrícola. (Antoniuos, 2012; Castillo et al., 2008; Tortella et al., 2013).

En países de Europa como Suecia, Francia, Reino Unido, Italia, Bélgica, Grecia y Dinamarca, se ha implementado el uso de este sistema, con la característica de la utilización de diferentes tipos de materiales locales, propios de la región como componentes de la biomezcla (Antoniuos, 2012; Castillo et al., 2008; Karanasios et al., 2012, Tortella et al., 2013). En Latinoamérica, países como Perú, Ecuador, Guatemala, El Salvador y Chile han trabajado con este tipo de sistemas, pero con la características de tener climas e ingredientes diferentes a los europeos (Castillo et al., 2008).

En Costa Rica también se han investigado los sistemas de biopurificación. Chin-Pampillo y colaboradores (2015a) adaptaron varias biomezclas hechas con componentes locales como suelo de la zona, compost o turba y como material lignoceluloso utilizó residuos de madera, fibra de coco, bagazo de caña de azúcar, papel y granza de arroz, para la degradación de carbofurán y dos de sus metabolitos, 3-cetocarbofurán y 3-hidroxicarbofurán, plaguicidas de la familia de los carbamatos. El mejor resultado obtenido fue para la mezcla de suelo, compost y fibra de coco con resultados de vida media de 2,7 días.

También se han hecho investigaciones sobre comportamiento del carbofurán en una biomezcla compuesta por granza de arroz, compost o turba y suelo preexpuesto y bioaumentada con el hongo ligninolítico, *Trametes versicolor* (Madrigal-Zúñiga et al., 2016), con resultados de vida media de 8,1 días, menor al resultado obtenido por Chin y colaboradores (2015a) de 16,8 días. Rodríguez-Rodríguez y colaboradores (2017) también evaluaron la biomezcla de granza de arroz, bioaumentada con el hongo, para la degradación de aldicarb, metomil y metiocarb, con resultados de degradación de 99% en tiempos inferiores a los 10 días para aldicarb y metomil. En un estudio reciente se trabajó la biomezcla compuesta por fibra de coco, compost y suelo de forma bioamuentada con el mismo hongo, para degradar triazinas y organofosoforados (Lizano-Fallas, Masís-Mora, Espinoza-Villalobos, Lizano-Brenes & Rodríguez-Rodríguez, 2017).

La técnica de cromatografía líquida de alta resolución acoplada a espectrometría de masas es útil en la determinación de residuos de plaguicidas porque es adecuada para trabajar con moléculas polares, con alto peso molecular, que sean termolábiles y que no requieran una derivatización o la incorporación de un compuesto que le brinde características diferentes necesarias para su detección, además de proporcionar resultados con una gran sensibilidad, especificidad, precisión y exactitud (Quintela et al., 2005). Esta técnica de análisis ha sido ampliamente utilizada para la determinación de plaguicidas y múltiples familias químicas en metodologías denominadas como multirresiduales (Alder, Greulich, Kempe & Vieth, 2006; García-Reyes, Molina-Díaz & Fernández-Alba, 2007; Lehotay et al., 2005).

Debido a que la metodología multirresidual debe ser adaptada a las condiciones de diferentes laboratorios, es necesario realizar la validación de la metodología a utilizar con las variaciones respectivas. La validación se realiza para asegurar que los resultados reportados sean confiables y el método es el indicado para la medición de los analitos deseados. Los parámetros de mérito o de desempeño a evaluar son límite de detección y cuantificación, exactitud, precisión, sensibilidad (de calibración) y robustez (Fajgelj & Ambrus, 2000; Miller & Miller, 2004). Esto permite considerar si una metodología analítica es aceptable para un propósito, puesto que debe ser mantenida bajo control estadístico en todo momento (Fajgelj & Ambrus, 2000).

En este trabajo se evaluará la degradación de 43 plaguicidas en forma residual aplicada sobre la biomezcla propuesta por Chin-Pampillo et al., (2015b), cumpliendo las etapas de validación de la metodología de extracción multirresidual y el análisis de los estas moléculas en el SBP. La técnica de detección será por cromatografía de líquidos acoplada a un espectrómetro de masas tipo tándem. Esto permitirá generar una metodología analítica apropiada para estimar el ámbito de aplicación de la biomezcla frente a diferentes plaguicidas.

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CAPITULO I

Validation of a methodology by LC-MS/MS for the determination of triazine, triazole and organophosphorus pesticide residues in biomixtures

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RESUMEN

Los sistemas de biopurificación (SBP) ayudan en el manejo de los residuos de plaguicidas y brindan la opción de depositar los desechos de los equipos utilizados en el campo para aplicar los plaguicidas. El análisis de los residuos en la biomezcla del SBP es necesario para identificar si se produce una degradación de las moléculas con resultados confiables. En este estudio, la metodología de extracción se optimizó y validó en una biomezcla compuesta de fibra de coco, compost y suelo, para determinar un total de 43 moléculas, distribuidas entre triazinas, triazoles y organofosforados mediante la técnica de cromatografía líquida acoplada a un espectrómetro de masas de triple cuadrupolo (LC-MS/MS). Para la validación, se evaluaron los parámetros de linealidad, efecto matriz, LOD, LOQ, especificidad, selectividad, precisión, veracidad y robustez en la biomezcla propuesta. Una vez que se validó la metodología, se evaluó la degradación de 8 moléculas en un SBP con el mismo tipo de biomezcla utilizada para la validación.

Palabras clave: sistemas de biopurificación, plaguicidas, metodología de validación, biomezcla, LC-MS/MS

ABSTRACT

Biopurification systems (BPS) are useful in the management of pesticide residues and provide an option to dispose wastewaters of agricultural origin derived from pesticide application practices. The analysis of pesticide residues in the BPS biomixture is necessary to determine whether the removal of the target compounds occurs with reliable results. In this study the extraction

methodology was optimized and validated in a biomixture composed of coconut fiber, compost and soil, to determine a total of 43 molecules, distributed among triazines, triazoles and organophosphates by means of the liquid chromatography coupled technique to a triple quadrupole mass spectrometer (LC-MS/MS). For the validation, the parameters of linearity, matrix effect, LOD, LOQ, specificity, selectivity, precision, trueness and robustness in the proposed biomixture were evaluated. Once the extraction methodology was validated, the removal of eight molecules was assayed in a BPS employed for the simultaneous treatment of a mixture of pesticide comercial formulations.
Keywords: biopurification system; pesticides; validation methodology, biomixture, LC-MS/MS

Highlights

- Multiresidue method was developed for pesticides in biopurification system (BPS)
- Extraction methodology optimized for the analysis of 43 pesticides in a biomixture
- Forty-one molecules meet the acceptance criteria for validation parameters
- The removal of several pesticides (>80%) after 53 days was estimated in the BPS

1. Introduction

The advances in agricultural science (e.g., improved soil and water management practices and the use of agrochemicals, organic fertilizers, biological control and pesticides) allowed the enhancement of food production. Although the use of pesticides was initially intended to minimize the effect of pests on crops and enhance its productivity; its application may also cause undesirable effects on human health and the environment as they inevitably reach non–target organisms [1–4].

The presence of pesticides in the environment is worrisome because of their toxicity and persistence [2,3]. The effects on humans and other organisms are compound-specific and vary depending on toxicity, route and time of exposure [2,3]. In the environment, aerial fumigations, superficial runoff, and infiltrations to groundwater are some common causes of contamination by pesticides [5–12]. Implementing good management practices and adequate treatment systems can mitigate point-source pollution by pesticides, hence, reducing its environmental impact. For instance, in situ treatment can reduce pesticides residues remaining in knapsack sprayers. However, this requires treatment systems that are accessible to farmers and easy to operate [6,9].

Bioremediation is regarded as a feasible treatment of wastewaters containing high loads of pesticides [13–16].

Particularly, biopurification systems (BPS) stand out for their low cost and maintenance, easy construction and versatility. BPS include biofilters, Phytobac® and biobeds [6,8–10,12,17,18]. These configurations use a biological active matrix that retains the contaminants and stimulate the rapid degradation of the compounds by microbial activity [6,7,9,10,18,19]. The biological matrix is a biomixture that comprises soil, compost or peat and lignocellulosic material in a 1:1:2 volume ratio [6,7,9]. Soil is the main source of degrading microorganisms; thus, it is desirable to use soils pre-exposed to the study target pesticides. Compost or peat is added for enhancing the absorption capacity, helping to control the temperature and humidity of the system. The lignocellulosic material is a lignin rich source for microorganisms, which favors the growth and activity of ligninolytic fungi, known for its wide capacity to degrade organic pollutants, in this respect, the use different components such as straw, bagasse, coconut fiber, citrus peel, branches, olive leaves, wood curl, paper, rice pellets, among others [6,7,9,10,16,20], has been applied.

BPS are effective degrading carbamates [21–23], organophosphorus [24–27] and triazines [4,26,28–31]. This suggests that BPS can degrade molecules with different action modes. Also, pesticides containing such molecules are not applied simultaneously, but rather in cycles in each crop. Therefore, pesticides active ingredients and their co-formulated materials can be treated in the BPS throughout the season as they are used in the crops [10,32]. Thus, the objectives of this study were i) to develop and validate a LC-MS/MS multiresidue methodology for the determination of more than 40 pesticides in a conventional biomixture, and ii) to evaluate the efficiency of a BPS during the treatment of wastewater containing commercial formulations of diverse pesticides, applying the validated methodology for the biomixture matrix...

2. Materials and methods

2.1 Chemicals and reagents

The analytical standards anilophos (98.9 %), azinphos-methyl (98.8 %), cadusafos (97.2 %), chlorpyrifos (99.5 %), dichlorvos (98.4 %), dimethoate (99.5 %), edifenphos (98.5 %), ethoprophos (98.8 %), fenamiphos (99.0 %), phoxim (99.4 %), heptenophos (98.6 %), isazofos (99.2 %), isofenphos (99.5 %), malathion (99.5 %), methamidophos (99.5 %), monocrotophos (99.5 %), pirimiphos-methyl (99.5 %), cyromazine (99.5 %), prometon (99.5 %), prometryn (99.5 %), simetryn (99.5 %), terbutryn (98.1 %), bitertanol (99.5 %), cyproconazole (99.5 %), epoxiconazole (99.5 %), fenbuconazole (99.5 %), flusilazole (98.6 %), hexaconazole (99.3 %),

myclobutanil (98.0 %), tebuconazole (98.0 %), triadimefon (99.5 %), triadimenol (98.7 %) were purchased from ChemService (Penssylvania, U.S.). Standards acephate (99.0 %), coumaphos (99.0 %), fenthion (99.0 %), triazophos (80.0 %), amethryn (98.0 %), atrazine (99.0 %), cyanazine (98.5 %), simazine (98.0 %), terbuthylazine (98.5 %), difenoconazole (98.7 %), paclobutrazole (98.5 %), propiconazole (99.0 %), carbofuran-d_3 (98.0 %) and linuron-d_6 (98.5 %) were acquired from Dr. Ehrenstorfer (Augsburgo, Germany). Commercial formulation of atrazine (Atranex®, 90 % w/w), ametryn (Agromart®, 50 % w/v), chlorpyrifos (Solver ™ 48 % w/v), diazinon (Zinoncoop 60 EC, 60 % w/v), malathion (Bioquim malation, 5 % w/w), tebuconazole/triadimenol (Silvacur® Combi 30 EC, 22.5 % and 7.5 % w/v respectively) and terbutryn (Terbutrex®, 50 % w/v) were acquired at local markets.

Distilled and deionized (DDI) water (<18 mΩ) was produced in the laboratory, formic acid (ACS, ISO, Reag. Ph Eur, 98-100 %), glacial acetic acid (ACS, ISO, Reag. Ph Eur 100 %), acetonitrile (LichroSolve®, >99.8%) and methanol (LichroSolve®, 99.8%) were purchased from Merck (Darmstadt, Germany). Magnesium sulfate ($MgSO_4$) anhydrous (> 99.5 %) and sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$, > 99.5 %) were obtained from Sigma-Aldrich (St Louis, MO, U.S.), bondesil-PSA (40 µm particle size) from Agilent (Santa Barbara, CA, U.S.), Sepra-C18 from Phenomenex (Torrance, CA, U.S.) and sodium chloride from JT Baker (PA, U.S.).

2.2 Analytical solutions

Stock solutions of individual analytes ranging from 700 to 3800 mg/Lwere prepared, depending on their solubility, in methanol or acetonitrile. Primary dilution standards (PDS) at 10 mg/L were prepared using acidified acetonitrile (0.1 % formic acid). Calibration standards from 1 to 500 µg/L were prepared in a mixture (1:1 v/v) of acidified acetonitrile-water acidified with 0.1 % formic acid, 1:1 v/v, and in the matrix extract (matrix-matched standards). The stock solutions were stored at -15 °C in a freezer while the PDS and the calibration standards at < 6 °C.

2.3 Samples and sample preparation

The biomixture employed consisted of coconut fiber, compost and soil (45:12:43). This biomixture was previously optimized for the removal of carbofuran [21]. The biomixture samples were fortified with the target pesticides during the optimization and validation of the method. Pesticides were extracted following a QuEChERS procedure described elsewhere [21]. Carbofuran-d_3 and linuron-d_6 were used as a surrogate and as an internal standard. Quality controls included blank samples (pesticide-free biomixture), blanks for calibration curve (pesticide-

free biomixture without surrogate or internal standards; extract used for the calibration curve in the matrix) and solvent reference (procedure reagents without sample).

2.4 Chromatographic conditions

LC-MS/MS analyses were carried out using an Agilent 1290 Infinity II LC System (Santa Clara, CA, U.S.) Ultra-high performance liquid chromatography (UHPLC) coupled to an Agilent 6460 triple quadrupole mass spectrometer. Chromatographic separation was done at 40 °C injecting 6 µL of the sample (2 µL loop) in a Poroshell 120 EC-C18 column (100 mm x 2.1 mm i.d., particle size 2.7 µm) and using a binary mobile phase consisting of acidified water (formic acid 0.1 % v/v, solvent A) and acidified methanol (formic acid 0.1 % v/v, solvent B) at a flow rate of 0.3 mL/min. The conditions were as follows: 30 % of solvent B for 3 min, 15 min linear gradient to 100 % solvent B, 4 min at 100 % solvent B, 0.1 min gradient back to 30 % of solvent B, and 5 min at initial conditions. The mass spectrometer used a jet stream (electrospray) ionization source operating at gas temperature 300 °C; gas flow 7 L/min, nebulizer 45 psi; sheath gas temperature 250 °C; sheath gas flow 11 L/min. The other conditions were capillary voltage 3500 V; nozzle voltage 500 V; heater MS1 and MS2 100 °C. Data acquisition was performed using the MassHunter software (Santa Clara, CA, U.S.).

2.5 Optimization of the transitions for each molecule

Each molecule was injected individually into the LC-MS/MS system to optimize the fragmentor voltage and the collision cell energy for all the transitions. The optimization was done in five acquisition modes including i) MS2 Scan to find the precursor ions (in positive and negative electrospray ionization), ii) Product Ion to find the optimized fragmentor voltage (ranging from 50 to 210 V) and the main fragments, iii) multiple reaction monitoring (MRM) for optimizing the collision cell energies (range of 1 to 45 V) of each fragment, all those methods were done without column; iv) MRM with column to find the retention time, and v) dynamic MRM (dMRM) to define a specific acquisition time range, this acquisition method was applied with the chromatographic gradient conditions..

2.6 Optimization of the analytical method

A 2^3 full-factorial design was used to study the effects of stirring (manual vs. automated), amount of water added to the sample (5 mL vs. 10 mL) and the amount of magnesium sulfate added for cleaning up (450 mg vs. 900 mg) on the extraction process. Each experiment was performed in duplicates. The recovery for each evaluated condition was the measured response.

2.7 Method validation

The validation of the method was conducted following guidelines of the European Commission – Directorate General for Health and Food Safety [33]. Analytical parameters evaluated included linearity, limit of quantification (LOQ), limit of detection (LOD), matrix effects, trueness, precision, robustness, scope of work, specificity and selectivity. Every sample was analyzed applying the methodology described in 2.3. The linearity of the calibration curves was evaluated with analytical standard solutions at ten concentration levels (5, 10, 20, 50, 90, 135, 170, 200, 250 and 500 µg/L). The standards were prepared in blank biomixture extract and in acidified (0.1 % formic acid) water-acetonitrile (1:1). Each calibration curve was prepared in triplicate. LOD and LOQ were estimated on a signal to noise (S/N) ratio > 3 and S/N >10, respectively. LOQ was the lowest spike level with good criteria of trueness and precision. For LOD seven blank samples were spiked at 2 µg/kg and 5 µg/kg. LOQ was tested with the same number of spike samples at 10 µg/kg.

Matrix effects were calculated as the correlation percentage between the slopes of the solvent calibration curve and the calibration curves prepared with blank biomixture extract.

Trueness and precision were evaluated spiking the blank biomixture at four different concentrations (10, 50, 150 and 350 µg/kg), with seven replicates for each spiked level (n = 7) and three analysts, for a total of 21 samples. Trueness and precision were determined as recovery and relative standard deviation percentages (RSD), respectively.

The robustness was measured applying Youden-Steiner test [34]. It was applied at two effect levels of seven factors or conditions. To apply the Youden test, seven changing factors in two conditions were employed (Table 1). This study was developed according to the experiment guide in Table 2. Capital letters indicate the experiment was applied with the values of the condition "HIGH", and the lower-case letters with the value of the condition "low".

The robustness was calculated by comparing the difference of the values of each factor, according to the experiment, in relation to the value calculated as a critical value, which depends on the total standard deviation of the experiment. To evaluate selectivity and specificity, five blank samples and five spikes samples at 25 µg/kg were prepared, then, the signals derived from both kinds of samples were compared to differentiate between signals provided by the matrix and the analyte, respectively.

The scope of work was calculated at 2000 µg/kg to evaluate the method efficiency to achieve good results at high concentrations.

2.8 Determination of pesticide removal in a functional BPS

Biomixture samples from a functional BPS applied in the field were collected to test the performance of the method at environmental relevant concentrations. The final volume of the BPS was 104 L. The biomixture was placed in a plastic container and allowed to stand for one week (BPS). A pesticide solution containing commercial formulations of ametryn, atrazine, chlorpyrifos, diazinon, malathion, tebuconazole, terbutryn and triadimenol was incorporated and mixed with the biomixture. Samples were collected at 0, 9, 14, 21, 28, 38, 47 and 53 d after pesticide disposal. Sampling of the biomixture from the BPS was performed with a basin and a shovel; composite samples of 200 g were collected combining small portions from the upper, intermediate and lower part of the biomixture. At least 100 g of the biomixture was kept in custody and stored at -20 °C. The remaining biomixture was reincorporated into the BPS. When possible, removal data for each compound was modeled according to a first order model (SigmaPlot 14.0) to estimate removal half–life (DT_{50}) values.

3. Results and discussion

3.1 Validation of the methodology to determine pesticide residues in biomixture samples

3.1.1 Optimization of pesticide molecules

The physicochemical properties of each compound were used to decide how the optimization experiment should be developed (Table S1) and to define the ionization mode, precursor ion and the solubility of the pesticide in the organic solvent of the methodology. Then, the fragmentor voltages and the collision cell energies were optimized for the precursor and the product ions for each molecule. The optimization results are shown in Table 3.

Each compound was first injected individually without the analytical column for identifying the best working conditions. MS2 Scan acquisition mode was conducted to identify the precursor ion of each molecule at positive or negative electrospray ionization mode (ESI^+ or ESI^-). All tested molecules showed better results in ESI^+ and worked with the protonated molecules $[MH]^+$. The use of adducts of sodium, potassium or ammonium was avoided, as they produce a lower sensibility in the next optimization steps [35] (Figure 1).

Then, the main fragments of the precursor ion were determined in Product Ion acquisition mode. Likewise, the fragmentor voltage that gives a signal with a greater intensity was selected. At least two product ions were selected for each molecule (Figure 1).

A third acquisition mode, MRM without column, was used to ensure the product ions or main fragments were selected at the maximum voltage signal (Figure 1). Finally, the MRM mode

with column was applied with the chromatographic column conditions and a proposed mobile phase gradient, at optimized values for the individual injection of each molecule. Thus, the retention time of each molecule and the evaluation of the transitions, as well as possible interference signals for other molecules were obtained. Table 3 shows the optimized values for all molecules.

3.1.2 Method optimization

The matrix of study was a biomixture, a sample with high organic and low water content (< 30 %). Pesticide extraction from this type of matrix with low water content was previously evaluated with QuEChERS methodologies [4,7,21,22,26,36].

A 2^3 full-factorial design was used to determine the method with best recovery for more molecules in the same experiment. Three critical factors of the methodology were evaluated: the amount of water added to 5 g of the biomixture (5 mL vs. 10 mL), the type of agitation employed during extraction (automated vs. manual), and the amount of magnesium sulfate added for sample drying in the cleaning stage (450 mg vs. 900 mg). The experimental conditions are shown in Table S2 (Supplementary Material). The examination of the effects of the main factors and the interactions between factors revealed that the individual factors produce the greatest inference on the analysis methodology.

The first factor evaluated was the amount of water added to the matrix (Figure 2); the recoveries exhibited differences of up to 5 % for triazines and triazoles and up to 19 % for organophosphates. The addition of 10 mL of water enhanced the extraction recovery rates of 3-5 % out of 37 molecules, particularly organophosphates molecules. This effect was attributed to the time of hydration of the matrix during the extraction, since it allows the opening of the pores of the matrix and that there is subsequently a better extraction of the molecules by the organic solvents [37,38].

Recoveries for cyromazine, cyproconazole, acephate and methamidophos decreased as the volume of water was increased. This is attributed to the high solubility in water of these molecules, which causes a greater affinity to the aqueous phase and subsequent losses by the addition of sodium sulfate to dry the sample. However, other molecules with higher water solubility like monocrotophos did not exhibit this effect.

When comparing the agitation types (Figure 2) (either with a programmable automated agitation equipment vs. manual agitation), variability between both methodologies was below 2 % for most of the pesticides. This finding implies that manual agitation can be used for this type of

samples, as usually employed for the traditional QuEChERS methodology applied to vegetable matrices [39–41]. The application of manual agitation in each extraction stage produced similar results to the use of agitation for 30 min at 2500 rpm. Cyproconazole was the only molecule that showed an improvement of 15 % when working with the programmable agitator. Considering the availability of the programmable shaker, that allows the simultaneous processing of several samples, the use of programmable agitation was selected for the purpose of the proposed method.

The third factor of study was the amount of magnesium sulfate used in the cleaning stage (Figure 2). Magnesium sulfate is added to remove the excess of water in the samples, since the proposed methodology requires a subsequent step of concentration to dryness. Also, it reduces the number of co-extracts due to the decrease in polarity in the extraction acetonitrile phase [39]. The addition of magnesium sulfate did not show significant differences in the groups of triazines and organophosphates; however, an increase in the recoveries between 1-5 % was observed when using the 900 mg of magnesium sulfate. Yet, most of the triazoles were favored when using 900 mg of magnesium sulfate. This is expected as this is the group with the lowest water solubility reported. Based on this finding, 900 mg of magnesium sulfate were used for improving the recovery of most of the studied molecules.

3.2 Parameters for the methodology validation

After optimizing the aforementioned factors, the methodology proposed employed 5 g of sample and the addition of 10 mL of DDI water, 15 mL of acetonitrile acidified (1% v/v acetic acid), 6 g of magnesium sulfate, 1 g of sodium chloride and 2.6 g of sodium acetate trihydrate followed by shaking for 30 min, 2500 rpm, and centrifuge (4000 rpm, 10 ºC, 7 min). After centrifugation, an aliquot of 3 mL of the extract was placed in a tube with 900 mg of magnesium sulfate, 150 mg of PSA and cleaned with 75 mg of C18. The sample was then stirred and centrifuged again at the same conditions of the first time. A sample of 1.5 mL of the supernatant was gently dried with a nitrogen stream and finally reconstituted to 1.5 mL with acidified (0.1 % v/v formic acid) water-acetonitrile mixture (1:1).

The subsequent validation of the method included selectivity, specificity, precision, intermediate precision, LOD, LOQ, trueness, linearity, scope of work, matrix effect and robustness.

3.2.1 Selectivity and specificity

Selectivity is the ability of the method to discriminate between the analyte of interest and other molecules present in the matrix. While specificity is the ability to obtain a negative result, when the samples do not have the analyte [33]. By coupling the care to choose the solvents and

reagents of the analytical method for the extraction, to the properties of the LC-MS/MS technique to identify compounds according the optimization of their mass, the results will be most of the time selective. The retention times, transitions and fragmentation and collision cell voltages of individual molecules were optimized (Table 3). Then, a working solution containing the 43 analytes, carbofuran-d_3 and linuron-d_6 was injected. Also, the following 53 additional molecules oxamyl, carbendazim, carbendazim-d_4, amitraz, methomyl, thiamethoxam, thiabendazole, imidacloprid, picloram, imazapyr, pirimicarb, 3-hydroxycarbofuran, acetamiprid, cymoxanil, imazapic, aldicarb, 3-ketocarbofuran, monuron, metribuzin, bromacil, propoxur, hexazinone, carbofuran, thiophanate- methyl, pyrimethanil, bentazone, metsulfuron-methyl, carbaryl, imazalil, metalaxyl, isoproturon, diuron, thiophanate, linuron, azinphos-methyl, azoxystrobin, propanil, methiocarb, molinate, dimethomorph, myclobutanil, fenarimol, prochloraz, fipronil, kresoxim-methyl, haloxyfop, pyraclostrobin, triflumuron, buprofezin, haloxyfop-p-methyl, fluazifop-p-butyl, teflubenzuron and pendimethalin were added to the previous mixture at a concentration of 200 µg/kg each. None of these additional 53 molecules (excluded from the validation) showed interference signals on the optimized triazines, triazoles and organophosphorus transitions, thus demonstrating the method is selective.

The specificity of the method was studied with five blank samples and five spiked samples; their comparison confirmed the absence of false positives for the studied molecules, thus suggesting it is a specific method. In the case of ametryn, atrazine, cyromazine, cyproconazole, tebuconazole, triadimenol, chlorpyrifos and fenamiphos, slight signals were identified in their respective transitions, which implies a slight interference for subsequent detections. The detected signals showed S/N ratios < 3 (LOD criteria). The S/N ratios of the spiked samples were higher to the LOQ and the LOD, which does not affect their selectivity when applying the methodology (Table S3).

3.2.2 LOD and LOQ

Since the method is intended for biomixtures used for treating pesticides, the expected concentrations in this matrix are quite high, in the order of more than 10 mg/kg (particularly in the moment of the disposal of pesticide-containing wastewater). The LOD and LOQ were determined based on the detector response that achieved S/N ratios > 3 and >10, respectively. Each concentration value was evaluated to ensure that low concentrations (in the order of µg/kg) were detected.

The LOQ is identified as the lowest level of spike sample with acceptable recovery and precision; in some cases, it can be equaled to the maximum limit of residues (MRL); however, there is no MRL for this type of matrix. The criteria to accept the LOQ was RSD ≤ 20 %. The same protocol was applied for the LOD but at a lower spiking concentration (5 µg/kg).The acceptance criterion was the detection of the two transitions and the ion response ratios from the sample and the average of the calibration standards lower than ± 30 % [33]. The results for these experiments are presents in Table 4.

Cyromazine was the only molecule in the LOQ study that presented a RSD greater than 20 %, which is justified as it is a basic ionic molecule, that may be adsorbed to soil due to known sorption of the triazines to the humic groups of soil [42]; however, during the extraction process, the sample reaches an approximate pH value of 4.5, due to the acetate buffer that was made in situ [42,43], which favors the extraction of the other triazines, but not the cyromazine that has been shown to require a greater amount of acid for better extraction [44]. Some organophosphates such as cadusafos, ethoprophos, fenthion and malathion had an RSD value close to 15 %. For these molecules, the greater variability at lower concentrations is not due to pH or p*K*a, but rather to the hydrogen bonds that are formed between pesticides and ionic compounds in the soil [42].

When analyzing the LOD, many of the studied molecules showed RSD values greater than 20 %, which did not guarantee good variability at low concentrations (< 5 µg/kg). For 42 of the molecules evaluated, the presence of both transitions was observed at concentration of 5 µg/kg and had a good RSD (< 20 %), which is why it complies with LOD calculations [45–47]. Only acephate had a different concentration for the LOD (25 µg/kg). In general, the triazines exhibited the lowest RSD in the LOD calculation; overall, among the studied groups, they were most sensitive to the method [48]. A greater variability was observed for organophosphates and triazoles at lower concentrations.

3.2.3 Precision as repeatability and intermediate precision

After detecting the concentrations of 5 µg/kg and 10 µg/kg for the LOD and LOQ, respectively, it was necessary to work at three higher concentrations (50, 150 and 350 µg/kg) to establish the precision of the methodology. The critical criterion was an RSD < 20 %. The precision as repeatability was determined with the results of one of the analysts (n = 7); intermediate precision was determined with three analysts who performed the methodology at different days (n = 21). The results are shown in Table 4.

After the evaluation of 43 molecules, cyromazine and dichlorvos showed the greatest dispersion data along the three concentration levels of intermediate precision parameter (> 40 %

and > 25 %, respectively). The precision as repeatability and intermediate precision had an RSD higher than 20 %, which exceeds the acceptance criterion of SANTE. The results for these molecules suggest the need to consider the behavior of the other parameters to determine whether the methodology is appropriate for their analysis. Thus, it is considered that the multiresidue method proposed is not precise for these molecules, or in the worst-case scenario, it would be better to test other methodologies for these molecules.

The remaining 41 molecules exhibited higher coefficients of variation at low concentrations, which decreased at higher concentrations. All organophosphates but dichlorvos presented values results that met the acceptance criteria. From the remaining 19 molecules, chlorpyrifos showed the highest RSD value. Acephate and methamidophos exhibited a different behavior, as their RSD increased with the concentration. The polarity of both molecules and the possibility to have greater hydrogen bonding between the molecules and the biomixture, may favor their greatest data dispersion; nonetheless, the dispersion was not greater than that allowed by the validation criterion in this case. For the case of cadusafos no precision was considered at low concentration (RSD = 21.77 % at 50 µg/kg). All the triazoles presented acceptable values of standard deviation, as the other nine triazines.

The RSD values were in general higher for intermediate precision than repeatability. This is expected because as data executed by three different analysts on different days was employed, which consequently gives a greater variability to the method.

3.2.4 Trueness

The trueness of the method is applied as a validation parameter in the absence of an interlaboratory test for the biomixture matrix. The trueness is understood as the average recovery of the concentration levels evaluated with a recovery percentage between 70-120 % [33]. The trueness of the method was determined using a low evaluated concentration, that is, the recovery value of 50 µg/kg.

When comparing these results with those obtained in Table 4, it was observed that dichlorvos (47.1 %) and cyromazine (49.4 %) presented results below the acceptance criteria (70-120 %). For acephate and methamidophos, the recoveries were quite good compared to other methodologies applied to fruits, vegetables, meats and soils, since they showed valuesof 92 % and 78 % respectively [49–52]. The organophosphates anilophos (90 %) and heptenophos (89 %) also exhibited relatively low recoveries, thus, these molecules should be carefully revised in a control chart to verify that this average is maintained. The recovery values obtained for the triazoles were within the acceptance criterion.

More than 80 % of the molecules showed recoveries > 100 %, a finding that can be ascribed to the ionization technique (electrospray system) applied in the LC-MS/MS, which had the effect of signal improvement; this behavior is classified by some authors as a cause of matrix effect [53–55].

3.2.5 Linearity

Three independent (not consecutive) calibration curves were prepared with 10 concentration levels each in the blank extract sample, including concentrations from the LOQ value (10 µg/kg) to 1000 µg/kg. Three acceptance criteria were considered: i) correlation coefficient greater than 0.99; ii) the percentage of residuals for each level must be less than 20 %; and iii) the slope ratio between the three calibration curves should be higher than 80 %. Table 5 shows the results of the calibration curves of the study molecules.

Every single molecule yielded acceptable results for each criterion. However, it is important to consider that, for acephate, azinphos methyl, coumaphos, cyromazine, dichlorvos, fenamiphos, fenbuconazole, methamidophos, and pirimiphos methyl, one of the work levels reached residual values close to or greater than 10 %, which implies that care must be taken in estimating the curve, as this could lead to an increase of LOQ if lower levels need to be eliminated to assure linearity range.

3.2.6 Matrix effect

The matrix effect (ME) is the comparison between the response of the calibration levels in organic solvent and the matrix [33,56–59]. The acceptance criterion was the ME (%) = $((slope_{matrix}/slope_{solvent})-1) \times 100$, lower than 20 %. The curve that was prepared in the matrix presented the same characteristics as the final extract (a phase of water-acetonitrile (1:1), acidified with formic acid 0.1%). The results are shown in Table 5.

Cyromazine (17 %), methamidophos (9.2 %) and phoxim (7.6 %) presented the greatest matrix effect; throughout the validation these molecules have shown the least favorable performance in the multiresidue methodology. The other molecules evaluated in the methodology presented ME less than 4 %. However, none of the molecules failed to meet the acceptance criteria of ME < 20 %.

The fact that no considerable matrix effect was observed by the biomixture matrix was due to the change in solvent, since there was a change in polarity of the sample and this caused a decrease in the amount of co-extracts before injection [53]. This finding was also supported by the direct observation of less particles remaining in the sample container and the filter used.

The decrease in co-extracts favors the formation of ions [56,57,59], since it reduces the ionic suppression effect normally achieved in the ionization technique by electrospray (which decreases the signal), compared to the chemical ionization technique at atmospheric pressure (which increase the signal) [53–55,59]. This factor avoids the production of high concentration of other compounds, which may increase the surface tension, the viscosity of the droplets in the nebulizer, and the proton affinity between the analytes and the co-extracts [54].

3.2.7 Scope of work

When an unknown sample is processed, there is a risk that the concentration of the analyte surpasses that of the highest level in the calibration curve; this implies that the sample must be diluted so that the analyte concentration remains within the validated parameters. In order to corroborate the method properly extracts and detects higher concentrations than those validated with satisfactory recovery, a two part experiment was performed. For the first one, the methodology was applied to five spiked samples with a concentration of 2000 µg/kg. The final extracts were diluted to an intermediate value of the calibration curve, and then quantified. The criteria for this parameter was the recovery (>70%, <120%) and the RSD (<20%).

Only cyromazine and dichlorvos presented recoveries below 70 % after dilution (Table 5). The remaining 41 molecules were extracted from the matrix at a concentration of 2000 µg/kg and still had a recovery between 70 % and 120 %, with an RSD less than 20 %. This implies that, although there is no linear relationship between concentration and response at high concentrations of the analyte, the method allows to work at such concentrations using proper dilutions. Moreover, the sample dilution provides the desired effect of an additional decrease of the matrix effect.

3.2.8 Robustness

The robustness test was carried out to demonstrate that the methodology is still reliable and accurate, after the variation of several extraction conditions (factors) (Table S4). Several experimental designs are used to evaluate the robustness of analytical methods [60–64]; the Youden-Steiner test was performed for this work. This test consists of a fractional factorial design of resolution III, which is represented by the mathematical model 2_{III}^{7-4}. It works at two levels of effect, in which seven factors or conditions that can result in significantly different results are considered (but not their interactions), for a total of eight experiments [34,61,65,66].

The robustness was calculated by comparing the difference of the values of each factor, according to the tables of experiment (Tables 1 and 2) in relation to the value calculated as critical,

which depends on the total standard deviation of the experiment [34,61,62]. The results are shown in Table S4 of the Supplementary Material.

Forty molecules met the robustness criterion. In particular for amethryn, simazine, dimethoate, bitertanol, hexaconazole the seven proposed factors did not statistically affect the behavior of the molecules. Four analytes (chlorpyrifos, coumaphos, cyromazine and methamidophos) showed high critical values (>20) that represented their high variability; nonetheless they passed the robustness test. This finding represents a drawback of the statistical method, as the critical factor increases with RSD >20 %, this method may mask the effect of conditions in the case of molecules with poor precision. Other two molecules, dichlorvos and fenamiphos, showed high critical values of 14 and 16, but they are still considered as acceptable.

Only cyproconazole, dichlorvos, fenamiphos and methamidophos failed the robustness test for one condition. In the case of cyproconazole, the condition was the temperature of the water bath; as the temperature increased, a lower recovery of the molecule was obtained, which implies that temperature control must be used during sample concentration with nitrogen. In the case of fenamiphos, the critical factor was the agitation time with acetonitrile, as a decrease greater than 20 % in the percentage of recovery was obtained with less agitation. For methamidophos, a greater variability was obtained as a cause of a shorter agitation time in the centrifuge, which does not allow an adequate phase separation. On the other hand, dichlorvos was affected by the time it was in contact with water, however, this molecule consistently exhibited lower recoveries and unsatisfactory RSD.

3.2.9 Summary of validation parameters

Every analyte showed satisfactory results for the determination of LOD, LOQ, linearity, specificity and selectivity. Besides these parameters, the proposed methodology exhibited low matrix effects; only cyromazine had a value out of the acceptance criterion.

For the parameters of trueness and precision, cyromazine and dichlorvos were the only two molecules that showed unsatisfactory results, with values of recovery < 70 % and RSD > 20 %. The lack of precision and accuracy suggests the use of another methodology to work with these molecules.

The parameter of robustness demonstrated that for most of the molecules slight changes do not affect the methodology performance. Only four molecules exhibited unsatisfactory results for one different methodology condition each.

Overall, the proposed methodology, except for dichlorvos and cyromazine, met the acceptance criteria for the determination of 41 of the evaluated molecules in the biomixture.

3.3 Removal of pesticide-containing wastewater in a BPS

The methodology was applied to monitor the removal capacity of a 104 L BPS. The synthetic wastewater applied on the biomixture contained eight pesticides (ametryn, atrazine, chlorpyrifos, diazinon, malathion, tebuconazole, terbutryn and triadimenol), described previously at similar concentrations to those expected after field application, according to the recommendation in the commercial formulations. Initial concentrations in the BPS ranged from 51.1 mg/kg to 3.9 mg/kg (tebuconazole and malathion, respectively). Triadimefon, which was not added as part of the wastewater, was initially detected at 0.09 mg/kg; its origin is likely due to contamination in the formulation containing tebuconazole/triadimenol, as triadimenol is a known transformation product of triadimefon [67].

Although most of the compounds were at least partially removed, none of them was eliminated at levels below the LOQ. The triazole tebuconazole and the organophosphate diazinon were not significantly removed. Previous works on BPS report unsuccessful elimination of triazoles, including tebuconazole and triadimenol [27,68,69]; nonetheless, in this case triadimenol was partly removed at the end of 53 d (up to 51.8 %). Many investigations indicate that triadimenol is a metabolite of triadimefon [67,70]; however, in this experiment triadimefon was not added and an increase in its levels was observed (up to 5.14 mg/kg). This finding could be due to an oxidation of the triadimenol in the biomixture, favored by the conditions of temperature and humidity, a scarcely studied reaction described by Deas & Clifford [71] in transformations with fungi. Other biomixtures have shown the ability to remove diazinon, at DT50 values from 4.9 d to 10.8 d, with an accelerated effect after successive applications, although in peat-based biomixtures [72].

Contrary to diazinon, the other organophosphates were removed from the biomixture at different rates; chlorpyrifos at an estimated DT50 of 10.5 d, while malathion concentration decreased to only 1.9 % after nine days of treatment (DT_{50} = 1.6 d). The removal of chlorpyrifos was significantly faster than data from soil (DT50 27-386 d) [73] and slightly faster than those achieved in other biomixtures, for which DT50 values are within the range 15-59 d [69,74–76]. The fast elimination of malathion in biomixtures was also described in a peat-based matrix ((DT50 3.8 d) [24] and a compost-based mixture (DT_{50} 7.1 d). From the three triazines tested, atrazine exhibited the faster removal (estimated DT50 11.2 d), followed by ametryn (DT_{50} 13.4 d) and terbutryn (DT_{50} 19.4 d). The removal of atrazine has been widely described in biomixtures, with DT50 values ranging from as low as < 10 d (after single or repeated applications) [29,31,69] to more than 20 d [68,77]. Comparable removal patterns have been achieved for ametryn [4] and terbutryn [28] in compost-based biomixtures.

4 Conclusions

The modified QuEChERS methodology was validated for the analysis of pesticides in a solid matrix (biomixture) made up of soil, compost and coconut fiber, aimed to remove pesticides from wastewater of agricultural origin. The method was proved under several validation parameters, where the results were satisfactory for most of the triazines, triazoles and organophosphates evaluated, except for dichlorvos and cyromazine, which did not meet the acceptance criteria for some parameters.

The developed LC-MS/MS methodology allows working with low and high concentrations, with good recovery percentages between 70 % and 120 %, coefficients of variation of less than 20 %, with linearity conditions that exceed the coefficient of determination value of 0.99, and with a matrix effects lower than 20 %, for 41 out of the 43 evaluated molecules.

The validated methodology was successfully applied to determine the efficiency of a pilot scale biopurification system, employed for the removal of wastewater residues containing eight pesticides from commercial formulations.

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Figuras y tablas del artículo del Capítulo I

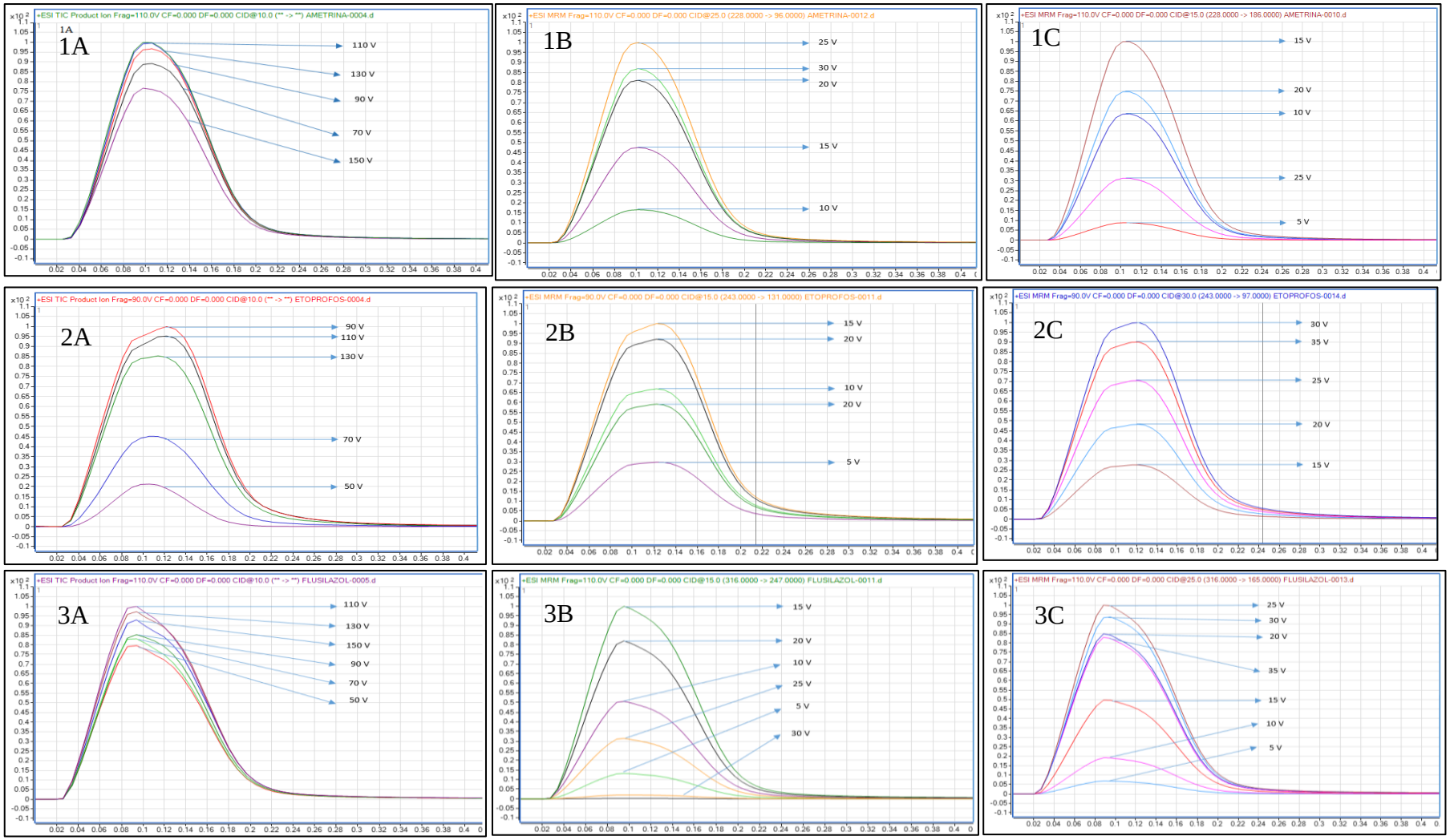

Figure 1. Optimization of voltage fragmentor and precursor ion (A); the quantification product ion (B); and confirmation product ion (C) for ametryn (1), ethoprophos (2), and flusilazol (3)..

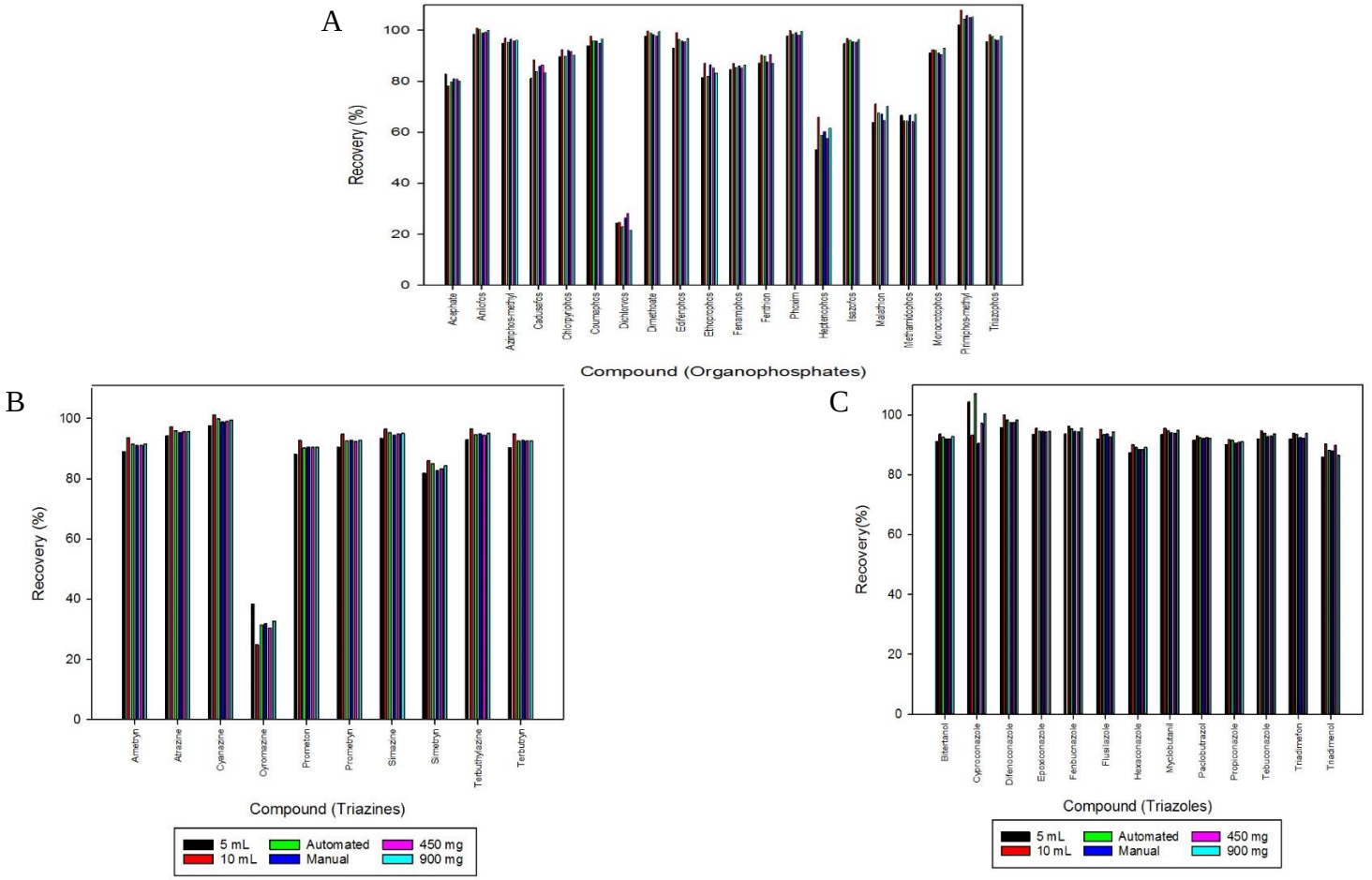

Figure 2. Recovery obtained from the design of comparative experiments 2^3 during the optimization of methodological factors: i. addition of water (5 mL vs. 10 mL) during the extraction; ii. automated or manual agitation during extraction; and iii. the amount of sulphate magnesium (450 mg vs. 900 mg) added in the cleaning step for: (A) Organophosphates; (B) Triazines, (C) Triazoles.

Table 1. Experimental design for the determination of the robustness test using the Youden-Steiner test.

ID Factor	Factor	Condition 1	Condition 2
F1	Water rest time (min)	20 (A)	30 (a)
F2	Acetonitrile stir time (min)	2 (B)	5 (b)
F3	Type of magnesium sulfate (brand)	Sigma (C)	Fluka (c)
F4	Shaker agitation time (min)	30 (D)	15 (d)
F5	Centrifuge time (min)	7 (E)	3 (e)
F6	Centrifuge speed (rpm)	4000 (F)	2500 (f)
F7	Water bath temperature (°C)	30 (G)	40 (g)

Table 2. Distribution of the experimental conditions of the study factors for the Youden-Steiner test

ID Factor	E1	E2	E3	E4	E5	E6	E7	E8
F1	A	A	A	A	a	a	a	a
F2	B	B	b	b	B	B	b	b
F3	C	c	C	c	C	c	C	c
F4	D	D	d	d	d	d	D	D
F5	E	e	E	e	e	E	e	E
F6	F	f	f	F	F	f	f	F
F7	G	g	g	G	g	G	G	g
RESULT	**s**	**t**	**u**	**v**	**w**	**x**	**y**	**z**

Table 3. Optimization of the transitions for the quantification (Q) and the confirmation (q) of the studied analytes in a LC-MS/MS.

Compound	Transition		Fragmentor (eV)	Collision energy (eV)	Retention time (min)	Type of transition
	Precursor ion	**Product ion**				
Acephate	184	143	60	5	0.91	Q
		95		25		q
Amethryn	228	186	106	17	8.05	Q
		96		25		q
Anilophos	368	199	70	10	13.58	Q
		171		15		q
Atrazine	216	174	106	17	9.42	Q
		96		25		q
Azinphos-methyl	318	132	60	13	10.65	Q
		125		17		q
Bitertanol	338	99	82	13	14.05	Q
		269		5		q
Cadusafos	271	159	70	5	14.32	Q
		131		20		q

Chlorpyrifos	350	97	90	30	15.74	Q
		198		15		q
Coumaphos	363	277	116	25	13.67	Q
		307		13		q
Cyanazine	241	214	100	15	7.05	Q
		104		30		q
Cyproconazole	292	70	110	15	12.42	Q
		125		30		q
Cyromazine	167	85	104	17	0.87	Q
		60		21		q
Dichlorvos	221	109	104	13	7.40	Q
		79		29		q
Difenoconazole	406	251	126	25	14.42	Q
		337		13		q
Dimethoate	230	199	70	3	3.45	Q
		125		20		q
Edifenphos	311	111	90	20	13.46	Q
		283		10		q
Epoxiconazole	350	121	106	25	12.75	Q
		101		40		q
Ethoprophos	243	97	84	33	12.52	Q
		131		17		q
Fenamiphos	304	217	138	21	13.13	Q
		234		13		q
Fenbuconazole	337	70	116	17	12.95	Q
		125		40		q
Fenthion	279	247	104	9	13.83	Q
		105		25		q
Flusilazole	316	165	110	25	13.14	Q
		247		15		q
Hexaconazole	314	70	116	21	13.85	Q
		159		33		q
Heptenophos	251	127	110	10	10.23	Q
		109		30		q
Isazofos	31	120	94	29	12.25	Q
		162		13		q
Malathion	331	99	82	25	11.83	Q
		127		9		q
Methamidophos	142	94	90	10	0.98	Q
		125		10		q
Monocrotophos	224	127	62	13	1.85	Q
		193		5		q
Myclobutanil	289	70	106	17	11.86	Q
		125		37		q
Paclobutrazol	294	70	110	15	11.81	Q
		125		35		q
Phoxim	321	192	94	9	13.88	Q
		115		21		q
Pirimiphos-methyl	306	164	90	20	13.38	Q
		108		30		q
Prometon	226	142	116	21	7.15	Q
		184		17		q
Prometryn	242	200	126	17	9.71	Q
		158		21		q
Propiconazole	342	159	126	29	13.61	Q
		69		21		q

Simazine	202	124	106	17	7.42	Q
		104		25		q
Simetryn	214	124	106	17	6.08	Q
		96		25		q
Tebuconazole	308	70	106	21	13.55	Q
		125		40		q
Terbythylazine	230	174	104	13	11.33	Q
		96		29		q
Terbutryn	242	186	96	17	9.83	Q
		91		29		q
Triadimefon	294	197	94	13	11.74	Q
		69		21		q
Triadimenol	296	70	72	9	12.35	Q
		99		13		q
Triazophos	314	162	100	15	12.25	Q
		119		35		q
Linuron-d6 (IS)	255	160	92	17	11.12	Q
		185		13		q
Carbofuran-d3 (SS.)	225	165	86	9	7.67	Q
		123		21		q

IS: internal standard; SS: surrogate standard.

Table 4. Results of average RSD calculated for the precision parameter at three spiked levels (n = 7) and intermediate precision (n = 21); average recovery (%) calculated for trueness (n = 7), and the LOD (n = 7) and LOQ (n = 7) for the method proposed to determine triazines, triazoles and organophosphates in a biomixture composed by soil/compost/coconut fiber.

Compound	Precision (as repeatability) (RSD < 20%)			Intermediate precision (RSD < 20%)			Trueness (Recovery % n = 7)	LOD 5 µg/kg (RSD)	LOQ 10 µg/kg (RSD)
	50 µg/kg	150 µg/kg	350 µg/kg	50 µg/kg	150 µg/kg	350 µg/kg	50 µg/kg		
Acephate*	5.06	6.16	8.72	12.25	6.07	6.89	92.1	27.8	11.4
Amethryn	3.27	7.77	2.80	4.44	5.04	10.74	106.0	5.8	4.2
Anilophos	4.17	4.63	3.47	6.77	5.27	8.39	90.0	24.3	6.4
Atrazine	3.83	7.06	3.30	5.72	4.60	7.75	111.5	3.7	5.7
Azinphos-methyl	3.25	7.58	2.35	5.90	5.15	6.85	107.6	22.9	5.5
Bitertanol	4.70	7.88	3.14	5.07	5.10	13.47	108.0	28.8	4.8
Cadusafos	6.92	7.48	5.06	21.77	8.34	12.42	99.9	7.2	17.6
Chlorpyrifos	7.81	10.66	3.99	12.76	7.53	10.97	103.2	27.4	9.7
Coumaphos	4.50	8.81	5.12	8.43	7.01	12.92	110.2	21.7	7.9
Cyanazine	5.19	7.32	3.16	5.38	4.73	10.01	112.1	5.1	5.3
Cyproconazole	6.27	8.59	5.25	7.67	6.10	12.24	116.4	33.6	7.4
Cyromazine	23.66	17.38	28.57	61.15	40.95	54.86	49.4	20.1	38
Dichlorvos	43.14	20.31	19.09	40.19	25.56	25.80	47.1	17.9	12.4
Difenoconazole	3.34	7.38	4.53	4.94	4.76	15.64	112.5	15.4	4.6
Dimethoate	3.76	7.78	4.65	5.45	5.18	5.57	110.4	6.8	5.6
Edifenphos	4.19	4.47	4.40	4.33	6.08	8.30	114.7	20.7	4.1
Epoxiconazole	2.75	7.62	2.88	4.19	5.13	13.67	108.2	23.3	4.1
Ethoprophos	7.32	10.09	2.78	11.05	9.29	9.93	97.0	28.2	14.5
Fenamiphos	4.72	6.47	3.88	8.15	6.39	8.78	105.3	16.2	8.1
Fenbuconazole	4.60	7.78	4.40	5.95	4.96	12.78	107.0	11.9	5.6
Fenthion	11.60	6.72	2.94	14.47	11.60	14.25	101.3	44.1	13.1
Flusilazole	4.01	6.77	3.84	5.33	4.76	13.23	108.1	11.5	5.2
Hexaconazole	3.63	8.09	3.67	4.29	5.39	12.80	102.1	13.1	4.2
Heptenophos	10.24	5.21	3.43	9.83	6.92	6.73	89.3	8.9	7.2
Isazofos	3.03	6.79	2.65	5.39	4.69	8.84	112.1	9.4	5.2
Malathion	8.28	4.76	3.41	19.41	9.18	8.54	93.7	19.8	13.4
Methamidophos	5.68	7.63	8.19	10.83	8.18	14.30	78.2	3.2	9.8
Monocrotophos	4.54	6.96	4.68	6.77	4.84	6.99	107.7	13.4	6.8
Myclobutanil	3.35	7.26	3.09	5.62	5.03	11.73	108.1	14.9	5.5
Paclobutrazol	3.94	7.89	3.24	4.16	6.08	10.06	106.7	6.6	4.0
Phoxim	3.35	5.52	5.14	8.63	5.86	8.73	114.2	23.2	8.1

Pirimiphos-methyl	2.74	7.78	3.32	6.74	5.14	13.36	113.0	11.9	6.3
Prometon	3.40	7.98	3.15	4.71	5.10	10.04	105.2	6.1	4.6
Prometryn	3.32	7.66	2.95	4.50	4.77	10.40	108.5	3.9	4.4
Propiconazole	4.16	7.55	3.38	5.43	5.17	14.10	103.7	29.5	5.1
Simazine	3.53	7.42	2.50	5.24	4.87	9.52	109.5	11.6	5.1
Simetryn	2.72	7.59	1.77	5.13	5.13	11.03	103.7	6.6	4.6
Tebuconazole	11.70	6.74	2.78	15.66	6.65	16.54	107.7	7.8	13.9
Terbuthylazine	3.28	7.09	3.26	5.07	4.48	10.14	109.3	8.3	4.9
Terbutryn	3.10	8.15	2.90	4.36	5.31	10.88	108.1	7.6	4.2
Triadimefon	2.52	7.56	2.64	4.35	5.21	12.86	109.9	12.1	4.2
Triadimenol	4.84	7.46	4.04	7.90	6.68	10.63	105.5	11.3	7.6
Triazophos	4.61	7.40	2.82	6.55	4.75	8.06	113.4	8.3	6.4

LOD: Limit of detection; LOQ: Limit of quantification. * Acephate had a LOD of 25 µg/kg and LOQ of 50 µg/kg

Table 5. Results of the parameters validation of linearity, scope of work and matrix effect for the method proposed to determine triazines, triazoles and organophosphates in a biomixture composed by soil/compost/coconut fiber.

Compound	Linearity (3 replicates. 10 calibration levels)			Scope of Work = Spiked at 2 mg/kg (RSD % with n = 21. diluited)	Matrix effect (Dif < 20%)
	r^2	Highest % of residuals detected < 20%	Calibration curve		
Acephate	0.994	9.99	y = 0.00043749 x – 0.00000017	102.5 (7.0)	0.24
Amethryn	0.999	5.38	y = 0.004057 x – 0.000042	106.6 (5.0)	1.57
Anilophos	0.996	7.88	y = 0.0002312 x – 0.0000019	108.4 (6.5)	0.22
Atrazine	0.992	6.49	y = 0.002688 x – 0.000031	108.2 (3.3)	0.48
Azinphos-methyl	0.996	10.0	y = 0.00006759 x – 0.00000063	106.2 (4.1)	2.20
Bitertanol	0.991	7.50	y = 0.0003322 x – 0.0000027	109.8 (4.9)	0.79
Cadusafos	0.995	7.89	y = 0.0006059 x – 0.0000046	94.1 (3.8)	1.22
Chlorpyrifos	0.994	6.31	y = 0.00009017 x – 0.00000088	103.8 (4.3)	1.19
Coumaphos	0.991	10.38	y = 0.0001379 x – 0.0000019	118.1 (7.8)	4.00
Cyanazine	0.998	8.41	y = 0.0004952 x – 0.0000066	115. 8 (4.7)	0.48
Cyproconazole	0.991	6.16	y = 0.0008256 x – 0.0000026	110.1 (4.4)	1.00
Cyromazine	0.997	14.33	y = 0.00043203 x + 0.00000018	29.4 (49)	17.29
Dichlorvos	0.995	9.85	y = 0.0002714 x – 0.0000019	61.7 (12.4)	1.64
Difenoconazole	0.990	6.90	y = 0.0009981 x – 0.0000095	99.4 (6.7)	2.06
Dimethoate	0.998	5.60	y = 0.00040682 x – 0.00000034	100.7 (3.9)	0.92
Edifenphos	0.997	5.56	y = 0.0002329 x – 0.0000021	113.3 (4.0)	1.05
Epoxiconazole	0.993	8.44	y = 0.000928 x – 0.000011	109.1 (3.8)	1.87
Ethoprophos	0.995	6.09	y = 0.0002974 x – 0.0000025	103.0 (3.7)	1.10
Fenamiphos	0.991	12.03	y = 0.0002977 x – 0.0000041	108.1 (5.9)	2.71
Fenbuconazole	0.992	9.70	y = 0.0005668 x – 0.0000077	111.4 (4.9)	0.10
Fenthion	0.995	8.65	y = 0.00005111 x – 0.000000095	102.7 (6.8)	1.32

Flusilazole	0.993	8.78	y = 0.001284 x – 0.000016	110.2 (4.4)	0.50
Hexaconazole	0.993	4.91	y = 0.001271 x – 0.000015	108.8 (4.3)	0.02
Heptenophos	0.996	7.48	y = 0.0002239 x – 0.0000016	100.2 (3.5)	2.85
Isazofos	0.995	7.89	y = 0.001438 x – 0.000011	109.4 (4.0)	0.66
Malathion	0.995	5.84	y = 0.000245 x – 0.000013	95.2 (4.3)	1.87
Methamidophos	0.997	10.44	y = 0.00017877 x – 0.00000098	102.8 (17.2)	9.15
Monocrotophos	0.996	5.62	y = 0.0003956 x – 0.0000032	109.1 (4.7)	2.57
Myclobutanil	0.994	4.74	y = 0.0009990 x – 0.0000084	111.0 (4.3)	0.62
Paclobutrazol	0.995	6.58	y = 0.002872 x – 0.000031	112.1 (5.7)	2.99
Phoxim	0.997	8.43	y = 0.00008963 x – 0.00000027	98.5 (4.5)	7.59
Pirimiphos-methyl	0.994	11.07	y = 0.003116 x – 0.000039	117.8 (4.2)	0.89
Prometon	0.993	5.11	y = 0.004279 x + 0.000054	105.1 (3.2)	0.65
Prometryn	0.994	5.97	y = 0.005715 x – 0.000071	107.5 (4.6)	0.13
Propiconazole	0.992	5.73	y = 0.0007152 x – 0.0000092	111.2 (4.1)	1.55
Simazine	0.993	5.07	y = 0.000963 x – 0.000012	108.3 (4.5)	0.56
Simetryn	0.994	4.89	y = 0.001779 x – 0.000021	101.7 (4.6)	0.83
Tebuconazole	0.994	6.09	y = 0.0015468 x – 0.00000088	107.5 (4.0)	1.66
Terbuthylazine	0.994	7.66	y = 0.006191 x – 0.000071	111.3 (4.0)	0.64
Terbutryn	0.993	7.24	y = 0.006781 x – 0.000088	106.3 (4.5)	0.20
Triadimefon	0.995	6.57	y = 0.0007691x – 0.0000069	109.2 (4.1)	0.30
Triadimenol	0.992	5.28	y = 0.001368 x – 0.000011	103.2 (3.9)	1.24
Triazophos	0.996	5.68	y = 0.001367 x – 0.000011	105.4 (4.2)	0.02

Información suplementaria del artículo del Capítulo I

Validation of a methodology by LC-MS/MS for the determination of triazine, triazole and organophosphorus pesticide residues in biomixtures

M. Masís-Mora, W. Beita-Sandí, J. Rodríguez-Yañez, C. Rodríguez-Rofríguez. Validation of a methodology by LC-MS/MS for the determination of triazine, triazole and organophosphorus pesticide residues in biomixtures, Anexo I)

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Table S1. Physicochemical properties of the investigated triazines, triazoles and organophosphates.

Molecule	Action mode	Molecular weight (g/mol)	Chemical formula	Water solubility at 20 °C (mg/L)	K_{oc} (mL/g)
Ametryn	Triazine	227.12	$C_9H_{17}N_5S$	200	316
Atrazine	Triazine	215.68	$C_8H_{14}ClN_5$	35	100
Cyanazine	Triazine	240.69	$C_9H_{13}ClN_6$	171	190
Cyromazine	Triazine	166.18	$C_6H_{10}N_6$	13000	765 [1]
Prometon	Triazine	225.3	$C_{10}H_{19}N_5O$	620	43.2
Prometryn	Triazine	241.36	$C_{10}H_{19}N_5S$	33	400
Simazine	Triazine	201.66	$C_7H_{12}ClN_5$	5	130
Simetryn	Triazine	213.30	$C_8H_{15}N_5S$	450	200
Terbuthylazine	Triazine	229.71	$C_9H_{16}ClN_5$	6.6	151-514 [1]
Terbutryn	Triazine	241.36	$C_{10}H_{19}N_5S$	25	2432
Bitertanol	Triazole	337.42	$C_{20}H_{23}N_3O_2$	3.8	1782-2238 [2]
Cyproconazole	Triazole	291.78	$C_{15}H_{18}ClN_3O$	93	900 [1]
Difenoconazole	Triazole	406.26	$C_{19}H_{17}Cl_2N_3O_3$	15.0	3200-7734 [1]
Epoxiconazole	Triazole	329.76	$C_{17}H_{13}ClFN_3O$	7.1	1073
Fenbuconazole	Triazole	336.82	$C_{19}H_{17}ClN_4$	2.47	2100-9000 [2]
Flusilazole	Triazole	315.39	$C_{16}H_{15}F_2N_3Si$	41.9	1664
Hexaconazole	Triazole	314.21	$C_{14}H_{17}Cl_2N_3O$	18	1040
Myclobutanil	Triazole	288.78	$C_{15}H_{17}ClN_4$	132	950
Paclobutrazol	Triazole	293.8	$C_{15}H_{20}ClN_3O$	22.9	400
Propiconazole	Triazole	342.22	$C_{15}H_{17}Cl_2N_3O_2$	150	1086
Tebuconazole	Triazole	307.82	$C_{16}H_{22}ClN_3O$	36	470-6000 [1]
Triadimefon	Triazole	293.8	$C_{14}H_{16}ClN_3O_2$	70	300
Triadimenol	Triazole	295.76	$C_{14}H_{18}ClN_3O_2$	72	750
Acephate	Organophosphate	183.17	$C_4H_{10}NO_3PS$	790000	302
Anilofos	Organophosphate	367.85	$C_{13}H_{19}ClNO_3PS_2$	9.4	4563 [3]
Azinphos-methyl	Organophosphate	317.32	$C_{10}H_{12}N_3O_3PS_2$	28	1112
Cadusafos	Organophosphate	270.39	$C_{10}H_{23}O_2PS_2$	245	77.5-621.43 [1]
Chlorpyrifos	Organophosphate	350.58	$C_9H_{11}Cl_3NO_3PS$	1.05	5509
Coumaphos	Organophosphate	362.77	$C_{14}H_{16}ClO_5PS$	1.5	18000
Dichlorvos	Organophosphate	220.98	$C_4H_7C_{12}O_4P$	18000	50
Dimethoate	Organophosphate	229.26	$C_5H_{12}NO_3PS_2$	25900	5.2-50 [1]
Edifenphos	Organophosphate	310.37	$C_{14}H_{15}O_2PS_2$	56	1863
Ethoprophos	Organophosphate	242.3	$C_8H_{19}O_2PS_2$	1300	70
Fenamiphos	Organophosphate	303.36	$C_{13}H_{22}NO_3PS$	345	446.2
Fenthion	Organophosphate	278.33	$C_{10}H_{15}O_3PS_2$	4.2	1500
Phoxim	Organophosphate	298.3	$C_{12}H_{15}N_2O_3PS$	1.5	686
Heptenophos	Organophosphate	250.6	$C_9H_{12}ClO_4P$	2200	163

Isazofos	Organophosphate	313.74	$C_9H_{17}ClN_3O_3PS$	69	155
Malathion	Organophosphate	330.36	$C_{10}H_{19}O_6PS_2$	148	1800
Methamidophos	Organophosphate	141.13	$C_2H_8NO_2PS$	200000	1.0
Monocrotophos	Organophosphate	223.16	$C_7H_{14}NO_5P$	818000	19
Pirimiphos-methyl	Organophosphate	305.33	$C_{11}H_{20}N_3O_3PS$	11	1100
Triazophos	Organophosphate	313.3	$C_{12}H_{16}N_3O_3PS$	35	358

Table S2. Number of experiments for the optimization of the extraction of pesticides according to the experimental 2^3 full-factorial design.

Experiment	Factor A	Factor B	Factor C
1	10 mL	Automated equipment	900 mg
2	10 mL	Automated equipment	450 mg
3	10 mL	Manual	900 mg
4	10 mL	Manual	450 mg
5	5 mL	Automated equipment	900 mg
6	5 mL	Automated equipment	450 mg
7	5 mL	Manual	900 mg
8	5 mL	Manual	450 mg

Table S3. Results of the selectivity tests for n = 5 replicates at 50 µg/kg for the investigated triazines, triazoles and organophosphorus.

Pesticide	Blank signal (Area)	Spike signal	Recovery (%) at 50 µg/kg
Acephate	No signal	> 10 S/N (LOQ)	90.8
Amethryn	< 500	> 10 S/N (LOQ)	95.0
Anilophos	No signal	> 10 S/N (LOQ)	93.5
Atrazine	< 500	> 10 S/N (LOQ)	97.0
Azinfós-mehyl	No signal	> 10 S/N (LOQ)	91.5
Bitertanol	No signal	> 10 S/N (LOQ)	93.5
Cadusafos	No signal	> 10 S/N (LOQ)	90.2
Carbofuran-d3(SS)	< 500	> 10 S/N (LOQ)	97.1
Chlorpyrifos	<500	> 10 S/N (LOQ)	80.4
Coumaphos	No signal	> 10 S/N (LOQ)	86.8
Cyanazine	No signal	> 10 S/N (LOQ)	95.7
Cyproconazole	< 500	> 10 S/N (LOQ)	97.1
Cyromazine	< 500	> 10 S/N (LOQ)	93.1
Dichlorvos	No signal	> 10 S/N (LOQ)	42.5
Difenoconazole	No signal	> 10 S/N (LOQ)	94.2
Dimethoate	No signal	> 10 S/N (LOQ)	100.1
Edifenphos	No signal	> 10 S/N (LOQ)	93.7
Epoxiconazole	No signal	> 10 S/N (LOQ)	95.4
Ethoprophos	< 500	> 10 S/N (LOQ)	95.0
Fenamiphos	< 500	> 10 S/N (LOQ)	97.0
Fenbuconazole	No signal	> 10 S/N (LOQ)	90.2
Fenthion	No signal	> 10 S/N (LOQ)	95.7
Flusilazole	No signal	> 10 S/N (LOQ)	97.1
Heptenophos	No signal	> 10 S/N (LOQ)	94.9
Hexaconazole	No signal	> 10 S/N (LOQ)	97.5
Isazofos	No signal	> 10 S/N (LOQ)	95.7
Linuron-d6 (IS)	No signal	> 10 S/N (LOQ)	94.2
Malathion	No signal	> 10 S/N (LOQ)	95.0
Methamidophos	No signal	> 10 S/N (LOQ)	88.6
Monocrotophos	No signal	> 10 S/N (LOQ)	94.8
Myclobutanil	No signal	> 10 S/N (LOQ)	94.1
Paclobutrazol	No signal	> 10 S/N (LOQ)	94.7
Phoxim	< 500	> 10 S/N (LOQ)	93.1
Pirimiphos-methyl	No signal	> 10 S/N (LOQ)	94.2
Prometon	No signal	> 10 S/N (LOQ)	94.9
Prometryn	No signal	> 10 S/N (LOQ)	95.7
Propiconazole	No signal	> 10 S/N (LOQ)	91.5
Simazine	No signal	> 10 S/N (LOQ)	95.0
Simetryn	No signal	> 10 S/N (LOQ)	88.6
Tebuconazole	< 500	> 10 S/N (LOQ)	73.8
Terbuthylazine	No signal	> 10 S/N (LOQ)	94.8
Terbutryn	No signal	> 10 S/N (LOQ)	94.2
Triadimefon	No signal	> 10 S/N (LOQ)	94.9
Triadimenol	< 500	> 10 S/N (LOQ)	88.3
Triazophos	No signal	> 10 S/N (LOQ)	93.5

SS: Surrogate standard, IS: Internal standard

Table S4. Robustness parameter results according to Youden-Steiner test for the factors of water contact time (A-a), stirring time with acetonitrile (B-b), type of magnesium sulfate (C-c), shaker stirring time (D-d), centrifuge time (E-e), centrifuge speed (F-f) and water bath temperature (G-g).

Molecule	**Critical Value**	**Factors evaluated**						
		A-a	B-b	C-c	D-d	E-e	F-f	G-g
Acephate	9.39	0.31	3.25	-6.99	-5.98	3.71	-2.42	6.28
Amethryn	5.65	-1.20	4.55	-1.02	-2.14	1.19	-1.59	4.91
Anilophos	9.39	0.31	3.25	-6.99	-5.98	3.71	-2.42	6.28
Atrazine	5.22	-0.09	4.54	-0.21	-1.76	1.34	-1.45	4.47
Azinphos-methyl	7.69	-0.82	4.15	-5.54	-4.75	1.11	-4.72	2.96
Bitertanol	5.80	-1.62	2.43	-3.58	-3.31	2.91	-3.16	2.86
Cadusafos	9.34	-4.56	6.10	0.26	-6.43	-3.31	-1.13	6.41
Chlorpyrifos	34.99	-13.41	8.94	-14.54	-25.67	4.66	-2.56	31.37
Coumaphos	27.39	-8.52	-18.08	24.80	0.10	7.12	-15.65	1.67
Cyanazine	6.42	1.01	2.71	-1.40	-3.64	4.39	-4.56	2.91
Cyproconazole	7.41	0.91	3.74	-1.23	-3.03	1.59	-3.31	7.55
Cyromazine	20.42	2.52	1.97	-3.41	-14.04	-19.23	11.81	-1.15
Dichlorvos	14.32	-14.91	5.14	2.25	-7.98	1.18	-5.53	3.07
Difenoconazole	8.15	2.96	-1.87	-1.38	-2.04	-0.01	-7.13	-6.87
Dimethoate	6.67	-2.44	5.04	-1.27	-0.97	3.43	-3.56	4.42
Edifenphos	7.20	1.10	1.22	-0.92	-5.05	6.31	-0.63	4.64
Epoxiconazole	8.96	1.44	0.13	-5.54	-0.18	5.72	-4.62	7.31
Ethoprophos	10.14	-11.76	3.41	-4.07	0.48	2.43	1.88	-1.92
Fenamiphos	16.34	-0.13	16.40	2.42	-6.29	-2.45	11.97	-1.90
Fenbuconazole	6.32	-1.60	2.99	-2.77	-3.75	3.10	-3.65	3.73
Fenthion	8.81	3.09	8.24	-0.38	1.05	-4.39	-0.31	6.15
Flusilazole	8.42	0.24	0.50	-3.22	-2.74	4.71	-5.92	6.99
Hexaconazole	5.76	0.17	1.34	-2.74	-2.10	4.22	-2.43	4.54
Heptenophos	8.38	-6.80	4.49	-0.46	-3.10	1.48	-3.49	5.68
Isazofos	6.15	-1.78	1.09	-3.22	-3.06	2.22	-3.19	5.18
Malathion	8.23	-2.93	5.96	-2.33	-5.36	2.84	-2.90	4.87
Methamidophos	55.77	-6.42	3.85	11.79	15.80	65.26	-18.58	19.86
Monocrotophos	6.04	-0.02	3.80	-1.60	-1.94	4.08	-3.53	3.75
Myclobutanil	5.97	-0.28	3.68	-2.57	-4.02	3.23	-2.62	2.95
Paclobutrazol	6.21	-1.71	1.70	-0.89	-4.16	5.17	-2.89	2.92
Phoxim	10.31	-6.17	2.25	-3.82	-3.15	6.20	1.77	8.77
Pirimiphos-methyl	7.82	-2.73	5.16	-2.52	-3.67	-0.80	-1.24	7.14
Prometon	5.76	-0.42	4.20	-1.17	-2.37	2.18	-1.97	-0.42
Prometryn	5.46	-0.42	4.27	-0.89	-2.49	1.49	-1.74	4.65
Propiconazole	7.00	-0.70	0.39	-2.10	-3.65	4.27	-1.54	6.83
Simazine	4.99	-1.38	4.82	-0.44	-2.34	0.67	-0.99	3.37
Simetryn	5.17	-0.38	3.90	-0.26	-2.45	1.49	-1.72	4.49
Tebuconazole	6.69	1.24	2.60	-2.67	-3.81	3.33	-4.53	4.09
Terbuthylazine	5.56	-1.29	4.52	-1.11	-2.42	1.48	-2.06	4.31
Terbutryn	5.82	-0.09	4.44	-0.35	-1.46	1.93	-1.64	5.56
Triadimefon	10.57	-1.62	3.51	3.12	-8.08	2.50	-3.56	9.31
Triadimenol	7.81	1.39	5.02	0.80	-5.26	1.19	-4.23	5.65
Triazophos	5.95	-0.86	1.78	-2.19	-4.37	1.31	-4.18	3.87

References

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CAPÍTULO II

Removal of triazines, triazoles and organophophates in biomixtures and application of a biopurification system for the treatment of laboratory wastewaters

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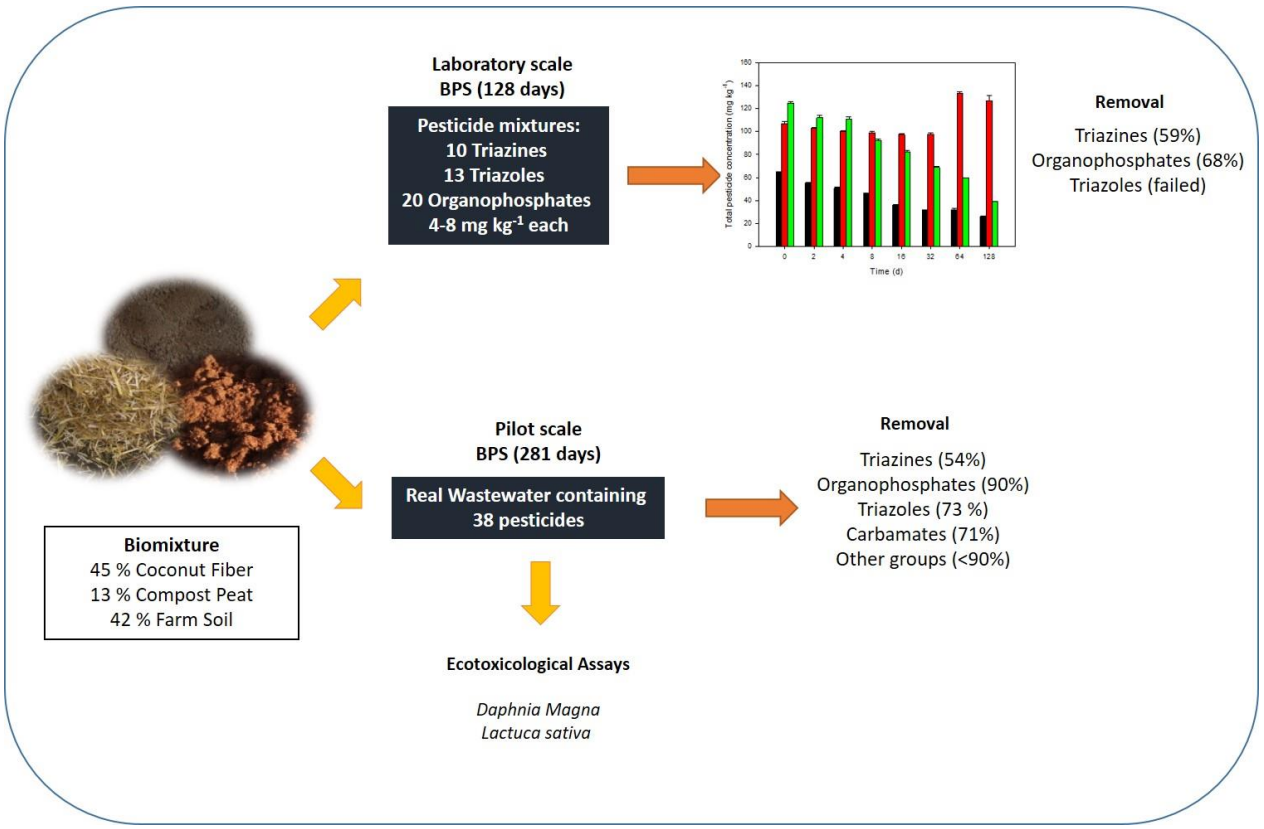
RESUMEN

Los sistemas de biopurificación (SBP) apenas se han explorado para eliminar mezclas complejas de plaguicidas. En este estudio, se presenta el potencial de una biomezcla para eliminar simultáneamente una mezcla de herbicidas (triazinas), fungicidas (triazoles) e insecticidas (organofosforados). Además, se usó un SBP que usaba la misma mezcla biológica para tratar un agua residual de laboratorio de análisis de pesticidas que contenía una mezcla de 38 compuestos. Se realizaron ensayos ecotoxicológicos en los elutriados de SBP para investigar la desintoxicación de la mezcla. Una mezcla (concentraciones de 4–8 mg kg^{-1}) realizada en un sistema de biomezcla a pequeña escala (SBPE) durante 128 días mostró una eliminación del 59.3% de triazinas, 68.5% de organofosfatos y ninguna eliminación de triazoles. El tratamiento de las aguas residuales de laboratorio (0.0036–0.25 mg/kg) en el BPS a escala piloto durante 281 d resultó en el patrón de eliminación de organofosforados (90.0%)> triazoles (73.4%)> carbamatos (71.3%)> triazinas (54.3 %). La desintoxicación completa hacia *Daphnia magna* y la desintoxicación parcial en la germinación de semillas de *Lactuca sativa* ocurrieron en el SBP. Aunque la complejidad de la mezcla de plaguicidas es mayor en el SBP, las concentraciones más

bajas encontradas en esta matriz podrían explicar las diferencias de eliminación entre SBPE y SBP y la inhibición aparente en la eliminación de carbamatos y algunas triazinas observadas en este último. Estos hallazgos sugieren que la eliminación de las aguas residuales de laboratorio que contienen plaguicidas debe hacerse en contenedores separados, de acuerdo con los grupos químicos antes de su tratamiento en BPS separados, para reducir los períodos de tratamiento. Se recomienda encarecidamente monitorear el proceso de tratamiento en el BPS con una batería de pruebas ecotoxicológicas.
Palabras clave: Plaguicidas; toxicidad; remoción; degradación; agua residual

ABSTRACT

Biopurification systems (BPS) have been barely explored for removing complex mixtures of pesticides. In this study, the potential of a biomixture to remove simultaneously a mixture of herbicides (triazines), fungicides (triazoles) and insecticides (organophosphates) is presented. Also, a BPS using the same biomixture was used for treating a pesticide testing laboratory wastewater containing a mixture of 38 compounds. Ecotoxicological assays were conducted on the BPS elutriates to investigate the mixture detoxification. A mixture (concentrations of 4–8 mg/kg) run in a small–scale biomixture systems (SSB) for 128 d showed 59.3% removal of triazines, 68.5% of organophosphates and no elimination of triazoles. The treatment of the laboratory wastewater (0.0036–0.25 mg/kg) in the pilot-scale BPS for 281 d resulted in the elimination pattern of organophosphates (90.0%) > triazoles (73.4%) > carbamates (71.3%) > triazines (54.3%). Complete detoxification towards *Daphnia magna* and partial detoxification in *Lactuca sativa* seeds germination occurred in the BPS. Although the pesticide mixture complexity is higher in the BPS, the lower concentrations found in this matrix, could explain removal differences between SSB and BPS and the apparent inhibition in the elimination of carbamates and some triazines observed in the latter. These findings suggest that disposal of pesticide–containing laboratory–wastewater should be done in separate containers, according to chemical groups before their treatment in *separate* BPS, in order to reduce treatment periods. Monitoring the treatment process in the BPS with a battery of ecotoxicological tests is strongly recommended.
Key words: pesticides; toxicity; removal; degradation; wastewater.



Highlights:

Small scale biomixtures (SSB) removed mixtures of triazines or organophosphates

BPS removed 77.2% pesticides from laboratory-wastewater containing 38 compounds

Triazoles removed in biopurification systems (BPS) but not in SSB

Differences in removal (SSB vs BPS) related to pesticide concentration and diversity

BPS proved efficient to mostly detoxify pesticide-containing laboratory-wastewater

1. Introduction

Agricultural activities worldwide are highly dependent on the use agrochemicals. Pesticides are among the diverse group of compounds used in agriculture and their application and handling are of high environmental concern due to their toxic effects on non-target populations. Pesticide contamination of environmental compartments may occur by diffuse or point–source processes. Even though diffuse contamination is difficult to control, it is only partially responsible of the pesticides found in surface and groundwater (De Wilde et al., 2007). On the contrary, point–source contamination (e.g., disposal of

application residues, preparation of application solutions, handling of wastewater from washing of application equipment) accounts for the main contamination events (De Wilde et al., 2007; Vischetti et al., 2004) but fortunately, it can be more easily controlled.

The approaches for pesticide elimination from wastewater mostly comprise physical processes (e.g., sorption onto granular activated carbon) (García Portillo et al., 2004) or chemical treatments with advanced oxidation processes (AOPs) (e.g., Fenton and ozonation) (Kusvuran et al., 2012; Mitsika et al., 2013). However, the cost-effectiveness of these methods for application in small farms requires further research; moreover, AOPs may produce transformation products of unknown toxicity, a drawback that may limit their application.

Biopurification systems (BPS), also known as biobeds, were developed in the 1990s as an eco-friendly, low cost biotechnological tool for treating pesticide containing wastewater of agricultural origin (Castillo et al., 2008). The degrading capacity of BPS relies on the biological matrix (biomixture) that is theoretically able to remove pesticides faster than in other environments (e.g., soil). The biomixture contains a lignocellulosic substrate, a humic-rich material and soil mixed at a (typical) volumetric ratio of 2:1:1. The lignocellulosic substrate provides an adequate matrix for the growth and activity of ligninolytic fungi (Karanasios et al., 2012), organisms known to be responsible for the transformation of several organic pollutants due to their unspecific extracellular (i.e., lignin modifying enzymes) and intracellular (i.e., cytochrome P450) enzymatic complexes (Yang et al., 2013). The humic-rich material favors the retention of pesticides in the matrix, in order to avoid their accumulation at the bottom of the system (in case of highly mobile compounds); peat was initially employed for this purpose; nonetheless, in the adaptation of BPS to different geographic areas, compost has raised as a proper substitute material for peat (Chin-Pampillo et al., 2015a, 2015b; Coppola et al., 2007). Finally, soil provides adapted degrading microbiota, reason why it should be ideally pre-exposed to the target pesticide(s) to be treated in the BPS (Sniegowski et al., 2012).

The use of BPS has shown promising results in the elimination of diverse pesticides (Rodríguez-Rodríguez et al., 2013). Several peat-based or compost-based biomixture configurations consistently report relatively fast removal of triazines (Castro-Gutiérrez et al., 2018; Huete-Soto et al., 2017b; Tortella et al., 2013a), organophosphates (Bozdogan et al., 2014; Fogg et al., 2003; Murillo-Zamora et al., 2017) and some carbamates (Chin-Pampillo et al., 2015b; Rodríguez-Rodríguez et al., 2017) using diverse materials as lignocellulosic substrates, such as coconut fiber, woodchips, rice husk, barley straw or wheat straw. On the other hand, these systems are not particularly efficient at removing the triazole fungicides (Castillo Diaz et al., 2016; Huete-Soto et al., 2017b; Spliid et al., 2006) or the neonicotinoid insecticides (Huete-Soto et al.,

2017b; Rodríguez-Castillo et al., 2018). Nonetheless, the simultaneous removal of multiple compounds (> 10) (Spliid et al., 2006), a situation likely to occur in BPS, has been scarcely evaluated. Co-existence of multiple pesticides may occur in a farm BPS in the cases of simultaneous application of several formulations, when frequent applications of different pesticides take place, when more persistent molecules are not yet degraded before subsequent applications, or in a combination of these situations. Most of the pesticides-containing wastewaters come from agricultural labors; however, research laboratories also produce considerable amounts of these residues, which may contain dozens of molecules, depending on the disposal protocols. Thus, the objectives of this work were (1) to determine the efficiency of a biomixture to treat simultaneously triazines, triazoles and organophosphates, (2) use a pilot scale BPS for treating real wastewaters from a pesticide analyses laboratory, and (3) investigate the extent of detoxification of a BPS using ecotoxicological tests. This is the first report that describes the use of BPS for treating wastewater of an origin different than agricultural activities.

2. Materials and methods

2.1 Chemicals and reagents

The analytical standards anilophos (98.9%), azinphos-methyl (98.8%), bentazone (99.5%), bitertanol (99.5%), cadusafos (97.2%), chlorpyrifos (99.5%), cyproconazole (99.5%), cyromazine (99.5%), dichlorvos (98.4%), dimethoate (99.5%), diuron (99.5 %), edifenphos (98.5%), epoxiconazole (99.5%), ethoprophos (98.8%), fenamiphos (99.0%), fenbuconazole (99.5%), flusilazole (98.6%), heptenophos (98.6%), hexaconazole (99.3%), imazalil (99.5%), isazofos (99.2%), malathion (99.5%), methamidophos (99.5%), monocrotophos (99.5%), myclobutanil (98.0%), phoxim (99.4%), pirimicarb (99.5%), pirimiphos-methyl (99.5%), prometon (99.5%), prometryn (99.5%), propanil (99.5%), pyraclostrobin (99.5%), pyrimethanil (99.5%), simetryn (99.5%), tebuconazole (98.0%), terbutryn (98.1%), thiametoxam (99.5%), triadimefon (99.5%) and triadimenol (98.7%) were purchased from ChemService (West Chester, PA, USA). Standards acephate (99.0%), amethryn (98.0%), atrazine (99.0%), buprofezin (99.0%), carbaryl (99.0%), carbendazim (99.0%), carbofuran (98.5%), carbofuran-d3 (98.0%), coumaphos (99.0%), cyanazine (98.5%), difenoconazole (98.7%), fenthion (99.0%), imidacloprid (99.5%), linuron-d6 (98.5%), methiocarb (99.5%), paclobutrazol (98.5%), propiconazole (99.0%), simazine (98.0%), terbuthylazine (98.5%), thiabendazole (99.0%) and triazophos (80.0%) were acquired from Dr. Ehrenstorfer (Augsburg, Germany).

Methanol and acetonitrile were HPLC grade, LiChrosolv® from Merck (Darmstadt, Germany). Dichloromethane (Suprasolv® for Gas Chromatography), formic acid (98–100%) and

glacial acetic acid (99.7%) were purchased from Merck (Darmstadt, Germany). Magnesium sulfate anhydrous and sodium acetate trihydrate from Sigma-Aldrich (St Louis, MO, USA); bondesil-PSA, 40 µm (Varian, PA, USA), Sepra-C18 (Phenomenex, CA, USA) and sodium chloride (JT Baker, PA, USA).

2.2 Experimental setup

2.2.1 Removal of mixtures of triazines, triazoles or organophosphates in a biomixture

Experiments were conducted in small scale biomixture systems (SSB) with 5 g of biomixture in 50 mL polypropylene tubes. The biomixture (pH 6.4; C 4.83%; N 0.32%; C/N 15.2; P 0.22%; Ca 0.48%; Mg 0.71%; K 0.19%; S 0.07%; Fe 31 192 mg kg-1; Zn 91 mg kg-1; Mn 521 mg kg-1; B 66 mg kg-1; EC 0.6 mS cm-1) consisted of coconut fiber, compost and farm soil at a volumetric composition of 2:1:1 (Chin-Pampillo et al., 2015a, 2015b). The biomixture was moistened to approximately 75% of the maximum water-holding capacity of the barrel and aged at 25 °C during one month with weekly homogenization prior to use. A total of 72 systems were separated in three sets; each set was spiked with a mixture of either, triazines, triazoles or organophosphates, at a final concentration of 4-8 mg kg-1 (each pesticide). The content of the tubes was manually homogenized and incubated in the dark at (25 + 1) °C for 128 d; selected concentrations correspond to those potentially found in a BPS (at the level of mg kg-1), considering application indications and the biomixture mass in a 200 L container. Every time samples were collected in triplicate tubes and subsequently analyzed to determine pesticide concentrations.

2.2.2 Removal of pesticide–containing wastewater in a biopurification system

A pilot-scale BPS was employed for the treatment of laboratory wastewater. The pilot-scale BPS consisted of a 204 L plastic barrel containing 140 L (~75.6 kg) of the biomixture, and was placed outside of the laboratory. The pilot-scale BPS was exposed to a temperature ranging from 12 to 33 °C and it was covered with plastic lid to protect the biomixture against the rain. Forty liters of laboratory-wastewater were poured and thoroughly mixed in the biomixture. The wastewater corresponded to the content of a disposal bin where residues from a Laboratory of Pesticide Analysis are collected; it contained most of the triazines, triazoles and organophosphates employed in section 2.3.1, plus additional pesticides from other chemical groups; characterization of the pesticide content in the wastewater is shown in Table S1 of the Supplementary Information. Composite samples were obtained from the BPS by withdrawing small portions of biomixture with basin and shovel from the upper, middle and lower parts of the system and then pooled to collect

around 120 g. Duplicate composite samples were collected over a period of 281 d, stored at -20 °C, and used for pesticide analysis and ecotoxicological tests, as described in section 2.3.2.

2.3 Analytical procedures

2.3.1 Extraction and quantification of pesticides

Extraction of pesticides from the BPS samples (biomixture) was carried out with water and acidified acetonitrile (formic acid 1% v/v) as described elsewhere (Chin-Pampillo et al., 2015a), with the modification that extraction mixture (sample, solvents and extraction salts) was shaken during 30 min at 2500 rpm in a multitube vortexer (BenchMixerTM, Benchmark Scientific, NJ, USA). Carbofuran-d3 and linuron-d6 were added as a surrogate and as an internal standard, respectively. Analyses were performed on an Agilent 1290 Infinity LC System (Santa Clara, CA, USA) Ultra High Performance Liquid Chromatograph (UHPLC) coupled to a Agilent 6460 triple quadrupole mass spectrometer. Chromatographic separation conditions are provided in Text S1 of the Supplementary Information. Selected transitions, limits of detection (LOD) and limits of quantification (LOQ) for the analytes are presented in Table S2 of the Supplementary Information. Conditions of the mass spectrometry detector are described elsewhere (Chin-Pampillo et al., 2015b, 2015a); extraction efficiencies were in the range of 70–120% for every compound. When possible, removal data for each compound was modeled according to a first order model (SigmaPlot 11.0) to estimate degradation half–lives (DT50) values.

2.3.2 Ecotoxicological assays

2.3.2.1 Immobilization test on D. magna

Sample elutriates for toxicity tests were prepared according to U.S. Environmental Protection Agency (EPA) protocol (U.S. EPA, 2001). The acute toxicity test of D. magna immobilization was performed using neonates, as described in the methodology EPA-821-R-02-012 (U.S. EPA, 2002). The concentration producing 50% of immobilization in the daphnids (EC50) was calculated with the software ToxCalc™ (Version 3.0, Tidepool Scientific Software, CA, USA) using the binomial method. Toxicity results and their 95% confidence intervals were expressed as toxicity units (TU): $TU = (EC50)^{-1} \times 100$.

2.3.2.2 Seed germination test on *L. sativa*

The phytotoxicity of the BPS biomixture during pesticide treatment was monitored on sample elutriates by seed germination tests with lettuce (L. sativa var. Georgia) (U.S. EPA, 1996). Germination indexes (GI) were determined using triplicate assays as previously described

(Lizano-Fallas et al., 2017). One-way analysis of variance followed by Holm-Sidak multiple comparisons were used to compare the GI respect to the initial GI value at the moment of wastewater disposal in the BPS.

3. Results and discussion

3.1 Removal of triazines, triazoles and organophosphates in biomixtures

The removal of three chemical groups of pesticides was independently assayed in SSB by simultaneous co-application of several compounds per group, for a total of 43 pesticides. The simultaneous elimination of ten triazine herbicides is shown in Figure 1.

Nine out of the ten triazines were removed with DT50 values ranging from 4.7 d to 330 d (Table 1); only prometon failed to exhibit a clear elimination. This could correlate with its slow degradation reported in soils (>500 d) (Lewis et al., 2016). Cyanazine, simazine and atrazine were swiftly degraded with DT50 of 4.7 d, 6.9 d and 7.8 d, respectively. These removal patterns were between 4- and 13-fold faster than those reported in soils (Lewis et al., 2016). Terbuthylazine also showed fast removal (DT50 = 11 d) that ranged from 2– to 7–fold faster than described in soil (Lewis et al., 2016). These four compounds were almost completely eliminated after 62 d of treatment. Atrazine elimination in biomixtures has been widely described; the lowest DT50 values reported were slightly below 10 d (Castro-Gutiérrez et al., 2018; Tortella et al., 2013a, 2013c), although other authors described values ranging from 21 to 24 d (Huete-Soto et al., 2017a, 2017b). Simazine elimination was reported in fungally bioaugmented biomixtures composed of soil and wood chips; however, the efficiency was lower and 27–48% of the removal was achieved in 84 d (Fragoeiro and Magan, 2008). In the case of terbuthylazine, an analogous biomixture removed this compound at a similar rate to that achieved in this work (DT50 = 8.1 d) (Gikas et al., 2018). These biomixtures seem markedly more efficient to remove terbuthylazine than those previously described in the literature, with DT50 of 30 d to > 200 d (Karanasios et al., 2010) and > 99 d (Kravvariti et al., 2010). No scientific reports of biomixture removal were found for cyanazine.

Contrary to the swiftly removed triazines, cyromazine and simetryn showed DT50 values of around 100 d, while values over 200 d were determined for ametryn, prometryn and terbutryn. All of these DT50 were higher than available values reported for dissipation in soil (Lewis et al., 2016). Surprisingly, similar biomixtures have been able to remove ametryn and terbutryn at faster rates, DT50 28-44 d (Cambronero-Heinrichs et al., 2018; Huete-Soto et al., 2017a, 2017b) and 34-51 d (Cambronero-Heinrichs et al., 2018), respectively; furthermore, (Lizano-Fallas et al., 2017) described the deceleration of terbutryn elimination in a compost-based biomixture (from

32% after 20 d to 35% after 60 d). Overall elimination of triazines after 128 d accounted for 59.3% (Figure 2). Microbial degradation of triazines usually takes place via enzyme-catalyzed successive hydrolysis reactions (i.e., a dechlorination, two dealkylations, a ring cleavage, a biuret deamination and an allophanate hydrolysis), where removal of the substituents on the s-triazine ring results in the production of cyanuric acid or melanine an intermediate products (Udikoviç-Koliç et al., 2012; Wackett et al., 2002). Interestingly, in our case, the slower removal occurred in the triazines with a S-containing substituent in the s-triazine ring. However, different degradation pathways of S-triazines have been reported depending on the bacterial strain, which could correlate with the different removal patterns observed. In this regard, Arthrobacter sp. and Nocardioides sp. are able to degrade cyromazine with the formation of N-cyclopropylammeline as the main metabolite, without the production of cyanuric acid or melanine (Hatakeyama et al., 2016). On the other hand, amino acid sequences of TrzN (gene coding for triazine hydrolase) can vary between s-triazine degrading strains, therefore changing their substrate specificities and resulting in different degradation capabilities (Yamazaki et al., 2008).

Simultaneous triazole removal was attempted with 13 compounds at initial concentrations ranging from 5 to 8 mg kg-1 each; however, the biomixture failed to clearly reveal significant elimination of the fungicides (Figure S1); consequently, no net decrease in the total triazole concentration was observed (Figure 2). Triazoles are particularly known for their persistence in the environment; hence, for many compounds their DT50 in soil are usually over 100 d and in some cases reaching values over 300 d (Table 1). Previous treatments in biomixtures have revealed similar results with negligible removal for epoxiconazole, fenbuconazole, triadimenol and tebuconazole, even using fungally bioaugmented matrices in periods from 50 d to 115 d (Huete-Soto et al., 2017b; Murillo-Zamora et al., 2017). Other studies described slow elimination in biomixtures of compounds such as propiconazole (DT50 = 160 d) (Spliid et al., 2006), difenoconazole (75% in 120 d) (Pinto et al., 2016) and epoxiconazole (70% in 254 d) (Fogg et al., 2004). Contrastingly, DT50 values for tebuconazole in biomixtures of different compositions were as short as 19 d and up to 152 d (Delgado-Moreno et al., 2017); similarly a 20–30% removal after 30 d was achieved using biomixtures bacterially bioaugmented (Castillo Diaz et al., 2016). Metabolic biodegradation pathway of tebuconazole by fungi and some bacteria (e.g., Pseudomonas, Enterobacter and Serratia) was reported to include the oxidation of the t-butyl group and opening of the triazole ring (Li et al., 2015; Obanda et al., 2008; Sehnem et al., 2009; Wang et al., 2018).

The simultaneous removal of 20 organophosphates was investigated in the biomixture (Figure 3). Enzymatic hydrolysis of the P-O or P-S bond is the most common biological reaction

for the initial transformation of these compounds (Kumar et al., 2018). Dichlorvos, fenamiphos, fenthion, heptenophos and malathion showed DT50 values below 11 d (Table 1); among these compounds, fenthion (DT50 = 5.9 d) showed accelerated removal with respect to typical values in soil (DT50 = 22–34 d) (Lewis et al., 2016). Chlorpyrifos was the only compound from the organophosphates with DT50 values between 18 d and 100 d that exhibited a clear enhanced removal (DT50 = 56 d) compared to typical values in soil (up to 386 d) (Lewis et al., 2016). Elimination patterns for edifenphos, pirimifos-methyl and triazofos were quite similar to those in soil; however, DT50 values for azinphos-methyl, phoxim and methamidophos increased from 2-fold to 13-fold with respect to field studies (Lewis et al., 2016). Acephate, cadusafos, coumaphos, dimethoate, ethoprophos and monocrotophos were highly persistent in the system with DT50 values over 100 d and in some cases even exceeding 250 d (acephate, coumaphos and and ethoprofos). The elimination of all of these compounds, except coumaphos (DT50 in soil of 152 d), was rather slow compared to their removals in soil that were fairly fast (Lewis et al., 2016). For instance, the reported DT50 values for acephate, dimethoate and monocrotophos were below 10 d (Lewis et al., 2016).

Just less than half of the 20 assayed organophosphates have removals from biomixtures documented in the literature. Similar removal rates to those from this work were determined for azinphos-methyl and methamidophos (Briceño et al., 2014; Lemerhyeratte et al., 2010). A conventional soil/peat/wheat straw matrix was similarly efficient to our matrix in the elimination of fenthion (DT50 = 5.9 d vs. 1.6 d) and malathion (DT50 = 7.1 d vs. 3.8 d) but less efficient removing dichlorvos (DT50 = 1.7 d vs. 15.5 d) (Bozdogan et al., 2014). Elimination of edifenphos was achieved at faster rates in a compost-based biomixture (DT50 = 3.2 d) (Murillo-Zamora et al., 2017); nonetheless, the removal of dimethoate was significantly slower (DT50 = 173 d vs. 5 d) than a previous study (Fogg et al., 2003). Chlorpyrifos, one the most commonly studied pesticides in BPS, had a DT50 within the range of 15–69 d so far reported in diverse matrices (Kravvariti et al., 2010; Tortella et al., 2012; Vischetti et al., 2008). Interestingly, co-disposal of antibiotics of agricultural use (at BPS relevant concentrations) has revealed accelerated mineralization of 14C-chlorpyrifos in compost-based biomixtures (Castillo-González et al., 2017; Huete-Soto et al., 2017a). Total organophosphate removal was continuous throughout the experimental period, reaching 68.5% after 128 d, the highest among the evaluated chemical groups. This global elimination was adjusted to a first order model with a DT50 of 66 d ($r = 0.9713$).

Removal in BPS is usually reported for individual pesticides or mixtures of few active ingredients. In this work, the simultaneous presence of more than ten compounds (also considering the relatively high pesticide concentrations employed) may easily result in a more

intensive affectation of microbial communities (Chu et al., 2008) including degrading populations, thus translating into removal delays for some compounds. The presence of pesticides that are highly toxic to soil microbial communities (such as tebuconazole and chlorpyrifos) hinders their own biodegradation, and consequently, their persistence increases with the application of high concentrations (Muñoz-Leoz et al., 2011). Adverse effects produced by the presence of pesticides in soil include the decrease of viable microbial counts, biomass, diversity and some enzymatic activities, the inhibition of some microbial groups, and changes in community composition (Hussain et al., 2009; Imfeld and Vuilleumier, 2012); nonetheless, opposite results such as enhanced enzymatic activities or stimulation of specific microbial groups have been also reported. In the particular case of this work, the complex mixtures of pesticides employed represent an ecotoxicological scenario in which several synergistic effects might take place (Cedergreen, 2014; Munkegaard et al., 2008), thus translating into the disturbance of microbial communities. This aspect may be critical in the case of fungicides and their deleterious effect on fungal degrading populations (Sigler and Turco, 2002; Smith et al., 2000). Moreover, different DT50 values have been obtained for some pesticides in biomixtures of the same composition, but in different chemical and biochemical environments due to the presence of different pesticide mixtures. Similarly, delays in the removal of some pesticides did not necessarily correlate with more complex pesticide combinations co-applied in the BPS (Huete-Soto et al., 2017a, 2017b).

3.2 Removal of pesticide–containing wastewater in a biopurification system

Wastewater from a laboratory devoted to pesticide residue analysis was treated in a pilot scale BPS. A total of 38 pesticides were detected after wastewater disposal in the BPS (Table 1), including 23 of the 43 compounds studied in the mixtures of section 3.1; the additional 15 compounds included pesticides from diverse chemical families, such as carbamates and neonicotinoids, among others. The initial concentrations ranged from 0.0036 mg kg-1 (cyromazine) to 0.25 mg kg-1 (chlorpyrifos) that, due to the nature of the wastewater, were below than expected concentrations in BPS used for the treatment of wastewaters of agricultural origin (> 5 mg kg-1).

Three of the five triazines detected (ametryn, terbutryn and prometon) decreased their DT50 values with respect to the behavior observed in the system containing the mixture of triazines alone, including prometon that was negligibly removed in that case. On the contrary, terbutryn and cyromazine reduced their removal rates; moreover, the latter compound was not significantly eliminated in the BPS. Total triazine elimination reached 54.3% at the end of the assay (281 d); nonetheless, most of the elimination was already achieved by the day 111 of treatment

(Figure 4). Contrary to the findings in the triazole containing SSB (small scale biomixture system), all of the triazoles detected in the BPS exhibited some degree of removal. DT50 values ranged from 78 d (cyproconazole) to 224 d (myclobutanil and paclobutrazol); triadimefon and tebuconazole, whose removal patterns could not be fitted to a first order model, showed low removal values (< 25%) by the end of the assay after 281 d. Although the efficiency improved with respect to the SSB, elimination was faster than reported in soil only in the case of cyproconazole. In the SSB and another report (Murillo-Zamora et al., 2017), negligible elimination was achieved for epoxiconazole, fenbuconazole, triadimenol and tebuconazole; nonetheless in these cases higher concentrations were applied (from 1.4 mg kg-1 to 15 mg kg-1 each compound), which could hinder microbial removal. Overall, 73.4% of the total triazole concentration was attained in the BPS (Figure 4).

Organophosphates showed an interesting behavior in the BPS; all of the eight detected compounds were mostly eliminated within the first days of treatment. Consequently, data could not be fitted for the whole experimental period, and final removal values are reported as a percentage in Table 1. After 2 d, 87.1% removal was achieved; additional elimination was barely accomplished, and by the end of the monitoring, 90.0% of the total organophosphate concentration was eliminated from the matrix (Figure 4). Therefore, global removal of organophosphates was more efficient at these concentrations in the BPS than in the SSB.

Four carbamates (i.e., carbaril, carbofuran, methiocarb and pirimicarb) were detected and removed in the BPS with DT50 values ranging from 114 d to 347 d. Nonetheless, in every case their removal was slower than reported in soil (DT50 3–86 d) (Lewis et al., 2016). The removal of carbamates in biomixtures has shown promising results, particularly for carbofuran, for which DT50 values as low as 1.6 d (Jiménez-Gamboa et al., 2018) and the elimination of toxic metabolites 3-hydroxycarbofuran and 3-ketocarbofuran (Chin-Pampillo et al., 2015b) have been reported. In the case of methiocarb, previous removal rates in biomixtures were comparable to those in soil; however, the same matrix was also capable to swiftly remove other carbamates such as methomyl and aldicarb (Rodríguez-Rodríguez et al., 2017). Total removal of carbamates after the treatment reached 71.3% (Figure 4). These findings advice against using the biomixture proposed in this work for carbamate removal, or at least not to treat the complex combination of pesticides present in the laboratory–wastewater.

The BPS exhibited slower elimination of other compounds from different chemical groups, with respect to their elimination in soil: bentazone, pyrimethanil, imazalil, diuron and buprofezin (Lewis et al., 2016). Remarkable are the cases of bentazone and propanil, for which negligible elimination was achieved; DT50 for bentazone in soil is < 20 d (Lewis et al., 2016) and 0.5 d in a

biochar-based biomixture (Mukherjee et al., 2016) while propanil DT50 in soil is only 0.4 d (Lewis et al., 2016). For these compounds (besides bentazone), efficient biomixtures (faster removal than in soil) have been described for imazalil (Omirou et al., 2012), diuron (Delgado-Moreno et al., 2017) and buprofezin (Karanasios et al., 2010). Similarly, elimination of pyrachlostrobin (23 % after 281 d) was slower than reported in a biomixture composed of olive leaves and compost (95.5% after 90 d) (Vischetti et al., 2012). On the contrary, the proposed biomixture was efficient to remove several compounds that are particularly persistent in soil, including the neonicotinoids, imidacloprid and thiamethoxam (typical DT50 values of up to 3000 d and 353 d, respectively, in aerobic soil conditions) (Goulson, 2013) and the benzimidazole thiabendazole (typical DT50 500–1000 d) (Lewis et al., 2016). Contrary to other assays in biomixtures that failed to remove imidacloprid and thiamethoxam (Huete-Soto et al., 2017b), or showed very slow elimination (Rodríguez-Castillo et al., 2018), the matrix used in this work achieved DT50 of 58 d and 35 d, respectively. Comparable results (DT50 = 24 d) were obtained in a matrix containing soil, olive pruning and wet olive mill cake (Delgado-Moreno et al., 2017); similarly compost–based biomixtures also succeeded to accelerate thiabendazole elimination (DT50 = 26.2–89.5 d) (Omirou et al., 2012). Although carbendazim elimination profile could not be modeled, it was completely removed by day 89 of treatment. Several reports described efficient elimination (compared to soil) with DT50 of around 10 d in biomixtures (Murillo-Zamora et al., 2017; Tortella et al., 2013b), that was enhanced after repeated applications, reaching DT50 of 2 d or less (Murillo-Zamora et al., 2017; Yu et al., 2009).

3.3 Detoxification of the biopurification system during the treatment of pesticide-containing wastewater

Ecotoxicological analyses were performed to estimate the detoxification of the BPS during the treatment of pesticide–containing laboratory-wastewater. An acute test on D. magna revealed the highest toxicity at the moment of wastewater disposal on the biomixture (2.3 TU) (Table 2).

This toxicity level was relatively low compared to that detected in other biomixtures used for the elimination of wastewater containing insecticides (though at higher concentrations than those initially detected in the BPS). These toxicity levels are expected to cause major affectation on the daphnids since the EC50 for all the insecticides detected (except anilophos and imidacloprid) are in the range of 0.00010–0.5 mg L-1 (Lewis et al., 2016). In the case of fungicide treatment, low toxicity towards this bioindicator was also found in biomixtures at high concentrations (Murillo-Zamora et al., 2017). The treatment in the BPS reduced the toxicity within the first four days and continued decreasing until the end of the assay. Moreover, the toxicity was

< 1 TU by the day 228. Accordingly, this initial decrease could be linked to the removal of organophosphates (mostly insecticides) that underwent extremely fast in the first days of treatment. Similarly, germination tests in L. sativa seeds also showed the highest toxicity at the beginning of the assay (GI = 24.0%). Subsequent analysis during the treatment in BPS revealed significant detoxification in the matrix ($p < 0.001$) at most of the sampling points (Table 2); however, detoxification was not continuous in this case, as indicated by the behavior observed in the GI. Such variations might be due to the transient formation of transformation products and their interactions with residual pesticides (or other transformation products) during the removal process, which might exhibit combinations of cumulative, synergistic or antagonist effects (Cedergreen, 2014; Cedergreen et al., 2008; Munkegaard et al., 2008) that are difficult to describe or predict considering the complexity of the pesticides mixture treated in the BPS. Decrease in toxicity may be ascribed to the elimination of the parent pesticides but also to their strongest adsorption to the components of the biomixture during the aging of the system, which translates into decreased bioavailability, particularly for pesticides of slow degradation (Castillo et al., 2008).

Conclusions

The BPS was able to remove 77.2% of the pesticides contained in the laboratory-wastewater after 281 d of treatment; nonetheless, the most significant elimination was already achieved in 89 d (62.5%). Moreover, detoxification of the matrix, though incomplete, was demonstrated towards two bioindicators. Contrary to the behavior observed in the SSB, the treatment in BPS was successful to significantly remove triazoles, probably due to the lower concentrations applied in the system. Similarly, accelerated organophosphate elimination was achieved and only 12.9% of their total concentration remained in the BPS. On the other hand, the removal of triazines was less efficient in the BPS compared to the SSB; likewise, the elimination of carbamates was much slower than typically reported in biomixtures. The differences in the removal observed in the SSB versus the BPS might be related to the lower concentrations applied in the latter (which likely exerted lower toxicity on degrading microbiota), but also to the different combination of pesticides, which might partially explain the delayed elimination of triazines and carbamates in the BPS. In this respect, different removal patterns have been described for the same pesticides under different chemical environments depending on the co-applied compounds in the same biomixture. The findings from this work suggest that disposal of pesticide–containing laboratory–wastewater should be done in separate containers according to chemical groups before their treatment in separate BPS (even though triazoles and organophosphates can be co-disposed in the biomixture used in this work), in order to reduce treatment periods; such BPS

should ideally contain different optimized biomixtures aimed at the removal of specific target pesticide groups or usual pesticide combinations. The authors strongly suggest the monitoring of the treatment process in the BPS with a battery of ecotoxicological tests.

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Figuras y tablas del artículo del Capítulo II

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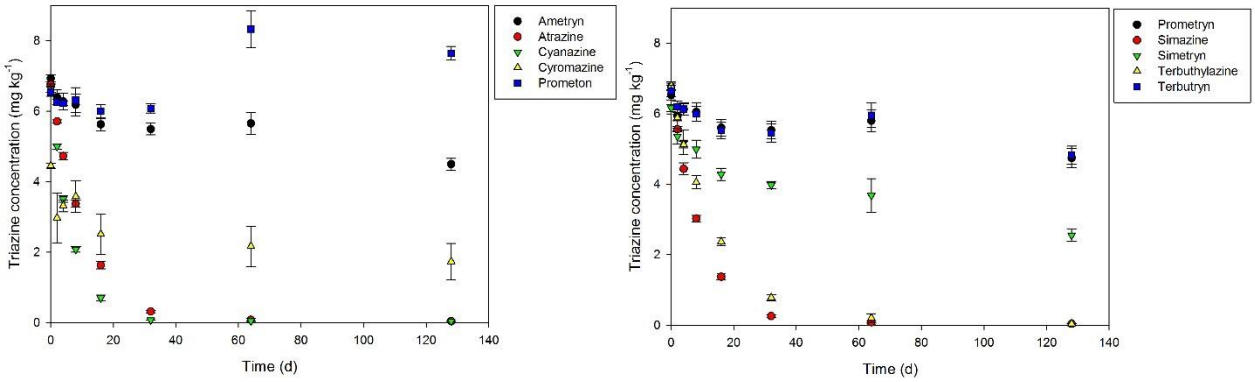

Figure 1. Removal profile of ten triazine pesticides during 128 d after their simultaneous application in a biomixture. Values plotted are means ± SD for triplicate systems.

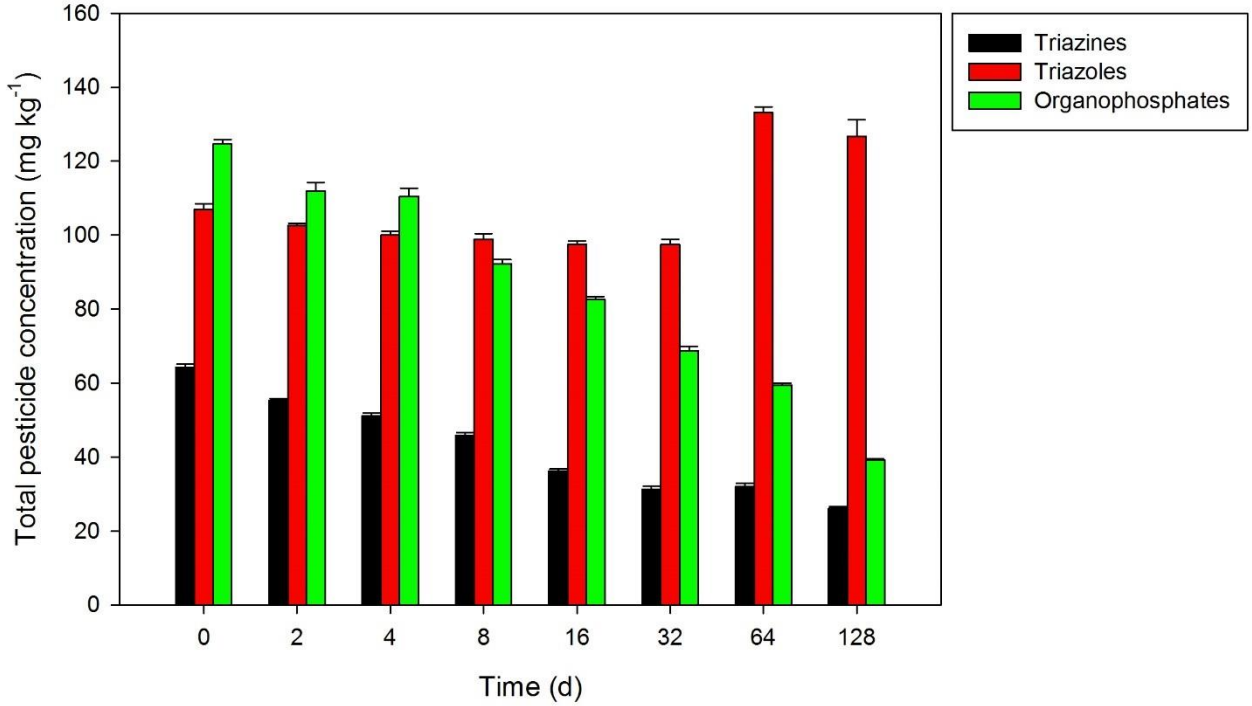

Figure 2. Total removal of organophosphate, triazine and triazole pesticides in a biomixture during individual treatment of each chemical group. Values plotted are means ± SD for triplicate systems.

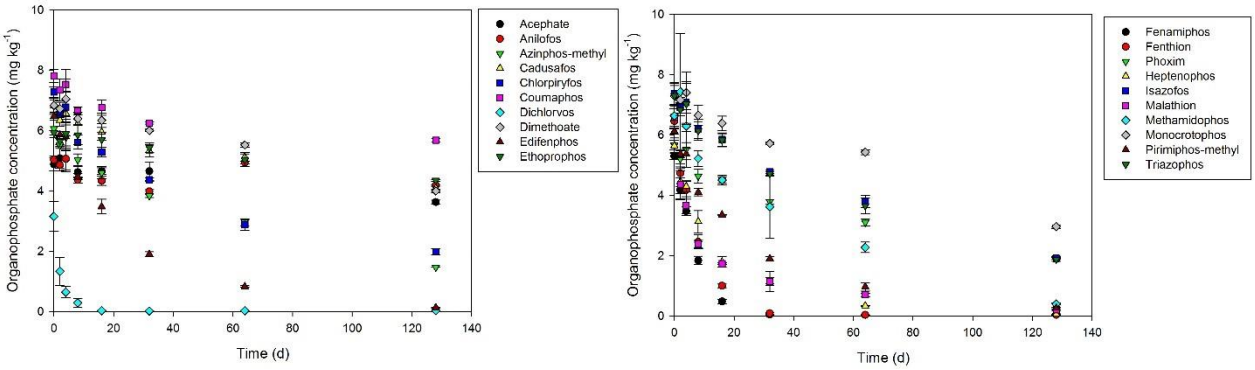

Figure 3. Removal profile of twenty organophosphate pesticides during 128 d after their simultaneous application in a biomixture. Values plotted are means ± SD for triplicate systems.

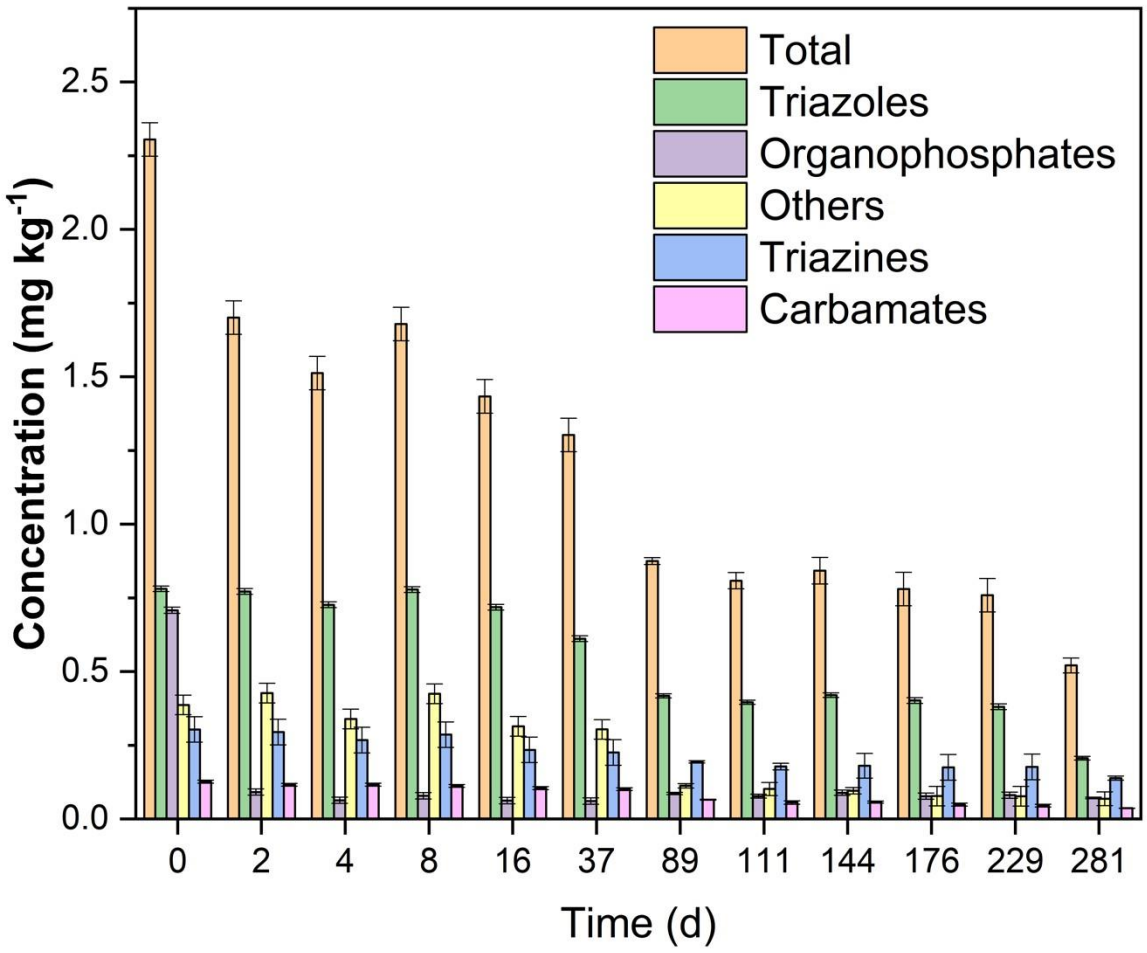

Figure 4. Elimination of organophosphates, triazines, triazoles, carbamates and others from laboratory–wastewater, during simultaneous treatment in a 140 L BPS with a biomixture of coconut fiber-compost-soil (2:1:1 v/v).

Table 1. Removal of triazines, triazoles and organophosphates in SSB and removal of pesticides from laboratory-wastewater in a BPS. When DT_{50} could not be estimated by fitting to a first order model in the BPS, removal values at the end of the treatment are reported. Removal values reported in soil (Lewis et al., 2016) and other BPS are included for the pesticides employed in this work.

	Removal in SSB			Removal in BPS					Removal in soil (according to PPDB [a])			Removal reported in other BPS			
Compound	**r**	**k (d^{-1})**	**DT_{50} (d)**	**Cn_i (mg kg^{-1})**	**r**	**k (d^{-1})**	**DT_{50} (d)**	**Removal after 281 d (%)**	**DT_{50} typical (d)**	**DT_{50} lab 20°C (d)**	**DT_{50} field (d)**	**Biomixture**	**DT_{50} (d)**	**Removal (%) (after X d)**	**Reference**
Triazines															
Ametryn	0.9115	0.0030	231	0.18	0.9327	0.0047	147	NC	37	60	37	Coconut fiber, compost, soil (45:13:42)	40.3	NA	(Huete-Soto et al., 2017b)
Atrazine	0.9998	0.0889	7.8	ND	NC	NC	NC	NC	75	66	29	Coconut fiber, compost, soil (45:13:42)	<10		(Castro-Gutiérrez et al., 2018; Tortella et al., 2013b, 2013a)
Cyanazine	0.9995	0.1485	4.7	ND	NC	NC	NC	NC	16	16	NA	NA	NA	NA	NA
Cyromazine	0.8167	0.0068	102	0.0036	NC	NC	NC	0.0	93	31.8	9.7	NA	NA	NA	NA
Prometon	NC	NC	NC	0.049	0.9526	0.0042	165.0	NC	500	932	NA	NA	NA	NA	NA
Prometryn	0.9105	0.0021	330	ND	NC	NC	NC	NC	41	41	NA	NA	NA	NA	NA
Simazine	0.9999	0.1009	6.9	ND	NC	NC	NC	NC	60	NA	90	Soil-woodchips; FB (*Phanerochaete chrysosporium*) Soil-woodchips; FB (*Trametes versicolor*)		34-38 (84) 27-48 (84)	(Fragoeiro and Magan, 2008)
Simetryn	0.9330	0.0066	105	0.038	0.9256	0.0038	182.4	NC	60	NA	NA	NA	NA	NA	NA
Terbuthylazine	0.9997	0.0657	11	ND	NC	NC	NC	NC	75.1	75.1	22.4	Coconut fiber, compost, soil (45:13:42) Coconut fiber, compost, soil (45:13:42); FB (*Trametes versicolor*)	8.1 NA	NA 30.4 (20)	(Gikas et al., 2018) (Lizano-Fallas et al., 2017)
Terbutryn	0.8682	0.0022	315	0.029	0.9396	0.0037	187.3	NC	74	74	52	Coconut fiber, compost , soil (45:13:42); FB (*Trametes versicolor*)	34-51		(Cambronero-Heinrichs et al., 2018)
Triazoles															
Bitertanol	NC	NC	NC	ND	NC	NC	NC	NC	23	8.5	23	NA	NA	NA	NA
Cyproconazole	NC	NC	NC	0.042	0.9444	0.0089	77.1	NC	142	142	129	NA	NA	NA	NA

Difenoconazole	NC	NC	NC	ND	NC	NC	NC	NC	130	130	85	Cork-biomixture; FB (*Lentinula edodes*)	NA	75 (120)	(Pinto et al., 2016)
Epoxiconazole	NC	NC	NC	0.041	0.9245	0.0037	187.3	NC	354	226	120	Topsoil, peat-free compost, barley straw (1:1:2)	61 or 24.4 (2nd application)	NA	(Fogg et al., 2003)
												Topsoil, peat-free compost, barley straw (1:1:2)	NA	70 (254)	(Fogg et al., 2004)
Fenbuconazole	NC	NC	NC	0.058	0.9364	0.0058	119.5	NC	60	152	61	Coconut fiber, compost, soil (45:13:42); FB (*Trametes versicolor*)		No removal	(Murillo-Zamora et al., 2017)
Flusilazole	NC	NC	NC	ND	NC	NC	NC	NC	300	427	94	NA	NA	NA	NA
Hexaconazole	NC	NC	NC	0.017	0.9075	0.0037	187.3	NC	122	NA	225	NA	NA	NA	NA
Myclobutanil	NC	NC	NC	0.14	0.9317	0.0031	223.6	NC	560	365	35	NA	NA	NA	NA
Paclobutrazol	NC	NC	NC	0.019	0.8219	0.0031	223.6	NC	112	120	29.5	NA	NA	NA	NA
Propiconazole	NC	NC	NC	0.090	0.9295	0.0044	157.5	NC	71.8	71.8	35.2	Chopped wheat straw, *Sphagnum* (moss), soil (2:1:1)	160	NA	(Spliid et al., 2006)
Triadimefon	NC	NC	NC	0.027	NC	NC	NC	19.4	26	NA	NA	NA	NA	NA	NA
Triadimenol	NC	NC	NC	0.021	0.9112	0.0051	135.9	NC	250	136.7	64.9	Coconut fiber, compost, soil (45:13:42)		No removal	(Murillo-Zamora et al., 2017)
Tebuconazole	NC	NC	NC	0.057	NC	NC	NC	25.0	63	365	47.1	Soil, vermicompost, tomato wastes (1:2:1); FB (*Acremonium* sp.)	NA	20-30 (30)	(Castillo Diaz et al., 2016)
												Soil, olive tree pruning, raw wet olive cake (1:2:1)	19	NA	(Delgado-Moreno et al., 2017)
Organophosphates															
Acephate	0.9556	0.0024	289	ND	NC	NC	NC	NC	3	NA	3	NA	NA	NA	NA
Anilofos	NC	NC	NC	0.023	NC	NC	NC	50.9	38	NA	NA	NA	NA	NA	NA
Azinphos-methyl	0.9900	0.0112	62	ND	NC	NC	NC	NC	10	31	NA	Straw, peat, soil (2:1:1)		89 (120)	(Briceño et al., 2014)
Cadusafos	0.9678	0.0039	178	0.17	NC	NC	NC	96.7	38	56.7	39	NA	NA	NA	NA
Chlorpyrifos	0.9772	0.0123	56	0.25	NC	NC	NC	97.2	386	386	27.6	Coconut fiber, compost, soil (45:13:42)		75.3 (20)	(Lizano-Fallas et al., 2017)
Coumaphos	0.8368	0.0022	315	ND	NC	NC	NC	NC	152	NA	NA	NA	NA	NA	NA
Dichlorvos	0.9979	0.4003	1.7	ND	NC	NC	NC	NC	2	2	NA	Soil, peat, wheat straw (1:1:2)	15.5	NA	(Bozdogan et al., 2014)
Dimethoate	0.9829	0.0040	173	ND	NC	NC	NC	NC	2.5	2.5	7.2	Topsoil, peat-free compost; wheat straw (1:1:2)	5	NA	(Fogg et al., 2003)
Edifenphos	0.9972	0.0375	18	ND	NC	NC	NC	NC	21	NA	NA	Coconut fiber, compost, soil (45:13:42); FB (*Trametes versicolor*)	3.2	NA	(Murillo-Zamora et al., 2017)

Ethoprophos	0.9853	0.0024	289	0.062	NC	NC	NC	89.7	13.6	13.6	1.3	NA	NA	NA	NA
Fenamiphos	0.9980	0.1299	5.3	ND	NC	NC	NC	NC	0.85	0.85	1.8	NA	NA	NA	NA
Fenthion	0.9983	0.1174	5.9	ND	NC	NC	NC	NC	22	34	NA	Soil, peat; wheat straw (1:1:2)	1.6	NA	(Bozdogan et al., 2014)
Phoxim	0.9797	0.0088	79	0.053	NC	NC	NC	90.5	6	NA	NA	NA	NA	NA	NA
Heptenophos	0.9925	0.0621	11	ND	NC	NC	NC	NC	1.4	NA	1.4	NA	NA	NA	NA
Isazofos	0.9921	0.0107	65	0.040	NC	NC	NC	89.5	40	34	NA	NA	NA	NA	NA
Malathion	0.9611	0.0977	7.1	ND	NC	NC	NC	NC	0.17	0.17	1	Soil, peat; wheat straw (1:1:2)	3.8	NA	(Bozdogan et al., 2014)
Methamidophos	0.9805	0.0203	34	ND	NC	NC	NC	NC	3.5	4	4	Soil, bleaching earth, straw (700g: 300g: 50g)		60 (42)	(Lemerhyeratte et al., 2010)
Monocrotophos	0.9778	0.0063	110	ND	NC	NC	NC	NC	7	NA	30	NA	NA	NA	NA
Pirimiphos-methyl	0.9940	0.0347	20	0.084	NC	NC	NC	66.4	39	12	39	NA	NA	NA	NA
Triazophos	0.9929	0.0109	64	0.027	NC	NC	NC	87.0	44	44	9	NA	NA	NA	NA
Carbamates															
Carbaril	NT	NT	NT	0.019	0.9298	0.0038	182.4	NC	16	16	NA	NA	NA	NA	NA
Carbofuran	NT	NT	NT	0.051	0.9906	0.0061	113.6	NC	29	12.8	14	Coconut fiber, compost, soil (45:13:42)	1.6	NA	(Jiménez-Gamboa et al., 2018)
Methiocarb	NT	NT	NT	0.045	0.9561	0.0058	119.5	NC	2.94	2.94	35.2	Rice husk, compost, soil (30:43:27); FB (*Trametes versicolor*)	6.5	NA	(Rodríguez-Rodríguez et al., 2017)
Pirimicarb	NT	NT	NT	0.012	0.9161	0.002	346.6	NC	86	86	9	Chopped wheat straw, *Sphagnum* (moss), soil (2:1:1)	93	NA	(Spliid et al., 2006)
Other															
Bentazone	NT	NT	NT	0.002	NC	NC	NC	0.0	20	20	7.5	Digestate, biochar, topsoil (5%: 5%: 90%)	0.5	NA	(Mukherjee et al., 2016)
Buprofezin	NT	NT	NT	0.012	0.8648	0.002	346.6	NC	50	135.4	45.6	Olive leaves, olive leaves compost, soil (2:1:1)	30-50	NA	(Karanasios et al., 2010)
Carbendazin	NT	NT	NT	0.033	NC	NC	NC	100.0	40	34.3	22	Soil, peat, wheat straw (1:1:2)	6.2	NA	(Tortella et al., 2013b)
Diuron	NT	NT	NT	0.037	0.89	0.0042	165.0	NC	75.5	75.5	89	Soil, peat, straw (1:1:2)	6	NA	(Delgado-Moreno et al., 2017)
Imazalil	NT	NT	NT	0.019	0.9364	0.0035	198.0	NC	76.3	76.3	6.4	Topsoil, compost of grape vine prunings, Straw (1:1:2)	15.5	NA	(Omirou et al., 2012)
Imidacloprid	NT	NT	NT	0.079	0.985	0.0119	58.2	NC	191	187	174	Soil, pruning, raw wet olive cake (1:2:1)	24	NA	(Delgado-Moreno et al., 2017)
Propanil	NT	NT	NT	0.0081	NC	NC	NC	13.2	0.4	0.4	NA	NA	NA	NA	NA

Pyraclostrobin	NT	NT	NT	0.015	NC	NC	NC	23.0	32	62	32	Pruning residues 2 years, pruning residues 5 years, fresh wheat straw (40%: 40% 20%)	NA	95.5 (90)	(Vischetti et al., 2012)
Pyrimethanil	NT	NT	NT	0.013	0.8921	0.0022	315.1	NC	55	55	29.5	Biochar, topsoil (1%: 99%)	65	NA	(Mukherjee et al., 2016)
Thiabendazole	NT	NT	NT	0.14	0.9465	0.0196	35.36	NC	500	1000	724	Topsoil, compost of winery by products, straw (1:2:1)	26.2	NA	(Omirou et al., 2012)
Thiamethoxan	NT	NT	NT	0.034	0.9939	0.0196	35.36	NC	50	121	39	Coconut fiber, compost, soil (45:13:42)		No removal	(Huete-Soto et al., 2017b)

[a] Pesticide Properties Data Base, University of Hertfordshire (Lewis et al., 2016)
SSB: small scale biomixture system; BPS: biopurification system
Cn_i: initial concentration detected in the BPS after wastewater disposal
FB: fungal bioaugmentation
NA: data not available
NC: not calculated
ND: not detected in the BPS
NT: not treated in SSB

Table 2. Ecotoxicological monitoring of a BPS during the treatment of laboratory-wastewater, as determined by acute toxicity tests on *D. magna* and germination tests in *L. sativa*. Significant difference ($P < 0.001$) in the GI with respect to the value at time zero is denoted by "*".

Time of treatment (d)	EC_{50} 48 h, % (Toxic units, TU)	Germination Index, GI (%)
0	43.8 (2.3)	24.0 ± 0.7
4	70.7 (1.4)	40.4 ± 5.5 *
8	73.2 (1.4)	55.9 ± 7.9 *
16	66.5 (1.5)	43.0 ± 8.1 *
38	92.3 (1.1)	56.5 ± 5.3 *
88	70.7 (1.4)	31.5 ± 0.6
110	70.7 (1.4)	37.5 ± 2.3 *
143	76.3 (1.3)	46.0 ± 3.2 *
175	75.2 (1.3)	34.8 ± 7.6
228	> 100 (< 1)	49.2 ± 8.9 *
281	> 100 (< 1)	54.3 ± 5.9 *

Información suplementaria del artículo del Capítulo II

Validation of a methodology by LC-MS/MS for the determination of triazine, triazole and organophosphorus pesticide residues in biomixtures

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Text S1. Analytical procedure

Chromatographic separation was done at 40 °C by injecting 6 µL samples in a Poroshell 120 EC-C18 column (100 mm x 2.1 mm i.d., particle size 2.7 µm), and using acidified water (formic acid 0.1% v/v, A) and acidified methanol (formic acid 0.1% v/v, B) as mobile phases. The mobile phase flow was 0.3 mL min^{-1} at the following conditions: 30% B for 3 min, followed by a 15 min linear gradient to 100% B, 4 min at 100% B and 0.1 min gradient back to 30% B, followed by 4 min at initial conditions.

Table S1. Characterization of the pesticide content in the wastewater of laboratory origin before its treatment in a 140 L BPS.

Compound	Substance group	Concentration (µg L^{-1})	LOD (µg L^{-1})	LOQ (µg L^{-1})
3-ketocarbofuran	Carbamate metabolite	4.803	0.009	0.018
3-hydroxycarbofuran	Carbamate metabolite	9.747	0.009	0.0018
Acephate	Organophosphate	0.040	0.028	0.055
Acetamiprid	Neonicotinoid	<0.009	0.009	0.017
Aldicarb	Carbamate	<0.009	0.009	0.018
Amethryn	Triazine	76.64	0.011	0.022
Amitraz	Amidine	<0.15	0.15	0.25
Anilophos	Organophosphate	<0.025	0.025	0.050
Atrazine	Triazine	0.110	0.011	0.021
Azinphos-methyl	Organophosphate	0.973	0.046	0.090
Azoxystrobin	Strobilurin	1.223	0.009	0.018
Bentazone	Benzothiazinone	3.970	0.014	0.028
Bitertanol	Triazole	3.149	0.009	0.019
Bromacil	Uracil	11.544	0.075	0.140
Buprofezin	Unclassified	5.564	0.008	0.015
Cadusafos	Organophosphate	2.563	0.014	0.028
Carbaryl	Carbamate	15.744	0.013	0.026
Carbendazim	Benzimidazole	10.659	0.006	0.013
Carbofuran	Carbamate	7.867	0.006	0.012
Chlorpyrifos	Organophosphate	0.315	0.040	0.079
Clethodim	Cyclohexanedione	<0.015	0.015	0.030
Coumaphos	Organophosphate	0.081	0.030	0.058
Cyanazine	Triazine	<0.013	0.013	0.026
Cymoxanil	Cyanoacetamide oxime	0.454	0.010	0.020
Cyproconazole	Triazole	0.117	0.012	0.024
Cyromazine	Triazine	<0.014	0.014	0.028
Dichlofluanid	Sulphamide	<0.033	0.033	0.064
Dichlorvos	Organophosphate	<0.017	0.017	0.034
Difenoconazole	Triazole	<0.009	0.009	0.018
Dimethoate	Organophosphate	3.932	0.003	0.007
Dimethomorph	Morpholine	2.068	0.029	0.057
Diuron	Phenylamide	21.656	0.010	0.021
Edifenphos	Organophosphate	<0.011	0.011	0.022
Epoxiconazole	Triazole	4.015	0.008	0.015
Ethoprophos	Organophosphate	0.165	0.017	0.034
Etofenprox	Pyrethroid	<0.011	0.011	0.021
Fenamiphos	Organophosphate	<0.009	0.009	0.018

Fenarimol	Pyrimidine	<0.029	0.029	0.057
Fenbuconazole	Triazole	34.737	0.014	0.028
Fenoprop	Phenoxipropionic acid	<0.183	0.183	0.347
Fenthion	Organophosphate	<0.052	0.052	0.100
Fipronil	Phenylpyrazole	0.435	0.025	0.049
Fluazifop-P-butyl	Aryloxyphenoxypropionate	1.044	0.011	0.023
Flusilazole	Triazole	<0.011	0.011	0.021
Haloxyfop	Aryloxyphenoxypropionate	<0.193	0.193	0.371
Haloxyfop-P-methyl	Aryloxyphenoxypropionate	<0.012	0.012	0.023
Heptenophos	Organophosphate	<0.014	0.014	0.028
Hexaconazole	Triazole	5.946	0.012	0.023
Hexazinone	Triazinone	<0.010	0.010	0.019
Imazalil	Imidazole	15.338	0.008	0.016
Imazapic	Imidazolinone	8.221	0.008	0.017
Imazapyr	Imidazolinone	3.605	0.009	0.018
Imidacloprid	Neonicotinoid	57.580	0.009	0.018
Isazofos	Organophosphate	0.050	0.008	0.016
Isoproturon	Urea	3.202	0.010	0.019
Kresoxim-methyl	Strobilurin	1.947	0.027	0.052
Linuron	Urea	<0.006	0.006	0.012
Malathion	Organophosphate	<0.033	0.033	0.064
Metalaxyl	Phenylamide	7.531	0.012	0.023
Methamidophos	Organophosphate	<0.013	0.013	0.026
Methiocarb	Carbamate	14,609	0.010	0.020
Methomyl	Carbamate	<0.012	0.012	0.024
Metribuzin	Triazinone	<0.011	0.011	0.022
Metsulfuron-methyl	Sulfonylurea	<0.019	0.019	0.038
Molinate	Thiocarbamate	<0.011	0.011	0.021
Monocrotophos	Organophosphate	<0.008	0.008	0.016
Monuron	Phenylurea	<0.009	0.009	0,019
Myclobutanil	Triazole	1.523	0.007	0.015
Oxamyl	Carbamate	<0.010	0.010	0.019
Paclobutrazol	Triazole	0.101	0.012	0.024
Pendimethalin	Dinitroaniline	<0.017	0.017	0.033
Phoxim	Organophosphate	<0.029	0.029	0.056
Pirimicarb	Carbamate	6.779	0.009	0.018
Pirimiphos-methyl	Organophosphate	<0.012	0.012	0.024
Prochloraz	Imidazole	0.421	0.005	0.009
Prometon	Triazine	36.589	0.010	0.020
Prometryn	Triazine	1.657	0.011	0.021
Propanil	Anilide	3.198	0.054	0.102
Propiconazole	Triazole	66.501	0.010	0.020
Pyraclostrobin	Strobilurin	14.752	0.017	0.034
Pyrimethanil	Anilinopyrimidine	3.057	0.010	0.020
Pyriproxyfen	Unclassified	<0.031	0.031	0.061
Simazine	Triazine	0.144	0.014	0.028
Simetryn	Triazine	37.784	0.009	0.018
Tebuconazole	Triazole	36.486	0.012	0.024
Teflubenzuron	Benzoylurea	<0.011	0.011	0.022
Terbuthylazine	Triazine	<0.008	0.008	0.016
Terbutryn	Triazine	9.796	0.011	0.022
Thiabendazole	Benzimidazole	23.407	0.006	0.013
Thiamethoxam	Neonicotinoid	23.105	0.012	0.024
Thiophanate	Benzimidazole	<0.013	0.013	0.025
Thiophanate-methyl	Benzimidazole	<0.009	0.009	0.017
Triadimefon	Triazole	6.553	0.008	0.016

Triadimenol	Triazole	0.286	0.011	0.022
Triazophos	Organophosphate	0.043	0.008	0.016
Tridemorph	Morpholine	<0.081	0.081	0.159
Triflumuron	Benzoylurea	<0.014	0.014	0.028

Table S2. Selected transitions and other parameters in the detection of pesticides in the biomixture (SSB and BPS), using the dynamic multiple reaction monitoring (dMRM) method.

Compound	Transition		Fragmentor (V)	Collision energy (V)	Retention time (min)	Type of transition	LOD (µg kg $^{-1}$)	LOQ (µg kg^{-1})
	Precursor ion	Product ion						
Acephate	184	143 95	60	5 25	0.9	Q q	6	12
Amethryn	228	186 96	106	17 25	8.05	Q q	6	12
Anilophos	368	199 171	70	10 15	13.58	Q q	3	7
Atrazine	216	174 96	106	17 25	9.42	Q q	8	17
Azinphos-methyl	318	132 125	60	13 17	10.65	Q q	10	20
Bentazone [a]	239	132 197	116	25 17	8.14	Q q	3	7
Bitertanol	338	99 269	82	13 5	14.05	Q q	10	21
Buprofezin	306	201 116	94	9 13	14.41	Q q	7	15
Cadusafos	271	159 131	70	5 20	14.32	Q q	9	18
Carbaryl	202	145 127	60	9 29	8.34	Q q	10	19
Carbendazim	192	160 132	94	17 33	1.55	Q q	6	14
Carbofuran	222	123 165	82	21 9	7.67	Q q	6	12
Chlorpyrifos	350	97 198	90	30 15	15.74	Q q	18	36
Coumaphos	363	227 307	116	25 13	13.67	Q q	14	28
Cyanazine	241	214 104	100	15 30	7.05	Q q	6	13
Cyproconazole	292	70 125	110	15 30	12.42	Q q	9	18
Cyromazine	167	85 60	104	17 21	0.87	Q q	8	16
Dichlorvos	221	109 79	104	13 29	7.40	Q q	5	10
Difenoconazole	406	251 337	126	25 13	14.42	Q q	8	16
Dimethoate	230	199 125	70	3 20	3.45	Q q	5	10

Diuron	233	72 160	90	20 25	10.05	Q q	6	12
Edifenphos	311	111 283	90	20 10	13.46	Q q	8	16
Epoxiconazole	330	121 101	106	25 40	12.75	Q q	6	12
Ethoprophos	243	97 131	84	33 17	12.52	Q q	6	12
Fenamiphos	304	217 234	138	21 13	13.13	Q q	3	7
Fenbuconazole	337	70 125	116	17 40	12.95	Q q	6	12
Fenthion	279	247 105	104	9 25	13.83	Q q	20	38
Flusilazole	316	165 247	110	25 15	13.14	Q q	6	12
Hexaconazole	314	70 159	116	21 33	13.85	Q q	6	12
Heptenophos	251	127 109	110	10 30	10.23	Q q	5	10
Isazofos	314	120 162	94	29 13	12.25	Q q	5	10
Imazalil	297	159 69	116	21 17	8.68	Q q	7	15
Imidacloprid	256	209 175	72	13 17	2.55	Q q	8	17
Malathion	331	99 127	82	25 9	11.83	Q q	12	25
Methamidophos	142	94 125	90	10 10	0.98	Q q	4	8
Methiocarb	226	121 169	72	17 5	11.32	Q q	14	27
Monocrotophos	224	127 193	62	13 5	1.85	Q q	3	6
Myclobutanil	289	70 125	106	17 37	11.86	Q q	7	15
Paclobutrazol	294	70 125	110	15 35	11.81	Q q	9	18
Phoxim	321	192 115	94	9 21	13.88	Q q	23	45
Pirimicarb	239	72 182	104	21 13	3.38	Q q	9	18
Pirimiphos-methyl	306	164 108	90	20 30	13.38	Q q	9	18
Prometon	226	142	116	21	7.15	Q	8	17

		184		17		q		
Prometryn	242	200	126	17	9.71	Q	3	7
		158		21		q		
Propanil	218	162	110	10	11.02	Q	14	27
		127		25		q		
Propiconazole	342	159	126	29	13.61	Q	5	10
		69		21		q		
Pyraclostrobin	388	194	94	9	13.90	Q	8	17
		163		25		q		
Pyrimethanil	200	107	138	25	8.35	Q	5	10
		82		29		q		
Simazine	202	124	106	17	7.42	Q	4	7
		104		25		q		
Simetryn	214	124	106	17	6.08	Q	15	29
		96		25		q		
Tebuconazole	308	70	106	21	13.55	Q	9	19
		125		40		q		
Terbuthylazine	230	174	104	13	11.33	Q	3	6
		96		29		q		
Terbutryn	242	186	96	17	9.83	Q	7	15
		91		29		q		
Thiabendazole	202	175	138	25	1.85	Q	18	35
		131		37		q		
Thiamethoxam	292	211	82	9	1.73	Q	5	10
		181		21		q		
Triadimefon	294	197	94	13	11.74	Q	9	18
		69		21		q		
Triadimenol	296	70	72	9	12.35	Q	6	13
		99		13		q		
Triazophos	314	162	100	15	12.25	Q	5	11
		119		35		q		
Linuron-d_6 (i.s.)	255	160	92	17	11.12	Q	NA	NA
		185		13		q		
Carbofuran-d_3 (s.s.)	225	165	86	9	7.67	Q	NA	NA
		123		21		q		

[a] Measured in negative mode
Q: quantification transition, q: qualifier transition
i.s.: internal standard; s.s.: surrogate standard

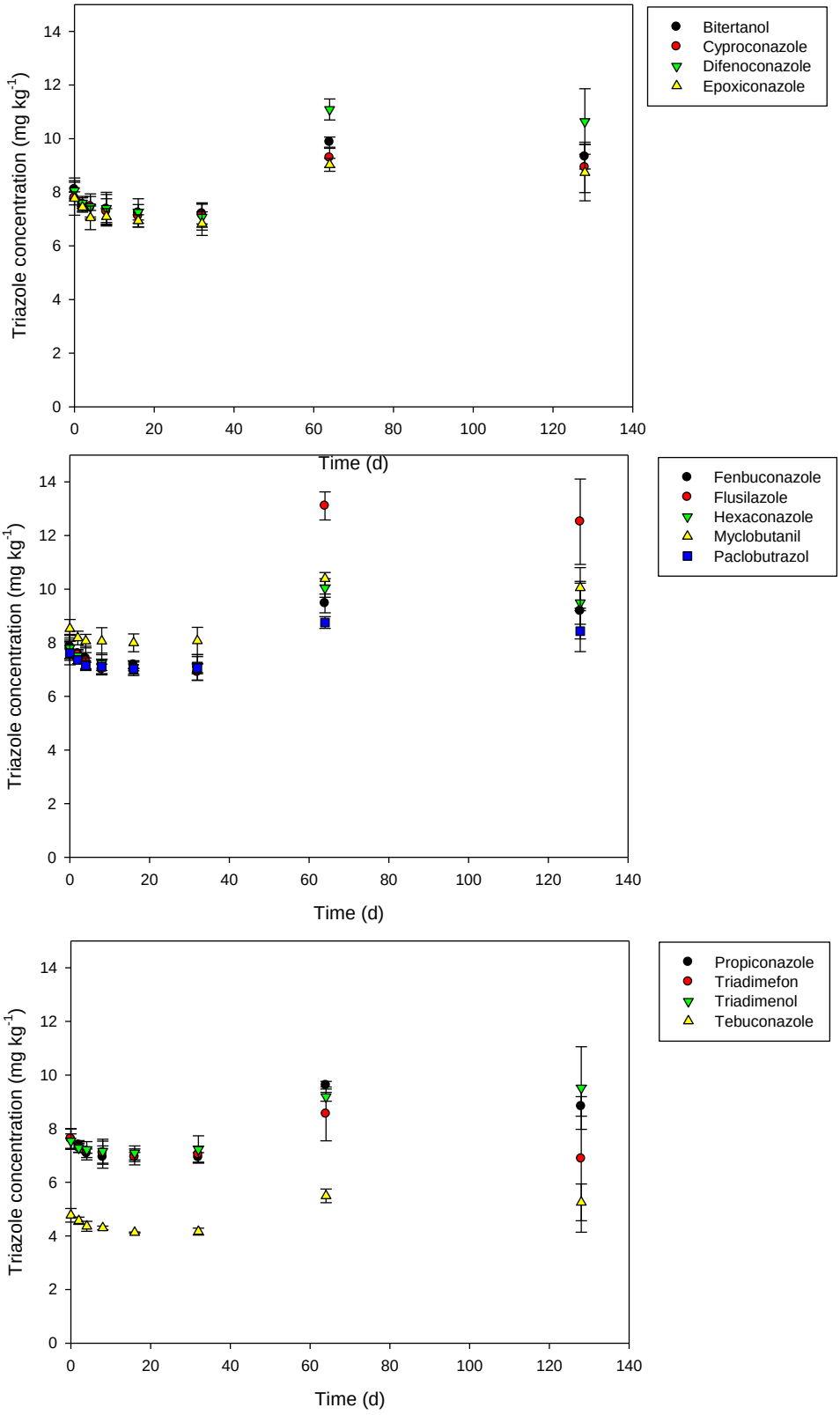


Figure S1. Concentration profile of triazole pesticides in a biomixture during simultaneous application. Values plotted are means ± SD for triplicate systems.

CONCLUSIONES Y RECOMENDACIONES

Las etapas de la investigación comprendieron la optimización de metodología de extracción, optimización en el equipo de detección, determinación de la degradación de las moléculas de estudio en la biomezcla y la evaluación de su funcionamiento con mezclas de formulados de plaguicidas y desechos de laboratorio con contenido de residuos de plaguicidas.

Con la optimización de la metodología de análisis para la determinación de las moléculas de estudio, se demostró que al adicionar una mayor cantidad de agua y de sulfato de magnesio a la muestra sólida, se da una mejor extracción de los plaguicidas (mayores porcentajes de recuperación); además la metodología propuesta puede aplicarse en condiciones en las que se cuente con un equipo automatizado de agitación o en el caso de que se deba hacer manual, ya que no se encontraron diferencias significativas al aplicar ambas variaciones metodológicas. Esto es beneficioso porque amplía el número de laboratorios en los que se puede replicar la metodología, independiente de contar o no con el equipo automatizado de agitación.

Una vez optimizados algunas condiciones analíticas de la metodología de extracción, se validó bajo los parámetros de mérito de especificidad, selectividad, límite de detección, límite de cuantificación, linealidad, precisión, veracidad, robustez, efecto matriz y ámbito de trabajo. La metodología propuesta presentó resultados satisfactorios para la mayoría de las moléculas evaluadas, con respecto a los criterios de aceptación de estos parámetros. Las excepciones fueron ciromazina y diclorvos, que presentaron valores de porcentaje de recuperación inferiores a 70% y de la desviación estándar relativa mayores de 20 %. Para el caso de estas dos moléculas, se concluye que no se cumple con los criterios de aceptación de precisión y veracidad indicados para esta metodología, por lo que se recomienda evaluar una metodología alternativa para su cuantificación en la matriz de estudio.

Se detectaron cuatro etapas de la metodología que deben considerarse críticas y de cuidado en su ejecución, ya que pueden causar una variabilidad en los resultados de detección de algunas moléculas. Los factores identificados fueron el tiempo de contacto con el agua, la agitación con acetonitrilo, el tiempo de centrifugación y la temperatura del baño-maría, por lo tanto, estos parámetros deben aplicarse tal y como se hizo la validación, para evitar pérdidas de las moléculas.

Se concluye que la metodología propuesta para la determinación de plaguicidas en la biomezcla de estudio es una metodología robusta, precisa y exacta, la cual se puede trabajar con valores de límite de detección y de cuantificación de 5 μg/kg y 10 μg/kg respectivamente, con la característica de ser una metodología específica y selectiva, con una linealidad superior a un coeficiente de determinación de 0,99 y en donde el efecto matriz se obtuvo cercano o inferior al 10 %, gracias al cambio de disolvente y la consecuente disminución de los co-extractos.

Con la metodología validada, se evaluó el comportamiento de degradación de los plaguicidas triazinas, triazoles y organofosforados (un total de 43 moléculas), para tres tipos de sistema: i. biomezcla a pequeña escala (SSB), bajo condiciones de temperatura y humedad controlada; ii. para una biomezcla utilizada en un sistema de purificación escala piloto, para el tratamiento de aguas residuales de un laboratorio; y iii. una biomezcla utilizada en un sistema de purificación escala piloto para el tratamiento de formulaciones de plaguicidas, con concentraciones cercanas a los residuos de aplicación en campo.

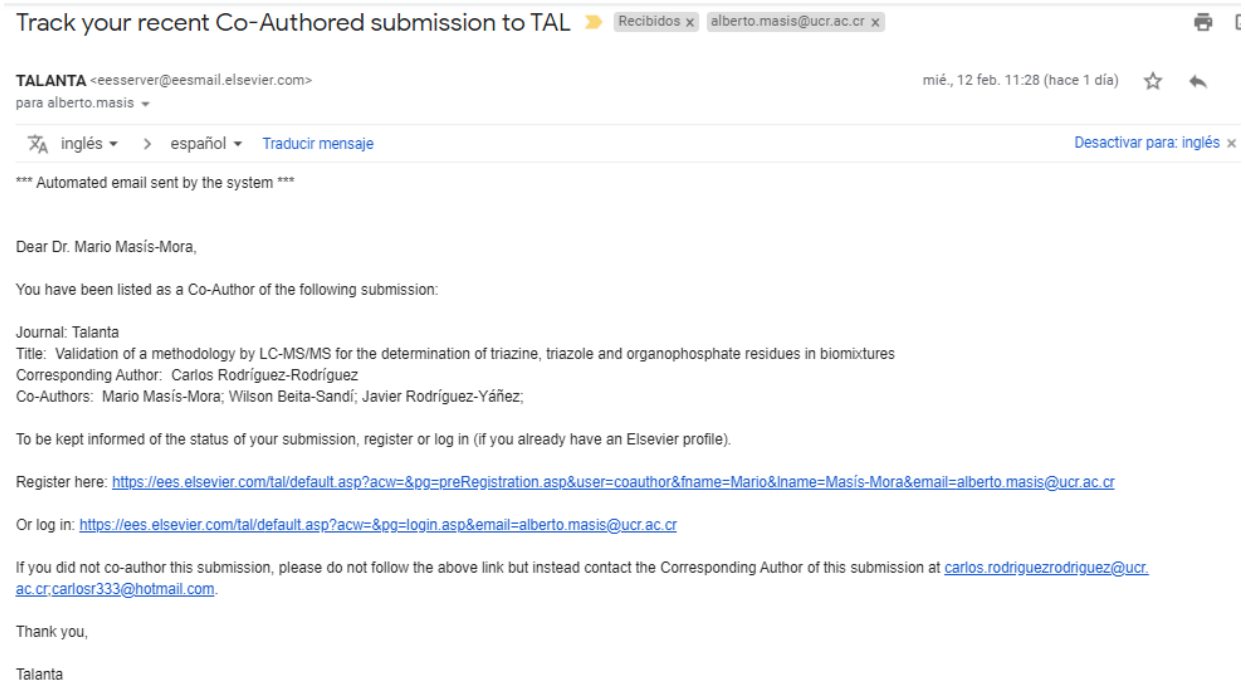
En el SSB se observó que las triazinas y los organofosforados lograron disminuir su concentración inicial en mayor porcentaje que los triazoles, llegando algunas moléculas a tener valores de vida media menores a los reportados en literatura, aunque en algunos casos se obtuvieron valores más altos. En general, los triazoles no presentaron un comportamiento de degradación satisfactorio.

En el sistema de biopurificación (BPS) al que se le agregó aguas residuales de laboratorio (conteniendo triazinas, triazoles, carbamatos y organofosforados, entre otros), se logró eliminar el 77,2% de los plaguicidas contenidos después de un periodo de 281 d de tratamiento; sin embargo, la eliminación más significativa se logró a los 89 días con un total de 62.5% de remoción. La degradación de estas moléculas se confirmó con la aplicación de pruebas ecotoxicológicas. Para moléculas como los triazoles, contrario al comportamiento observado en el SSB, el tratamiento en BPS fue parcialmente exitoso, probablemente debido a la aplicación de concentraciones menores en las aguas residuales de laboratorio. En el caso de los organofosforados presentes en la misma mezcla de residuos de laboratorio, se obtuvo un valor total de remoción de 81,9 % al final del experimento. La eliminación de triazinas y carbamatos fue menos eficiente en el BPS en comparación con el SSB para el caso de las triazinas y en comparación con datos reportados. Estas diferencias se podrían deber a las concentraciones bajas aplicadas o al hecho de presentar una mayor diversidad de plaguicidas, con sus consecuentes interacciones entre ellas y con los componentes de la biomezcla.

En el BPS usado para el tratamiento de formulados de plaguicidas, se observó que al igual que en los otros dos sistemas, las triazinas y los organofosforados presentaron una eficiente/parcial degradación en la biomezcla propuesta, mientras que Los triazoles presentaron una mayor persistencia, por lo que se recomienda separar los desechos de formulados y buscar una metodología de tratamiento/descarte alternativa para estos compuestos

ANEXOS

ANEXO 1: CARTA DE RECEPCIÓN DE LA REVISTA CIENTÍFICA TALANTA



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