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Multiple stressors on aquatic ecosystems under Mediterranean conditions

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Multiple stressors on aquatic ecosystems under Mediterranean conditions

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Informe del Director de Tesis Doctoral

Estimados miembros de la Comisión Académica del Programa de Doctorado en Hidrología y Gestión de los Recursos Hídricos:

La tesis de Alba Arenas Sánchez se ha desarrollado en el campo de la ecología acuática, la ecotoxicología y la evaluación del riesgo ambiental de la contaminación. En concreto, dicha tesis se ha centrado en investigar el impacto de los factores de estrés químico y los factores de estrés relacionados con la escasez de agua en los ecosistemas acuáticos de la región Mediterránea. Aunque la tesis se ha centrado una región concreta del planeta, la temática y resultados de este trabajo son de gran relevancia para las regiones áridas o semiáridas, particularmente para la mejora de los sistemas de evaluación del riesgo de la contaminación, los cuales no suelen tener en cuenta la variación hidrológica y los impactos adicionales generados por ésta. Además hay que tener en cuenta que la escasez de agua y la contaminación son dos de los principales problemas ambientales que están relacionados con el aumento de la presión demográfica y el cambio climático global, y por tanto, el escenario futuro en relación a estas dos variables no es muy alentador si no se toman las medidas de previsión y de gestión adecuadas.

La tesis de Alba Arenas Sánchez aborda dicha problemática desde varios ángulos. Por un lado describe la bibliografía disponible e identifica las lagunas de conocimiento respecto al efecto combinado de la contaminación y la sequía en los diferentes componentes del ecosistema acuático. Por otro lado desarrolla un extenso trabajo de monitoreo ambiental dedicado a evaluar la presencia de contaminantes orgánicos e inorgánicos a nivel de cuenca, identifica los principales factores de estrés químico, y evalúa su variabilidad espacial y temporal en relación a la variación hidrológica y la respuesta estructural y funcional del ecosistema. Además, esta tesis incluye un estudio de laboratorio usando microcosmos para evaluar la interacción entre el aumento de la temperatura, la sequía y un insecticida sobre poblaciones y comunidades de invertebrados. Finalmente, la tesis discute sus resultados en relación a otros estudios recientes publicados en temáticas similares y propone directrices claras para la mejora del monitoreo y evaluación del riesgo de los contaminantes en un escenario de creciente escasez de agua y de cambio climático global.

Los diseños experimentales y métodos implementadas por Alba Arenas Sánchez son complejos y de un carácter marcadamente multidisciplinar. Hay que destacar su gran destreza para el diseño

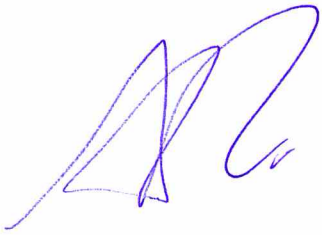
de experimentos y campañas de monitoreo ambiental, su capacidad para la medición de las principales variables físicas y químicas que afectan al estado ecológico de los ríos, su gran habilidad para la identificación taxonómica de plantas acuáticas y de invertebrados, así como su dominio de la química ambiental, los sistemas de información geográfica y la estadística univariante y multivariante para la evaluación de datos biológicos y ambientales.

Asimismo hay que resaltar el esfuerzo y dedicación que ha puesto en la redacción de este trabajo, que ha dado lugar a cuatro artículos científicos JCR en revistas de alto impacto (3 Q1 y 1 Q2), de los que en dos es primera autora y en otros dos primera autora a título compartido. Además es de esperar que el quinto capítulo de la tesis de lugar a otro artículo científico. Este resultado indica que su capacidad para el análisis y publicación de resultados científicos se ha visto fortalecido sustancialmente durante el periodo predoctoral.

Durante el periodo de ejecución de la tesis Alba Arenas Sánchez ha realizado una estancia de tres meses en el LEHNA (Laboratorio de Ecología de los Hydrosistemas Naturales y Antrópicos), en la Université Claude Bernard Lyon (Francia), y ha participado en varios seminarios y cursos relacionados con el tratamiento estadístico de datos, la redacción de artículos científicos, el uso de especies modelo en ecotoxicología y el uso de rasgos biológicos (traits) para el análisis de comunidades. Además, ha participado en cuatro conferencias internacionales exponiendo los resultados de su trabajo y ha colaborado en diversas actividades de difusión científica (Feria por la Ciencia y la Innovación, Noche Europea de los Investigadores, Semana de la Ciencia), las cuales han tenido una gran aceptación.

Por último, me gustaría resaltar el compromiso adquirido por la doctoranda en la mejora de la ciencia y la regulación ambiental, y las grandes dotes de Alba Arenas Sánchez para llevar a cabo proyectos científicos en equipos pluriculturales y multidisciplinares.

Por tanto, emito informe favorable para la presentación y defensa de la tesis doctoral.



Fdo: Dr Andreu Rico Artero

Universidad de Alcalá

D. Eloy García Calvo, Coordinador de la Comisión Académica del Programa de Doctorado en Hidrología y Gestión de Recursos Hídricos,

HAGO CONSTAR que la Tesis Doctoral titulada *Multiple stressors on aquatic ecosystems under Mediterranean conditions*, presentada por Dª Alba Arenas Sánchez, bajo la dirección del Dr. Andreu Rico Artero, ha sido realizada por compendio de artículos, reuniendo los requisitos exigidos a este tipo de tesis, así como los requisitos científicos de originalidad y rigor metodológicos para ser defendida ante un tribunal. Esta Comisión ha tenido también en cuenta la evaluación positiva anual del doctorando, habiendo obtenido las correspondientes competencias establecidas en el Programa.

Para que así conste a los efectos del depósito de la tesis, se firma en Alcalá de Henares a 5 de junio de 2019.

Fdo.: Eloy García Calvo

A mis padres, y a mi hermano

“Lo esencial es invisible a los ojos”

(*Le Petit Prince*, Antoine de Saint-Exupéry, 1943)

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Summary

Water scarcity and chemical pollution are two of the main problems affecting aquatic communities in Mediterranean ecosystems under a global change scenario. The naturally variable flows in these systems are expected to be strongly altered by reduced annual rainfall, pronounced drought periods, frequent and intense floods, as well as an increasing trend in water abstraction to sustain human population demands. Moreover, the potential risk of a wide range of pollutants resulting from growing demographic pressure and intensification of industrial and agricultural activities is high in these hydrologically variable Mediterranean systems. Still, understanding the vulnerability of Mediterranean aquatic communities to this multiple stressed scenario (i.e. water stress, their associated physico-chemical changes and pollution stress) needs further assessment.

The aim of this thesis was to contribute to a better understanding of the individual and combined effects of high hydrological variability (including desiccation periods or potential water scarcity conditions) and chemical stress in aquatic ecosystems of (semi-)arid Mediterranean regions.

This thesis begins with a literature review of existing knowledge on the potential responses of aquatic communities in (semi-)arid regions to the combined effect of water scarcity and chemical stress (Chapter 2). This chapter confirmed that the knowledge and number of studies in this topic was reduced and highlighted the need of: (1) experimental studies on different biota groups and life stages, with particular attention to those including traits relevant for the adaptation to water scarcity;(2) more studies on the effects of pesticides on edge-of-field water bodies affected by water scarcity; (3) more knowledge on population and community recovery capacity to assess its vulnerability; (4) combining field monitoring and experimental studies to reach more conclusive, causal relationships on the effects of co-occurring stressors; (5) and implementing results from these studies to develop ecological scenarios and models recommended for further developments on prospective aquatic risk assessment of chemicals in (semi-)arid areas, as well as to support the update of regulatory approaches for the assessment of the ecological status of Mediterranean surface waters.

In Chapter 3, the concentration of a wide range of pesticides and point source chemicals (e.g. pharmaceuticals, cosmetics, industrial compounds) were detected and quantified in the upper Tagus river basin, with marked Mediterranean conditions. The methodological approach followed showed that although a qualitative screening method was a helpful tool in the selection of target compounds to be quantified, this type of approaches are subject to uncertainties, as some false positive and false negatives were be encountered on the basis of LC-MS/MS analytical verifications. To minimize these uncertainties further work should be done on the availability of updated libraries with exact mass data for different groups of chemicals and rely on a larger number of chemical standards. Grab samples proved not to be fully suitable for contaminants with discontinuous exposure such as pesticides, while the use of passive sampling methods (e.g. POCIS) are recommended. This study showed that some sites of the upper Tagus river basin, primarily dominated by agricultural and/or urban land use, are highly polluted. Some insecticides, herbicides, and fungicides were measured at high concentrations; and point source contaminants such as paracetamol, ibuprofen, some

antibiotics and life-style compounds (caffeine, paraxanthine, nicotine), were detected at especially high concentrations downstream urban areas or small villages without wastewater treatment facilities.

In Chapter 4, an ecological risk assessment was performed on the basis of the organic micropollutants quantified in Chapter 3, and a range of monitored metals, which showed that acute toxicity is likely to occur for some metals (copper and zinc) in the most impacted sites. Low acute toxicity was determined for organic contaminants on the basis of grab samples. However, the assessment performed based on POCIS measurements resulted in potential acute risks for primary producers due to diuron exposure, and to invertebrates and fish due to chlorpyrifos exposure. Several chemical mixtures that may result in chronic toxicity for freshwater biodiversity were also identified, which include some herbicides (for primary producers), and some insecticides and point-source chemicals (for invertebrates and fish). The inclusion of these potentially toxic compounds present in mixtures should be considered in future management plans at a basin level. This study also showed that some metals and pesticides exceeded the Water Framework Directive (WFD) regulatory thresholds. Assessment of the chronic effects of point-source chemicals on behavioral, reproductive or developmental dysfunctions is recommended. Chapter 4 also shows a major influence of land use on chemical pollution status and slight seasonal differences in physico-chemical parameters and the concentration of some insecticides according to the contraction phase (i.e. summer), as well as to application patterns. Despite refinement of monitoring designs and sampling methods are needed to obtain more robust results on temporal variability, this seasonal variation should be considered to increase the efficiency of management actions in Mediterranean basins.

In Chapter 5, the impact of hydrological stress on biological responses to pollution was assessed, evaluating the composition of macroinvertebrate communities at a taxonomic and functional (trait-based) level. Seasonal differences were observed on macroinvertebrate taxonomic and functional composition. Taxonomic and functional richness were significantly lower in the polluted sites in summer (i.e. drought period) and autumn (i.e. early expansion period). Moreover, richness, functional richness and functional diversity were more severely affected in sites impaired by both pollution and drought stress, leading to simplified communities dominated by generalist taxa. Asexual reproduction, reproduction by clutches, cocoons and plurivoltinism, were connected to highly polluted sites whereas reproduction by isolated eggs, semivoltinism or respiration by gills were more frequent in lowly polluted sites. Other traits such as dispersal, substrate relation and feeding habits showed clearer responses in summer and autumn and responded to pollution (e.g. interstitial organisms, burrowers, deposit feeders), but also to drought (e.g. aerial dispersal) and to the combined effects of drought and pollution (e.g. diapause). Attention should be paid to trait correlations, but these results support the development of monitoring and risk assessment procedures to identify vulnerable taxa in water stressed and highly polluted Mediterranean rivers.

In Chapter 6, a controlled laboratory microcosm (model-ecosystem) study was performed to interpret the causal relations between stressors related to water scarcity (i.e. increased temperatures and drought) and chemical stress (the insecticide lufenuron), and zooplankton responses at population and community level. The results show that the community exposed to lufenuron at 28˚C had a faster response and recovery than the community at 20˚C. The

combined effects of lufenuron and temperature resulted in a synergistic effect on some taxa (*Daphnia sp*., Cyclopoida). The tested zooplankton community had a high resilience to drought, although some particular taxa were severely affected after desiccation (Calanoida). Interactions between drought and lufenuron were not statistically significant. However, rewetting after desiccation contributed to lufenuron remobilization from sediments, which could be related with the slight Cyclopoida population decline at high exposure concentrations. This study shows how environmental conditions related to water scarcity in (semi-)arid regions may influence chemical fate and the vulnerability of zooplankton communities to chemical stress.

Finally, in Chapter 7, the overall results of this thesis are discussed in a broader context, aiming (1) to assess the contribution of this thesis and other new studies to the gaps identified in Chapter 2, (2) to evaluate potential toxicity risk of regulated and unregulated pollutants in Mediterranean basins for which studies are available, (3) to assess the degree of protection of biological communities affected by chemical stress under drought conditions in Mediterranean regions in current regulatory procedures, and finally (4) to provide recommendations for improving remaining knowledge gaps and identified weaknesses at a regulatory level.

Based on the findings of this thesis, it can be concluded that hydrological conditions influence water quality status and responses of Mediterranean aquatic invertebrate communities, with drought or water scarcity periods intensifying the detrimental effects of pollution. On the other hand, Mediterranean zooplankton communities seem to have a high recovery capacity to water scarcity and chemical pollution. However, more experimental studies (micro- and mesocoms) attending to the impact of pesticides (with different mode-of-action and persistence) under different drought levels and timing of stressors, and better understanding of community responses and food web interactions, are needed. At a regulatory level, priority substances frequently detected above the regulatory threshold, especially chlorpyrifos or Hg, require urgent management measures. The inclusion of non-priority substances identified as having potential risk at a basin level should considered in specific management plans, after proper cost-effective validation through monitoring. The most toxic compounds identified were metals and pesticides, but the potential ecological risk of point source chemicals should be evaluated carefully, attending to their specific mode-of-action and sub-lethal effects (e.g. growth, behavioral effects) on appropriate biological endpoints (e.g. bacteria, vertebrates). Moreover, variability of reference conditions between seasons in Mediterranean rivers based on taxonomic and functional indexes, and biological sampling schemes are recommended to be revised as well, with the aim of covering real worst-case conditions (i.e. during drought periods) related to the ecological disturbance of aquatic communities. Under a prospective point of view, the microcosm study of this thesis is one of the most novel high-tier studies considering hydrological variation and complete desiccation. In that sense, risk assessment procedures should invest in the development of ecological scenarios and models considering the impact of high hydrological variability in (semi-)arid aquatic ecosystems on the fate and effect of chemicals.

Resumen

La escasez de agua y la contaminación química son dos de los principales problemas que afectan a las comunidades acuáticas de ecosistemas mediterráneos en un escenario de cambio global. Se espera que los caudales naturalmente variables en estos ecosistemas se vean fuertemente alterados debido a la reducción de las precipitaciones anuales, los periodos de sequía pronunciados, las frecuentes e intensas inundaciones, así como por una tendencia creciente en la extracción de agua en respuesta a las demandas de la población. Además, el riesgo potencial del amplio rango de contaminantes resultantes de la creciente presión demográfica y la intensificación de las actividades industriales y agrícolas, es alto en estos ecosistemas mediterráneos hidrológicamente variables. Aun así, la comprensión de la vulnerabilidad de las comunidades acuáticas mediterráneas en este escenario de estrés múltiple (es decir, estrés hídrico, los cambios físico químicos asociados y estrés por contaminación) requiere de una investigación detallada.

El objetivo principal de esta tesis ha sido contribuir a una mejor comprensión de los efectos individuales y combinados de la alta variabilidad hidrológica (incluyendo periodos de sequía o potenciales condiciones de escasez de agua) y el estrés químico en ecosistemas acuáticos de regiones (semi-)áridas mediterráneas.

Esta tesis comienza con una revisión literaria sobre el conocimiento existente de las posibles respuestas de las comunidades acuáticas en regiones (semi-)áridas al efecto combinado de la escasez de agua y el estrés químico (Capítulo 2). Este capítulo confirma que el nivel conocimiento y la cantidad de estudios sobre este problema son reducidos, destacando la necesidad de: (1) estudios experimentales con diferentes grupos bióticos y estadios de desarrollo, con especial atención a aquellos con rasgos biológicos relevantes para la adaptación a la escasez de agua; (2) más estudios sobre los efectos de los pesticidas en cuerpos de agua cercanos a zonas agrícolas afectados por la escasez de agua; (3) más conocimiento sobre la capacidad de recuperación de las poblaciones y las comunidades para evaluar su vulnerabilidad; (4) combinar el monitoreo de campo y los estudios experimentales para establecer relaciones causales más concluyentes sobre los efectos de factores de estrés coexistentes; (5) y la implementación de los resultados de estos estudios para desarrollar escenarios y modelos ecológicos recomendados para futuras mejoras en la evaluación prospectiva del riesgo de sustancias químicas en ecosistemas acuáticos de áreas (semi-)áridas, así como para apoyar la actualización de los procedimientos regulatorios para la evaluación del estado ecológico de las aguas superficiales mediterráneas.

En el Capítulo 3, se detectó y cuantificó la concentración de un amplio rango de pesticidas y otros productos químicos de emisión continua (e.g. productos farmacéuticos, cosméticos, compuestos industriales) en la parte alta de la cuenca del río Tajo, con marcadas condiciones mediterráneas. La metodología seguida mostró que a pesar de que un método de selección cualitativo puede ser una herramienta útil en la selección del compuesto objetivo a cuantificar, este tipo de enfoques están sujetos a incertidumbres, ya que se encontraron algunos falsos positivos y falsos negativos en base a las verificaciones analíticas con LC-MS/MS. Para minimizar estas incertidumbres, se debe trabajar más en la disponibilidad de librerías actualizadas con datos de masa exacta para diferentes grupos de productos químicos y contar

con un mayor número de estándares químicos. El muestreo puntual de agua demostró no ser totalmente adecuado para contaminantes con exposición discontinua, como pesticidas, mientras que en ese caso se recomienda el uso de métodos de muestreo pasivo (e.g. POCIS). Este estudio mostró que algunos puntos de la cuenca alta del río Tajo, principalmente dominados por el uso de suelo agrícola y/o urbano, están altamente contaminados. Algunos insecticidas, herbicidas y fungicidas se midieron en altas concentraciones; y se detectaron contaminantes de emisión continua como paracetamol, ibuprofeno, algunos antibióticos y compuestos de uso doméstico (cafeína, paraxantina, nicotina) en concentraciones especialmente altas, aguas abajo de grandes áreas urbanas o pueblos pequeños sin plantas de tratamiento de aguas residuales.

En el Capítulo 4, se realizó una evaluación de riesgo ecológico de los microcontaminantes orgánicos cuantificados en el Capítulo 3, y de una serie de metales monitoreados simultáneamente, que demostró que existe un riesgo de toxicidad aguda para algunos metales (cobre y zinc) en las zonas más impactadas por la presión antropogénica. La toxicidad aguda fue baja para los contaminantes orgánicos en base a muestras de agua puntuales. Sin embargo, la evaluación realizada en base a las mediciones de POCIS resultó en riesgos agudos potenciales para los productores primarios debido a la exposición al diurón, y a los invertebrados y peces debido a la exposición al chlorpyrifos. También se identificaron varias mezclas químicas que pueden resultar en toxicidad crónica para la biodiversidad en cuerpos de agua dulce, las cuales incluyen algunos herbicidas (para los productores primarios) y algunos insecticidas y productos químicos de emisión continua (para invertebrados y peces). La inclusión de estos compuestos potencialmente tóxicos presentes en mezclas debe considerarse en futuros planes de gestión a nivel de cuenca. Este estudio también muestra que las concentraciones de algunos metales y pesticidas estaban por encima de los límites regulatorios de la Directiva Marco del Agua (DMA). Se recomienda evaluar los efectos crónicos de los productos químicos de emisión continua en las disfunciones del comportamiento, la reproducción o el desarrollo. El Capítulo 4 también mostró una gran influencia del uso del suelo en el nivel de contaminación química, y leves diferencias estacionales en los parámetros físico químicos y la concentración de algunos insecticidas asociados con la fase de contracción (i.e. verano) y los patrones de aplicación. A pesar de la necesidad de revisar los diseños de monitoreo y los métodos de muestreo necesarios para obtener resultados más sólidos sobre la variabilidad temporal, esta variación estacional debe tenerse en cuenta para aumentar la eficiencia de las medidas de gestión en las cuencas mediterráneas.

En el Capítulo 5, se evaluó el impacto del estrés hídrico en las respuestas biológicas a la contaminación, evaluando la composición de las comunidades de macroinvertebrados a nivel taxonómico y funcional (basado en rasgos biológicos). Se observaron diferencias estacionales en la composición taxonómica y funcional de los macroinvertebrados. La riqueza taxonómica y funcional fue significativamente menor en los sitios contaminados en verano (i.e. el periodo de sequía) y en otoño (i.e. el periodo de expansión temprana). Además, la riqueza, la riqueza funcional y la diversidad funcional se vieron más gravemente afectadas en los sitios afectados por la contaminación y el estrés por sequía, lo que resultó en comunidades simplificadas dominadas por taxones generalistas. La reproducción asexual, la reproducción por colonias de huevos, la producción de capullos y el plurivoltinismo, se relacionaron con sitios altamente

contaminados, mientras que la reproducción con huevos aislados, el semivoltinismo o la respiración por branquias fueron más frecuentes en los sitios poco contaminados. Otros rasgos como la dispersión, la relación con el sustrato y los hábitos de alimentación mostraron respuestas más claras en verano y otoño, respondiendo a la contaminación (e.g. organismos intersticiales, escarbadores, consumidores de depósitos), pero también a la sequía (e.g. dispersión aérea) y a los efectos combinados de la sequía y contaminación (e.g. diapausa). Se debe prestar atención a las correlaciones entre rasgos, pero estos resultados apoyan el desarrollo de procedimientos de monitoreo y evaluación de riesgos para identificar taxones vulnerables en ríos mediterráneos afectados por la sequía y altamente contaminados.

En el Capítulo 6, se realizó un estudio controlado de microcosmos en laboratorio (ecosistema modelo) para interpretar las relaciones causales entre los factores de estrés relacionados con la escasez de agua (i.e. el aumento de la temperatura y la sequía) y el estrés químico (el insecticida lufenuron), y las respuestas del zooplancton a nivel población y comunidad. Los resultados muestran que la comunidad expuesta al lufenurón a 28°C tuvo una respuesta y recuperación más rápida que la comunidad a 20°C. Los efectos combinados del lufenurón y la temperatura dieron como resultado un efecto sinérgico en algunos taxones (*Daphnia* sp., Cyclopoida). La comunidad de zooplancton evaluada mostró una alta resistencia a la sequía, aunque algunos taxones se vieron gravemente afectados después de la desecación (Calanoida). Las interacciones entre la sequía y el lufenurón no fueron estadísticamente significativas. Sin embargo, la fase simulada de expansión (o lluvia) después de la desecación contribuyó a la removilización del lufenurón de los sedimentos, lo que podría estar relacionado con el ligero descenso de la población de Cyclopoida a altas concentraciones. Este estudio muestra cómo las condiciones ambientales relacionadas con la escasez de agua en las regiones (semi-)áridas pueden influir en la exposición química y la vulnerabilidad de las comunidades de zooplancton al estrés químico.

Finalmente, en el Capítulo 7, los resultados generales de esta tesis se discuten en un contexto más amplio, con el objetivo de (1) evaluar la contribución de esta tesis y otros estudios nuevos a las lagunas de conocimiento identificadas en el Capítulo 2, (2) evaluar el riesgo potencial de toxicidad de contaminantes regulados y no regulados en las cuencas mediterráneas para las que se dispone de estudios, (3) evaluar el grado de protección de las comunidades biológicas afectadas por el estrés químico en condiciones de sequía en las regiones mediterráneas en los procedimientos regulatorios actuales, y finalmente (4) proporcionar recomendaciones de mejora para las lagunas de conocimiento restantes y las debilidades identificadas a nivel regulatorio.

En base a los hallazgos de esta tesis, se puede concluir que las condiciones hidrológicas influyen en el estado de la calidad del agua y las respuestas de las comunidades de invertebrados acuáticos de ecosistemas mediterráneos, con una intensificación de los efectos perjudiciales de la contaminación en períodos de sequía o escasez de agua. Por otro lado, las comunidades mediterráneas de zooplancton parecen tener una alta capacidad de recuperación de la escasez de agua y la contaminación química. Sin embargo, se necesitan más estudios experimentales (micro- y mesocomos) que atiendan el impacto de pesticidas (con diferentes modos-de-acción y persistencia) bajo diferentes niveles de sequía y momento de exposición al estrés; así como una mejor comprensión de las respuestas de la comunidad y las

interacciones en la cadena alimentaria. A nivel regulatorio, las sustancias prioritarias que se detectaron con frecuencia por encima del límite regulatorio, especialmente chlorpyrifos o Hg, requieren medidas de gestión urgentes. La inclusión de sustancias no prioritarias identificadas como de riesgo potencial a nivel de cuenca debe considerarse en planes de gestión específicos, después de una validación adecuada y eficiente por medio de programas de monitoreo. Los compuestos más tóxicos identificados fueron los metales y los pesticidas, pero el riesgo ecológico de los productos químicos de emisión continua debe ser reevaluado, atendiendo a su modo de acción específico y los efectos sub-letales (e.g. crecimiento, efectos comportamentales) en los organismos biológicos apropiados (e.g. bacterias, vertebrados). Además, también se recomienda revisar la variabilidad de las condiciones de referencia entre estaciones en los ríos mediterráneos en base a índices taxonómicos y funcionales; así como los periodos de muestreo biológico, con el objetivo de cubrir las condiciones más desfavorables (i.e. durante los períodos de sequía) respecto al impacto de la contaminación y sequía en las comunidades acuáticas. Bajo un punto de vista prospectivo, el estudio de microcosmos de esta tesis es uno de los estudios más novedosos bajo condiciones ecológicamente realistas que consideran la variación hidrológica y la desecación completa. En este sentido, los procedimientos de evaluación de riesgos deberían invertir en el desarrollo de escenarios y modelos ecológicos, que consideren el impacto de la alta variabilidad hidrológica en los ecosistemas acuáticos (semi-)áridos sobre la exposición y el efecto de los productos químicos.

List of publications

Arenas-Sánchez, A., Rico, A., Vighi, M., 2016. Effects of water scarcity and chemical pollution in aquatic ecosystems: state of the art. Science of the Total Environment, 572: 390-403.

Rico, A., Arenas-Sánchez, A., Alonso-Alonso, C., López-Heras, I., Nozal, L., Rivas-Tavares, D., Vighi, M., 2019. Identification of contaminants of concern in the upper Tagus river basin (central Spain). Part 1: Screening, quantitative analysis and comparison of sampling methods. Science of the Total Environment, 666: 1058-1070.

Arenas-Sánchez, A., Rico, A., Rivas-Tabares, D., Blanco, A., Garcia-Doncel, P., Romero-Salas, A., Nozal, L., Vighi, M., 2019a. Identification of contaminants of concern in the upper Tagus river basin (central Spain). Part 2: Spatio-temporal analysis and ecological risk assessment. Science of the Total Environment, 667: 222-233.

Arenas-Sánchez, A., López-Heras, I., Nozal, L., Vighi, M., Rico, A., 2019b. Effects of increased temperature, drought, and an insecticide on freshwater zooplankton communities. Environmental Toxicology and Chemistry, 38: 396-411.

General introduction

1. Climate Change and water scarcity in Mediterranean aquatic ecosystems

Mediterranean (semi-)arid regions have been described as one of the most vulnerable regions to the effects of Climate Change (IPPC, 2012, 2014). Freshwater aquatic ecosystems in these regions are characterized by a pronounced seasonal variation related to heavy rainfalls occurring in spring and autumn, alternated with drier conditions in winter, and especially in summer (Gasith and Resh, 1999; Robson et al., 2011). However, these areas are currently suffering severe alterations in such hydrological patterns, due to reduced annual precipitation, more pronounced and prolonged droughts and higher flood frequency, which are expected to become more recurrent in the near future (EEA, 2008; Sabater and Tockner, 2010; IPPC, 2012, 2014). Additionally, in a context of global change, these regions are subject to an increasing water abstraction pressure to satisfy growing human population demands (Barceló and Sabater, 2010; Petrovic et al., 2011). The changes in climatic patterns added up to the overexploitation of aquatic resources in these regions, result in a clear imbalance between available water resources and anthropogenic demands. This situation leads to a more recurrent and pronounced water scarcity situation (Figure 1), which is defined as a persistent condition in which water demand exceeds the exploitable water resources in a sustainable way (Barceló and Sabater, 2010; Navarro-Ortega et al., 2012). In line with that, the IPPC (2007) also concluded that the area affected by droughts had increased in many regions of the globe since 1970 and is likely to increase even more in the 21st century.

The impact of hydrological variability and flow intermittency on aquatic biodiversity of rivers and streams in (semi-)arid regions, such as the Mediterranean ones, has been largely studied (Stanley et al., 1997; Bonada et al. 2007a; Sabater and Tockner, 2010). A reduction in flow, also called 'ecosystem contraction', is associated with harshened water quality conditions, such as increased water temperatures, lower oxygen concentrations or lower dilution potential for suspended particles, nutrients or dissolved organic matter (DOC) (Hamilton et al., 2005; Barceló and Sabater, 2010; Carere et al., 2011), which might be exacerbated in periods of water scarcity. Such changes in environmental conditions are usually associated to a spatial modification and/or reduction in the aquatic habitat, which may also interfere with food availability and interaction among aquatic species. Several studies refer to a natural adaptation and resilience capacity of aquatic communities in these hydrologically variable environments (Acuña et al., 2005; Williams, 2005; Bonada et al., 2007b). This capacity is explained by the development of specific physiological and behavioral traits such as aerial respiration to resist low oxygen concentrations, drought-resistant reproduction forms, high dispersal abilities or migration to dry-season refuges (Lahr, 1997; Robson et al., 2011; Storey and Quinn, 2013). However, adapted aquatic communities might be negatively affected by the expected increase in extreme climatic and hydromorphological changes resulting from climate and global change, with consequent impacts on their resilience capacity to other human-related impacts, such as pollution.

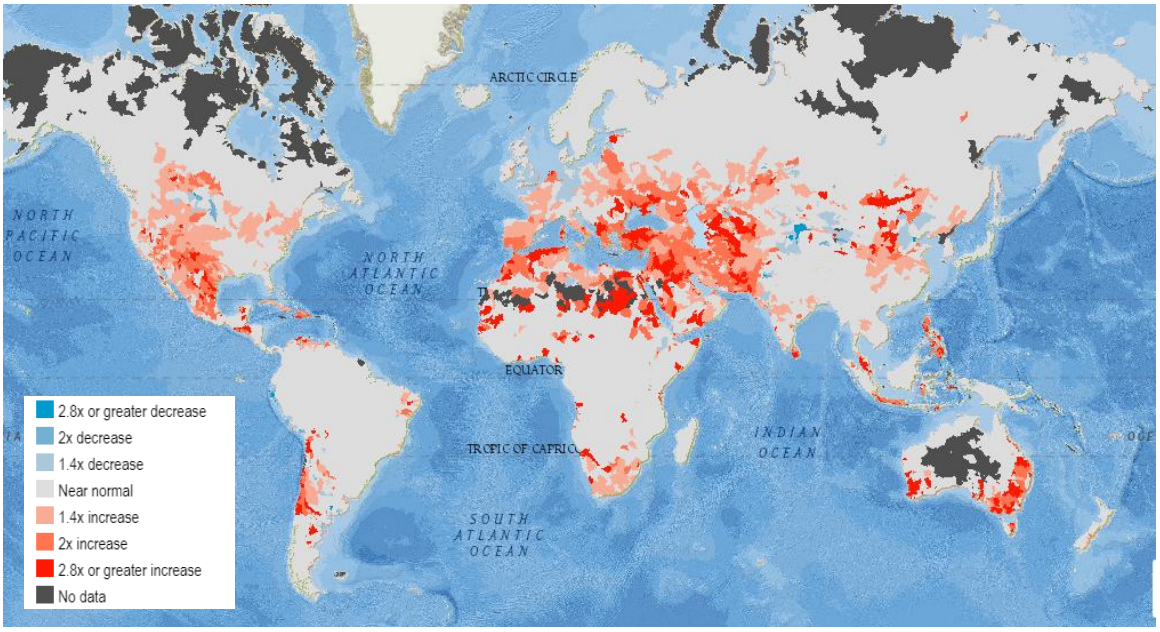


Figure 1. Projected change in water stress from baseline to 2040 business as usual scenario. Projected change in water stress shows how development and/or climate change are expected to affect water stress, the ratio of water use to supply. The "business as usual" scenario (SSP2 RCP8.5) represents a world with stable economic development and steadily rising global carbon emissions. Accessed from: World Resources Institute, 2019; Source: Luck et al., (2015).

2. Detection, prioritization and regulation of contaminants in European surface waters

A wide range of pollutants can be found in the aquatic environment (e.g. pesticides, pharmaceuticals, life-style compounds, home-care products), which are normally present in complex mixtures that can result in lethal and sub-lethal effects on aquatic organisms (Schwarzenbach et al., 2006; Malaj et al., 2014). The number of contaminants registered for commercial use in Europe is increasing exponentially as a result of the growing demographic pressure and the intensification of industrial and agricultural activities (Figure 2). All of those substances have a potential risk to reach surface waters at different concentrations via drift after application of pesticides, run-off or drainage waters going over polluted soils, or effluents (treated or untreated) from urban or industrial settlements.

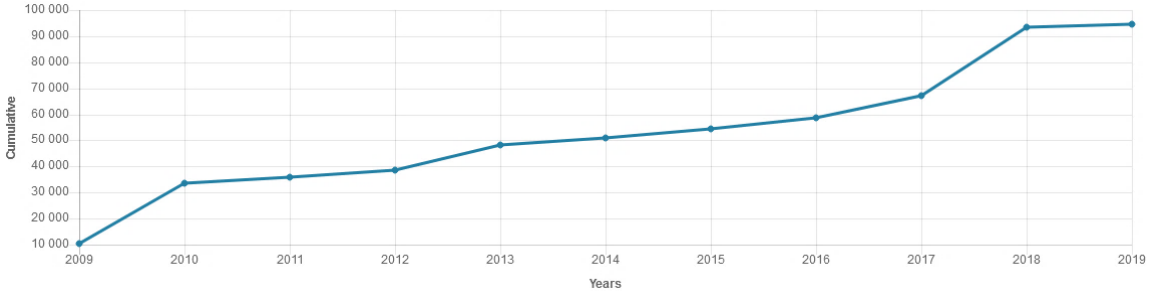


Figure 2. Total number of chemical products registered in the EU from 2009. Source: ECHA (2019)

The Water Framework Directive (WFD; Directive 2000/60/EC) constitutes the most extensive legislative framework for the protection of surface waters in Europe. With regards to the

assessment of the chemical status of surface waters, it provides Environmental Quality Standard (EQS) that must be met for 45 organic and inorganic compounds identified as priority (hazardous) substances. It also advocates for the additional monitoring of substances of national or regional concern by the different member states (EC, 2003; Directive 2013/39/EU). However, the wide range of chemicals that can be currently detected in surface waters due to development in monitoring and analytical techniques suggest that the WFD priority substances may only constitute a small fraction of potentially toxic compounds to aquatic organisms (Barceló and Petrovick, 2007; Silva et al., 2015; Tsaboula et al., 2016).

Mediterranean watersheds are known to be exposed to high pollution levels due to their seasonally lowered water flows and high urban, agricultural and industrial pressures, resulting in concentration levels most often above those found in other European basins (Petrovic et al., 2011; López-Doval et al., 2013). However, the number of studies assessing the risks of regulated and emergent chemicals in Mediterranean rivers is limited (e.g. Ginebreda et al., 2010; López-Doval et al., 2012; Kuzmanović et al., 2015). Further research is needed to better understand the temporal and spatial distribution of chemical contaminants and their mixtures in these ecosystems, and to assess their potential risks to freshwater organisms.

3. Vulnerability of Mediterranean aquatic ecosystems to multiple stressors

From the above described conditions affecting aquatic ecosystems, it is important to note that negative effects of chemical pollutants may be influenced by the prevailing environmental conditions (Noyes et al., 2009; Schiedek et al., 2007). Apart from forming mixtures, chemical stressors can be found with other elements such as nutrients or metals, or under physico-chemical conditions that might act as stressors or influence their bioavailability to aquatic organisms (Hering et al., 2015; Rico et al., 2016a). These complex conditions are usually termed as ‘multiple stressors’, i.e. any combination of two or more biological, physical or chemical factors that exert stress on organisms (Crain et al., 2008). Assessing and predicting the effects of multiple stressors on aquatic biodiversity and vulnerability (i.e. exposure, sensitivity and recovery capacity; Ippolito et al., 2010) of aquatic communities can be a difficult task, since when stressors co-occur their interaction could result in ecological surprises. This means that their combined effect are not always additive, but could be larger (synergistic) or smaller (antagonistic) than expected (Ormerod et al., 2010; Schäfer et al., 2016). Moreover, it should be noted that, while organisms are the biological units that react first to the stressor on the basis of their specific traits, vulnerability of aquatic ecosystems needs to be understood on the basis of the impacts observed at the population and community level. Particularly, population responses and the interactions among them can be difficult to predict, but they are the key to understand changes produced in community structure and the ecological functions they mediate (Ippolito et al., 2010).

To date, the number of studies assessing the interaction between climate-related stressors, particularly those related to water scarcity, and chemical stress at community level is very limited. Some authors have discussed that aquatic communities regularly affected by harsh environmental conditions, such as regular desiccation periods, may display a lower functional redundancy and consequently have lower resilience to chemical stress (Moe et al., 2013). However, other studies suggest that the degree of specialization obtained in communities

adapted to extreme climatic conditions is positively correlated to a higher resilience to short-term chemical exposure, but also depends on the time of exposure, the mode of action of the chemical, the level of drought reached, and the adaptation of the impacted communities to it (Lahr, 1997; Stampfli et al., 2013). Since these responses seem to be context dependent and are still not completely understood, the combined impact of hydrological variation, water scarcity, its correlated physico-chemical changes and pollution on aquatic Mediterranean communities need to be further assessed (Petrovic et al., 2011; Osorio et al., 2014; Sabater et al., 2014). Disentangling the causal relations between these multiple factors and biological responses should be based on: (1) a combination of field studies, contributing to get an estimation of the main factors dominating the response; and (2) experimental studies (preferably model-ecosystems which allow the evaluation of responses at the population and community level), in which the interaction between stress factors can be better simulated and studied under controlled conditions (Sabater et al., 2007; López-Doval et al., 2010; Ricart et al., 2010).

Moreover, current regulatory procedures for the risk assessment of chemicals seem to generally neglect the influence of hydrological variability and its related physico-chemical changes on biological communities responses to chemical stress; being most of the monitoring and management measures based on permanently flowing water bodies (Gallart et al., 2012; EFSA, 2013; Prat et al., 2014). At a national level, several Mediterranean river types have been established for the assessment of ecological status of surface waters as part of the WFD (Directive 2000/60/EC; RD 817/2015). However, the level of protection of aquatic ecosystems to chemical pollution under a water scarcity scenario should be revised closely.

4. Research objectives and scope

The aim of this thesis is to contribute to a better understanding of the individual and combined effects of high hydrological variability (including desiccation periods or potential water scarcity conditions) and chemical stress in aquatic ecosystems of (semi-) arid Mediterranean regions.

The specific research objectives of this thesis are:

1. To evaluate the existing knowledge regarding the impact of hydrological and chemical stress in aquatic ecosystems of (semi-)arid regions, identifying knowledge gaps and developments needed in this research field.
2. To identify the main chemical stressors which potentially influence aquatic ecosystems in Mediterranean regions.
3. To assess the response of aquatic communities to multiple chemical stressors under hydrological stress conditions in Mediterranean regions.
4. To analyze under controlled conditions the sensitivity and recovery capacity of aquatic populations and communities of Mediterranean regions to chemical, thermal and hydrological stress; determining possible stressor interactions (additive, synergistic or antagonistic).
5. To evaluate the degree of inclusion of the obtained results in current chemical risk assessment regulatory frameworks, suggesting further steps for improvement in case large mismatches are found.

5. Thesis outline

This thesis begins with an overview of existing knowledge on the potential biological responses of aquatic communities to the combined effects of chemical stress and water scarcity in (semi-) arid regions. Chapter 2 provides a description of the impact of variable hydrological conditions, typical of Mediterranean regions, on abiotic components of water quality, as well as on biotic communities. The results from existing laboratory and field studies assessing the combined effects of hydrological variability related to water scarcity and chemical pollution on the structural and functional characteristics of aquatic communities are evaluated. In addition, knowledge gaps on this research field and on current regulatory risk assessment of chemicals in scenarios of water scarcity are identified, and suggestions for further research are provided.

In Chapter 3, the organic micropollutants more frequently detected in surface waters, with high toxicity potential and use, were identified and quantified. The study area was the upper part of the Tagus river basin, with marked Mediterranean conditions. The samples were obtained from an extensive monitoring field work performed in spring, summer and autumn of 2016. A novel methodology was applied that combines the establishment of a priority criterion for the selection of target compounds detected in a screening analysis, which were further analyzed by means of a quantitative method. Moreover, two sampling methods (grab and passive sampling) were used and compared in this chapter. In Chapter 4, the correlation of the quantified chemicals in Chapter 3 with other physico-chemical water quality parameters and metals was assessed, as well as the influence of land use and time (i.e. seasonal variation) on their distribution. Additionally, the potential most toxic pollutants in the study area were determined by means of a prioritization approach based on a preliminary ecological risk assessment on the cumulative toxicity of pollutants to the main groups of aquatic organisms (algae, invertebrates, fish) considered in regulatory processes.

Chapter 5 evaluates the impact of hydrological stress on invertebrates' responses in different pollution scenarios, taking into account taxonomic as well as functional (trait-based) responses. Samples collected corresponded to the same monitoring campaigns as for Chapter 3 and 4. Macroinvertebrates were selected for this work as they are the most commonly used biotic indicators in stream management, and they can provide valuable information on past stress events. Changes in taxonomic and functional biotic indexes (e.g. richness, diversity, functional richness or functional diversity) were assessed for each sampling season, as well as more specific changes in taxonomic and trait composition. Additionally, traits associated to taxa tolerating drought, pollution or the combination of both, are identified.

In Chapter 6, the results from a controlled laboratory microcosm (model-ecosystem) study are presented. This study contributes to the required link between field and laboratory studies to establish causal relations between stressors and biotic responses. Thus, the response of zooplankton communities to the individual and combined effects of chemical stress (the highly persistent insecticide lufenuron) and two physical stressors related with water scarcity under Mediterranean conditions (i.e. high temperature and drought) are assessed in this chapter. The assessment was performed at a community and population level, and recovery was also evaluated. Zooplankton was selected for this experiment since they are good biotic indicators of the ecological status of lentic, slow-flowing and intermittent waters. As an additional point to

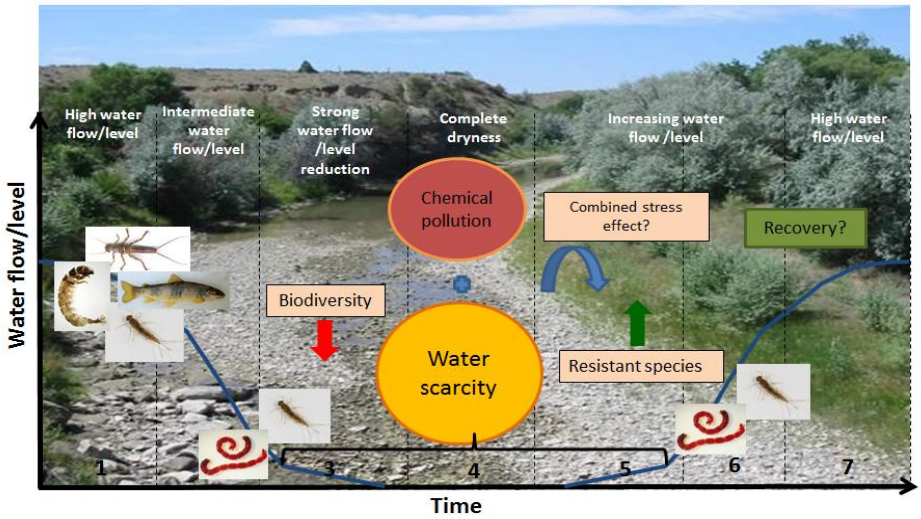
understand possible consequences of changing environmental conditions on chemical exposure, the fate of the insecticide under the different environmental scenarios simulated in this study, was also evaluated.

Finally, in Chapter 7, the results of this thesis are discussed, providing a broad view of the current state of the art on the assessment of the impact of pollution in water stressed Mediterranean aquatic ecosystems, identifying remaining knowledge gaps and providing recommendations for further studies in this line. Moreover, a comparative assessment of pollutants potentially hazardous to the aquatic environment in the Tagus river basin is done with respect to other studies performed in Mediterranean basins, with the aim of identifying risk trends or specific pollutants posing a high risk. Recommendations for improving the efficacy of this type of assessments are provided, as we well as some suggestions for updating chemical monitoring programs and management measures, in light of the results found. Finally, an evaluation of the applicability of current prospective and retrospective chemical regulatory procedures to Mediterranean freshwater ecosystems affected by water scarcity is presented.

Chapter 2

Effects of water scarcity and chemical pollution in aquatic ecosystems: state of the art

Alba Arenas-Sánchez, Andreu Rico, Marco Vighi



Abstract

Water scarcity is an expanding climate and human related condition, which drives and interacts with other stressors in freshwater ecosystems such as chemical pollution. In this study we provide an overview of the existing knowledge regarding the chemical fate, biological dynamics and the ecological risks of chemicals under water scarcity conditions. We evaluated a total of 15 studies dealing with the combined effects of chemicals and water scarcity under laboratory conditions and in the field. The results of these studies have been elaborated in order to evaluate additive, synergistic or antagonistic responses of the biological communities. As a general rule, it can be concluded that, in situations of water scarcity, the impacts of extreme water fluctuations are much more relevant than those of an additional chemical stressor. Nevertheless, the presence of chemical pollution may result in exacerbated ecological risks in some particular cases. We conclude that further investigations on this topic would take advantage on the focus on some specific issues. Experimental (laboratory and model ecosystem) studies should be performed on different biota groups and life stages (diapausing eggs, immature stages), with particular attention to those including traits relevant for the adaptation to water scarcity. More knowledge on species adaptations and recovery capacity is essential to predict community responses to multiple stressors and to assess the community vulnerability. Field studies should be performed at different scales, particularly in lotic systems, in order to integrate different functional dynamics of the river ecosystem. Combining field monitoring and experimental studies would be the best option to reach more conclusive, causal relationships on the effects of co-occurring stressors. Contribution of these studies to develop ecological models and scenarios is also suggested as an improvement for the prospective aquatic risk assessment of chemicals in (semi-)arid areas.

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1. Introduction

Water scarcity, defined as a structural, persistent reduction on water availability is one of the main problems faced by societies in the 21st century. Water scarcity problems have increased in many regions since the 70's and it is likely that they continue over this century due to the increasing human population, accelerated economic activity and land-use changes (Stocker et al., 2013; Herrera-Pantoja and Hiscock, 2015). Arid and semi-arid regions, which occupy more than one third of the planet's land surface and host about 30% of the world population, are particularly vulnerable to the increasing pressure on water resources (Safriel et al., 2005). These regions have been described as the most exposed to the impacts of climate change by the Intergovernmental Panel on Climate Change (IPPC), with prospects of increasing average temperatures and reduced annual precipitation, leading to prolonged drought periods (IPPC 2012, 2014). Moreover, the exploitation of aquatic resources in these regions results in a clear imbalance between anthropogenic demand and available water resources (Barceló and Sabater, 2010; Navarro-Ortega et al., 2012). For instance, in the northern part of the Iberian Peninsula, water abstraction represents 4-7% of the total resource available, while in watersheds of the semi-arid Mediterranean basins the demand ranges from 55% to 224% (Sabater et al., 2009). In Europe, about 45% of the extracted water is used for industry, 41% for agriculture and 14% for domestic use (Sabater and Tockner, 2010). Predictions for water abstraction in Europe are expected to increase from 415 km^3 to approximately 660 km^3 by 2070 (as a reference: average annual discharge of the Rhine River is 73 km^3) (Henrichs and Alcamo, 2001; Sabater and Tockner, 2010). It must also be noted that the problem of water scarcity is not only relevant in (semi-)arid regions. For example, in the Alpine and Subalpine areas, water abstraction due to hydroelectric power production represents one of the major factors of alteration of water bodies (CIPRA, 2005). A decrease of water quantity is directly related to a decrease of the capacity of freshwater ecosystems to dilute anthropogenic contaminants, and can influence the physico-chemical and biological characteristics of the ecosystem (Barceló and Sabater, 2010, Petrovic et al., 2011). Thus, water scarcity, along with water quality deterioration problems resulting from a global change scenario, have become two of the most important threats for the sustainability of aquatic ecosystems in (semi-)arid areas and in other regions with excessive water abstraction (Davis et al., 2010; Vörösmarty et al., 2010; Petrovic et al., 2011).

In arid and semi-arid regions, natural hydrological variation leads to severe drought periods that alternate with wet phases and occasional floods in a periodic basis (García-Roger et al., 2011; Robson et al., 2011; Boix et al., 2010). Water bodies presenting periodic wet and complete drought cycles are defined as 'temporary' water bodies. Permanent and variable low volume water bodies can be also found within these areas, providing a mosaic of habitat types (Williams, 2005; Ademollo et al., 2011; Robson et al., 2011). On the other hand, the hydrological alterations resulting from an expanding water scarcity scenario are expected to modify current hydrological patterns and associated ecosystems' functioning (Barceló and Sabater, 2010). The expected increase in frequency, intensity and duration of drought periods associated with climate change (Verdonschot et al., 2010; IPPC, 2014) and an increasing anthropogenic water demand (Verdonschot et al., 2015; Navarro-Ortega et al., 2012) may lead to substantial changes in the aquatic landscape configuration and in the hydrological connectivity between water bodies of these regions (Robson et al., 2011; Snelder et al., 2013; Datry et al., 2016). This can lead to changes in biogeochemical processes (Petrovic et al., 2011; Corcoll et al., 2015),

geomorphological dynamics and habitat structure and availability for aquatic organisms (Barceló and Sabater, 2010). As a consequence, this can result in aquatic communities suffering dramatic changes in their structure and functioning, through the adaptation to new hydrological conditions (Robson et al., 2011).

The impact of low flows and complete drying on the aquatic community has been largely studied (Stanley et al., 1997; Sabater and Tockner, 2010; Verdonschot et al., 2015; Datry et al., 2016). Several studies refer to an extraordinary adaptation and recovery capacity of natural communities in these environments (Yount and Niemi, 1990; Acuña et al., 2005; Williams, 2005). This capacity is explained by the prevalence of specific physiological and behavioral traits such as resistance to oxygen depletion, drought-resistant eggs, dispersal abilities or migration to dry-season refuges (Lahr, 1997; Robson et al., 2011; Storey and Quinn, 2013). It is questionable, however, whether the expected increase in the frequency and magnitude of extreme events and the increase in water abstraction, will go beyond the tolerance range of most species characteristic of those ecosystems. Whether these species will be affected by other human-related impacts such as those directly or indirectly related to chemical pollution is another remaining question under these constantly changing and multiple stressed scenarios.

The relationship between water quality and quantity has been recognized in the European Water Framework Directive (WFD; Directive 2000/60/EC), addressing the importance of managing water quantity to ensure a good water quality status. Also, several scientific committees (SCHER, SCENIHR and SCCS) have highlighted the need of developing more ecologically realistic scenarios, as well as the inclusion of multiple stressors on ecological risk assessment (EC, 2013). More specifically, studies focused on arid and semi-arid regions suggest that the combined impact of water scarcity, its correlated physico-chemical changes and pollution on the community need to be further considered (Petrovic et al., 2011; Osorio et al., 2014; Sabater et al., 2014). However, there is still a big uncertainty on how hydrological variation and chemical pollution affect aquatic communities under varying environmental conditions (Petrovic et al., 2011; Navarro-Ortega et al., 2014). In this study, we provide a detailed description on the fate of contaminants in aquatic ecosystems under water scarcity conditions and on the characteristics of aquatic communities inhabiting them. Furthermore, we review the current knowledge on the combined effects of chemical pollution and the environmental stressors associated to water scarcity on the structural and functional characteristics of aquatic communities. With this review we attempt to identify and describe the perceived gaps on current ecological risk assessment of chemicals in scenarios of water scarcity and provide recommendations for future research.

2. Influence of water scarcity on chemical exposure

The physico-chemical status of water bodies is directly related with water quantity (Barceló and Sabater, 2010), and can therefore result in alterations on the exposure of contaminants, as summarized in Figure 1. Decreased water availability corresponds to a reduced current velocity, reduced thermic stability and modified evaporation patterns in lotic (Hamilton et al., 2005) and lentic waters (e.g. ponds) (Lahr, 1997). Simultaneously, these changes generate an impact on other physico-chemical variables. In warm climates, temperature increase is directly related to lower oxygen solubility, as well as to an acceleration of metabolic processes, resulting on an

overall reduction of oxygen levels (Carere et al., 2011) and on lower pH values. These pH changes can generate a direct impact on chemical exposure by releasing sequestrated metals in bottom sediments (Arnott et al., 2001). Moreover, low flows are directly related with a lower dilution capacity, resulting in an increase in concentration of pollutants in water (Ricart et al., 2010; Boxall, 2011; Osorio et al., 2014).

Reduced water availability may also influence turbidity and light penetration. In flowing waters, where turbidity is mainly due to suspended solids, reduced flow results in lower turbulence and higher deposition rates (Kirkby and Froebrich, 2006, cited in Ademollo et al., 2011). This, associated with lower depth, results in a deeper light penetration which influences the photolysis of contaminants (Lam et al., 2005; Ademollo et al., 2011). However, in particular cases, high turbidity levels may occur due to stirring up of bottom materials associated with rapid temperature inversions and kinetic energy transfer by wind (Williams, 2005). This phenomenon is more relevant in lentic ecosystems, despite turbidity is mainly related to the presence of phytoplankton. In these systems, lower water level may also increase nutrient re-suspension, increasing algal growth. In flowing waters, reduced velocity also reduces oxygen levels by impeded reaeration processes (Petrovic et al., 2011) as well as higher organic matter deposition rates (Ademollo et al., 2011; Petrovic et al., 2011). This may modify exposure patterns to contaminants because suspended solids with high organic carbon and specific physico-chemical properties can act as a sink of hydrophobic chemicals (Van den Berg et al., 2001; Zoumis et al., 2001).

Intense and frequent floods can have an impact on sediment resuspension and transport, generating remobilization of suspended solids and previously absorbed hydrophobic pollutants (Obermann et al., 2009). Moreover, an increase in turbidity due to particulate matter resuspension can also reduce the photolytic degradation of pollutants. More turbulent conditions have also been related with exposure of anoxic sediments to oxic conditions, generating a change in the chemical properties of the sediment-contaminant complex that causes mobilization and transfer of chemicals to the water layer (Calmano et al., 1993; Zhuang et al., 1994). Furthermore, sediments can be completely air exposed during periods where the flow or water level is extremely reduced or stops, as it is typical of (semi-)arid areas. The decrease in moisture leads to a positive change in the redox potential (Eh), and a decrease in sediment pH, generating changes in pollutant mobilization, volatilization and degradation (Eggleton and Thomas, 2004). Drying processes have been proved to increase sediment capacity for sequestrating organic pollutants and reduce compounds volatilization as well as microbial activity (Ademollo et al., 2011). Conversely, replicated drying/rewetting cycles generally lead to desorption of the leachable and mobile fraction of contaminants by changes in physico-chemical conditions. Nevertheless, continuous and fast cycles can also lead to activated microbial activity and consequent pollutant uptake and biodegradation. On the other hand, microbial activity can degrade chemical compounds but also be the cause of increased toxicity in waters by transforming parent molecules into polar, more soluble metabolites (Ademollo et al., 2011).

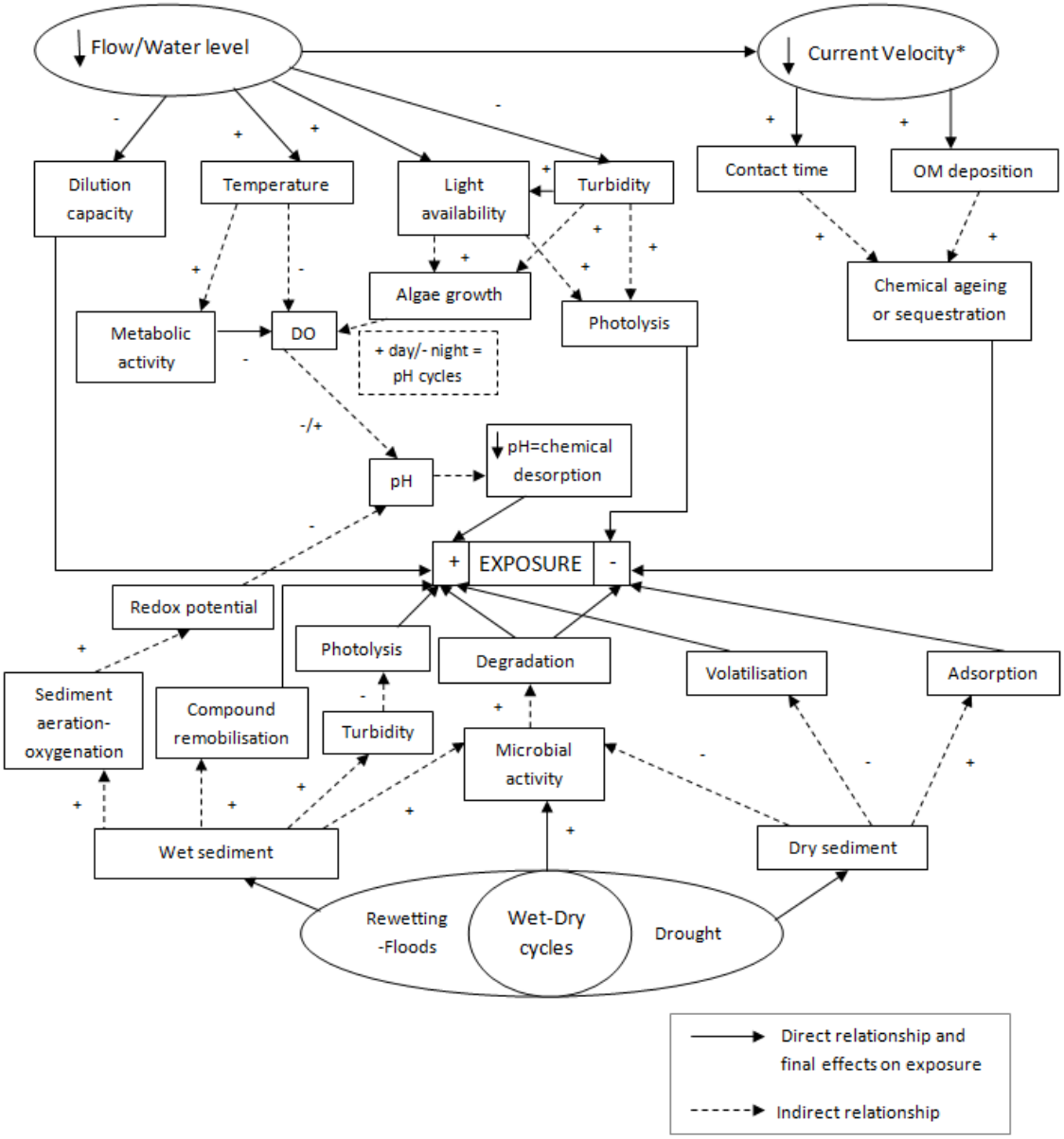

Figure 1. Physico-chemical variables affected by water scarcity and processes influencing the fate and dissipation of contaminants in aquatic ecosystems. +: positive influence on a variable or final exposure; -: negative influence on a variable or final exposure; -/+: positive or negative influence on pH depending on the prevalence of physico-chemical or biological processes (i.e., day/night pH and dissolved oxygen –DO- cycles); OM: organic matter *: processes only applicable in lotic systems.

In conclusion, water scarcity and drying/rewetting events result in complex interactions among hydrological and physico-chemical processes that affect chemical exposure patterns in aquatic ecosystems. At this stage it is difficult to conclude on whether water scarcity and associated hydrological events will necessarily result in an increasing level and duration of chemical exposure to aquatic organisms. The prevalence of some processes above others can vary according to the physico-chemical properties of the contaminant and the characteristics of the evaluated environmental scenario. Furthermore, the knowledge in which we can build these conclusions is based on existing fate studies relating physico-chemical processes to the observed dissipation of chemical substances. However, a very limited number of formal studies evaluating the fate of contaminants in scenarios of severe water fluctuation or intermittent flow conditions are available.

3. Influence of water scarcity on the structure and functioning of aquatic ecosystems

Species distribution, community structure and succession of aquatic communities are directly associated with variables that depend on the system's hydrology, such as physico-chemical characteristics, habitat type and hydrological connectivity (Fritz and Dodds, 2004; Acuña et al., 2005; Fritz and Dodds, 2005). Figure 2 shows a schematic overview of the biological changes occurring in different phases of water availability in lentic and lotic systems. Decreases in water availability are usually associated (at least in temperate/warm climate) to increased water temperatures, high nutrient and suspended solid concentrations, and decreases on dissolved oxygen concentrations (DO) (Stanley et al., 1997; Golladay et al., 2002). Under these circumstances, only species capable of tolerating higher turbidity and more eutrophic-like conditions usually persist (Acuña et al., 2005; Williams, 2005). Additionally, in unshaded lentic water, the build-up of nutrients, high temperatures and solar radiation facilitate the formation of algae blooms (Dahm et al., 2003) and consequent formation of anoxic water layers underneath with extreme pH and oxygen daily fluctuations (Williams, 2005). This last concept is included in Figure 1 as an indirect physico-chemical factor affecting not only biota, but also chemical exposure due to changes in pH.

As water level is reduced the riparian edges of the river dry-up. The consequence is not only a reduction of the space covered by the river but also a loss of riparian habitats for aquatic species. This phenomenon is usually called 'ecosystem contraction' (Lake, 2003), with the opposite process occurring during the rewetting period, also known as 'ecosystem expansion' (Stanley et al., 1997). In the contraction phase, the remaining wet parts usually show an increase in total density (Datry et al., 2016) and species richness but a reduction in total biomass (Boulton and Stanley, 1995; Clinton et al., 1996). In this regard, Acuña et al. (2005) suggest that ecosystem contraction can induce maximum habitat heterogeneity and biotic diversity at some intermediate levels (phase 2 in Figure 2). On the other hand, such habitat reduction usually leads to space and food resource limitations, resulting in an increase of predation and intra- and inter-specific competition (Lake, 2011; Datry et al., 2016). In the long term, such high predation and competition stress, together with harsh physico-chemical conditions, usually contribute to the extinction of the most vulnerable species and the establishment of simplified food webs (phase 3 in Figure 2) (Lahr, 1997; Acuña et al., 2005). For example, Acuña et al. (2005) found that slight flow reduction resulted in an increase of macroinvertebrate density but reduced biodiversity. Taxa with low DO requirements (e.g. Naididae, Lumbriculidae, *Chironomus* sp., and adult Dytiscidae and Hydraenidae beetles) increased significantly as flow decreased, but taxa with high DO requirements (e.g. *Ancylus fluviatilis* and *Bithynia* sp.) rapidly disappeared. In general, as aquatic ecosystems are affected by flow intermittency, generalist species dominate over specialist ones, with the consequent simplification of the food web (Southwood et al., 1974, cited in Bonada et al., 2007a; Corti and Datry, 2015).

Complete dryness constitutes an inflexion point in the ecosystem dynamics as implies the disappearance of aquatic forms and the majority of the organisms that are not capable to tolerate droughts (phase 4 in Figure 2). Aquatic communities in arid and semi-arid regions, such as the Mediterranean, usually exhibit a wider range of adaptation patterns to tolerate drought than communities in humid regions (Bonada et al., 2007b). The adaptation patterns vary considerably among taxonomic groups and range from the modification of cellular structures, in

the case of primary producers, to the occurrence of particular egg, larvae or adult physiological forms as well as specific behaviors capable to tolerate periods of dryness (Table 1; Williams, 2005; Bonada et al., 2007b; Bogan and Lytle, 2011; Storey and Quinn, 2013). Dominance of species with short life cycles, small body size, specialized respiration techniques, diapause stages and capable of producing resistant eggs or juveniles are usually found in these systems. Avoidance of the dry phase by air dispersal, migration to higher flow sections or 'resting' in sheltered places or pools (refugia) are some of the behavioral patterns typically used to prevent total dryness (Williams, 2005; Datry et al., 2016). Strategies to recolonize after drought (phase 5 in Figure 2) are mainly based on behavioral avoidance, more specifically on avoidance by aquatic (e.g. fish) or aerial migration (e.g. hemipterans and coleopterans) and subsequent recolonization after water flow is re-established (Williams, 2005; Bogan and Lytle, 2011). Several authors have related community adaptation to a constantly changing environment, as it is the case of temporary water bodies typically found in (semi-)arid regions, with an overall high taxa richness and diversity in these systems (Williams, 2005; Bonada et al., 2007b). A summary of the major taxonomic groups with traits that are capable to resist or recolonize these highly variable environments is presented in Table 1. This table summarizes the most relevant features of each taxonomic group, however these features are not necessarily applicable to all the species included in each group and differences can be observed between species. This is the case of cladocerans, which normally exhibit a wide tolerance to pH, temperature and dissolved oxygen, but yet some species can be restricted to very narrow ranges of these abiotic parameters. Calanoida, Cyclopoida and Harpacticoida are the main Copepoda species found in these environments, however, adaptation patterns differ among them. For example, harpacticoids and calanoinds are capable of producing subitaneous eggs (hatching within few days) and resting eggs (long periods of dormancy), while cyclopoids produce only subitaneous eggs but may enter a resting stage as late copepodites (Williams, 2005). Also, not all fish species are capable of resisting extreme conditions related to these environments. Only migratory species or the highly specialized ones such as lungfishes, capable of aestivating at the bottom wet mud during the dry phase (Williams, 2005) are usually found in these environments.

Biological traits have an important influence on resistance or resilience to extreme water fluctuations or desiccation. Nevertheless, temporal dynamics in rewetting and spatial characteristics of the landscape play a substantial role in the recovery of populations and communities after the rewetting period (Lytle and Poff, 2004; Acuña et al., 2005; Williams, 2005; Bonada et al., 2007b) (phase 5 and phase 6 in Figure 2). For example, populations recovering too quickly to rewetting could be subject to mass mortality if a summer rainfall causes a false start of the rewetting period. Similarly, slow responses can result in a loss in the competition advantage with other species or insufficient time to effectively recolonize the system before other species dominate (Williams, 2005; Storey and Quinn, 2013). The degree of isolation of ponds or streams with respect to other water bodies, represented by the effective distance to permanent or wet aquatic ecosystems and the landscape permeability, is known to influence the opportunities for effective aerial recolonization (Galic et al., 2013) and post-drought recovery.

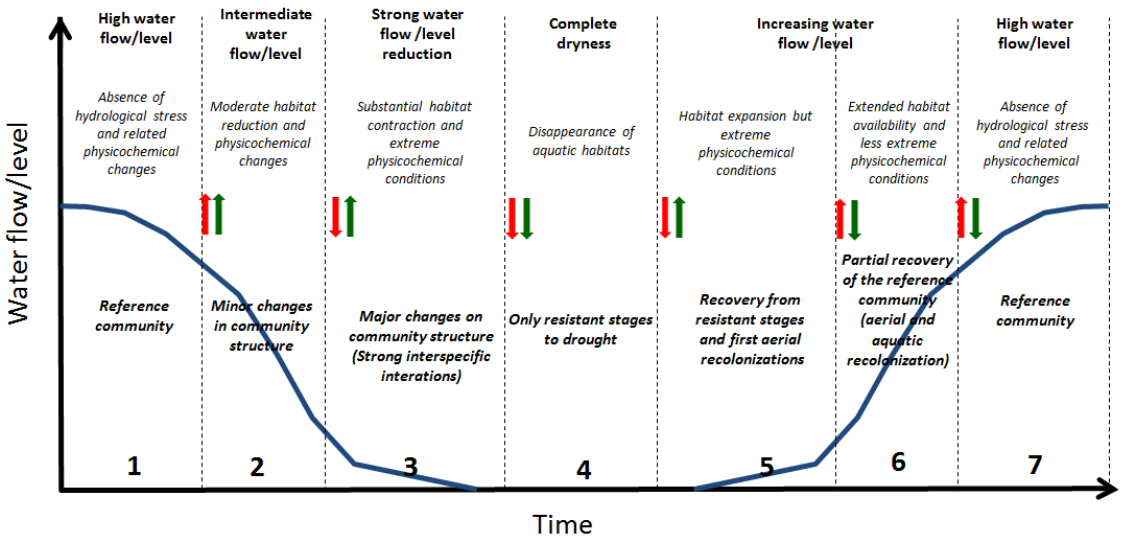

Figure 2. Conceptual description of the effects of water flow (lotic systems) or water level fluctuations (lentic systems) on the characteristics of aquatic communities. Numbers from 1 to 7 represent the different phases with respect to hydrology, physico-chemical changes and community composition. Red and green arrows refer to the positive (upwards) or negative (downwards) trend in biodiversity and density of organisms, respectively, based on available literature (see text).

4. State-of-the-art on the combined effects of water scarcity and chemical pollution on aquatic ecosystems

A literature search was performed in order to evaluate and discuss some examples of laboratory and field studies dealing with the combined effects of water scarcity and chemical exposure on aquatic ecosystems. The databases Web of Knowledge and Scopus were used to search for relevant studies. The following search formula was introduced: ("Aquatic organism" OR Biofilm OR Alga OR "Aquatic invertebrate" OR "Aquatic community") AND (Ecotoxicology OR Pollutant OR Chemical OR Contaminant OR "Multiple stressors") AND ("Water scarcity" OR Drought OR Temporary). This search was used as an initial data source. However, due to the reduced amount of articles fitting the selection criteria, relevant references found in these articles were selected as well. Only peer-reviewed papers describing experiments or field monitoring studies that evaluated water scarcity itself (and/or its consequences on the alteration of physico-chemical variables) together with pollution stress were evaluated. From this literature search, 15 papers were selected. From those, only six consist of experimental studies using laboratory or semi-field (mesocosms) designs in which the hydrological conditions and chemical exposure patterns are controlled (Table 2). A classification based on the cumulative response of the interaction between stressors in these studies was performed. This classification is based on the approach described in Piggot et al. (2015), in which the effect resulting from the interaction between two stressors can be described as additive, synergistic or antagonistic depending on the statistical significance of the interaction and the magnitude and direction of the individual and combined effects caused by the evaluated pair of stressors. The interaction is defined as additive when there is no significant interaction between factors and as positive or negative synergistic when there is a significant two-factor interaction and the effects are more positive or negative than predicted additively, respectively. The same statistical criterion is followed for positive or negative antagonistic effects, but in this case the effects are

less positive or negative than predicted additively, respectively (Table 2). The rest of the articles selected are based on field studies in which the inter- and/or intra-annual hydrological variation (and consequent physico-chemical changes) was monitored *in-situ* in (semi-)arid regions of Southern Europe and Africa (Table 3). These field studies are based on comparative assessments of different time points or rivers with assumed similar properties but different flow and pollution conditions. A pollution gradient was generally covered in a spatial scale along the selected rivers. The biota groups assessed in these studies were: fungi, bacteria, algae, zooplankton, macroinvertebrates and fish. Bacteria and algae were mostly studied as components of biofilm structures. In the next sub-sections, a brief description of the outcomes of these studies for each taxonomic group is provided.

4.1 Fungi and bacteria

The effects of water scarcity and chemical pollution on fungi and bacteria have been evaluated under controlled conditions making use of artificial streams, consisting of glass or methacrylate straight channels in which water was recirculated during the whole experiment. Pesce et al. (2016) evaluated the individual and combined effects of flow intermittency and the fungicide tebuconazole (20 µg/L constant exposure) on leaf-associated fungi and bacterial communities. In their study, flow intermittency significantly influenced the fungal and bacterial community structure and increased its biomass, while reducing its enzymatic activity and their leaf litter decomposition capacity. The fungicide tebuconazole only affected the structure of the fungal community. The combination of water flow intermittency and tebuconazole exposure resulted in a slight increase on the responses observed in the flow intermittency treatment as compared to the continuous flow treatment. However, differences were not significant in this case (additive effect). Results also suggested that flow intermittency had a stronger influence on the microbial community and mediated functions than the tested tebuconazole concentration. A possible explanation for the absence of drastic effects of tebuconazole is that the concentration used in the study was lower than the concentrations that proved to result in significant microbial effects in previous studies, which ranged from 33 to 500 µg/L (Bundschuh et al., 2011; Zubrod et al., 2011; Artigas et al., 2012).

Proia et al. (2013) found that flow reduction and flow intermittency increased the biofilm bacterial enzymatic activity but reduced their survival capacity (i.e. life-to-dead ratio). The observed increase in bacterial enzymatic activity contrasts with the results observed by Pesce et al. (2016). Nevertheless, different enzymes were analyzed in these studies. Proia et al. (2013) based their analysis on extracellular alkaline phosphatase activity. Alkaline phosphatase enables the release of inorganic phosphorous available for microbial uptake, especially when inorganic phosphate is limiting (Allison and Vitousek, 2005), as it was the case of this study. The enzyme β-glucosidase was analyzed by both Corcoll et al. (2015) and Pesce et al. (2016), however the first study also found an increase in enzymatic activity. They suggested that the increase in enzymatic production could be associated with a co-tolerance to short-term exposure to pharmaceuticals after a drought period. On the other hand, Pesce et al. (2016) associated the decrease in enzymatic activity after drought stress with a trade-off between stress tolerance and cell functioning, with more energy allocated to cell maintenance than to enzymatic production. In the studies by Corcoll et al. (2015) and Proia et al. (2013) the isolated effects of chemicals were also evaluated. Corcoll et al. (2015) found that a pharmaceuticals

mixture (total nominal concentration of 5000 ng/L, constant exposure) containing compounds with antimicrobial mode of action significantly reduced bacterial taxa richness, whereas Proia et al. (2013) found that the bactericide triclosan (110 µg/L pulse exposure) reduced the bacterial survival rate, but increased enzymatic activity. In the study by Proia et al. (2013), the combined effect of triclosan and drought significantly decreased the bacterial survival and prolonged the recovery of the phosphorus uptake rate (negative synergistic effect).

Field studies also presented some general insights on the bacterial community response under different water scarcity and pollution scenarios. For example, Sabater et al. (2016) found that flow variability and the associated changes in physico-chemical variables had a stronger influence on the structure of the bacterial community than chemical pollution, measured based on a group of 157 organic micropollutants. However, the combined impact of both stressor groups had a larger explanatory power in the evaluated community responses, and resulted in negative effects on the measured bacterial enzymatic activity. Conversely, Ponsatí et al. (2016) described that industrial pollution, herbicides and pharmaceuticals were more strongly correlated with bacterial density and enzymatic activity as compared to hydrological changes. It must be noted, however, that the hydrological variability in the later study was only measured for 15 days before sampling (once per year) which may lead to an underestimation of the actual variability in flow conditions. In this case, the combined effect of both stressor groups explained a larger proportion of the bacterial variability. Furthermore, in these two last studies, instead of changes in total bacterial density, shifts on relative abundances and trophic simplification were associated with increasing flow variability and pollution levels. This was also applicable to the algae present in the studied biofilms. Osorio et al. (2014) studied the combined effect of flow variability and pharmaceuticals, and found that chemicals concentration had a significant inverse relationship with flow. They also observed that bacterial enzymatic activity was higher in conditions with high chemical concentrations and stable low flows, than in conditions with variable and high flows. However, the direct correlation between hydrology and bacterial community responses was not assessed. They also found that floods have a negative effect on biofilm biomass, structure and recovery capacity, and antibiotics significantly reduced bacterial survival, independently of hydrology (Osorio et al. 2014).

4.2 Algae

Algae were one of the most largely studied groups, together with bacterial communities. The negative impact of low flows and flow intermittency on biomass, taxa richness and net primary production was described by Corcoll et al. (2015) and Proia et al. (2013). A positive influence on green algae and cyanobacteria proliferation was described as well in these studies. A negative effect was also observed on diatom survival rate. In these studies, the effects of isolated chemical exposure resulted in a reduction of cyanobacterial abundances and photosynthetic activity for pharmaceuticals (Corcoll et al. 2015), and a strong but not statistically significant decrease in diatom survival in the case of triclosan (Proia et al. 2013). Furthermore, the combined effect of flow variability and chemical exposure (i.e. pharmaceuticals mixture at 5000 ng/L constant exposure or triclosan at 110 µg/L pulse exposure) showed greater dominance of green algae over cyanobacteria and diatom communities. An increase in primary production after combined exposure to flow intermittency and pharmaceuticals mixture as compared to the isolated effect of flow intermittency (negative antagonistic effect) was found in

Corcoll et al. (2015). Proia et al. (2013) also found a significant negative effect on the diatom survival rate after exposure to combined stress conditions, despite the isolated effect of flow intermittency showed stronger effects (negative antagonistic effect). Recovery, with respect to diatom survival rate, was not achieved within the study period under flow intermittency or combined stress conditions. However, algae recovered in the treatments that were exposed to triclosan as single stressor. The study performed by Sabater et al. (2002) showed that low flows and exposure to Cu^{2+} (15 µg/L) had a significant negative effect on algae biomass and photosynthetic activity. However, these authors also found the combined effect of low flow velocities and Cu^{2+} exposure resulted in a less negative effect on these endpoints than the sum of both individual stressors (negative antagonistic effect). Apart from that, Sabater et al. (2002) highlighted the high tolerance capacity of algal communities after being exposed to a chemical stressor for a prolonged time, which agrees with the PICT (Pollution Induced Community Tolerance) concept introduced by Blanck (2002). The PITC concept assumes that toxicants exert a selective pressure on the biological community capable of provoking a replacement of species or inducing a phenotypic adaptation of individuals (Blanck, 2002). Accordingly, Sabater et al. (2002) found higher tolerance of the algae species *Achantes minutissima* and *Stigeoclonium tenue* to Cu^{2+} at higher flow velocities, which dominated over the sensitive species *Synedra ulna*. The authors explain that this increase in algae tolerance to Cu^{2+} may be related with increased Cu^{2+} bioavailability over time due to damage on the boundary layer of biofilms at higher current velocities.

Regarding field studies, several algae responses have been observed. Sabater et al. (2016) found that flow variability was the most important variable compared to a wide range of organic micropollutants, similarly to what was observed for bacterial constituents of the biofilm. Nevertheless, the combined impact of both groups of stressors was a better predictor of the variability observed in the community structure, resulting in less diverse communities dominated by tolerant species. On the other hand, Ponsatí et al. (2016) concluded that pollution was the most important stressor influencing the community structure, resulting in an increase of algae biomass and a decrease in tolerance to radiation excess, as compared to hydrological changes. However, the hydrological data analysis in this study might have some weaknesses as explained previously (section 4.1). Osorio et al. (2014) also found high algae biomass related with pollution increase and, despite the direct correlation between community and flow variability was not evaluated, the beneficial effect of stable flows on algae biomass was also observed. Ricart et al. (2010) and Brix et al. (2012) discussed the results of the same sampling campaign on the Llobregat basin. Ricart et al. (2010) found that herbicides were the main stressors influencing structure and functions of the diatom community. In particular, pesticides were responsible for >91% of the variance of chlorophyll-a response. More surprisingly, Brix et al. (2012), working on the same samples, determined that a group of alkylphenolic compounds (APCs), known as endocrine disruptors (EDCs), were the known factors explaining the largest fraction of variance with respect to the diatom community distribution. This result is in contrast with the fact that a direct effect of EDCs on the diatom community is unlikely to occur due to the lack of endocrine systems in photosynthetic organisms. However, in the same study, it is also stated that a large fraction of unexplained variance still exists. Therefore, these effects may result from the exposure to associated compounds deriving from different pollution sources (i.e., urban, industrial, agricultural) which were not analyzed in the study. A correlation between

pesticides studied in the first paper (Ricart et al., 2010) and EDCs studied in the second (Brix et al., 2012) was not performed. Some other physico-chemical variables which might be related with water scarcity (e.g. temperature) were related with algae metabolic activity and green algae/cyanobacterial abundances. Nevertheless, despite the experimental design in both studies was relevant to assess seasonal hydrological variation and its influence on aquatic communities, no direct hydrological analysis was applied in either of them. With respect to insecticide application combined with high hydrological variation in temporal ponds, Fayolle et al. (2015) found that community structure and organism density was determined by hydrological conditions rather than by insecticide application (*Bti* serotype H14).

4.3 Zooplankton

Martin et al. (2014) studied the combined effects of decreasing water depth and two contamination treatments of fire-retardants on zooplankton population abundance and community structure using mesocosms. In this study it was not possible to identify a significant isolated effect of the hydrological and physico-chemical variables associated to the water depth decrease, but a significant reduction on diversity and an increase in total density related with the fire-retardants exposure at the highest concentration treatment was found. The combined effect of decreasing water depth and fire-retardants contamination resulted in a statistically significant decrease in diversity (negative synergistic effect) and an increase in total density (negative antagonistic effect) at low concentrations, reaching similar values to those resulting from the isolated effect of the chemical exposure at high concentrations. The increase in density was related with the dominance of some tolerant rotifera species such as *Brachionus urceolaris*, which most likely benefited from the competition with other populations that less capable to tolerate reduced flow conditions, such as *Brachionus calyciflorus*. Also, *Ceriodaphnia quadrangula* showed variable responses depending on the chemical treatment (Martin et al., 2014).

The impact of several fire-retardant treatments on the recovery of the zooplankton community after desiccation was studied by Angeler et al. (2005) by means of an indoor microcosm study in which sediment from a dried out wetland was used. These authors found that abundances of Ostracoda, Cladocera and Rotifera species were significantly higher in the chemical controls after the return of the aquatic phase than in the chemical treatments. Treatments with the lowest exposure concentration showed a significant decrease in community diversity as compared to the chemical control, with a significant dominance of bdelloid rotifers towards the end of the experiment. Maximum diversity values were reached after three weeks for this treatment and the control. Treatments at the medium and high application rates had a significant negative effect on species abundances and diversity, with no recovery during the whole experiment. Therefore, it could be concluded that pollution had significant impact on community structure. However, to draw clearer conclusions on the isolated or combined effect of stressors, treatments with no desiccation or pre-desiccation phase and treatments combining no-desiccation and fire-retardant exposure, would be needed.

Table 1. Relation of key of biological traits for surviving and recolonizing aquatic ecosystems under water scarcity conditions. The traits described are characteristic of the indicated taxonomic group but do not compulsorily apply to all the species within the group. Some notes have been included in the text (Section 3) for better clarification. The different phases indicated in the table correspond with the low water flow/level (phase 3), desiccation (phase 4) and rewetting (phase 5) periods as described in Figure 2. [1]

		Adaptation traits to low water flows (levels) and drought																																				
		Resistance strategies																																		*Recolonization*		
		Phases 3 - 5		Phases 4- 5																																Phase 5		
		Inmature and adult stages		Cellular structures					Eggs and pupating structures						Larvae and juvenile stages				Methamorphic intermediate stages				Adult stages												Inmature and adult stages		
Taxonomic groups		Resistance to high temperatures	Resistance to oxygen depletion	Modified vegetative cells	Mucilaginous sheaths	Oil accumulation in cells	Heat-resistant asexual cyst	Resistant cyst	Dormant eggs	Diapausing eggs	Resistant eggs	Ephipial eggs	Gelatinous egg mass	Cocoons	Resistant larvae	Larvae deep in substrate	Inmatures near groundwater	Juveniles in burrows at groundwater table	Resistant pupae	Terrestial pupae	Semi-terrestial pupae	Resistant nymphs	Adults in burrows at groundwater table	Adult or near adult survival in moist soil	Dehydrated individuals	Secretion of protective cysts	Ephigram of dried mucus across shell	Refugia under algal mats	Construction of small mucus-lined 'cells'	Dormant adults in substrate	Burrowing aduls	Aerial migration (adults)	Aquatic migration	Larvae migration through parasiting
Microorganisms	Algae/flagellated protozoans	■		■	■	■	■																															
	Cyliophora	■																																■		
Lower invertebrates	Cnidaria	■																																		
	Plateminta							*	■					■																						
	Nematoda	■																																		
	Gastrotrichia	■																																■		
	Rotifera	■																							■	■								■		
Annelids	Oligochaeta	■	■					*	■																											
	Hirudinea	■																						■					■							
Molluscs	Bivalvia	■																																		
	Gasteropoda	■																								■	■									
Aracnids	Acarina													■																						■
	Araneida	■																																■		
Crustaceans	Anostraca	■									■																							■		
	Notostraca										■																									
	Conchostraca										■																									
	Cladocera	■	■									■												■												
	Copepods	■						**		■					■																■			■		
	Cyclopida	■																																■		
	Ostracoda										■													■											
	Amphipoda/Isopoda	■															■																	■		
	Decapoda	■																■					■												
	Other crustacea	■																																■		
Insects	Collembolla										■																									
	Megaloptera	■																																■		
	Plecoptera	■													■																			■	■	
	Ephemeroptera	■									■																							■	■	
	Odonata	■																				■												■		
	Lepidoptera	■	■																															■		
	Hemiptera	■	■																															■	■	
	Hymenoptera		■																																	
	Neuroptera		■																																	
	Coleoptera	■	■								■										■													■		
	Tricoptera	■								■			■			■				■														■	■	
	Chironomidae	■	■								■			■	■																			■	■	
	Simuliidae	■	■																															■		
	Culicidae	■	■								■				■				■															■		
	Other Diptera	■	■								■				■				■															■	■	
Vertebrates	Fishes										■																				■					
	Amphibians																														■					

*Cysts enclosing young, adults or fragments of animals. **Cysts enclosing adults. [1] The table has been built using biological trait information from Lahr (1997), Tachet et al. (2000), Williams (2005) and Rico and Van den Brink (2015).

Only one field study has been performed investigating the zooplankton community (Lahr et al., 2000). Lahr et al. (2000) demonstrated a high sensitivity of cladocerans to insecticides (fenitrothion, diflubenzuron, deltamethrin and bendiocarb) after application during the wet season. A qualitative analysis of the hydrological seasonality based on data from previous monitoring campaigns was used in this study. The study showed that recovery capacity of some species after insecticide application may be influenced by changes in intensity and duration of hydrological cycles. Depending on their drought resistant capacity, some populations were found to recover faster than others during the post-drought treatment phase, consequently affecting the final community structure. For example, cladocerans showed a high sensitivity to insecticides but a fast recovery (3 to 6 weeks) was observed due to their capacity to reproduce continuously by parthenogenesis and depositing resting eggs during the dry season. This capacity allowed them to recover even when the population was dramatically reduced after the insecticide application during the wet season (Lahr et al., 2000).

4.4 Macroinvertebrates

Macroinvertebrates have only been studied at the individual level in controlled laboratory studies. Pesce et al. (2016) described indirect effects in *Gammarus fossarum* feeding rates caused by alterations in the fungal community due to drought and fungicide stress. As fungal biomass increased with flow intermittency, *Gammarus* decreased its feeding rates (measured as leaf surface ingested). However, when leaf surface ingested was converted to fungal biomass, no significant differences with continuous flow systems was found, which suggest a 'compensatory feeding mechanism' to reach nutritional requirements.

Several field studies have been found investigating the combined effects of toxic pressure and hydrological conditions on macroinvertebrates. As for primary producers (i.e. diatoms) constituting the biofilm surface, Sabater et al. (2016) concluded that flow variation had a significantly stronger influence on macroinvertebrate community structure than pollutants. However, once more, the combined effect of both stressor groups resulted in a higher explanatory power of the observed community variability than the influence of each stressor separately. Less diverse communities with dominance of tolerant species (e.g. *Chironomus* sp., *Branchiura sowerbyi*, *Limnodrilus hoffmeisteri* or *Caenis luctuosa*) were correlated with the increase in hydrological and chemical impairment along the selected rivers. Lahr et al. (2000) concluded that fairy shrimps (*Streptocephalus* spp.) and backswimmers (*Anisops* spp.) were the most vulnerable species to the application of four insecticides in temporary ponds. The impact of hydrological changes was not directly measured as in the case of zooplankton populations, but it was discussed as a key factor influencing the recovery capacity of different species. *Streptocephalus* spp. needs ponds to dry out before a new generation can be stablished from resting eggs. Therefore, this population showed difficulties to recover after the insecticide application done in the rainy season due to impeded hatching since no complete desiccation occurred before that application. *Anisops* spp. showed fast recovery by aerial recolonization when the rewetting phase was reestablished after the dry period. Other studies also found that flow variability and related physico-chemical factors were more important for macroinvertebrate community changes than herbicides, pharmaceuticals or even insecticides (Crosa et al., 2001; Ricart et al., 2010; Brix et al., 2012). Oppositely, Bollmohr and Schulz (2009) observed that the only variable having a significant negative effect on the community structure

were the organophosphates (OP), more specifically chlorpyrifos and azinphos-methyl; despite low flows had a significant impact on the increase of Ephemeroptera abundance and a positive correlation with total abundance. Most of the taxa, apart from Megaloptera, were indicated to be negatively correlated to OP. At the most polluted site, the shift in community structure was mainly due to the significant decrease in Ephemeroptera and Trichoptera, and the increase in Megaloptera densities. *Demoreptus* sp. and *Castanophlebia* sp. were significantly the most sensitive Ephemeroptera species to chemical pollution during the low flow period (Bollmohr and Schulz, 2009). Nevertheless, Brix et al. (2012) and Ricart et al. (2010) concluded that the combined influence of both groups of stressors (i.e. flow related physico-chemical variables and pollutants) had a strong influence on the benthic invertebrate community variability in the assessed systems, despite flow variability itself was not explicitly included in the analysis.

4.5 Fish

Only one field study evaluated the possible combined effect of hydrological seasonality and insecticide pollution on fish populations (Crosa et al., 2001). In this study, the effects of the direct insecticide (permethrin) applications to control populations of the blackfly *Simulium damnosum* in a Guinean river were monitored, together with hydrological and biological parameters to assess the impact of these treatments on non-target species (Crosa et al., 2001). In this study, no significant effects after insecticide application were found. However, short-term evaluations showed that temporal trends in fish catches were related to changes in river discharge, with high densities being found during dry periods and lower densities during wet periods. In the long-term, it was observed that the increase in mean annual fish catches was associated with an increase in volume of flowing water, but no influence of the insecticide was found (Crosa et al., 2001). These authors also concluded that invertebrates were better indicators of the short-term combined effects of chemical pollution and flow variability as compared to fish, due to their short life-cycles. The clear absence of short-term effects of permethrin on fish, which showed seasonal short-term variation mainly related to flow variation and their capacity to survive drought, is also a reason for this recommendation.

5. Concluding remarks and recommendations

The potential side-effects of chemical pollution in aquatic ecosystems has been widely recognized and studied by the scientific community. However, only a limited number of studies effectively describe the possible consequences of chemical pollution under water scarcity conditions. The available experimental studies have tested the effects of pharmaceuticals and home-care products, fungicides, metals and fire-retardants by means of indoor artificial channels and (micro-) mesocosms, using one (or at most three) exposure concentration(s) and following a factorial design. With respect to the experimental designs used, we found one study (Angeler et al., 2005) that evaluated the recovery of an invertebrate community in dried sediment from a lentic system, but missed a control treatment with no desiccation during the experiment. This made not possible to draw clear conclusions on the real effect of hydrological stress on the aquatic community as compared to the effect of chemicals (i.e. fire-retardants). It is recommended that future experiments evaluating the combined effects of both stressor groups include experimental units that include un-polluted controls with different hydrological conditions (e.g. regular flow vs decreased/ceased flow), as well as chemical treatments with the

same hydrological conditions, to evaluate the single and combined effects of each group of stressors.

The focus of the available studies has been on bacteria and algae forming biofilms, whereas only two studies have focused on zooplankton responses. Decreasing flow velocity or intermittency was artificially created in the experiments performed with biofilms, whereas in the experimental set-ups that comprised zooplankton, naturally water decreasing depth (up to desiccation) has been evaluated. From the outcomes of these studies it can be concluded that the effects of hydrological alterations on aquatic communities are, in most cases, higher than those of the tested chemical exposure concentrations. In the majority of the cases, intermittent droughts significantly influenced the biomass and the structure of the microbial communities (Sabater et al., 2002; Proia et al., 2013; Corcoll et al., 2015; Pesce et al., 2016), and affected important ecosystem functions such as leaf litter decomposition and photosynthetic activity (Sabater et al., 2002; Pesce et al., 2016). Decreasing water depth, however, has been found to yield no or very mild effects on the zooplanktonic community (Martin et al., 2014), whereas near to desiccation or desiccation cause significant changes in macroinvertebrate communities (Williams, 2005; Bonada et al., 2007a). Based on the approach described by Piggot et al. (2015), the majority of the endpoints studied in the selected experimental studies (Table 2) showed additive effects when exposed to water scarcity and chemical stress, being the first of the two stressors the greatest in magnitude. However, synergistic and antagonistic effects were also observed for some endpoints. The combined effects on diatom survival, primary production, total algae biomass and zooplankton total density described in four reviewed studies (Sabater et al., 2002; Proia et al., 2013; Martin et al., 2014; Corcoll et al., 2015) resulted in an antagonistic response. On the other hand, the authors of two studies (Proia et al., 2013; Martin et al., 2014) observed a more negative response on phosphorus uptake, bacterial survival and zooplankton biodiversity than the expected sum of individual stressors, yielding synergistic responses. It should be noted, however, that water level reductions or water intermittency influence several physico-chemical variables and habitat conditions at the same time, and therefore the so called ‘water scarcity stress’ must be regarded as a combination of stressors acting together (see sections 2 and 3). The observed responses are therefore context-dependent and are highly determined by the characteristics of the affected community and the tolerance range of each species to those varied stressors, including abiotic (e.g. nutrients, oxygen depletion) and biotic (i.e. intra- and interspecific competition, predation) variations, the timing of the stressors, and the landscape configuration that influence recolonization. For this reason, similar chemical exposure patterns may result in varied responses depending on the structure of the tested community and hydrological conditions. The recovery potential of biofilms to drought in a pollution scenario has only been evaluated in two of the selected studies (Proia et al., 2013; Corcoll et al., 2015), while three of the studies (Lahr et al., 2000; Crosa et al., 2001; Angeler et al., 2005) focused their recovery assessment on invertebrate communities. Due to their particular life-cycles and habitat requirements, these groups of organisms have been identified as good indicators of the ecological status of aquatic ecosystems after short-term (biofilms) and more persistent stress (invertebrates) conditions (Bonada et al., 2006; Sabater et al., 2007; Boix et al., 2010). Therefore, the development of new studies assessing recovery from water scarcity and chemical pollution should focus on these taxonomic groups.

Field monitoring studies have generally accounted for the combined impacts of intra and inter-annual hydrological variability in lotic systems and a wide array of chemical contaminants from urban, industrial and agricultural sources (e.g. metals, pesticides, pharmaceuticals, other endocrine disruptors). In most instances these studies made use of different ordination techniques to interpret the monitoring results. These studies have clear advantages as compared to the lab-based research since they were able to incorporate a more realistic view of the range of chemical and non-chemical stressors (e.g. physico-chemical alterations) that can affect biological communities in scenarios of limiting flow conditions. For instance, they were capable of assessing temporal and spatial variations in toxic stress pressure due to varying dilution potential of water bodies (Osorio et al., 2014), allowing the identification of correlations between chemical pollutants and other abiotic parameters, and contributing to a better understanding and identification of the multiple stressor groups that affect biological communities. Their major limitation resides at the establishment of clear causal relationships between the individual stressors that are grouped together and the biological community responses (for further discussions see Rico et al. 2016a). For this reason, they can be seen as hypothesis generating approaches, and several authors have referred to the need of complementary experimental approaches to disentangle the precise ecological effect of the field-based identified factors (Sabater et al., 2007; López-Doval et al., 2010; Ricart et al., 2010). Field studies have been crucial to identify the relative importance of hydrological variation in the invertebrate and the microorganism communities (Boix et al., 2010; Sabater et al., 2016), which are key for the study of the population and community dynamics and for the setting of reference conditions for further ecotoxicological assessments. In this regard, it is worth recognizing the contribution of the concluded SCARCE project and the on-going projects GLOBAQUA (Navarro-Ortega et al., 2012, 2014) and MARS (Hering et al., 2015) to the improvement of the existing knowledge of multiple stressors in (semi-)arid areas. It is expected that the outcomes of these projects help to elucidate the ecological processes that underpin the observed biological responses, and that an improved ecological framework can be obtained to understand the contribution of different stressors to ecological impairment in complex aquatic ecosystems (Kuzmanović et al., 2016; Sabater et al., 2016).

Based on the existing knowledge, we propose some suggestions for future research and for the development of an improved regulatory framework for the risk assessment of chemicals under water scarcity conditions:

1. Experimental studies: The development of more experimental laboratory and model ecosystem studies including communities that are representative of ecosystems vulnerable to both types of stressors is one of the most urgent steps. More studies focused on zooplankton and macroinvertebrate communities are needed, as they constitute important taxa on the basis of the trophic chain and are essential water quality indicators. Experiments covering a varied range of water scarcity pressures and chemical concentrations and life stages (diapausing eggs, immature stages) need to be performed. With respect to the chemical compounds studied, we suggest that more studies are performed with pesticides in model ecosystems simulating edge-of-field water bodies affected by water scarcity, particularly considering the discontinuous and intermittent character of their exposure patterns in relation to varying hydrological conditions. Studies focused on pharmaceutical and industrial compounds should be performed simulating ecosystems receiving waste water effluents from urban areas, where water level can become

highly variable (as well as chemical dilution) but rarely reach desiccation. Experimental research should also monitor the varying physico-chemical conditions and their influence on the fate of water contaminants in scenarios of severe water fluctuation or intermittent water flow conditions.

2. Field studies: The monitoring design and sampling techniques should be improved for lotic systems. One must be aware that the ecological characteristics of any stretch of a river are strictly depending on the conditions (natural or anthropogenic) occurring upstream. Therefore, the consequences of water scarcity and pollution, individually and in combination, should be studied considering the river (or a given segment of it) as a whole, investigating the impacts downstream of events occurred upstream. This kind of monitoring strategy is in agreement with the traditional concept of "river continuum" (Vannote et al., 1980) or with the more recent concept of "riverscape" proposed by Fausch et al. (2002). The advantages of such an approach may be a better understanding of the ecological dynamics of the river ecosystem and their modification due to combined stress factors, as compared to the approaches that are currently used. Moreover, monitoring of ecologically relevant river segments may allow assessing the impacts on more mobile components of the community (i.e. fishes). Some details on monitoring principles in this direction are proposed by Fausch et al. (2002).

3. Recovery assessment: Population and community recovery are critical to establish reference conditions after a chemical or non-chemical disturbance. Effects of chemical exposure and water scarcity may have different effects on population and community recovery depending on the characteristics of the disturbance, the traits of the affected and recolonizing communities, and the landscape configuration. The literature review performed by Gergs et al. (2016) determined that exposure to organic and inorganic pollution usually results in longer recovery times for macro-invertebrates in lotic ecosystems than droughts or flood events. They suggested that droughts may significantly affect the recovery of certain taxa and biological communities after a chemical disturbance (Gergs et al., 2016). It is important to dedicate more experimental and field monitoring studies to quantify the impact of water scarcity, including post-drought recovery, on chemical effects and to evaluate taxa and physiological traits that are affected by these stressor combinations. On the other hand, it is important to quantify the effects caused by the chemical legacy from pollution events that occur prior to a water scarcity scenario, which might have severe consequences for the monitored responses in a rewetting phase. The results of such studies may have consequences for risk-based management actions e.g. the establishment of pesticide buffer strips or safety margins for pesticides used next to temporary ponds or streams.

4. Risk assessment scenarios: The regulatory guidelines for the prospective risk assessment of chemicals are based on permanent water bodies. For instance, scenarios for the exposure assessment of pesticides in streams of southern Europe assume a minimum water level of 30 cm (FOCUS, 2001a, 2001b; EFSA, 2013). Thus, the need to protect small rivers or temporary ponds is not recognized, whereas peak exposure concentrations in these systems can be up to three times larger due to diffuse sources of pollution such as pesticide spray drift. The confirmation that water scarcity and the associated hydrological variability is increasing and expanding beyond arid and semi-arid regions (Barceló and Sabater, 2010; Acuña et al. 2014) supports the need of an improved consideration of these ecosystems in prospective risk assessments. Despite

the adaptation of exposure models and scenarios to water scarcity conditions, ecological scenarios and models should be developed to evaluate multiple stressor effects on the dispersal, survival and resilience of aquatic populations and communities -see Galic et al. (2010) and Rico et al. (2016b) for a description of ecological models and scenarios. Ecological scenarios should be developed including sensitive taxa to chemical pollution which encompass traits and life stages capable of surviving water scarcity conditions (Table 1). The dynamic physico-chemical (temperature, dissolved oxygen) and habitat features in the aquatic ecosystems associated to these conditions (i.e., ecosystem contraction and expansion) should be also considered in the development of these scenarios. The scenarios and models should account for different spatial configurations of chemical pollution and drying events in river networks and lentic water landscapes. It should be noted that intermittent aquatic ecosystems constitute mosaics of (lentic and lotic) aquatic and terrestrial ecosystems in which dispersal processes and environmental filtering influence (meta-) community dynamics (Datry et al., 2016). The usefulness of ecological models to assess long-term chemical risks and spatial-temporal extrapolations in these scenarios offers new opportunities to assess the combined effects of both types of stressors, as well as to evaluate the succession of aquatic and terrestrial communities and their energy fluxes.

5. Regulatory approaches: The need of more integrative and ecologically realistic, site-specific approaches is recognized in the WFD (Directive 2000/60/EC). Nevertheless, the problem of monitoring rivers that suffer from water scarcity is not fully addressed in the WFD, particularly in the definition of reference conditions (EC, 2003). The need for considering hydromorpholological characteristics for defining reference conditions is mentioned in the Annex II of the WFD: "type-specific hydromorphological and physico-chemical conditions shall be established representing the values of the hydro-morphological and physico-chemical quality elements specified". However, water flow is only mentioned as optional characteristic to be defined in the classification of river typologies, and no mention is made on the consideration of hydromorphological and physico-chemical seasonal variability in lentic systems. Moreover, only the quantity of river discharge (flow) is assumed as parameter and not its variability in the seasonal cycle, yielding variable results depending on the sampling time, the weather conditions, and the hydrological state of the river. It should be noted that a strong seasonal variability, up to intermittency, is a natural condition of water bodies in southern Europe. Moreover, due to the described expanding perspective of water scarcity, more regions may be affected by these hydrological conditions in the future. This condition strongly affects the quality of water and the potential effects of pollution and additional stress factors. Thus, understanding the functioning of highly variable or temporary systems, and describing adapted reference conditions and risk assessment approaches differing from those used for permanent ones is essential for reaching real 'good ecological status' (Gallart et al., 2012; Acuña et al., 2014). The establishment of reference conditions for temporary rivers presents a future challenge, principally because measures to establish habitat or community conditions under limited water flows (e.g. surviving forms in the hyporreic zone) are not fully developed and because the full ecotoxicological effects of chemical residues possibly found in river beds or pond sediments are not well understood. Suggestions for a better assessment of temporary rivers according to the requirements of the WFD have been proposed as part of the MIRAGE project (Prat et al., 2014). For instance Gallart et al. (2012) developed a novel approach for the definition of the (meso-)habitat occurring in a given reach of temporary rivers (i.e., flood, riffles, connected

pools, disconnected pools, dry) in a particular moment based on hydrological conditions. In a more recent study, Cid et al. (2016) used the aquatic state classification tool developed by Gallart et al. (2012) to define the taxonomic and biological-trait characteristics of the invertebrate community associated to some of these aquatic states (i.e., flowing, disconnected pools). The study by Cid et al. (2016) concluded that some families (e.g. Hydrophilidae, Simuliidae, Hydropsychidae) are important to classify the aquatic state, and some trait categories (e.g. feeding habits, food, locomotion, and substrate relation) provide even more accurate predictions of these aquatic states as compared to the taxonomic classification. This last study offers very relevant information for the establishment of aquatic states in reference temporary streams using routing biological monitoring information in the absence of hydrological data. Further studies should aim at refining the tools defined by Cid et al. (2016) including chemical exposure gradients in order to identify taxa and biological traits that are characteristic of a given hydrological condition and that are vulnerable to chemical stress. Through this, it might be possible to develop monitoring metrics and standards for hydrologically variable and temporary rivers affected by chemical pollution.

Table 2. Summary of selected experimental studies (laboratory, micro- and meso-cosms studies) dealing with the combined effects of water scarcity and chemical exposure in aquatic ecosystems. A more detailed version of this table, including the description of major findings per study, is provided as Supporting Information (Table S1).

Hydrological stressor	Chemical stressor	Experimental design	Taxonomic group	Biological endpoint	Stressors' interaction[1]	Reference
Flow intermittency	Fungicide (tebuconazole)	40 days artificial streams	Fungi and Bacteria	Biomass	AD (Fungi) AD (Bacteria)	(Pesce et al., 2016)
				Community structure	N/A	
				Leaf litter decomposition	AD	
				Enzymatic activity	AD	
		2 x 4 days *Gammarus* feeding assays	Macroinvertebrates	*Gammarus fossarium* feeding rate	AD	
Flow intermittency	Pharmaceuticals (1 psychiatric drug, 2 antibiotics, 2 β-blockers, 1 anti-inflamatories, 1 lipid regulator, 1 diuretic)	42 days artificial streams	Biofilms (Algae+Bacteria)	Total biomass	AD	(Corcoll et al., 2015)
				Net Primary Production (NPP)	-A	
				Community Respiration (CR)	AD	
				<u>Algae</u>		
				Biomass	AD	
				Photosynthetic activity (PA)	-A	
				Community structure	N/A	
				Algal taxa richness	AD	
				<u>Bacteria</u>		
				Bacterial density	AD	
				Bacterial Operational Taxonomic Unit (OTUs) richness	AD	
		2 x 24 hours acute toxicity test		Photosynthetic activity (PA)	-S	
				Bacterial enzymatic activity	AD	
Flow intermittency	Bactericide (triclosan)	47 days artificial streams	Biofilms (Algae+Bacteria)	Total biomass	N/E	(Proia et al., 2013)
				Enzymatic activity	AD	
				Phosphorus (P) uptake rate	-S	
				<u>Algae</u>		
				Community structure	N/A	
				Live-to-dead ratio	-A	
				Photosynthetic activity (PA)	AD	
				<u>Bacteria</u>		
				Live-to-dead ratio	-S	
Low flow velocity	Cu^{2+}	7 days artificial streams	Algae (in biofilms)	Biomass	-A	(Sabater et al., 2002)
				Community structure	N/A	
				Shannon-Wiener biodiversity index	N/E	
				Photosynthetic activity (PA)	-A	

Table 2 (cont.)

Hydrological stressor	Chemical stressor	Experimental design	Taxonomic group	Biological endpoint	Stressors' interaction[1]	Reference
Decreasing water depth	Fire-retardant (>90% ammonium polyphosphates and <1% yellow prussiate of soda)	1 year mesocosm	Zooplankton	Species richness	N/E	(Martin et al., 2014)
				Pielou´s evenness index	-S	
				Simpson diversity index	-S	
				Total density	-A	
				Community structure	N/A	
Desiccation	Fire-retardant (>90% ammonium polyphosphates and <1% yellow prussiate of soda)	4.5 months indoor microcosms (3 moths dry phase and chemical treatment, 1.5 months wet phase)	Zooplankton	Community structure	N/A	(Angeler et al., 2005)
				Shannon-Wiener biodiversity index	N/E	
				Eveness index	N/E	

[1] Classification based on Piggot et al. (2015). The acronyms refer to the five types of interactions between stressors described in this study. Depending on the direction of individual stressor effects and the direction the cumulative effect, the interactions can be: additive (AD), positive synergistic (+S, more positive than predicted additively), negative synergistic (-S, more negative than predicted additively), positive antagonistic (+A; less positive than predicted additively) and negative antagonistic (-A; less negative than predicted additively). N/A: not applicable classification since it is not possible to define interactive effects' direction and magnitude based on the indicated endpoint (i.e., community structure). N/E: not evaluated due to the absence of statistical effects between none of the tested stressors and the evaluated endpoint.

Table 3. Summary of selected field monitoring studies in which the combined impact of water scarcity and chemical exposure have been evaluated in aquatic ecosystems. A more detailed version of this table, including the description of major findings per study, is provided as Supporting Information (Table S2).

Hydrological stressor	Chemical stressor	Taxonomic group	Biological endpoint	Experimental design	Location	Reference
Inter-annual flow variation	157 organic micropollutants (urban, industrial and agricultural sources)	Biofilms (Algae+Bacteria) Macroinvertebrates	Algae Biomass Community structure Bacteria Enzymatic activity Community structure	19 sampling points in 4 rivers, pollution gradient, 2 sampling periods: end of summer on 2 consecutive years (wet-dry years)	North-East, East and South Spain	(Sabater et al., 2016)
Inter-annual flow variation	157 organic micropollutants (urban, industrial and agricultural sources)	Biofilms	Total density Algae Biomass Photosynthetic capacity (PC) Tolerance to excess light (NPQ) Community structure Bacteria Density Enzymatic activity	19 sampling points in 4 rivers, pollution gradient, 2 sampling periods: summer-autumn on 2 consecutive years (wet-dry years)	North-East, East and South Spain	(Ponsatí et al., 2016)
Intra-annual water level variation	Insecticide, larvicide (*Bti* : *Bacillus thuringiensis* var. *israelensis* serotype H14)	Algae	Total density Community structure	3 sampling sites in shallow Mediterranean temporary wetlands, 5 years monitoring, sampling after *Bti* application related with flooding	France	(Fayolle et al., 2015)
Intra-annual flow variation	73 pharmaceuticals	Biofilms (Algae + Bacteria)	Algae Biomass Photosynthetic activity (PA) Bacteria Enzymatic activity Live-to-dead ratio	2 sampling sites in one river, 2 sampling periods: winter-spring and spring-summer	North-East Spain	(Osorio et al., 2014)
Intra-annual flow variation	6 insecticides (azinphos-methyl, clorpyrifos, endosulfan, fenvalerate, cypermethrin and malathion)	Macroinvertebrates	Community structure Population dynamics	3 sampling sites in one river, 2 sampling periods: winter-spring and spring-summer	South Africa	(Bollmohr and Schulz, 2009)
Inter and Intra-annual flow variation	9 endocrine disruptors (Alkylphenolic compounds - APCs-)	Diatoms (in biofilms) Macroinvertebrates	Community structure Population densities	7 sampling sites in 2 rivers, pollution gradient, 4 sampling periods: late spring and Autumn on 2 consecutive years	North-East Spain	(Brix et al., 2012)

Table 3 (cont.)

Hydrological stressor	Chemical stressor	Taxonomic group	Biological endpoint	Experimental design	Location	Reference
Inter and Intra-annual flow variation	22 pesticides (18 herbicides and 4 insecticies)	Diatoms (in biofilms)	Biomass Community structure Photosynthetic activity (PA) Extracellular polysacaride content (EPS) Green algae/cyanobacteria ratio (F1/F3) Enzymatic activity	7 sampling sites in 2 rivers, pollution gradient, 4 sampling periods: late spring and autumn on 2consecutive years	North-East Spain	(Ricart et al., 2010)
		Macroinvertebrates	Community structure			
Inter and Intra-annual flow variation	Insecticides, larvicides (permethrin and organophosphates)	Macroinvertebrates	Community structure	12 year monitoring program in 2 rivers (several applications and several samplings per year)	West Africa	(Crosa et al., 2001)
		Fish	Species number Total weight per individual			
Inter and Intra-annual flow variation	4 insecticides (fenitrothion, diflubenzuron, deltamethrin and bendiocarb)	Zooplankton Macroinvertebrates	Community structure Population densities	16 sampling points in temporary ponds, 4 sampling periods: 4 consecutive years alternating treatment and non-treatment years, covering wet and dry period each year.	West Africa	(Lahr et al., 2000)

Inter-annual: several sampling periods on consecutive years, with at least one sampling time per year. Comparison of different flow conditions among years, at the same sampling period.

Intra-annual: several sampling periods covering wet and dry cycle along the year

Chapter 3

Identification of contaminants of concern in the upper Tagus river basin (central Spain). Part 1: Screening, quantitative analysis and comparison of sampling methods

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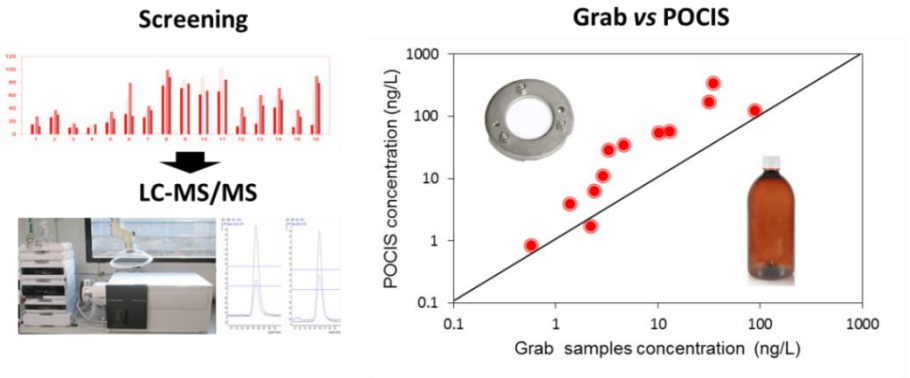

Abstract

Pesticides and point source contaminants (primarily pharmaceuticals) were monitored in 16 sampling sites of the upper Tagus river basin during spring, summer and autumn of 2016. A qualitative screening analysis was performed using a library of 430 compounds. Next, a novel method was implemented for the selection and quantification of contaminants with LC-MS/MS. The method is based on the frequency of detection in the screening, ecotoxicity data and the potential use in the watershed. Moreover, the efficacy of grab samples and passive samples (POCIS) in detecting compound-specific exposure patterns was compared during the summer sampling campaign. The screening method detected the presence of 268 compounds in the study area, out of which 52 were selected for the quantitative analysis (20 pesticides and 32 point source chemicals). Although very helpful in the prioritization exercise, the qualitative screening demonstrated some biases and the need for improvement by using more effective instruments for confirming positive results. Grab samples proved not to be fully suitable for contaminants with discontinuous exposure such as pesticides, which may be underestimated, but offer a sufficient basis for the characterization of contaminants coming from urban wastewaters. All selected chemicals showed a very high concentration variability due to differences among sampling sites, which are related to agricultural intensity and demographic pressure. Some insecticides (chlorpyrifos, dimethoate, imidacloprid), herbicides (diuron, metribuzine, simazine, terbuthylazine), and fungicides (carbendazim) were measured at concentrations exceeding 100 ng/L; while paracetamol, ibuprofen, some antibiotics (azithromycin, sulfamethoxazole, trimethoprim) and life-style compounds (caffeine, paraxanthine, nicotine) were found at very high concentrations (up to several µg/L). The results of this work represent the basis for the development of an ecological risk assessment for the aquatic ecosystem in the upper Tagus river basin and for the identification of basin-specific contaminant mixtures of environmental concern.

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1. Introduction

Monitoring studies have reported the presence of a large number of organic contaminants in water bodies across Europe (Houtman, 2010; Meffe and Bustamante, 2014; Busch et al., 2016; Rico et al., 2016a). However, the list of priority chemicals that are regularly controlled as part of the European Water Framework Directive (WFD; Directive 2000/60/EC) only includes a reduced number of organic compounds, some of which have been banned for several years (EC, 2012). Currently, hundreds of pesticides are registered for agricultural and other uses in Europe (Schwarzenbach et al., 2006), while thousands of pharmaceuticals may be used for human or veterinary purposes (Mossialos et al., 2004). This fact indicates that the number of chemicals that may be present in surface waters is enormous, with some of them possibly resulting in chemical mixtures and ecological impacts that are as yet unknown.

Pesticides and pharmaceuticals represent two classes of organic environmental contaminants whose exposure patterns and potential risks for the aquatic environment are very different. Pesticides may reach surface waters immediately after application (through aerial drift deposition) or by leaching and surface water runoff, and therefore are considered “diffuse” or “non-point source” contaminants. Exposure patterns in rivers are usually characterized by marked concentration peaks related to their application patterns and/or the intensity and frequency of rainfall events (e.g., Verro et al., 2009; Brock et al., 2010; O'Brien et al., 2016; Lorenz et al., 2017; Morselli et al., 2018). On the other hand, pharmaceuticals are mostly considered “point source” contaminants, reaching surface waters through localised emission points, such as effluents of treated or untreated urban discharges or animal rearing facilities (e.g. Zuccato et al., 2000, 2006; Fernandez et al., 2010). Although emissions of pharmaceuticals and lifestyle compounds may vary depending on population fluctuations, demographic structure or consumption patterns (Valcárcel et al., 2013; Arnold et al., 2014), their exposure patterns in river ecosystems are not as intermittent as for pesticides. The biological targets of most pesticides (e.g. photosynthesis or acetylcholinesterase enzymes) are shared in nature by a wide range of freshwater organisms, including primary producers, invertebrates and fish (e.g., McKnight et al., 2012; Schäfer et al., 2012). Conversely, pharmaceuticals are biologically active substances with very specific mode of action, usually designed to interfere with metabolic processes in vertebrates (mainly mammals). Therefore, many of them are not expected to result in adverse effects on aquatic organisms, although some exceptions exist such as structural and functional alterations of microbial communities caused by antibiotics (Rico et al., 2014; Välitalo et al., 2017) or fish behavioral effects caused by physichiatric drugs (Brodin et al., 2013; Brooks, 2014). The knowledge of the effects of complex mixtures of pesticides and pharmaceuticals in freshwater ecosystems is still very limited, and improved monitoring and management policies are still required to assess their combined exposure and to minimize their risks for aquatic ecosystems.

The design of an appropriate monitoring strategy for surface waters requires careful consideration of a number of relevant issues. One of the key challenges is the appropriate selection of compounds. Given the large number of organic chemicals that may be present in surface waters of European countries and the budgetary limitations to measuring all substances, there is a need for a preliminary selection of chemicals of concern regarding their potential toxicological effects (Von der Ohe et al., 2011; Tsaboula et al., 2016). Another key challenge is the selection of appropriate sampling times and suitable sampling techniques (Van den Brink et al., 2018). Currently, monitoring methods based on a limited amount of grab samples may not be a suitable approach for chemicals which are emitted discontinuously or those that are highly bioccumulative (Brack et al., 2017). The selection of

appropriate analytical tools is also important in order to target a range of contaminants with different molecular structure and physico-chemical properties (Kot-Wasik et al., 2007; Masiá et al., 2014a). In this regard, it is crucial to optimize sampling methods, as well as to develop suitable conceptual approaches and analytical techniques for the selection and quantification of a relevant number of compounds.

Mediterranean watersheds are characterized by a high vulnerability to agricultural and pharmaceutical contamination due to their high hydrological variability and high demographic pressure, and usually show concentration levels above those found in other European basins (Petrovic et al., 2011; Arenas-Sánchez et al., 2016). Pesticide concentrations are strongly related to river flow conditions, often peaking at the time of highest water scarcity (Ccanccapa et al., 2016). Pharmaceutical concentration levels may also be influenced by the dilution capacity of rivers; however, their concentration levels remain relatively stable over time. Slight seasonal fluctuations of pharmaceuticals in Mediterranean rivers have been related to environmental conditions such as temperature and their influence on the degradation of contaminants (Fernández et al., 2010; Valcárcel et al., 2013). So far, the number of studies assessing the occurrence and spatio-temporal patterns of organic contaminants in Mediterranean basins is rather limited. Further research is needed to identify water basin specific pollutants that need to be monitored to complement those already controlled under the WFD and to design appropriate chemical monitoring and abatement strategies.

The overarching goal of this study was to identify contaminants of concern that drive the risks for aquatic biodiversity in the upper Tagus river basin (Central Spain), beyond those that are regularly monitored as part of the WFD. This study has been divided into two parts (Part 1 and Part 2). In the current paper (Part 1) we assessed the occurrence of pesticides and point source contaminants (mainly pharmaceuticals) in different locations characterized by different level of anthropogenic impact, and tested the suitability of different sampling and analytical techniques for their determination. To do so, first we performed a qualitative screening analysis and implemented a novel method for the selection of target compounds to be analyzed with a quantitative analytical approach. Second, we compared the outcomes of a passive sampling technique with the traditional grab sampling method usually implemented for the chemical status assessment of surface waters according to the WFD. In this study we comparatively assess the results of the screening and the quantitative method, describe the outcomes of the grab sampling and the passive sampling technique, and report the measured environmental concentrations for the selected compounds. In the Part 2 of this study (see Arenas-Sánchez et al., 2019a) the measured chemical concentrations are used to perform an ecological risk assessment, to identify chemical mixtures of concern and to establish relationships with different land use and other abiotic variables.

2. Materials and Methods

2.1. Study area and sampling

This study was performed in the upper reach of the Tagus river basin. Sixteen sampling sites were selected along the tributaries of the Tagus River (Figure 1), covering a wide range of anthropogenic impacts. Sites 1 (Salado River), 2 (Henares River, upstream), 3 (Sorbe River, upstream reservoir), 4 (Sorbe River, downstream reservoir) and 5 (Henares River, midstream) are at the mouth of sub-basins with most surface area covered by natural land use (i.e. forested areas, grasslands). Sites 6

(Badiel River), 7 (Tajuña River), 12 (Melgar Stream, upstream), 13 (Melgar Stream, midstream), 14 (Melgar Stream, downstream) and 15 (Algodor Stream) are predomintantly impacted by agricultural activities. Finally, sites 8 (Henares River, downstream), 9 (Jarama River), 10 (Pantueña Stream), 11 (Manzanares River), 16 (Guaten Stream) are located in areas downstream of urban areas (Figure 2), including large cities such as Alcalá de Henares (site 8) and Madrid (sites 9 and 11). A more detailed description of the sampling sites, the watershed characteristics and the hydrological conditions of the sampling sites is provided in the Part 2 of this study (Arenas-Sánchez et al., 2019a).

Grab water samples were taken in spring (11-14th of April), summer (11-14th of July), and autumn (21-24th of November) of 2016 (i.e., one sample per site and season). Sampling was usually performed between 10h am and 16h pm. Water samples were collected in the middle section of the river, at a 20-40 cm depth, with 1 L amber glass bottles. Immediately after sampling, they were transported to the laboratory and stored at -20 °C until further analysis.

During the summer sampling campaign, passive samplers were placed in each sampling site for two weeks (14 days) and retrieved simultaneously with the grab samples. The passive samplers used were POCIS (Polar Organic Chemical Integrative Samplers) membranes purchased from USGS Technology (Columbia, MO, USA), containing an Oasis hydrophilic-lipophilic balance (HLB) sorbent. The membrane was fixed between two stainless steel washers with a circular opening of 41 cm^2 (effective sampling surface). The structure was attached to a stainless steel cage and submerged in the middle section of the river. On the sampling day, POCIS were collected, put into air-tight amber bottles with 50 mL of methanol, transported to the laboratory and stored at -20 °C until analysis. Only twelve POCIS were recovered, while four were lost for various reasons (e.g. vandalism or very high flow velocity). A blank POCIS, which was also transported to the field and stored in the same conditions as the rest, was used to rule out any potential source of contamination during transport, air exposure or manipulation.

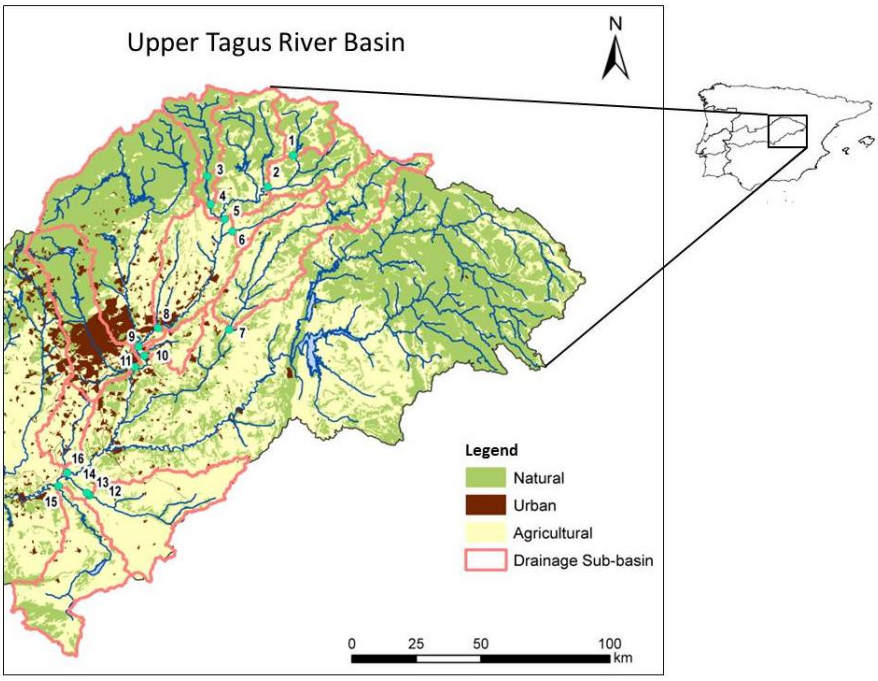

Figure 1. Map of the study area in the upper Tagus river basin and sampling sites (1-16). The three major categories of land use are also displayed.

2.2. Analytical procedures

2.2.1. *Reagents and chemicals*

The standards (purity ≥ 98-99%) for the quantitative analysis of all the chemicals listed in Table 1 were purchased from Sigma-Aldrich (St. Louis, MO, USA); except for citalopram (purity: 97%), which was provided by the Center of Applied Chemistry and Biotechnology of the University of Alcalá (Spain). Individual standard solutions of the targeted compounds were prepared at the concentration level of 2000 mg/L in different solvents (MeOH, MeOH:water or EtOH) and stored in amber glass vials at -20 °C in the dark. Working standard solutions were prepared by appropriate dilution of stock solutions in MeOH:water (10:90, v/v).

LC/MS-grade acetonitrile and methanol were supplied from Scharlau (Barcelona, Spain). Formic acid (purity ≥ 98%) and ammonium fluoride were obtained from Merck (Darmstadt, Germany). High purity water was obtained from a Milli-Q water purification system (Millipore, Mildford, MA, USA).

2.2.2. Sample treatment

River water grab samples were filtered thought a 0.7 µm glass fiber filter (Merck Millipore, Cork, IRL). The cleaning and pre-concentration were carried out following the off-line solid phase extraction (SPE) procedure described by Robles-Molina et al. (2014). SPE cartridges (Oasis HLB, 200 mg/6 cc, Waters, Mildford, MA, USA) were preconditioned with 4 mL of methanol and 8 mL of ultrapure water. Afterwards, water samples (200 mL) were passed through the SPE cartridges using a vacuum manifold (Phenomenex, Torrance, CA, USA). The cartridges were dried for 2 min under full vacuum (5 bar) to eliminate residual water. Analytes were eluted with two aliquots of 4 mL of methanol. The obtained extracts were evaporated to dryness at 45 °C, 0.2 Torr using a SpeedVac concentrator (Thermo Scientific, Massachusetts, USA), and then reconstituted with 1 mL of MeOH:water (10:90, v/v) and vortex stirring for 1 min. The reconstituted samples were filtered through 0.22 µm PVDF filters and transferred to an amber glass vial prior to analysis.

Regarding the POCIS samples, the 50 mL of methanol and the sorbent material contained in the amber glasss bottles were poured over a glass funnel placed on top of a 60 mL empty SPE polypropylene cartridge (Extrabond, Scharlab, Barcelona, Spain) with a high density polyethylene 20 µm frit (Agilent Technologies, Palo alto, CA, USA). Next, the sorbent membrane was rinsed with another 20 mL of methanol and collected in the same flask. The extract was evaporated to dryness and reconstituted with MeOH:water following the same conditions as described above.

2.2.3. Screening method

Screening of 430 multi-class pharmaceuticals, drugs of abuse, life-style compounds, pesticides, flame retardants and plasticizers was carried out by a high performance liquid chromatograph (Agilent Technologies 1260 Series, Palo alto, CA, USA) coupled to a 5600 TripleTOF mass spectrometer (SCIEX, Melbourne, Australia), abbreviated hereafter as LC-QTOF. The MS acquisition was performed in positive ionization mode with electrospray source (ESI) using information-dependent acquisition (IDA), which consists of two tests: a full scan mass spectrum between m/z 50–1000 and a product ion scan of precursor ions predefined by the user. Chromatographic conditions and LC-QTOF instrumental parameters are summarized in the Supporting Information (Table S1). Data acquisition and processing for screening was carried out using the software Peak view 1.2 (SCIEX) with the extraction ion chromatogram (XIC) Manager application. XIC Manager automatically calculates XICs and performs the identification of compounds, which are displayed in the chromatogram panel and

in a table. These tables show name, formula, adduct/modification, and retention time of the 430 compounds included in the database described in Robles-Molina et al. (2014). The results of mass, accurate mass error (ppm), isotope pattern match and MS/MS fragmentation were confirmed using a spectral library. It is important to note that MS/MS spectra of some compounds included in the database were not available in the library in our equipment. However, this was not adopted as excluding criterion, and in that case they were identified only with the mass spectrum (MS).

The criteria used for the identification of compounds were established according to SANCO (2009) and Masiá et al. (2014b):

- Accurate mass error, calculated as the difference between theoretical and experimental monoisotopic mass $[M+H]^+$, below 5 ppm and percentage difference of the isotopic pattern lower than 10 %.
- The comparison between experimental and theoretical MS/MS spectrum expressed as "purity score" was higher than 75%. This value is obtained by matching a MS/MS pattern from the library to an experimental MS/MS spectrum acquired, based on the relative intensity (isotopic profile) of the precursor and product ions.
- In the case standards were available in the laboratory, compounds were also confirmed by retention time (error lower than 5%). The compounds for which we had standards available were acetaminophen, amoxicillin, azithromycin, atenolol, carbamazepine, ciprofloxacin, citalopram, diclofenac, erythromycin, gemfibrozil, ibuprofen, imidacloprid, metronidazole, omeprazole, sulfamethoxazole, terbutryn, trimethoprim, estradiol (E2), estrone and caffeine.

The number of positive samples obtained as result of the screening analysis helped us to develop a scoring system, which was used to select the compounds for the analytical quantification (see next section).

2.2.4. Selection of compounds for the quantitative analysis

The selection of compounds for the quantitative analysis was performed following a novel method based on several criteria. The criteria and scoring system used for pesticides were:

- *Results of the screening analysis.* A score (S_{Sc}) for the results of the screening analysis was calculated for each compound using the following algorithm:

$$S_{Sc} = 10 \times \frac{N}{N_{max}} \qquad [1]$$

in which, "S_{Sc}" is the value of the score; "N" is the number of positive samples of the evaluated compound in the screening analysis; "N_{max}" is the maximum number of positive samples detected for a compound within the group i.e., pesticides, pharmaceuticals, antibiotics, hormones, life-style compounds or industrial chemicals (e.g., 42 for metolcarb in the pesticides group).

- *Toxicological relevance.* A score, based on the available toxicity data, was calculated using the following algorithm:

$$S_{Tox} = (2 - \log EC50) \times \frac{10}{(2 - \log EC50_{min})} \qquad [2]$$

in which, "S_{Tox}" is the value of the score; "EC50" is the value of the EC50 (mg/L) of the most sensitive organism among algae, invertebrates and fish (Tables 2 and 3). $EC50_{min}$ is the minimum value of EC50 among the list of considered chemicals. A value of EC50 higher than 100 mg/L was assumed as negligible, with a score equal to zero. In the observed range of toxicity data (minimum: 0.0001 mg/L;

maximum: 100 mg/L) the algorithm gives a score from 0 to 10. Details on the toxicity data sources for the different chemicals and on the procedure for their selection are described in the Part 2 of this study (Arenas-Sanchez et al., 2019a).

- *Possible uses in the watershed*. Ten crops were identified as relevant in the watershed (wheat, maize, barley, triticale, oat, sunflower, pea, almond, vineyard, olive) based on the published results of crop areas and production in 2015 in the regions of Madrid, Guadalajara and Toledo. All data was accessed from the webpage of the Spanish Ministry of Agriculture, Fisheries and Food (MAGRAMA, Madrid, Spain). Information on the main active compounds applied to each of these crops was also compiled with experts' collaboration from the Agronomic Institute of Castilla y Leon (ITACyL)(data not published). A score (S_{Crop}), from 0 to 10, was calculated using the following algorithm:

$$S_{Crop} = 10 \times \frac{C}{C_{max}} \qquad [3]$$

in which, "C" is the number of crops that may be treated with the pesticide; "C_{max}" is the maximum number of crops treated with one of the considered pesticides (C_{max}= 9).

The total score (S_{Total}) was calculated giving higher weight to the results of the screening analysis:

$$S_{Total} = (2\ x\ S_{Sc}) + S_{Tox} + S_{Crop} \qquad [4]$$

After the calculation of the total score and ranking, other specific issues (registration in Spain, fate properties, etc.) were evaluated on a case-by-case basis for the final pesticide selection.

For point source chemicals different criteria were used. First of all, the chemicals where divided into five groups: pharmaceuticals (excluding antibiotics and estrogens/steroids); antibiotics; estrogens and steroids; illicit drugs; stimulants and life-style compounds; and industrial chemicals. For each group, a scoring system was developed using the screening results and the toxicological relevance as main criteria, following the same procedure as described above for the pesticides. Other specific issues were evaluated on a case-by-case basis for the final selection, see section 3.2.

2.2.5. Quantitative method

The quantification of the selected compounds was carried out by high performance liquid chromatography HPLC (1200 Agilent series, Palo alto, CA, USA) coupled to an Agilent 6495 triple quadrupole (QQQ) mass spectrometer, equipped with an electrospray ionization (ESI) interface (Agilent Technologies, Palo alto, CA, USA), in positive and negative mode. Ions were generated using an electrospray ion source with Agilent Jet Stream Technology. A summary of the optimum chromatographic conditions, instrumental parameters for the LC-MS/MS system, and MRM transitions is provided in the Supporting Information (Tables S2 and S3). The performance of the analytical method was established according to the Directive 96/23/EC and validated according to SANCO (2011). The sensitivity of the method was estimated by establishing the limits of quantification (LOQs) and detection (LODs). The LOQs were determined as the lowest concentration whose quantification transition presented a signal-to-noise ratio (S/N) = 10, and qualification transition was detected accomplishing abundance criteria. The LODs were determined as the minimum detectable amount of analyte with a signal-to-noise ratio (S/N) = 3, maintaining abundance criteria between transitions. The precision of the LOQ levels was determined by repeatability (n=5) and was between 2% and 16% RSD (Relative Standard Deviation). The method linearity for each compound was established from the corresponding LOQ level to a maximum concentration of 10 µg/L, using external standards over a two concentration range: ng/L (for low concentration levels)

and µg/L (for high concentration levels). The standard regression line was obtained as the mean of three injections of each calibration point, which had a regression coefficient (R^2) > 0.99.

The efficiency of SPE protocol carried out to extract the organic contaminants from river waters was evaluated. A pool of different water samples, which presented different levels of dissolved organic carbon, suspended solids and nutrients, was used to obtain a representative river water sample. Accuracy of the method (n=5) was evaluated as recovery percentage using samples fortified at two concentration levels (15 ng/L and 150 ng/L). The precision, expressed as RSD, was <20 % for both concentration levels (see Table 1). The calculated recoveries were used to calculate the actual sample concentrations. The method quantification limits (MQLs) were determined taking into account the pre-concentration factor (LOQ/200) applied in the SPE protocol and the achieved recoveries. The performance of the analytical method, for the 52 selected chemicals is summarized in Table 1.

2.2. Calculation of water concentrations for POCIS samples

In the POCIS samples, the concentration in water was estimated from the concentration measured in the POCIS sorbent according to the following equation (Morin et al., 2012):

$$C_S = \frac{C_W \, x \, R_S \, x \, t}{M_S} \qquad [5]$$

in which, C_s is the concentration in the POCIS sorbent (ng/g) at the sampling time, C_w is the time weighted average (TWA) concentration in water (ng/L) during the exposure time, R_s is the sampling rate (L/d) for each chemical, M_s is the mass of POCIS sorbent (g), and t is the exposure time (14 days). From equation [5], the following relationship is obtained:

$$C_W = \frac{C_S \, x \, M_S}{R_S \, x \, t} = \frac{A_S}{R_S \, x \, t} \qquad [6]$$

in which, A_s is the amount of the chemical (ng) measured in the POCIS sorbent.

For the majority of the selected chemicals (75%), R_s values were available in the literature. Non available data were extrapolated using the Kow value of the substances. Selection and extrapolation procedures are described in the Supporting Information, while the estimated R_s values are listed in Table S5.

3. Results and discussion

3.1. Results of the screening analysis

In total, 268 different compounds where detected in at least one sample as result of the screening analysis: 129 pesticides and 139 point source chemicals. In the 60 samples (48 grab and 12 POCIS samples), many chemicals were just detected occasionally (56 chemicals present only in 1 sample), while 21 chemicals were detected in >50% of the samples. Further details on the results of the screening analysis are provided in Table S4 of the Supporting Information. The number of detected chemicals in the different sampling sites varied considerably and may be assumed as a preliminary indicator of the level of antrophogenic impact in the watershed (Figure 2).

Table 1. Validation parameters of the analytical method development for the 52 compounds quantified in this study: quantification limit (LOQ), detection limit (LOD), recovery (%) and RSD values (n=5).

Chemical name	LOQ, ng/L	LOD, ng/L	Recovery, % (RSD, %)	Recovery, % (RSD, %)
			15 ng/L	150 ng/L
Antibiotics				
Amoxicillin	60	20	51 (19%)	146 (13%)
Azithromycin	80	5	91 (17%)	97 (18%)
Ciprofloxacin	500	80	147 (5%)	99 (7%)
Erythromycin	10	3	56 (3%)	88 (17%)
Lincomycin	10	3	97 (2%)	107 (5%)
Metronidazole	300	80	97 (4%)	98 (3%)
Sulfamethoxazole	20	6	99 (5%)	102 (3%)
Trimethoprim	10	3	107 (8%)	103 (3%)
Tylosin	500	80	95 (17%)	90 (19%)
Analgesics and other pharmaceuticals				
Acetaminophen (paracetamol)	80	20	99 (8%)	95 (6%)
Atenolol	60	20	98 (9%)	118 (10%)
Carbamazepine	10	3	96 (5%)	93 (15%)
Citalopram	10	3	59 (18%)	59 (14%)
Diclofenac	300	100	92 (12%)	100 (11%)
Gemfibrozil	15	5	99 (4%)	101 (10%)
Ibuprofen	60	15	86 (7%)	120 (17%)
Ketoprofen	300	80	91 (4%)	108 (3%)
Loratadine	40	20	37 (3%)	55 (16%)
Naproxen	100	50	141 (5%)	71 (5%)
Omeprazole	10	3	83 (4%)	102 (11%)
Salbutamol	10	3	92 (2%)	98 (6%)
Valsartan	500	60	108 (7%)	108 (6%)
Venlafaxine	300	20	82 (9%)	99 (20%)
Steroids/Estrogens				
Estradiol	30	10	64 (13%)	87 (15%)
Estrone	30	10	75 (20%)	82 (8%)
Progesterone	150	40	76 (12%)	74 (8)
Testosterone	300	150	89 (14%)	88 (13%)
Drugs and Life-style compounds				
Amphetamine	80	40	50 (10%)	41 (20%)
Caffeine	150	40	109 (3%)	107 (9%)
Nicotine	150	20	70 (10%)	83 (20%)
Paraxanthine	300	150	127 (9%)	109 (9%)
Industrial chemicals				
TBP - Tributyl-phosphate	300	60	58 (7%)	70 (16%)
Insecticides				
Carbofuran	3	1	54 (17%)	96 (12%)
Chlorpyrifos ethyl	20	10	NR	NR
Diazinon	3	1	78 (12%)	83 (5%)
Dimethoate	5	2	42 (1%)	144 (7%)
Imidacloprid	10	5	106 (4%)	138 (11%)
Malathion	50	5	52 (10%)	48 (20%)
Metolcarb	100	3	33 (17%)	82 (3%)
Pirimicarb	3	1	75 (11%)	102 (6%)
Spinosyn-A	10	3	35 (10%)	33 (15%)
Herbicides				
Chlortoluron	10	3	83 (3%)	120 (6%)
Diuron	20	6	56 (7%)	89 (17%)
Metribuzin	3	1	78 (5%)	105 (4%)
Simazine	20	6	79 (2%)	95 (2%)
Terbutryn	10	3	111 (2%)	89 (17%)
Terbuthylazine	10	3	73 (3%)	103 (10%)

Table 1 (cont.)

Chemical name	LOQ, ng/L	LOD, ng/L	Recovery, % (RSD, %) 15 ng/L	Recovery, % (RSD, %) 150 ng/L
Fungicides				
Carbendazim	10	3	89 (3%)	106 (9%)
Kresoxim methyl	50	20	33 (7%)	37 (18%)
Propiconazole	20	5	62 (1%)	73 (19%)
Spiroxamine	20	5	30 (10%)	27 (15%)
Tebuconazole	20	5	78 (1%)	70 (17%)

NR: Not Recovered.

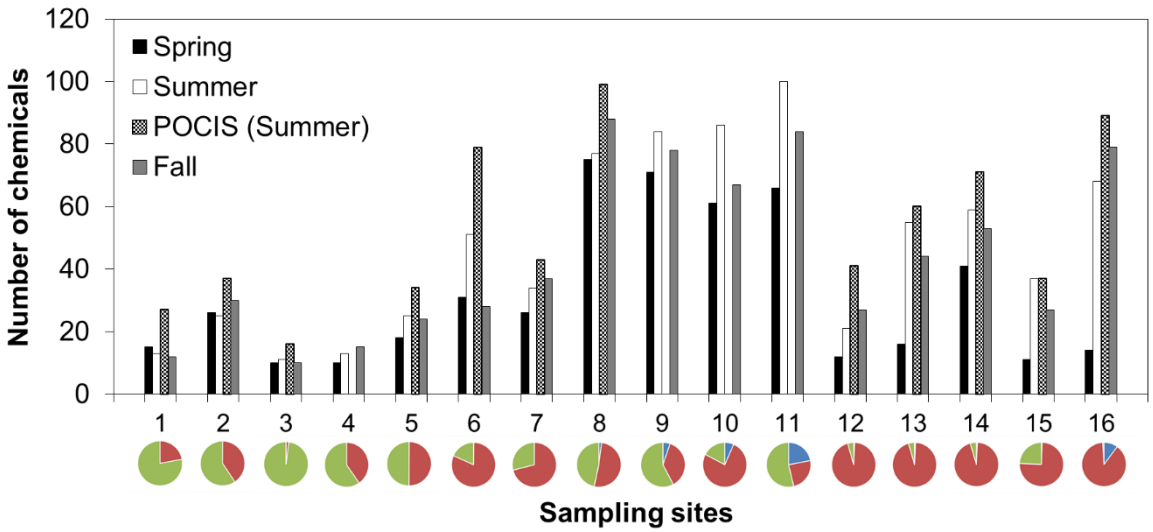

Figure 2. Number of detected chemicals in the screening analysis and percentage of land use cover in the different sampling sites (blue: urban; red: agricultural; green: natural). The POCIS samples in the sampling sites 4, 9, 10, 11 were lost. The maximum number of compounds screened in the grab and POCIS samples were 430.

3.2. Selection of compounds for the quantitative analysis

The ranking and the scoring of the 129 pesticides detected in the screening analysis is reported in Table S6 of the Supporting Information, while the 20 selected pesticides are shown in Table 2. The selection considered those that ranked in the top of the list, with some exceptions:

1. Cypermethrin was classified at the fifth position of the list, mainly due to its use on all the crops of the watershed and due to its toxicity for aquatic invertebrates. However, due to its physico-chemical properties (low water solubility, high affinity for soil, relatively low persistence) its presence in water at measurable concentrations is unlikely. In fact, it was detected in only one sample in the screening analysis. Therefore, it was not further considered in the quantitative analysis.
2. Several chemicals ranking within the top thirty of the list are not used in any of the crops present in the watershed and are not even authorized in Spain for agricultural use. Some of them (metalaxil, pyroquilon, isoprocarb, imazamethabenz methyl, thiabendazole) were not further considered. However, other non-authorized chemicals where selected, both because

they may be authorized for non-agricultural uses (e.g. urban disinfection, pest control in urban green areas) and to verify the reliability of the screening method.

Table 2. Pesticides selected for the quantitative analysis. The number of positive samples in the screening analysis is reported together with the calculated SSc value, followed by the EC50 values for the most sensitive aquatic organism, the calculated STox value, the uses in the watershed (i.e., number of crops in which the compound may be applied, maximum 10), the estimated SCrop value, the calculated STotal value (baed on SSc, STox and SCrop) and the authorization status in Spain.

Chemical name	Type	Positive samples	SSc	Toxicity value (mg/L)	STox	Uses	SCrop	STotal	Authorized in Spain[1]
Carbofuran	I	15	3.57	0.04[a]	5.66	0	0.00	12.81	No
Chlorpyrifos ethyl	I	3	0.71	0.0004[a]	9.50	9	10.0	20.93	Yes
Diazinon	I	14	3.33	0.001[a]	8.33	0	0.00	15.00	No
Dimethoate	I	12	3.10	0.20[a]	4.50	3	3.33	14.02	Yes
Imidacloprid	I	23	5.71	17[a]	1.28	3	3.33	16.04	Yes
Malathion	I	10	2.38	0.008[a]	6.83	0	0.00	11.59	Yes
Metolcarb	I	41	10.0	0.96[b]	3.36	0	0.00	23.36	Yes
Pirimicarb	I	25	6.43	0.0001[a]	10.0	3	3.33	26.19	Yes
Spinosyn-A	I	8	1.43	0.20[a]	4.80	4	4.44	12.10	Yes
Chlortoluron	H	6	1.67	0.032[c]	5.82	5	5.56	14.71	Yes
Diuron	H	14	3.33	0.007[c]	6.92	0	0.00	13.59	No
Metribuzin	H	5	0.71	0.04[c]	5.66	4	4.44	11.53	Yes
Simazine	H	24	6.19	0.06[c]	5.37	0	0.00	17.75	No
Terbutryn	H	19	4.52	0.008[c]	6.83	0	0.00	15.88	No
Terbuthylazine	H	11	2.38	0.02[c]	6.16	1	1.11	12.04	Yes
Carbendazim	F	16	3.33	0.09[a]	5.08	0	0.00	11.74	No
Kresoxim methyl	F	18	4.29	0.15[a]	4.71	2	2.22	15.50	Yes
Propiconazole	F	9	1.43	0.02[c]	6.16	6	6.67	15.69	Yes
Spiroxamine	F	28	6.67	0.01[c]	7.04	0	0.00	20.37	Yes
Tebuconazole	F	7	1.67	3.6[c]	2.33	7	7.78	13.44	Yes

I= Insecticides; H= Herbicides; F= Fungicides
[1]Information from MAGRAMA (2018).
[a] 48h EC50-Daphnia.
[b] 96h LC50-fish.
[c] 72h EC50-algae.

Point source chemicals may be considered as indicators of urban contamination. The ranking and the scoring of the 139 point source chemicals identified in the screening analysis included 67 pharmaceuticals, 30 antibiotics, 22 ilicit drugs, stimulants and life-style compounds (in the following referred to as life-style compounds), 7 steroids and estrogens, and 13 industrial chemicals, which are listed in Tables S7 to S11 of the Supporting Information. For pharmaceuticals (excluding antibiotics), 14 compounds where selected (Table 3 and S7). Seven chemicals, listed in the top twenty of the ranking, where excluded from the final selection: three metabolites, one nucleoside and three products for external application (one insect repellent, one antimicotic and one antiseptic). All the excluded chemicals showed very low toxicity (EC50>100 mg/L). The final selection included several types of pharmaceuticals of common use (i.e., analgesics, β-blockers, antidepressants, antihistaminics).

Nine compounds were selected from the antibiotics group (Table 3 and S8). Malachite green, an external disinfectant with several use restrictions, which ranked nine at the list, was excluded. It was substituted by the next compound in the priority list, ciprofloxacin, a more commonly used antibiotic

in human medicine recently included in the watch list of the European Commission (EC, 2018). In the other minor groups, the selection criteria were often considered on a case-by-case basis (Table 3 and Tables S9-S11): in the estrogen/steroid group the objective was to include two estrogens and two steroids (Table S9); in the drugs and life-style compound group, besides the three chemicals on the top of the list, the most abundant caffeine metabolite (paraxantine) and a widely used stimulant (amphetamine) were chosen; among the industrial compounds, TBP was preferred to TCPP because it is more toxic and more widely used as plasticizer. The main physico-chemical properties of all selected compounds are reported in the Supporting Information (Table S5).

Table 3. Point source chemicals selected for the quantitative analysis in the different groups. The number of positive samples in the screening analysis is reported together with the calculated SSc value. The acute EC50s for the most sensitive aquatic organism are reported together with the calculated STox value. Finally, the STotal (calculated with the SSc and Stox values) is provided.

Chemical name	Classification	Positive samples	SSc	Toxicity value (mg/L)	STox	STotal
Pharmaceuticals						
Acetaminophen	analgesic/anti-inflammatory	25	4.60	16 [a]	3.16	12.4
Atenolol	β-blocker	39	8.00	>100	0.00	16.0
Carbamazepine	antiepilectic	46	8.00	20 [b]	2.78	18.8
Citalopram	antidepressant	20	2.20	2 [c]	6.74	11.1
Diclofenac	analgesic/anti inflammatory	25	5.00	38 [c]	1.98	12.0
Gemfibrozil	hypolipidemic	24	5.00	6 [c Q]	4.88	14.9
Ibuprofen	analgesic/anti inflammatory	15	1.00	>100	10.00	12.0
Ketoprofen	analgesic/anti inflammatory	24	4.80	>100 [Q]	3.67	13.3
Loratadine	antihistaminic	12	2.20	0.7 [c Q]	8.53	12.9
Naproxen	analgesic/anti inflammatory	27	5.80	19 [b Q]	2.90	14.5
Omeprazole	gastro-protector	21	4.00	31 [b]	3.57	11.6
Salbutamol	antiasthmatic	31	6.40	>100 [Q]	0.00	12.8
Valsartan	antihypertensive	34	6.80	8 [c Q]	4.37	18.0
Venlafaxine	antidepressant	50	10.0	10 [a]	3.97	24.0
Antibiotics						
Amoxicillin	antibiotic	7	1.32	56 [c]	7.14	9.77
Azithromycin	antibiotic	18	3.16	36 [c]	6.35	12.7
Ciprofloxacin	antibiotic	15	2.63	6.7 [c]	3.73	8.99
Erythromycin	antibiotic	14	1.84	0.6 [c]	10.22	13.9
Lincomycin	antibiotic	9	1.84	0.07 [c]	10.02	13.7
Metronidazole	antibiotic	16	3.95	40 [c]	1.27	9.16
Sulfamethoxazole	antibiotic	44	10.0	>100	0.00	20.0
Trimethoprim	antibiotic	22	5.79	>100	0.00	11.6
Tylosin	antibiotic	6	1.84	>100 [Q]	5.62	9.30
Steroids/Estrogens						
Estradiol	estrogen	11	7.86	2.5 [a Q]	9.41	25.1
Estrone	estrogen	12	9.29	65 [b Q]	1.09	19.7
Progesterone	steroid	7	4.29	2 [c Q]	10.00	18.6
Testosterone	steroid	8	5.00	8 [c Q]	6.30	16.3
Drugs and Life-style compounds						
Amphetamine	nervous stimulant	17	2.0	>100 [Q]	0.58	4.66
Caffeine	nervous stimulant	49	10.0	>100	0.00	20.0
Nicotine	alcaloid	36	7.35	4 [b]	8.08	22.8
Paraxantine	metabolite	33	6.94	>100	0.00	13.9
Industrial chemicals						
TBP - Tributyl-phosphate	plasticizer	26	7.57	1.8 [c]	2.92	18.1

[a] 48h EC50-Daphnia.
[b] 96h LC50-fish.
[c] 72h EC50-algae.
[Q] QSAR (Quantiative Structure-Activity Relationship).
Note: values >100 mg/L refer to all organism groups.

3.3. Results of the quantitative analysis

The concentrations of all analysed compounds in the water and POCIS samples of the 16 sampling sites in the three seasons are provided in Tables S12 to S19 of the Supporting Information. A summary of the results obtained for pesticides and point source chemicals is provided in Table 4, and in Figures 3 and 4.

Regarding pesticides, four chemicals (carbofuran, malathion, metolcarb and kresoxim-methyl) were never detected in all samples (n=60) at concentrations above the LOQ, and one chemical (spiroxamine) was detected only in one grab sample. Spinosyn-A and chlorpyrifos were never detected in the grab samples, while they where detected in four and eight out of the twelve POCIS samples, respectively. For all other pesticides, the frequency of detection was relatively high, with four compounds present in >90% of the samples: the insecticide imidacloprid, the herbicides simazine and terbuthylazine, and the fungicide carbendazim. However, in many cases, measured concentrations were very low. For all chemicals, the first quartile includes concentrations very close to the minimum detected value (Figure 3). For thirteen chemicals, the median is below 5 ng/L (Table 4). The highest maximum concentrations in the grab samples were about several hundred ng/L for some herbicides (diuron, simazine) and for carbendazim. A similar situation was found in the POCIS samples, except for some maximum concentrations for some insecticides (e.g. chlorpyrifos, dimethoate, imidacloprid) and for the herbicide terbuthylazine, which were about several hundred ng/L. The variability of concentrations among sampling sites and times was very high, with differences between minimum and maximum values from three to four orders of magnitude. Generally, lower concentrations were detected in sites with lower urban or agricultural impact (sites 1 to 5, Table S12-S15). A more detailed description of the influence of sampling time and land use characteristics on the measured concentration dynamics is provided in the Part 2 of this study (Arenas-Sánchez et al. 2019b). The high frequency of detection could indicate that some agricultural activity is present in all sub-basins of the studied area, but the high variability indicates that the agricultural impact, although present, is very different in the different sub-basins.

Except for carbofuran, a high frequency of detection was found for some pesticides that are currently not authorized or restricted for agricultural uses (diazinon, diuron, simazine, terbutryn, carbendazim). The frequencies of detection varied between 44 and 94% in the grab samples, and between 75 and 100% in the POCIS samples. As previously mentioned, some of these compounds may be used for pest control in urban areas, and therefore they may reach surface water ecosystems by storm water runoff or by wastewater treatment plants. The exception is simazine, which had been banned in the EU for >10 years (EC, 2004), however it is a relatively persistent compound in agricultural soils so that runoff events can still contribute to their mobility to freshwater ecosystems. The pesticides that are included in the list of priority substances in the WFD (chlorpyrifos, diuron, simazine and terbutryn) were all below maximum allowable concentrations; with the exception of chlorpyrifos measured in the POCIS samples (0.396 µg/L), which exceeded by almost four times the regulatory threshold (0.1 µg/L).

Regarding point source chemicals, only one (amphetamine) was never quantified and two compounds (tylosin and progesterone) were found only once, in the POCIS samples, at very low concentrations (Table 4). For all other chemicals, the frequency of detection was very high, with almost one half of compounds (15 out of 32) present in >80% of the samples, and eight present in >90% of the samples. As for pesticides, the first quartile includes concentrations

Table 4. Concentration range (median, minimum-maximum) and percentage of occurrence in the 48 grab samples and in the 12 POCIS samples for all chemicals analysed (I= insecticides; H= herbicides; F= fungicides; Ph= pharmaceuticals; A= antibiotics; St= steroids and estrogens; Ls=drugs and life-style compounds; Pl= plasticizers). Concentrations are provided in ng/L. n.d.: not detected.

Chemical		Median (min-max), Occurrence		Chemical		Median (min-max), Occurrence	
		Grab	POCIS			Grab	POCIS
Carbofuran	I	n.d., 0%	n.d., 0%	Ibuprofen	Ph	11.2 (0.41 - 2761), 100%	11.2 (0.41 - 2761), 75%
Chlorpyrifos ethyl	I	n.d., 0%	126 (<0.1 - 396), 67%	Ketoprofen	Ph	31.44 (<1.5 - 356), 52%	486 (<1.5 - 2149), 42%
Diazinon	I	0.11 (<0.02 - 2.11), 44%	0.12 (<0.02 - 9.45), 92%	Loratadine	Ph	5.38 (<0.2 - 26.2), 13%	243 (<0.2 - 2977), 25%
Dimethoate	I	0.76 (<0.03 - 21.2), 63%	5.74 (<0.03 - 351), 83%	Naproxen	Ph	339 (<0.5 - 1404), 46%	2362 (<0.5 - 7960), 33%
Imidacloprid	I	2.68 (<0.05 - 21.2), 92%	32.1 (0.85 - 342), 100%	Omeprazole	Ph	1.06 (<0.05 - 392), 48%	121 (<0.05 - 1537), 33%
Malathion	I	n.d., 0%	n.d., 0%	Salbutamol	Ph	2.82 (<0.05 - 10.22), 65%	3.30 (<0.05 - 28.4), 50%
Metolcarb	I	n.d., 0%	n.d., 0%	Valsartan	Ph	154 (<2.5 - 3337), 90%	905 (7.95 - 22940), 100%
Pirimicarb	I	0.06 (<0.02 - 0.35), 29%	2.70 (<0.02 - 3.69), 25%	Venlafaxine	Ph	9.98 (<1.5 - 614), 85%	57.6 (<1.5 - 1407), 58%
Spinosyn-A	I	n.d., 0%	2.48 (<0.05 - 105), 33%	Amoxicillin	A	1.71 (<0.3 - 15.1), 17%	16.0 (<0.3 - 16.0), 8%
Chlorturon	H	1.07 (<0.05 - 20.0), 50%	7.95 (<0.05 - 98.0), 50%	Azithromycin	A	5.06 (<0.4 - 1032), 90%	8.23 (<0.4 - 73058), 75%
Diuron	H	22.9 (<0.1 - 109), 52%	42.5 (<0.1 - 995), 75%	Ciprofloxacin	A	8.70 (<2.5- 786), 79%	263 (<2.5- 1026), 33%
Metribuzine	H	0.48 (<0.02 - 15.3), 46%	15.6 (<0.02 - 439), 50%	Erythromycin	A	0.44 (<0.05 - 17.8), 81%	0.93 (<0.05 - 177), 42%
Simazine	H	1.85 (<0.1 - 261), 94%	6.74 (0.13 -159), 100%	Lincomycin	A	0.95 (<0.05 - 11.06), 58%	0.76 (<0.05 - 60.3), 75%
Terbutryn	H	0.71 (<0.05 - 45.4), 85%	3.92 (<0.05 - 77.5), 92%	Metronidazole	A	21.8 (<1.5 - 131), 46%	12.2 (<1.5 - 19.9), 17%
Terbuthylazine	H	0.32 (<0.05 – 16.1), 90%	1.57 (0.25 - 121), 100%	Sulfamethoxazole	A	9.42 (<0.1 - 5962), 88%	27.5 (<0.1 - 3043), 92%
Carbendazim	F	2.13 (<0.05 - 118), 92%	10.36 (0.78 - 273), 100%	Trimethoprim	A	4.42 (<0.05 - 1288), 83%	99.89 (<0.05 - 2283), 83%
Kresoxim methyl	F	n.d., 0%	n.d., 0%	Tylosin	A	n.d. - 0%	5.90 (<2.5 - 5.90), 8%
Propiconazole	F	4.96 (<0.25 - 21.8), 35%	2.62 (<0.25 - 27.5), 67%	Estradiol, 17-beta-(E2)	St	0.41 (<0.15 - 0.83), 17%	6.84 (<0.15 - 9.97), 33%
Spiroxamine	F	3.76 (<0.1- 3.76), 2%	n.d., 0%	Estrone	St	0.86 (<0.15 - 17.25), 79%	44.8 (4.21 - 276), 100%
Tebuconazole	F	1.67 (<0.1- 447), 85%	13.2 (0.21- 77.1), 100%	Progesterone	St	n.d. - 0%	12.4 (<0.75 - 12.4), 8%
Acetaminophen	Ph	10.8 (<0.8 - 9825), 94%	434 (<0.8 - 5606), 58%	Testosterone	St	3.68 (<1.5 - 4.15), 6%	15.7 (<1.5 - 34.6), 42%
Atenolol	Ph	29.3 (<0.3 - 673), 88%	43.9 (<0.3 - 833), 92%	Amphetamine	Ls	n.d. - 0%	n.d. - 0%
Carbamazepine	Ph	9.04 (0.06 - 342), 100%	127 (3.52 - 2880), 100%	Caffeine	Ls	107 (5.95 - 5870), 100%	1782 (330 - 14532), 100%
Citalopram	Ph	3.97 (<0.05- 25.7), 96%	25.4 (<0.05 - 442), 83%	Nicotine	Ls	39.3 (1.46 - 598.6), 100%	662 (57 - 5785), 100%
Diclofenac	Ph	35.4 (<1.5 - 440), 67%	562 (<1.5 - 2667), 67%	Paraxanthine	Ls	826 (2.20 - 57586), 100%	8796 (<5 - 17560), 83%
Gemfibrozil	Ph	40.0 (<0.05 - 798), 81%	2262 (<0.05 - 9093), 75%	Tributyl-phosphate	Pl	25.3 (<1.5 - 1075), 96%	62.2 (<16.1 - 708), 100%

very close to the minimum detected value (Figure 4) and the variability of data, assessed as the difference between the minimum and maximum detected values, is often higher than three orders of magnitude. This variability is mainly determined by differences between sites with low and high urban impact. For ten chemicals, the maximum detected concentration was higher than 1 μg/L (Table 4). It is noteworthy the high concentrations of acetaminophen (paracetamol) in sampling sites 13 and 14 (3.5-9.8 μg/L), downstream of a small village without wastewater treatment facilities, and the high concentrations of ibuprofen (up to several μg/L) in the same location as well as in the Henares and Manzanares rivers (sites 8 and 11), downstream of the cities of Alcalá de Henares and Madrid, respectively. The concentrations of acetaminophen are above those reported by previous monitoring studies in the region (Valcárcel et al. 2013; Fernández et al. 2010), while the concentrations of ibuprofen downstream of Alcalá de Henares are similar to those previously reported by Fernández et al. (2010) in a nearby sampling site (2.5 μg/L). Regarding the antibiotics, azithromycin, sulfamethoxazole and trimethoprim were measured in concentrations above 1 μg/L in the Henares River downstream of Alcalá de Henares during the autumn season. The concentrations of sulfamethoxazole in the grab sample (6 μg/L) and azithromycin in the POCIS sample (73 μg/L) in that sampling site were particularly high (Table 4). The maximum concentrations of these compounds are higher than those reported by Valcárcel et al. (2011, 2013) in nearby study sites. Interestingly, Valcárcel et al. (2011) also described some seasonality in the exposure concentrations of antibiotics in surface waters, with highest exposure concentrations in autumn, and identified these three compounds as priority substances due to their potential ecotoxicological hazard to invertebrates and primary producers.

Our study confirms caffeine and its metabolite paraxanthine as ubiquituous compounds in the Tagus river basin, with maximum exposure concentrations of 15 and 58 μg/L respectively (Table 4), which are well above the 95th percentile of the global surface water exposure concentrations reported by Rodríguez-Gil et al. (2018). In the case of paraxanthine, the maximum concentration reported in our study is above the highest maxima identified by Rodríguez-Gil et al. (2018), which was measured by Valcárcel et al. (2011) in the Manzanares River (Madrid, Spain).

3.4. Suitability of the analytical method

One of the main challenges in multi-residue analysis of organic contaminants in surface waters concerns the choice of the best SPE protocol (type of sorbent, hydrophobicity/hydrophilicity of elution solvent and sample volumes). The sample treatment step is critical to obtain acceptable recoveries for all compounds and, consequently, reliable quantitative data. In this study, we applied an SPE protocol using OASIS HLB sorbent, as its hydrophilic-lipophylic balance has proven to be versatile enough and efficient in the extraction of analytes of a wide range of polarities (see also Dinh et al., 2011; Jeong et al., 2017). Taking into account the large amount of contaminants included in this study and their different properties, we considered that the recovery percentages obtained were satisfactory. For the high spiking level, 88% of point source contaminants and 70% of pesticides presented recoveries between 70% and 120% (RSD ≤ 20%). Only three point source contaminants (amphetamine, citalopram and loratadine) and four pesticides (spiroxamine, malathion, kresoxim methyl, spinosyn-A) presented recoveries lower than 60% and 50%, respectively. As it was expected, the results obtained at the low spiking level were worse that those at the highest level. The number of compounds with acceptable recoveries decreased for point source contaminants (70%), and even more for pesticides (55%). It is important to note that, in these cases, the RSD values wer also lower

than 20%, which is a key variable to obtain accurate and reliable quantification data. Chlorpyrifos was the only compound that was not recovered with the selected SPE procedure, probably due to its high hydrophobicity. Both the elution from SPE cartridge and the extraction from POCIS samples could be improved by adding a second extraction with a non-polar solvent (acetonitrile, hexane, chloroform or dichloromethane), or by using C18 cartridges in SPE protocols as suggested by Ferrer and Thurman (2007).

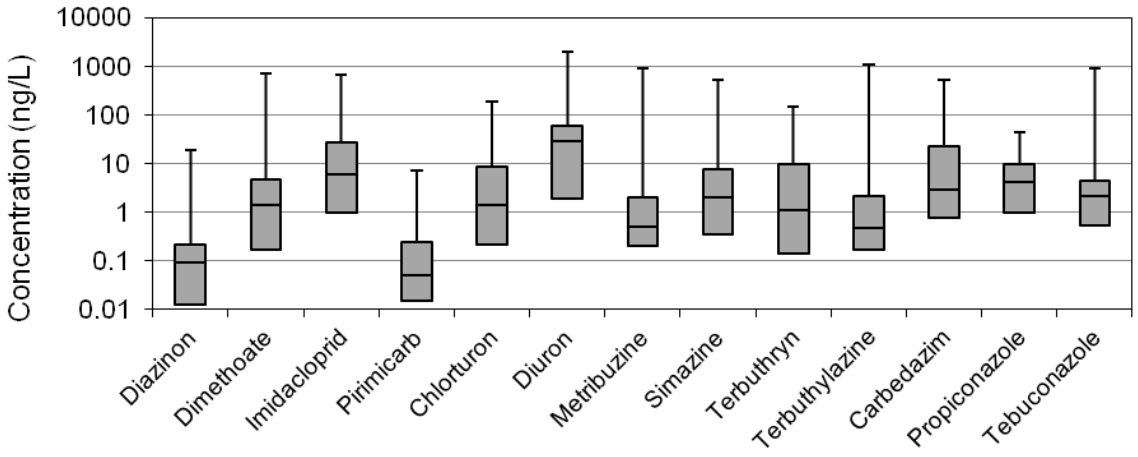

Figure 3. Box plots with the concentrations of pesticides in the POCIS and grab samples. Only chemicals with more than one positive sample are included. The box represents the 25th percenile, the median and the 75th percentile. The width of the lower wisker (first quartile) is generally too short to be seen in the figure.

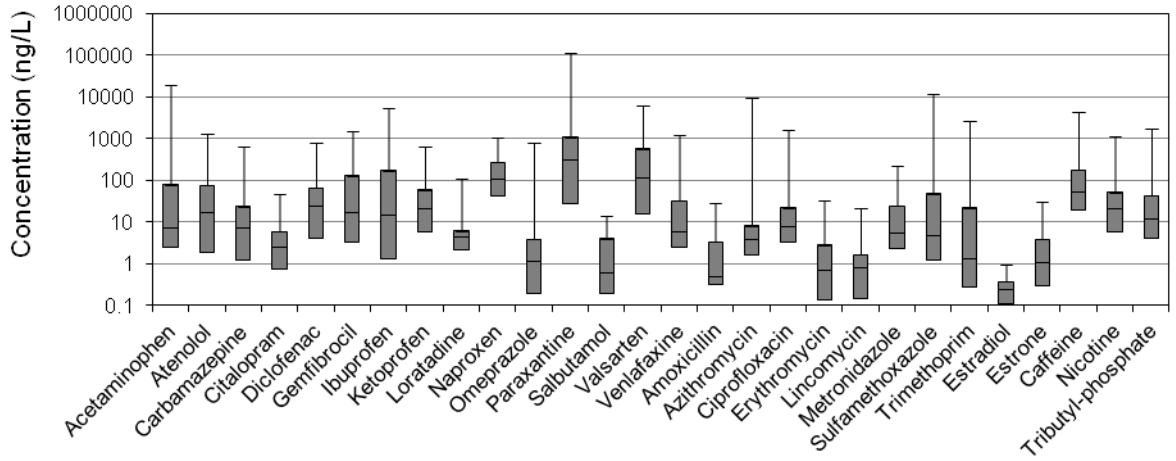

Figure 4. Box plots on the concentrations of point source chemicals in the POCIS and grab samples. Only chemicals with more than one positive sample are included. The box represents the 25th percenile, the median and the 75th percentile. The width of the lower wisker (first quartile) is generally too short to be seen in the figure.

In other cases, the observed recoveries were above 120%. Although SPE protocols are mainly designed as cleanup and extraction technique, sometimes the preconcentration factor applied in order to enhance the sensitivity of the analytical methodology can become a limiting factor. Apart from the target compounds, other matrix components can be absorbed on the SPE sorbent, leading to ionization suppression or, less frequently, to enhancement of the signal (Al-Odaini et al., 2010). This was the case of amphetamine. The low recovery obtained for this analyte may be due to a co-elution with another compound included in the samples with the same quantification transition but different qualifier. In the case of azythromycin, the matrix effect led to an important suppression on

signal intensity in the SPE extract (200 times preconcentrated) when compared with the measured signal in the same extract that was 10 and 50 times diluted. Signal suppression in SPE extract was the effect most commonly found for the majority of compounds in water samples with high levels of organic content and nutrients. In order to achieve a reliable and accurate quantification, we minimized this effect by diluting all samples with MeOH:water (10:90, v/v), before LC-MS/MS analysis. The accentuated matrix effect can also be related to some inconsistencies between the information provided by the screening approach and the quantification based on target MS/MS method, as only the sample extracts highly preconcentrated (200) were analysed by LC-QTOF.

In this study, we have carried out the quantification of target compounds by LC-MS/MS in positive and negative ionization mode. Some of the detected point source contaminants (e.g. gemfibrocil) showed higher ionization efficiency under negative conditions, so the optimization of both operating conditions was key in order to achieve an accurate and reliable quantification of target compounds. The LC-MS/MS instrument was equipped with Jet Stream and iFunnel technology, which allows increased ion transmission and greatly improves the signal to noise ratio of the analytes. In addition to instrumental specifications, the selection of the most characteristic and intense transitions, and the optimization of collision energies for each target compound in LC-MS/MS method, allowed the confirmation of "false negative" compounds at concentration levels close to the LOQs. TOF instruments offer high selectivity and sensitivity under full-scan conditions compared to other analyzers, but they are around one order of magnitude less sensitive to some compounds when compared with a triple quadrupole instrument used in the MRM mode (Martínez-Bueno et al., 2007).

In conclusion, the extraction procedure proposed in this study was adequate to obtain a monitoring of organic contaminants present in river waters. It is evident that some limitations, such as the poor recoveries in the case of some compounds, could be optimized by using different SPE protocols or by utilizing internal standards. Unfortunately, the unavailability of isotopically labeled standards for all compounds and their high cost hamper its use in multi-residue methods. In this case, the dilution of the samples was considered as a good alternative to avoid such problems.

3.5. Comparison between screening and quantitative analysis

The screening analysis represents a relatively rapid and economic method for the selection of chemicals likely to be present in surface waters and worth to be examined more carefully with a quantitative analysis. However, when the screening results were compared with the results of the quantitative analysis some mismatch was identified, leading to false positive and false negative results:

1. False positive results: screening results showed the presence of some compounds, which were not confirmed by the quantitative analysis. This could be due to the fact that their fragmentation spectra were not available in the library, and consequently, only accurate mass error (ppm) was taken into account for the detection and isotope pattern match. This happened more often for pesticides.
2. False negative: screening results showed the absence of compounds that were confirmed by quantitative analysis. In some cases, this could be due to the fact that these compounds were in the samples at low concentrations, and the use of specific MRM transitions (quantification and qualification) by LC-MS/MS improved the sensitivity of the method.

A detailed comparison between the results of the screening and the quantitative analysis for pesticides and for point source compounds are reported in Tables S12 to S15, and in Tables S16 to

S19, respectively; while a synthesis of these results is shown in Tables S20 and S21. For pesticides, the agreement between screening data and quantitative analysis was not completely satisfactory. Considering the 48 grab samples, the percentage of false negatives is 75%. This was expected as in many cases pesticide concentrations in the grab samples were very low (pg/L to few ng/L; Table 4). When only values 10 times above the LOD are included, the percentage of false negatives drops to 42%, confirming that the uncertainty increases at very low concentrations due to high sensitivity of the LC-MS/MS as compared to the LC-QTOF. False positives were 21%. Four chemicals frequently detected in the screening analysis (carbofuran, malathion, metolcarb, kresoxim methyl) were never found in the quantitative analysis. Higher percentages of false negative and false positive results were obtained with the POCIS samples (53% and 33% respectively). Particular cases are those of chlorpyrifos, which was detected in eight out of the twelve POCIS samples with only some signals in the grab samples (but not quantified); and spinosyn-A, never found in grab samples and detected in four out of the twelve POCIS samples (Table S15).

For point source chemicals, the match between the results of both methods was slightly better. The percentage of false negatives (higher than 10 times the limit of detection) and of false positives in the grab samples is 20% and 8% respectively (Table S21), and in the POCIS samples 26% and 11%, respectively.

In conclusion, the screening approach applied in this study needs improvement for the selection of priority chemicals. The combination of accurate mass measurements, retention time, isotopic pattern, along with characteristic fragmentation of each compound and standards provides the unequivocal identification of each compound. Therefore, more complete libraries are needed to increase the number of chemicals that can be reliably identified in future studies.

3.6. Comparison between grab and passive sampling methods

The comparison of concentrations found in grab samples with those calculated from POCIS samples is shown for some selected pesticides and point source chemicals in Figures 5 and 6, respectively. In the figures only values higher than 0.1 ng/L are reported, assuming that a comparison between extremely low values may be poorly reliable. Figures for the other compounds are shown in the Supporting Information (Figures S1 and S2).

For pesticides, concentrations in POCIS samples were, in general, slightly higher than in the grab samples. In sites 6, 13, 14 and 16, for some compounds (e.g. imidacloprid, diuron, simazine), TWA concentrations calculated for POCIS samples were found to be more than an order of magnitude higher than the concentrations measured in grab samples. Lower differences were observed in site 8. This is not surprising considering that sampling site 8 is on a relatively large river (Henares river) characterized by a high and relatively constant water flow, while the other sampling sites are located in small creeks, subject to higher chemical exposure and flow variability in relation to rainfall events.

Pesticide loadings to surface waters are regulated by episodic events such as spray drift and rainfall. Spray drift is particularly relevant for compounds applied directly on crops, such as insecticides and fungicides, which result in sequential pesticide pulses (Verro et al., 2009; Brock et al., 2010; Morselli et al., 2018). The results obtained for pesticides in our study, with higher concentrations in POCIS than in grab samples, and some peaks detected in POCIS, can be related to the land use and pesticide emissions in the sub-basins corresponding to the different sites and by the precipitation records obtained from the meteorological stations. For example, sub-basins corresponding to sampling sites

6, 10, 12, 13, 14, 15 and 16 are characterized by high agricultural land use (see Figure 1), which would explain the high concentrations of pesticides in these sites in at least one sampling time. Regarding precipitations, it must be noted that the studied area is characterized by semi-arid conditions and precipitations in summer are scarce. Nevertheless, in the two weeks of exposure of the passive samplers, some unusual rain events were registered in the meteorological stations of Siguenza and Mandayona (corresponding to sampling site 6), Alcala de Henares and Arganda del Rey (corresponding to sampling site 8), and Tembleque (corresponding to sampling sites 12, 13, 14, 16; see Figure S3).

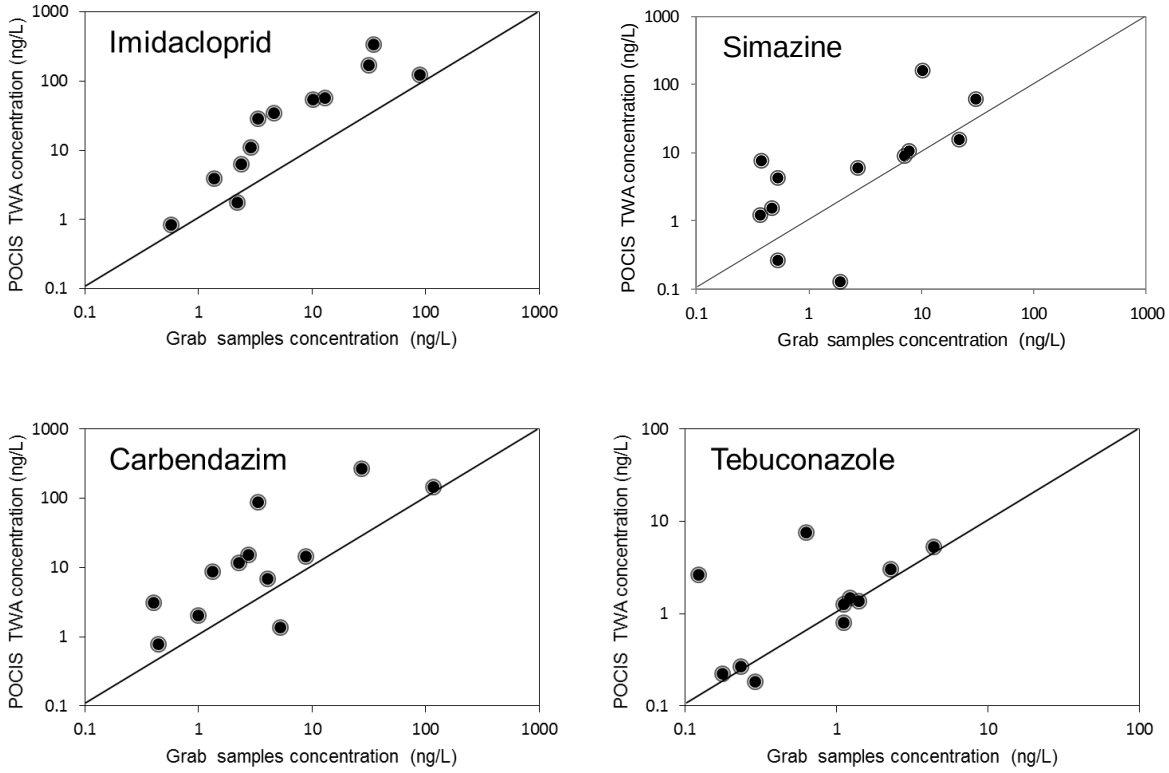

Figure 5. Comparison of pesticide concentrations measured in the grab samples and the TWA (time weighted average) concentrations calculated in the POCIS samples. The line represents the 1:1 correspondence between concentrations in grab and POCIS samples.

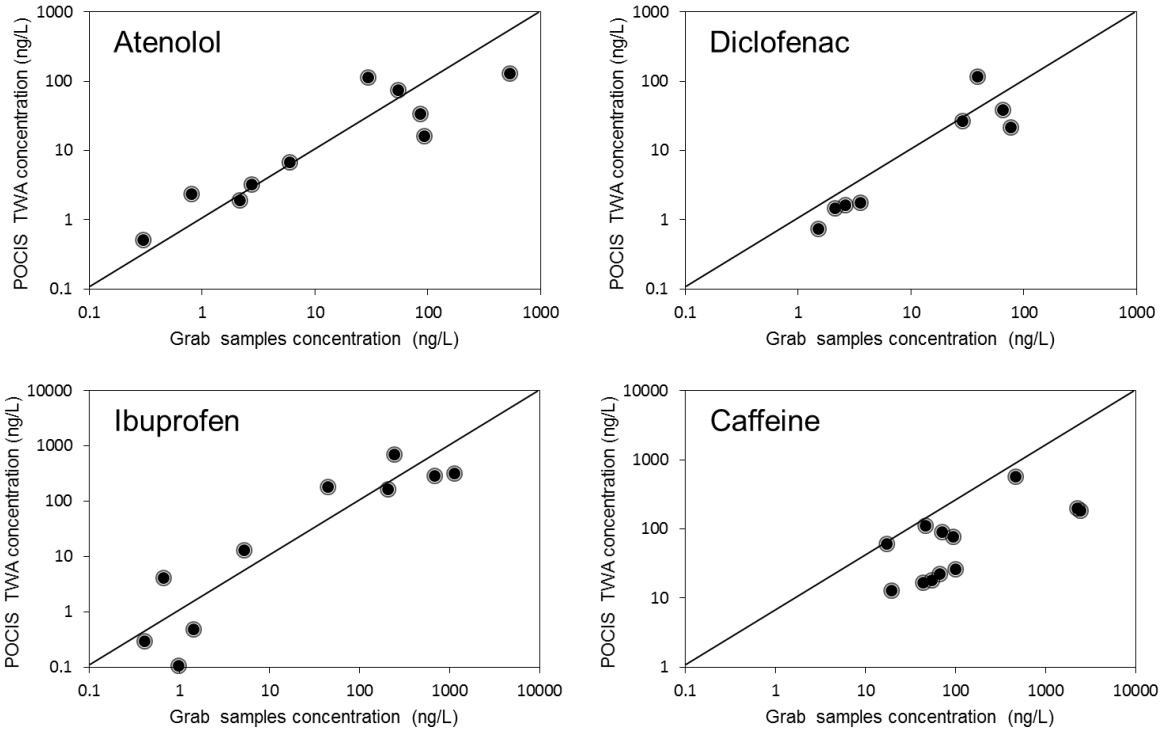

Figure 6. Comparison of point source chemical concentrations in the grab samples and the TWA (time weighted averages) calculated in the POCIS samples. The line represents the 1:1 correspondence between concentrations in grab and POCIS samples.

Considering the seasonal cycle, herbicides are mostly applied in spring, while the major application period for insecticides is usually late spring-summer and for fungicides late summer-autumn. POCIS samples were used only in summer (12 sites). However, some interesting results were observed. Chlorpyrifos was never detected in the grab samples but detected in eight out of twelve POCIS samples, with relatively high water concentrations in sites 8 (244 ng/L), 13 (396 ng/L), 14 (217 ng/L), and 16 (329 ng/L). Comparable outcomes were observed for spinosyn-A, although detected in less POCIS samples (four out of twelve) with lower water concentrations. These contrasting results are, at least partly, related to the performance of the extraction method from grab samples, which were relatively low for spinosyn-A and negligible for chlorpyrifos. Regarding crops in the region and the rainfall data reported by the meteorological stations close to those sampling sites the results regarding chlorpyrifos were expected, at least in POCIS samples.

For point source chemicals, the agreement between grab and POCIS samples was found to be very good, with concentrations differing less than order of magnitude. In some cases, data are quite scattered, but without clear trends above or below the 1/1 line (Fig. 6). This study shows that for the point source contaminants included in this study, grab samples offer sufficient precision to describe exposure levels and to assess the chemical status of surface waters, while seasonal samples may be required to describe the long-term exposure dynamics related to the fluctuation of the population and to the different seasonal use of some substances (e.g. antibiotics; see Section 3.3 and Valcárcel et al., 2013). On the contrary, for pesticides, a monitoring based only on grab samples may lead to substantial underestimation of the actual concentrations, at least in correspondence with application periods and rainfall events. This is particularly relevant in small creeks but may also occur, to a lesser extent, in medium-sized rivers.

4. Conclusions

The present study describes a novel method for the selection of contaminants to be quantified in freshwater samples. The method is based on a preliminary qualitative screening and criteria regarding the frequency of detection, the potential ecotoxicological hazard, and the possible use of the chemical in the watershed. In this study, the method has been applied to identify priority pesticides and point source chemicals in the upper Tagus river basin over three seasons (spring, summer, autumn). Fifty-two contaminants (20 pesticides and 32 point source chemicals, mainly pharmaceuticals) have been selected out of a preliminary list of 268 compounds, and their concentration levels have been determined. Moreover, the suitability of the current monitoring method based on grab samples has been compared with POCIS passive samples during the summer monitoring campaign. This study demonstrates that chemical screening approaches are subject to uncertainties, and that some false positives and false negatives may be encountered on the basis of LC-MS/MS analytical verifications. To minimize them, further work should be dedicated to increasing the availability of updated libraries with exact mass data for different groups of chemicals (drugs, pesticides, pharmaceuticals, antibiotics), and should rely on a larger number of chemical standards, for carrying out unequivocal confirmations (retention time, MS and MS/MS spectra). This study also shows that for chemicals characterized by discontinuous emissions, such as pesticides, a reduced number of grab samples may not be suitable to adequately characterize contamination patterns. These contaminants require alternative sampling procedures (e.g. POCIS devices), particularly in small rivers in which the temporal variability of concentrations is higher. The chemical monitoring performed in this study shows that some sites of the upper Tagus river basin, primarily dominated by agricultural and/or urban land use, are highly polluted. Some insecticides (chlorpyrifos, dimethoate, imidacloprid), herbicides (diuron, metribuzine, simazine, terbuthylazine), and fungicides (carbendazim) have been measured at concentrations exceeding 100 ng/L, while several point source contaminants have been detected at concentrations (well) above 1 µg/L. Particularly, paracetamol, ibuprofen, some antibiotics (azithromycin, sulfamethoxazole, trimethoprim) and life-style compounds (caffeine, paraxanthine, nicotine) have been detected downstream of urban areas or small villages without wastewater treatment facilities at concentrations one order of magnitude above the concentrations reported in previous studies.

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Chapter 4

Identification of contaminants of concern in the upper Tagus river basin (central Spain). Part 2: Spatio-temporal analysis and ecological risk assessment

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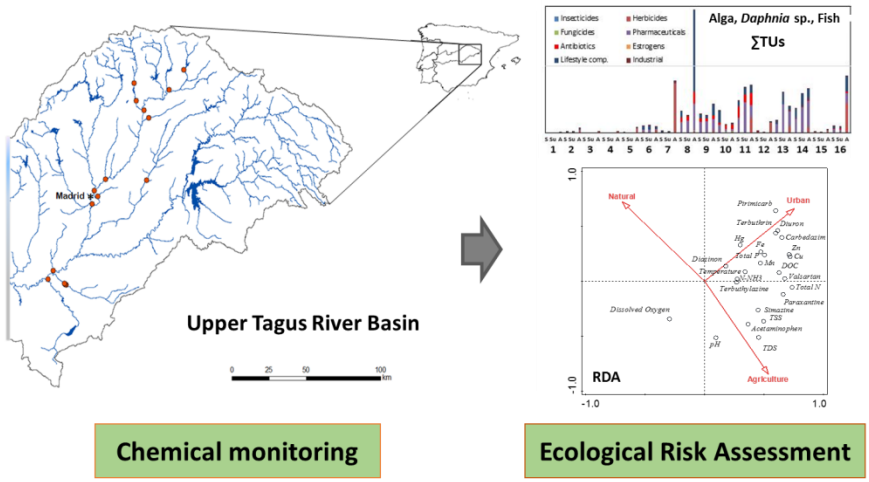

Abstract

This study provides a description of the water quality status in the tributaries of the upper Tagus River and a preliminary risk assessment for freshwater organisms. A wide range of physico-chemical parameters, nutrients, metals and organic contaminants (20 pesticides, and 32 point source chemicals, mainly pharmaceuticals) were monitored during spring, summer and autumn of 2016. Monitoring of organic contaminants was performed using conventional grab sampling and passive samples (POCIS). The variation of the different groups of parameters as regards to land use and sampling season was investigated. The prioritization of organic and inorganic contaminants was based on the toxic unit (TU) approach, using toxicity data for algae, invertebrates and fish. Finally, the compliance with the Environmental Quality Standards (EQS) set as part of the Water Framework Directive (WFD) was evaluated for the listed substances. This study shows that the land use characteristics had a large influence on the spatial distribution of the contaminants and other water quality parameters, while temporal trends were only significant for physico-chemical parameters, and marginally significant for insecticides. Acute toxicity is likely to occur for some metals (copper and zinc) in the most impacted sites (TU values close to or above 1). Low acute toxicity was determined for organic contaminants (individual compounds and mixtures) on the basis of grab samples. However, the assessment performed with POCIS samples identified diuron, chlorpyrifos and imidacloprid as potentially hazardous compounds. Several contaminant mixtures that may cause chronic toxicity and that should be considered in future regional chemical monitoring plans were identified. Our study also shows that some metals and pesticides exceeded the WFD regulatory thresholds and that only 30% of the sampled sites had a good chemical status. Further research is needed to identify chemical emission sources and to design proper abatement options in the Tagus river basin.

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1. Introduction

The number of contaminants that can be found in surface waters worldwide follows an exponential increase as a result of growing demographic pressures and the intensification of industrial and agricultural activities. The estimated number of substances commercially available in Europe is over 100,000 compounds (EINECS, 1990; ELINCS, 2017); and similar numbers hold for the USA (Muir and Howard, 2007). Chemical pollution can result in lethal and sub-lethal effects on aquatic organisms and significant losses of habitat and biodiversity (Schwarzenbach et al., 2006; Malaj et al., 2014). In this way, the elaboration of lists of chemicals that pose a threat to aquatic ecosystems plays a major role in environmental legislation for surface waters (Kuzmanović et al., 2015). The Water Framework Directive (WFD; Directive 2000/60/EC) constitutes the most extensive legislative framework for the protection of surface waters in Europe and aims at achieving a good ecological status of all European water bodies, by not only assessing the hydro-morphological and biological status, but also their chemical status. In this regard, the WFD has provided Environmental Quality Standard (EQS) that must be met for 45 compounds that have been identified as priority (hazardous) substances, and advocates for the additional monitoring of substances of national or regional concern by the different member states (WFD; Directive 2013/39/UE).

Current developments in monitoring and analytical techniques show that the WFD priority substances only constitute a small fraction of the whole plethora of chemicals that are found in surface water ecosystems (e.g. pharmaceuticals, life-style compounds, home-care products, other pesticides; Barceló and Petrovic, 2007; Silva et al., 2015). Moreover, organic and inorganic contaminants form complex mixtures, whose spatiotemporal dynamics and potential ecotoxicological side effects are still relatively unknown. Therefore, chemical risk assessment and prioritization approaches are needed to identify pollutants that should be included as part of basin-specific monitoring and management programs (Von der Ohe et al., 2011; Hering et al., 2015; Rico et al., 2016a; Tsaboula et al., 2016).

The number of studies assessing the risks of regulated and unregulated chemicals in Mediterranean rivers is limited (e.g. Ginebreda et al., 2010; López-Doval et al., 2012; Kuzmanović et al., 2015). Moreover, the assessment of the effect of multiple stressors related with anthropogenic contamination in these rivers is still a challenge. This is mainly due to the region's marked seasonal hydrological and climatological patterns, which interfere with chemical exposure and bioavailability, and with the characteristics of its biological communities (Arenas-Sánchez et al., 2016, 2019b). Studies are still needed to better understand the temporal and spatial distribution of chemical contaminants in these ecosystems and to assess theirs risks for freshwater organisms.

The Tagus River is the longest river in the Iberian Peninsula (1,092 km) and holds the third largest catchment (81,947 km^2). It flows from the central Spanish Plateau (Teruel region) up to Portugal (Lisbon). The basin is subject to a Mediterranean climate, characterized by hot and dry summers, and mild-to-cold winters, and with the majority of rainfall events occurring in spring and autumn (Benito et al., 2003). In its upper part, the Tagus watershed is characterized by forest and conservation areas and extensive agricultural production, while 150 km downstream it is characterized by a high degree of demographic pressure, primarily from Madrid and its surrounding cities, which host approximately 6.7 million inhabitants. Given the different land use influences, the Tagus River and its tributaries may be exposed to a wide range of contaminants. To date, the number of studies assessing the contamination patterns in the watershed and their potential ecotoxicological risks is limited. In the upper Tagus river basin (central Spain), most studies have focused on assessing contamination with

pharmaceuticals, illicit drugs and life-style compounds (Fernández et al. 2010; Valcárcel et al. 2011, 2013), while the impacts of pesticides have only been evaluated in the lower areas of the catchment (Portugal; Silva et al., 2015). Studies targeting at the identification of priority contaminants at a basin level, including pesticides, point-source chemicals (e.g. pharmaceuticals, life-style compounds) and other potentially hazardous substances, such as metals, are currently unavailable.

The overall aim of this study was to provide a description of the water quality status in the upper Tagus river basin and to identify contaminants that may pose a potential ecotoxicological hazard beyond those that are monitored under the WFD. This study has been divided into two parts (Part 1 and Part 2). Part 1 of this study is presented in Rico et al. (2019) and describes a chemical screening analysis, followed by a novel prioritization approach, which was used to select and quantify exposure concentrations for 52 pesticides and point-source chemicals (mainly pharmaceuticals) in the tributaries of the Tagus River. In the present paper (Part 2) the dataset has been supplemented with a wide range of physico-chemical, nutrient and metal analysis performed in the same sampling sites. The main objectives of the present paper are: (1) to explore the relationship of the monitored water quality parameters with land use and their seasonal variation, (2) to prioritize contaminants and contaminant mixtures regarding their potential ecotoxicological hazard, and (3) to assess the compliance of the measured concentrations of selected contaminants with the EQSs established as part of the WFD. Ultimately, we expect that this study contributes to the identification of basin-specific contaminants that are included as part of future monitoring plans and to the design of proper chemical abatement options in the Tagus river basin.

2. Materials and Methods

2.1. Description of the study area and land use data

Sixteen sampling sites were selected, covering a range of hydro-morphological conditions and different levels of anthropogenic impact (Figure 1). All sampling sites were close to the Tagus River Basin Authority monitoring flow gauges, from which flow data series for 2016 were obtained.

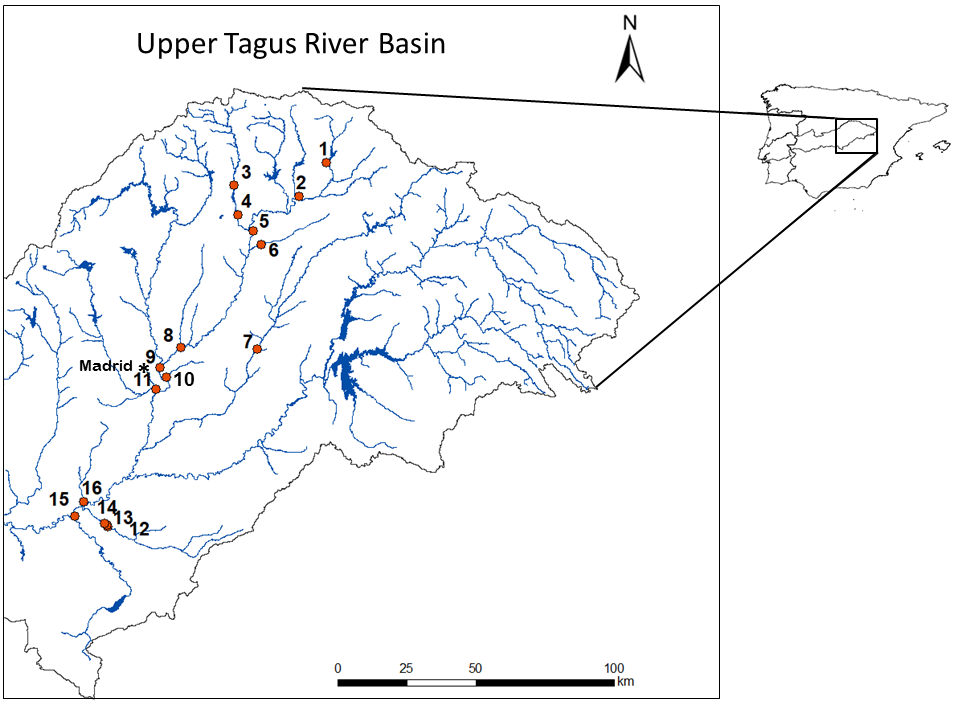

Figure 1. Map of the study area and sampling sites in the upper Tagus river basin.

The afferent drainage area of the associated sub-basins to each sampling point was extracted using GIS software (ArcGIS). The Hydrology tool of the Spatial Analyst Toolbox was implemented by using an algorithm that includes fill, flow direction and flow accumulation routines to delineate the watersheds using a 25 x 25 m Digital Elevation Map (DEM) provided by the Tagus River Basin Authority. Once the afferent drainage areas were defined, the associated land use was extracted from the Corine Land Cover layer (2006), downloaded from the Spanish National Center for Geographic Information (CNIG). A summary of the land use and average hydrological conditions of the sampled rivers is reported in Table 1. The 16 sub-basins have a diverse range of surface area (from 467 to >8 000 km^2). Similarly, the land use varies among sampling sites, with sites 1 to 5 being mainly surrounded by natural areas (up to 98% of natural surface), and site 7 having a mixed natural and agricultural land use. The other sites were characterized by a high (up to about 95%) agricultural impact (sites 6, 12, 13, 14, 15), and moderate to very high (up to >20%) urban impact (sites 8, 9, 10, 11, 16). The sampled water bodies ranged from medium sized rivers (annual average water flow that exceed 10 m^3/s) to very small creeks (annual average water flow <1 m^3/s). The water flow presents high variability, including seasonal and monthly variability, which ranges from about 1.6 (Manzanares and Guaten) up to about 300 (Sorbe upstream the reservoir) times difference between the maximum and minimum monthly averages.

Table 1. Area of the watersheds draining into the selected sampling sites, land use characteristics, and water flow parameters (annual average, minimum and maximum monthly averages).

	Watershed area (km^2)	Land use (%)			Water flow (m^3/s)		
		Urban	Agriculture	Natural	Annual average	Min. Mont.	Max Mont.
1 - Salado River	1273	0.03	22.1	77.9	0.27	0.07	0.48
2 - Henares River	2324	0.11	40.5	59.4	1.63	0.79	3.56
3 - Sorbe River (ups. res.)	1274	0.02	2.00	97.9	3.58	0.03	9.90
4 - Sorbe River (ds. res.)	2188	0.00	40.3	59.7	2.21	0.69	8.68
5 - Henares River (upper)	4782	0.10	50.0	49.9	4.82	2.04	12.9
6 - Badiel River	931	0.20	81.5	18.3	0.13	0.02	0.32
7 - Tajuña River	4888	0.10	70.9	28.9	1.52	0.99	2.49
8 - Henares River (lower)	5700	2.1	51.3	46.6	6.75	3.59	13.65
9 - Jarama River	8644	5.2	37.1	57.7	15.2	5.80	49.9
10 - Pantueña Stream	467	6.6	76.1	17.3	0.08	0.03	0.17
11 - Manzanares River	2370	21.9	24.3	53.7	10.4	8.58	13.8
12 - Melgar Stream (ups. d.p.)	3319	0.73	94.7	4.5	0.18	0.06	0.40
13 - Melgar Stream (d.p.)	3319	0.73	94.7	4.5	0.18	0.06	0.40
14 - Melgar Stream (ds. d.p.)	3319	0.73	94.7	4.5	0.18	0.06	0.40
15 - Algodor Stream	2452	0.40	75.3	24.3	0.20	0.01	0.67
16 - Guaten Stream	786	10.1	89.0	1.0	0.63	0.49	0.81

ups.res.: upstream of a reservoir; ds.res.: downstream of a reservoir; ups. d.p.: upstream of an urban discharge point; d.p.: next to the urban discharge point; ds. d.p.: downstream the urban discharge point.

2.2. Sampling methods

Water samples were taken in spring (April 11-14), summer (July 11-14) and autumn (November 21-24) of 2016, with one sample per site and season. All samples were collected in the middle section of the river by using: 1L plastic bottles for analysis of nutrients and dissolved organic carbon (DOC); 1L amber glass bottles for organic contaminants; 250 mL plastic bottles acidified (pH < 2) with nitric acid 69% (5 mL/L) for metals. Immediately after sampling, all samples were transported to the laboratory, where they were kept frozen at -20 ˚C until further analysis; except for metal samples, which were stored at 4˚C and analyzed within 72h. During the summer sampling campaign, passive samplers (POCIS: Polar Organic Chemical Integrative Samplers) were properly fixed with stainless steel cages and placed in the river bottom for two weeks in all sampling sites. After 14 days, POCIS were

collected, stored in air-tight containers and transported to the laboratory, where they were kept frozen at -20 ˚C until further analysis. Only 12 samplers were recovered out of the 16 that were deployed. The missing ones were lost for various reasons (e.g. vandalism, high water flow).

2.3. Nutrients and physico-chemical parameter analysis

Water temperature (˚C), pH, electrical conductivity (EC), total dissolved solids (TDS), dissolved oxygen (DO), total suspended solids (TSS) were measured *in-situ* using a portable multimeter probe (HANNA Instruments, Woonsocket, RI, USA, model HI98194). Nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+), orthophosphate (PO_4^{-3}) and total phosphorus were measured according to the methods described in the Standard Methods for the Examination of Water and Wastewater (APHA, 2005). The DOC concentration was measured on a Shimadzu TOC-VCSH/CSN coupled to an ASI-V autosampler (Shimadzu Corporation, Kyoto, Japan).

2.4. Metal analysis

The analysis of metals (Mn, Fe, Cu, Zn, Cd, Pb, Hg) was performed with a 7700 ICP-MS equipment (inductively coupled plasma mass spectrometry, with a MicroMist nebulizer) from Agilent Technologies (Palo alto, CA, USA). The plasma conditions were: forward power (1550 w), gas flow rate (15 L/min), auxiliary gas flow rate (0.9 L/min) and nebulizer gas flow rate (1.1 L/min). Standard regression lines were obtained as the mean of three injections of each calibration point, and the regression coefficient was >0.99. The concentration ranges used in the regression lines were 5-1000 ng/L and 0.005- 1000 µg/L in nitric acid 1 %, for Hg and the rest of metals, respectively.

2.5. Organic contaminant analysis

Several groups of organic contaminants were analyzed in the grab and in the POCIS samples, which are characterized by different use and emission patterns (i.e., point and non-point source contaminants). In total 52 chemicals were analyzed (Table S1 in the Supporting Information): 20 pesticides (9 insecticides, 6 herbicides, 5 fungicides), and 32 point source contaminants including 23 pharmaceuticals (9 of them antibiotics), 4 estrogens and steroids, 4 life-style compounds (alkaloids and other stimulants), and 1 industrial chemical. The procedure for selecting these chemicals is described in the Part 1 of this study (Rico et al., 2019). Organic chemicals were analyzed by liquid chromatography using an HPLC system (Agilent 1200 Series, Agilent Technologies) coupled to an Agilent 6495 triple quadrupole (QQQ) mass spectrometer (LC-MS/MS). Further details of the analytical procedure are provided in Rico et al. (2019). The physico-chemical properties of the selected organic contaminants are reported in the Supporting Information (Table S1).

2.6. Spatio-temporal analyses

A variance partitioning analysis was performed to evaluate the relative contribution of land use and sampling time (season) on the variability observed in the entire measured parameter dataset. Moreover, a variation partitioning analysis was performed for each group of parameters separately: physico-chemical parameters (temperature, pH, TDS, TSS, DOC); nutrients ($N\text{-}NH_3$, total N, total P); metals (Cd, Cu, Fe, Hg, Mn, Pb, Zn); pesticides (also separately for insecticides, herbicides and fungicides); and point source contaminants (also separately for pharmaceuticals excluding antibiotics, and for antibiotics). The variance partitioning analysis was performed with two groups of variables: land use variables (% agriculture, %urban and %natural) and season, as a single categorical value (spring, summer, autumn), under the Redundancy Analysis option (RDA).

Finally, an RDA was performed to assess the influence of land use on the variation of the physico-chemical parameters, and the most relevant metals and organic contaminants contributing to the toxicity of aquatic organisms (see section 2.7). To prevent an overrepresentation of the chemicals measured in summer due to the grab and POCIS sampling, only grab sample results were included in the analysis. All statistical analyses were performed with the CANOCO v.5 software (Ter Braak and Smilauer, 2012). Prior to any calculation, the parameter values were log(x+1) transformed in order to normalize parameters with different units and scales, and to approximate a normal distribution of the data.

2.7. Toxicity data mining and ecological risk assessment

An ecological risk assessment (ERA) for metals and organic contaminants was performed using acute toxicity data for freshwater organisms: algae, *Daphnia sp.* and fish. EC50 or LC50 values from laboratory studies were collected from the ECOTOX database (https://cfpub.epa.gov/ecotox/) as well as from other relevant data sources. In absence of experimental data for organic compounds, toxicity data were calculated using QSARs (Quantative Structure-Activity Relationships). Details on the selected endpoints and the QSARS used for the estimation of toxicity data are provided in the Supporting Information. It must be noted that QSAR equations are mainly reliable for narcotic-like compounds. When they are used for predicting effects likely to be specific (i.e. effects of herbicides on algae, insecticides on animals) the QSAR-derived toxicity data may underestimate risks. However, the vast majority of toxicity data used for pesticide evaluations was based on experimental data. QSARs were mainly employed to estimate toxicity data for some pharmaceuticals, hormones, and life-style compounds (see Table S1).

The ERA for individual organic and inorganic chemicals was performed following the Toxic Unit (TUs) approach for each taxonomic group (i.e., calculated as the ratio between the measured environmental concentration and the EC50 or LC50 value for the standard test species established for each taxonomic group). In case of concentrations below the analytical detection limit (LOD), TUs were calculated using LOD/2. Iron TUs were not calculated for algae, due to the lack of toxicity data. A refinement of the calculation could be made normalizing the data for bioavailable metals according to the BLM (Biotic Ligand Model) approach (Di Toro et al., 2001; De Schamphelaere and Janssen, 2002). However, since the complete set of data required for a sound application of the BLM was not available, metal TU values in this study were based on total dissolved data. TUs for chemical mixtures were calculated according to the concentration addition (CA) concept, as the sum of TUs for individual chemicals (Backhaus et al., 2000). Key organic contaminants were identified by selecting those compounds that contribute to the 90% of the total potency of the mixture in the grab and POCIS samples with TUs higher than 0.001. Moreover, contaminant mixtures in these samples were identified regarding the different taxonomic groups and seasons. This method is an adaptation of the prioritization approach developed by Von der Ohe et al. (2011). Finally, the Maximum Cumulative Ratio (MCR) was calculated as the ratio between the cumulative toxicity of the mixture and the maximum toxicity from one component of the mixture to assess the relationship between toxic potency and number of contaminants that contribute to it (Price and Han, 2011).

3. Results and discussion

3.1. Influence of land use and sampling season on the measured parameters

The variance partitioning analysis indicated that land use substantially affects the variance of the whole set of data, explaining 35% of the total variance, while the influence of time (season) was not

remarkable (Table 2). Splitting the analysis by groups of measured parameters, generally confirms these results. Land use alone explained from 34% (antibiotics) up to 55% (nutrients) of the variance of the different groups of parameters. In all cases, the effect of land use on the variance was highly significant (Monte Carlo p-value<0.01). Nevertheless, the seasonality effect was only significant for physico-chemical parameters (8.4% explained variance, Monte Carlo p-value<0.05) and marginally significant in the case of insecticides (8% explained variance, Monte Carlo p-value: 0.09).

Table 2. Variance partitioning analysis performed for each parameter group based on RDAs. LU ∪ SE is the total explained variance, LU| SE is the variance explained only by land use, SE| LU is the variance explained only by season, and LU ∩ SE is the shared variance between land use and season. At the end, LU and SE represent all variance explained by land use and season, respectively, together with the results of the significance test (Monte Carlo p-value). Results are expressed as percentage of explained variance.

Parameter group	LU ∪ SE	Residual variance	LU| SE	SE| LU	LU ∩ SE	LU	SE
All parameters	35.0	65.0	35.0	<0.1	<0.1	37.8**	4.7
Metals	39.1	60.9	36.5	2.6	<0.1	37.6**	5.2
Physico-chemicals	50.4	49.6	42.0	8.4	<0.1	42.4**	10.2*
Nutrients	55.3	44.7	55.3	<0.1	<0.1	55.0**	1.2
Pesticides	42.2	57.8	41.8	0.4	<0.1	44.3**	2.9
Insecticides	59.6	40.4	51.6	8	<0.1	53.7**	9.4[a]
Herbicides	40.2	59.8	40.2	<0.1	<0.1	41.1**	1.2
Fungicides	44.4	55.6	44.4	<0.1	<0.1	45.0**	2.3
Point-source contaminants	34.8	65.2	34.8	<0.1	<0.1	36.2**	2.2
Pharmaceuticals	36.1	63.9	36.1	<0.1	<0.1	37.4**	1.5
Antibiotics	34.4	65.6	34.4	<0.1	<0.1	35.9**	1.7

** p-value ≤ 0.01, * 0.05 ≥ p-value ≥ 0.01, [a] marginally significant 0.1 ≥ p-value > 0.05

3.1.1. Physico-chemical parameters

The influence of seasonality on physico-chemical parameters (water temperature, pH, TSS, DOC) depend on physical and biological factors (photosynthesis, microbial activity, dilution capacity) which generally follow predictable seasonal patterns. In >75% of the samples, DO was between 70% and 100% of saturation, which means that no remarkable oxygen depletion occurred at least at the time of sampling. Low oxygen values (<70%) were found in sites highly impacted by urban land use and wastewater discharges. However, no clear temporal trend could be determined for this parameter. pH values were in the range 6.2-9.6 (Table 3), which is considered as a regular range for freshwaters (Bundschuh et al., 2016). Overall, values were slightly higher in spring and summer, most likely due to a higher photosynthetic activity. Other parameters like TDS are more dependent on the natural geochemical characteristics of the watershed than on human or biological impact. This parameter showed relevant differences among sampling sites independently of the dominating land use in the sub-basin and/or sampling site. Thus, very low values were found in the Sorbe River (sites 3 and 4), which is mainly surrounded by forested areas, and very high values were monitored in the Salado River (site 1) or Melgar Stream (sites 12, 13 and 14), with natural and agricultural land uses, respectively. Despite slightly higher values could be observed in some sites suffering from reduced summer flows, the seasonal variability as compared to the spatial variability was low (Tables 2 and 3). All measured physico-chemical data are showed in Table S2 of the Supporting Information.

3.1.2. Nutrients

The influence of land use on this group of parameters was significant (Table 2), with highly impacted sites showing the highest values, including some remarkable ones. High concentrations of total inorganic nitrogen (>10 mg N/L), were measured in all sampling periods in sites 8 (Henares River

downstream) and 11 (Manzanares River), both downstream of large urban settlements. The major component of total N was ammonia (>10 mg N/L), particularly in spring samples. This can be understood as an indication of reducing conditions, confirmed by relatively low oxygen concentrations, particularly in site 11, with around 50% of oxygen saturation. Additionally, in site 8 very high nitrite concentration was measured in summer. All these data confirm the high impact of urban pollution. Relatively low levels of total inorganic nitrogen (<3 mg N/L), with low or negligible concentrations of ammonia nitrogen, were measured in sites 1 to 5, characterized by prevailing natural conditions in the watershed. In some sites, high values of ammonia, combined with elevated pH values and high summer temperatures, led to extremely high levels of unionised ammonia (NH_3) (e.g. higher than 100 μg/L), with a maximum value of >700 μg/L in site 9 (Jarama River) in summer (Table 3).

Total phosphorus concentration was also high in sites characterized by urban land use, particularly in site 16, where agricultural surface is also relevant, with possible additional contribution from fertilisers. Relatively low phosphorus concentrations, never higher than 50 μg/L, were measured in sites 1 to 5.

Nutrients did not show significant seasonal patterns although they may also reach surface waters trough runoff and are influenced by biological activity. However, our results indicated that in the selected sites, the impact of these temporal patterns may be outweighed by the contribution of wastewater discharges. All measured nutrient data are showed in Table S2 of the Supporting Information.

Table 3. Measured physico-chemical parameters, nutrients and metals in the different sampling points in spring, summer and autumn. Median (minimum-maximum).

	Spring	Summer	Autumn
Physico-chemical parameters			
Temperature (˚C)	12.1 (7.27-16.3)	19.5 (13.8-24.2)	9.75 (7.43-15.1)
pH	8.31 (7.26-8.61)	8.48 (7.09-9.62)	7.78(6.23-8.09)
Conductivity (μS/cm)	1545 (45.5-5315)	1778 (89.5-5114)	1527 (75.5-5001)
Alkalinity (mg $CaCO_3$/L)	227 (20.2-358)	189 (39.0-281)	225 (21.1-380)
TDS (mg/L)	791 (22.5-2656)	896 (44.5-2553)	764 (35.0-2542)
TSS (mg/L)	15.9 (1.60-167)	75.9 (0.20-365)	26.3 (0.40-74.7)
Dissolved oxygen (mg/L)	9.94 (5.05-11.2)	8.08 (2.20-10.6)	8.32 (5.54-10.2)
Dissolved oxygen (% Sat.)	89.5 (50-102)	86.5 (25-110)	75.0 (53-86)
DOC (mg/L)	5.40 (1.70-7.90)	4.90 (1.10-9.20)	4.59 (1.78-7.70)
Nutrients			
$N\text{-}NH_4^+$ $N\text{-}NH_3$ (mg/L)	0.73 (<0.001-15.3)	0.19 (0.03-6.60)	0.10 (<0.001-7.53)
$N\text{-}NH_3$ (mg/L)	0.05 (<0.001-0.21)	0.03 (<0.001-0.75)	0.008 (<0.001-0.05)
$N\text{-}NO_2$ (mg/L)	0.0076 (<0.001-0.23)	0.03 (0.001-1.67)	0.02 (<0.001-0.57)
$N\text{-}NO_3$ (mg/L)	2.57 (0.09-13.4)	3.98 (0.36-6.83)	3.58 (0.28-7.86)
N-Inorg. Tot. (mg/L)	3.76 (0.09-17.2)	4.78 (0.43-12.2)	4.20 (0.28-12.0)
$P\text{-}PO_4$ (mg/L)	0.04 (<0.003-0.65)	0.09 (<0.003-0.62)	0.11 (<0.003-0.96)
Total P (mg/L)	0.05 (0.002-0.31)	0.07 (0.01-0.95)	0.11 (<0.003-0.64)
N/P	217 (6.74-2089)	156 (14.2-1125)	75.5 (10.6-305)
Metals			
Mn (μg/L)	28.9 (3.00-118)	77.9 (3.03-163)	22.4 (0.41-801)
Fe (μg/L)	96.2 (17.0-676)	294 (20.1-1362)	186 (18.3-1074)
Cu (μg/L)	0.75 (0.35-9.48)	1.32 (0.39-7.41)	0.82 (0.29-14.5)
Zn (μg/L)	3.06 (0.70-68)	6.64 (0.71-56.2)	12.2 (<4.7-73.7)
Cd (μg/L)	0.01 (<0.005-0.22)	0.03(<0.005-0.07)	0.02 (<0.005-0.10)
Pb (μg/L)	1.25 (<0.73-6.05)	2.87 (<0.73-6.17)	2.60 (<0.73-5.75)
Hg (μg/L)	<0.058	0.09 (<0.058-0.14)	<0.058

3.1.3. Metals

In sampling sites 1 to 5 the measured metal concentrations were generally low, usually in the range of the natural background levels described by Crommentuijn et al. (1997); with the exception of few outliers (e.g. manganese in site 1 in autumn and iron in site 3 in summer). Remarkably high concentrations were measured in highly impacted sites, particularly in those downstream of urban areas (sites 8, 9, 10, 11 and 16). This spatial distribution confirms the results of the variance partitioning analysis indicating a highly significant effect of the land use. No regular trends could be identified in terms of seasonal variability (Tables 2 and 3), leading to not significant seasonal effect shown by the statistical analysis. The concentrations of selected metals are reported in Table S2 and Figure S1 of the Supporting Information.

3.1.4. Organic contaminants

For all groups of organic contaminants, the variance partitioning analysis indicated a highly significant land use effect (Table 2). Pesticides and point-source contaminants showed low or negligible concentrations in less impacted sites (e.g. sites 1 to 5) and much higher concentrations in the most impacted ones (e.g. 8 to 11, and 16). This is also evident from Figure 2, in which the concentrations in grab samples of some selected pesticides that were detected in >50% of the sites at levels higher than 1 ng/L are shown. Graphs for all other measured chemicals are available in the Supporting Information (Figures S2 and S3). The complete set of data on the concentrations of organic contaminants is reported in the Part 1 of this study (Rico et al., 2019).
A larger seasonal variation was expected for pesticides, due to their seasonal emission patterns. This is not supported by the statistical analysis, except for a marginally significant result obtained with respect to insecticide concentrations, which showed higher concentrations in the summer period. These results are in line with those described by Ccanccapa et al. (2016) which showed that higher pesticide concentrations occur during the time of the year with lower water flows in other Mediterranean rivers (Júcar and Turia).

In spite of that global result, a seasonal trend coherent with usual application patterns, may be observed for some particular compounds. For example, the highest concentrations for the majority of highly impacted sites were detected in summer for the insecticide imidacloprid. Also for some fungicides, there seemed to be an increase in some summer samples, but the pattern was not that clear (e.g. carbendazim, tebuconazole; Figure 2).

The variance partitioning analysis indicated that seasonality had no effect on point source contaminants as a whole, neither on the group of pharmaceuticals (excluding antibiotics) and antibiotics. This result confirms that the emission of these substances mainly depends upon relatively constant sources (e.g. urban wastewater; Osorio et al., 2012). However some seasonal trends were observed towards increasing concentrations of some antibiotics (azithromycin, sulfamethoxazole, trimethoprim) in autumn downstream of urban areas (Rico et al., 2019). The concentrations of pharmaceuticals and antibiotics (Figure S3) were, in general, relatively low in the sampling sites 1 to 5, 12 and 15. Other chemicals (such as caffeine, nicotine and tributyl phosphate) were detected at relatively high levels in almost all sampling sites, although relevant spatial variability was also present, with some sites reaching concentrations of several µg/L. Highly contaminated sites for most point-source contaminants were 8 to 11, 13 and 14. A detailed description of the quantitative analysis of organic contaminants (pesticides and point source chemicals), as well as the concentrations of all analyzed compounds in the grab and POCIS samples of the 16 sampling sites in the three seasons is reported the Part 1 of this study (Rico et al., 2019).

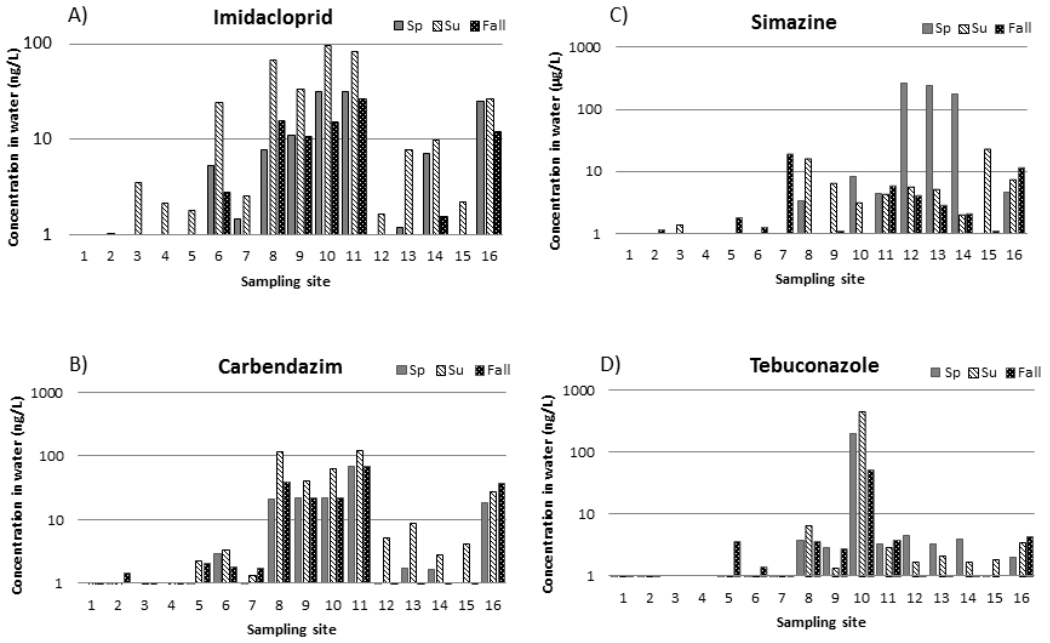

Figure 2. Concentrations for pesticides with high occurrence percentages in river water grab samples. Only values over 1 ng/L are shown for the 16 sampling stations and for each sampling period (spring, summer, autumn).

3.2. Ecological Risk Assessment

3.2.1. Metals

The TU values calculated for the mixture and for individual metals on different groups of organisms (algae, *Daphnia sp.*, fish) are shown in Figure 3, while the raw data are provided in Table S3. For metals, and in particular for those metals that are essential micronutrients, the concepts that are applied to organic contaminants (frequently xenobiotics) to calculate a PNEC (e.g. the application of an assessment factor of 1000 to an acute EC50) are not applicable. Indeed, in most cases, it would lead to values orders of magnitude below the natural background levels (Crommentuijn et al., 1997). Therefore, TU values calculated in these sampling sites may be assumed as negligible. In this way, the threshold was set at 0.1 for this group of compounds.

TU values for the mixture in sampling sites 1 to 7 were generally below 0.1. In other sampling sites (particularly 8, 9 10, 11 and 16), at all sampling times and for all organisms, metals represented a group of chemicals of high concern with high TU values for the mixture (>0.1). However, it should be noted that the concentration addition (CA) concept for metal mixtures is purely indicative, since different metals have different toxicological mode of action. Nevertheless, even considering metals individually, in site 16, TUs were found to be close to 1 (due to zinc toxicity to algae) and higher than 1 (due to copper toxicity for *Daphnia* sp.) during the autumn campaign, which indicates the possibility for acute toxic effects. Regarding the different taxonomic groups, toxicity to algae was clearly dominated by zinc. Toxicity to invertebrates was dominated by copper (and to a much lesser extent by the combination with iron and zinc); and toxicity to fish by a combination of copper and zinc (Figure 3). For all the other metals, acute TU values were below 0.1. However, for cadmium, mercury and lead, a risk for the aquatic community cannot be excluded since they are not essential micronutrients and have a high potential for bioaccumulation (Förstner and Wittmann, 2012). Only for manganese and iron, the concentrations measured and the TUs calculated (generally well below 0.1) can be assumed as below levels of concern for aquatic organisms in all sampling sites.

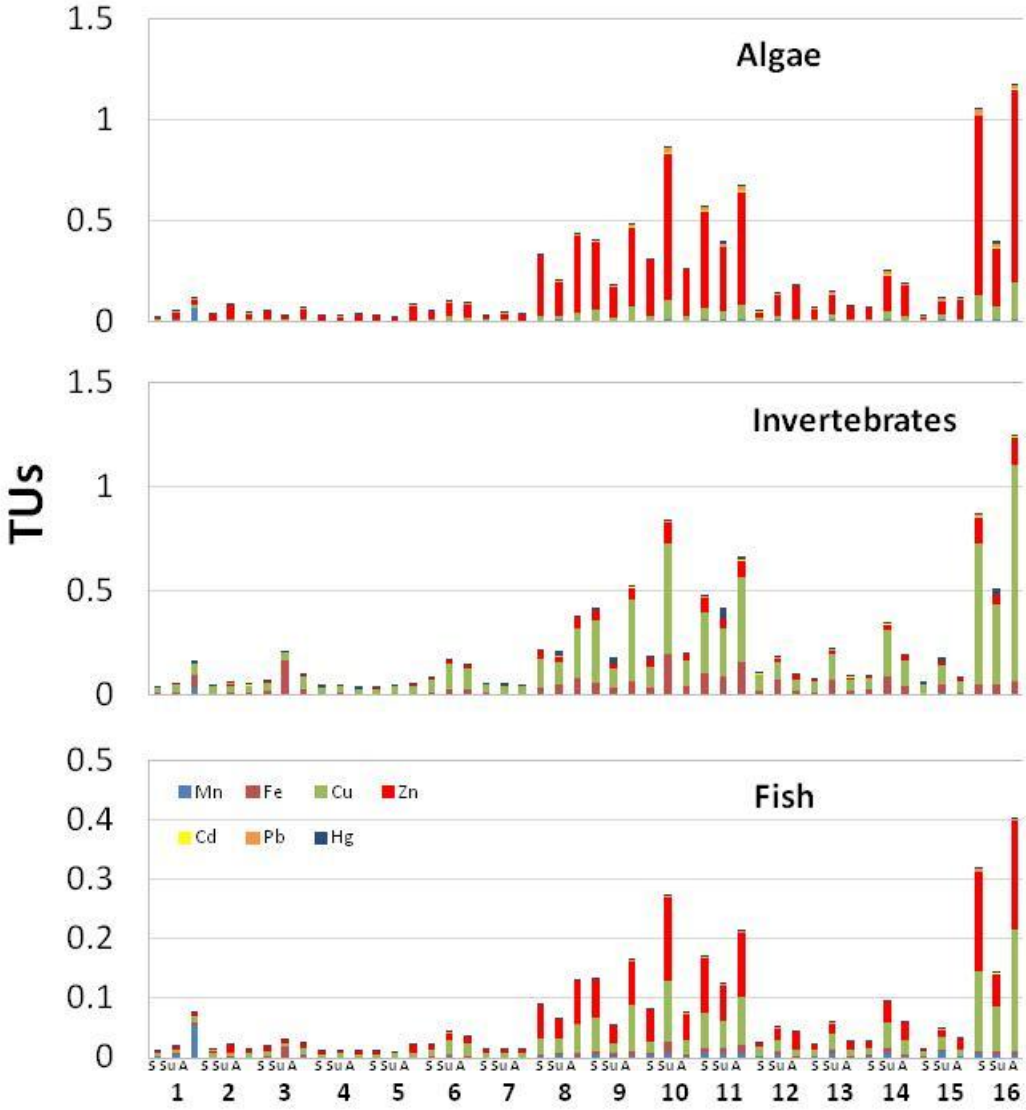

Figure 3. TU values for the mixture (as the sum of TUs for individual metals) and for individual metals calculated for the different taxonomic groups. For algae, iron was excluded due to a lack of toxicity data.

3.2.2. Organic contaminants

The TU values calculated for the total mixture and for the mixtures of the different groups of organic contaminants based on grab and POCIS samples are shown in Figure 4, while the TUs for individual chemicals are reported in Tables S4 and S5. The results indicate that in grab samples most TU values for individual chemicals, as well as for mixtures, are far below 0.01 indicating that acute toxicity is unlikely. Results obtained from the 14 d time weighted average (TWA) concentrations corresponding to POCIS samples (Figure 4) showed potential acute risks. In particular, a TU of 0.16 was calculated for algae in site 16 mainly due to high concentration of the herbicide diuron (0.1 µg/L). For invertebrates, high TUs (above 0.1) were calculated in sites 8, 13, 14 and 16 due to high concentrations of the insecticide chlorpyrifos (up to 0.4 µg/L), and for fish in sites 13 and 16 due to the presence of the same compound. Based on these results, it can be concluded that in some sites of the sampled watershed area, pesticides could be a reason for substantial concern regarding acute toxicity, which may be only identified through the use of POCIS sampling devices that capture

concentration peaks. On the other hand, the levels of individual point-source contaminants were far below a level of acute toxicity.

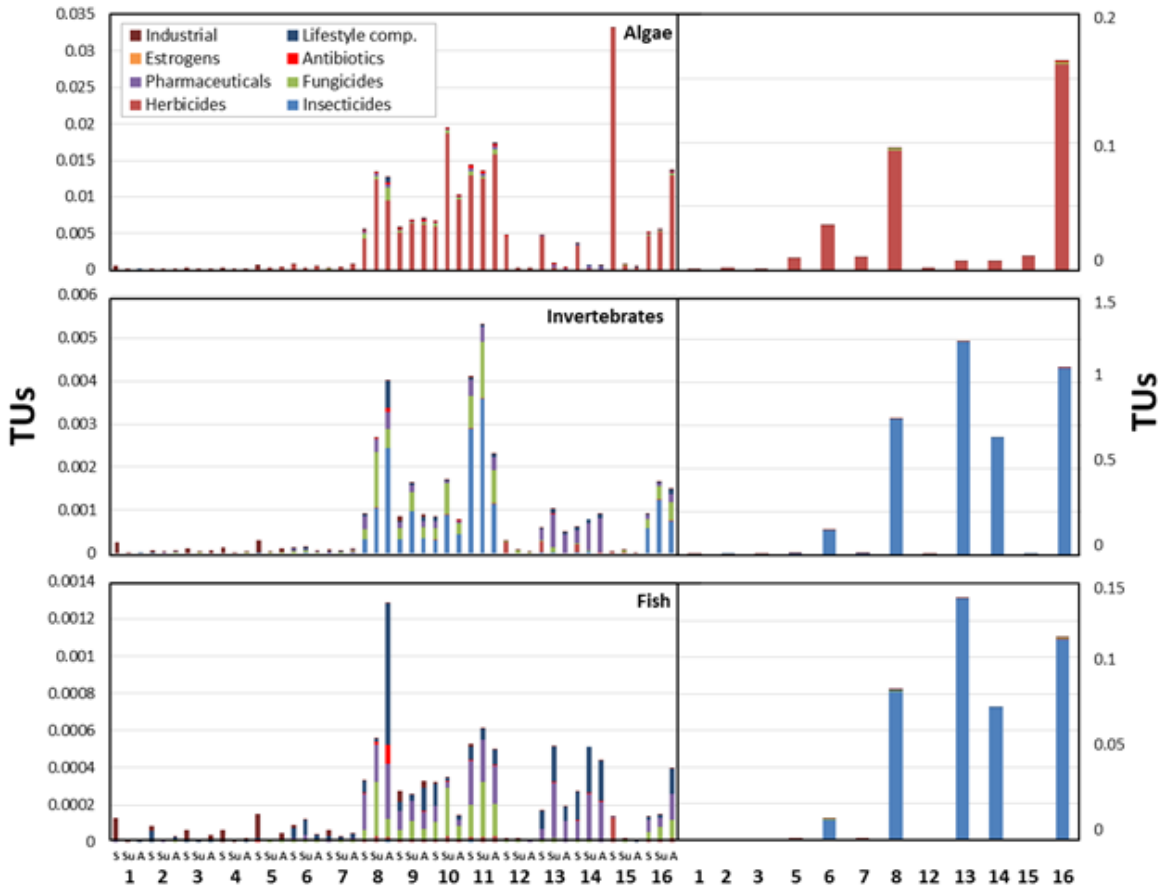

Figure 4. Values of TUs for the total mixture and the different groups of organic contaminants (as the sum of TUs for sub-groups of pesticides and point-source contaminants) calculated for the different taxonomic groups in grab samples (left side of the figure and left scale) and in POCIS samples (right side of the figure and right scale). Results from POCIS samples correspond to the summer campaign.

Despite the 0.01 threshold to identify potential acute risks, we used a threshold of 0.001 TUs to identify contaminants that may (jointly) result in chronic toxicity. Table 4 shows the relative contribution of the different compounds to the toxicity of these samples. Regarding algae, several grab samples exceeded the threshold, being the herbicide diuron the major contributor to the toxicity of the identified samples, followed by terbutryn, simazine and terbuthylazine (Table 4). As described above, toxicity to invertebrates on the basis of POCIS samples was dominated by chlorpyrifos. However, several grab samples, principally those in sites 8 and 11, exceeded the 0.001 threshold (Figure 4), mainly due to the presence of the insecticides pirimicarb and diazinon, the fungicide carbendazim, and to a lesser extent the analgesic acetaminophen, and the blood pressure regulator valsartan (Table 4). Similarly, toxicity to fish was dominated by chlorpyrifos in the POCIS samples, but a grab sample also exceeded the 0.001 threshold (Figure 4), due to the high paraxantine concentration (57.6 µg/L), in combination with other pharmaceuticals (Table 4).

It must be taken into account that the ecological risk assessment was performed on the basis of toxicity data for common standard test species, as surrogates of highly biodiverse taxonomic groups

(primary producers, invertebrates and fish). Generally, these species show a relatively high sensitivity to most organic and inorganic contaminants as compared to their counterparts, and an assessment factor of 10 is usually taken to consider possible interspecific sensitivity differences. However, interspecific sensitivity differences may exceed those factors for some compounds with a very specific mode of action. A clear example is the case of some insecticide groups, such as neonicotinoids, which are two-to-three orders of magnitude more toxic to some insect taxa than to *Daphnia magna* (Raby et al., 2018). Therefore, under such cases, the TU approach may underestimate ecological risks (Tsaboula et al., 2016). A recent study by our group indicates that Mediterranean freshwater ecosystems are sensitive to imidacloprid at concentrations below those that have been monitored in this study (0.3 µg/L; see Rico et al., 2019), with effects being mainly observed in mayfly nymphs and Diptera larvae (Rico et al., 2018a). Therefore, imidacloprid should also be considered as potential contaminant of concern in the Tagus river basin.

Table 4. Selected organic contaminants that explain >90% of the total potency of the mixture in the samples with TUs higher than 0.001. The percentages represent average values over the selected samples.

Algae		Invertebrates		Fish	
Chemicals	**% TUs**	**Chemicals**	**% TUs**	**Chemicals**	**% TUs**
Diuron	**55**	Chlorpyrifos	**31**	Chlorpyrifos	**82**
Terbutryn	**13**	Pirimicarb	**24**	Paraxantine	**8**
Simazine	**11**	Carbendazim	**15**		
Terbuthylazine	**11**	Acetaminophen	**10**		
		Diazinon	**7**		
		Valsartan	**6**		

The results of the chronic risk for pharmaceuticals estimated from acute toxicity data should be interpreted with caution. Pharmaceuticals are by definition biologically active compounds. Under long-term exposure conditions, effects such as reproductive, endocrine and developmental dysfunctions, that are not observed in acute tests, have been measured (Brooks, 2014; Crane et al., 2006). Therefore, further ecotoxicological characterizations taking into account their specific mode of action, their possible interactions, and sub-lethal effects derived from chronic toxicity studies are strongly recommended.

Maximum concentrations for antibiotics such as azithromycin, ciprofloxacin, sulfamethoxazole, and trimethoprim were found to range from about 1 µg/L to 73 µg/L (Rico et al., 2019). These concentrations are close to those that affect the growth of the cyanobacterium *Microcystis aeruginosa* and its interspecific competition with green algae (Guo et al., 2015, 2016; Rico et al., 2018b). It must also be taken into account that continuous exposure to antibiotics may contribute to the development of antibiotic resistance in environmental bacteria. Although this endpoint was not formally included in this study due to its yet unclear consequences for aquatic ecosystems, it has an important relevance for human health (Ashbolt et al., 2013). The maximum measured concentrations for the antibiotics azithromycin, ciprofloxacin, metronidazole and trimethoprim, which were generally found in the Henares River (downstream of Alcalá de Henares) were found to exceed the resistance thresholds proposed by Bengtsson-Palme and Larsson (2016) and Rico et al. (2017), and therefore, should be taken into account in further human health risk assessments.

Some of the priority organic chemicals identified in the current study have also been measured and listed as priority substances in other studies performed in the Iberian peninsula and other Mediterranean watersheds. For example, Kuzmanović et al. (2015) identified diuron as the primary

compound contributing to toxic effects to primary producers in the Llobregat, Ebro, Jucar and Guadalquivir basins. Chorpyrifos has been ranked as top priority compound in several monitoring studies performed in the Jucar river basin (Ccanccapa et al., 2016) and in other Spanish watersheds (López-Doval et al., 2012), and diazinon has also been considered as a relevant compound for freshwater ecosystems by Kuzmanović et al. (2015). Tsaboula et al. (2016) identified imidacloprid as one of the priority substances in the Pinios watershed (Greece) together with a long list of other pesticides. Valcárcel et al. (2011) identified caffeine as one of the most hazardous compounds for freshwater ecosystems of the Tagus basin due to its behavioral effects on fish. In our study, its major metabolite (paraxanthine), which has similar properties, was identified as priority substance.

3.2.3. Mixture composition

There is strong evidence in the literature showing that in realistically occurring mixtures the number of chemicals explaining a high percentage (80-90% or more) of the total mixture potency is low, even in mixtures composed by a very high number of chemicals (Boedeker et al., 1993; Henning-De Jong et al., 2008; Verro et al., 2009). Price and Han (2011) introduced the concept of Maximum Cumulative Ratio (MCR) as the ratio between the cumulative toxicity of the mixture and the maximum toxicity from one component. They demonstrated that MCR tend to decrease if the potency of the mixture increases, so in highly toxic mixtures, just one (or few) chemicals dominate.

The MCR values calculated with the results of this study are shown in Figure 5. If only organic contaminants were considered, in all grab samples with TU values higher than 0.001, the MCR value was lower than 3. This means that the most toxic chemical explained at least 33% of the total mixture potency. If POCIS samples were considered, with TU values higher than 0.1, the highest MCR value was 1.35 (the most toxic chemical explains 74% of the total mixture potency; Figure 5, left). Including metals in the TU calculations of grab samples, the toxic potency of the mixture strongly increased and the MCR decreased, being one or two metals the major responsible for the toxicity of the mixture (Figure 5, right).

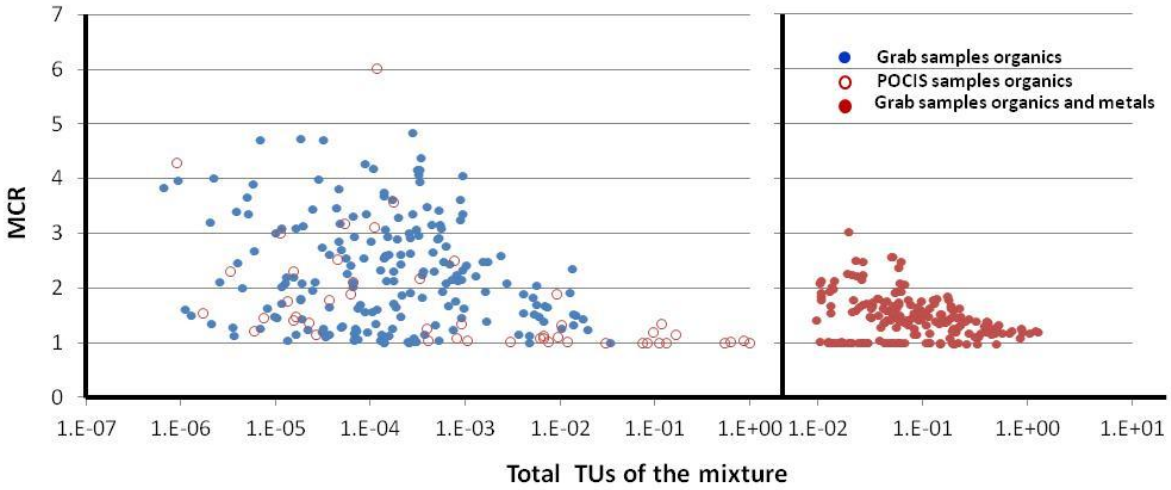

Figure 5. Values of the Maximum Cumulative Ratio (MCR) as a function of the total potency of the mixture expressed as toxic units (TUs). Three types of mixtures are considered: mixture of organic contaminants in grab samples; mixture of organic contaminants in POCIS samples; mixture of organic contaminants and metals in grab samples.

These results confirm the hypothesis that in most toxic mixtures a limited number of chemicals are the main contributors to the total toxicity value, supporting our results in section 3.2.2, in which no

more than six organic contaminants were identified as responsible for >90% of the total toxicity of the mixture to the different taxonomic groups (Table 4). When the toxic contribution of these compounds was assessed per taxonomic group and season, the number of representative compounds to be considered in the toxic mixtures was generally 3 or less, with a maximum of 5 (i.e., for fish in autumn: paraxantine, nicotine, valsartan, carbendazim, naproxen; Table 5). Overall we did not observe large seasonal changes in the (mixtures of) compounds that may affect the different taxonomic groups, except when toxicity is dominated by one single compound due to a peaked exposure pattern (i.e., chlorpyrifos in invertebrates and fish) or when one compound clearly dominates the toxicity of the sample (i.e., paraxantine for fish; Table 5).

Table 5. Main organic contaminants and contaminant mixtures contributing to the toxicity of the samples with TU > 0.001 per season and taxonomic group. n= the number of sites in which the total TU was >0.001 based on all identified key compounds (compounds contributing to 90% of the toxicity for at least one taxonomic group).

	Primary producers	Invertebrates	Fish
Spring	1. Diuron+Terbutryn 2. Diuron+Terbutryn+Terbuthylazine 3. Simazine 4. Terbuthylazine (n=9)	1. Pirimicarb+Carbendazim (n=1)	
Summer	1. Diuron+Terbutryn 2. Diuron+Terbutryn+Terbuthylazine 3. Diuron+Terbuthylazine (n=11)	1. Chlorpyrifos 2. Chlorpyrifos+Pirimicarb 3. Pirimicarb+Carbendazim 4. Valsartan+Acetaminophen (n=11)	1. Chlorpyrifos (n=6)
Autumn	1. Diuron+Terbutryn 2. Diuron+Terbutryn+Terbuthylazine (n=5)	1. Pirimicarb+Carbendazim+Valsartan 2. Diazinon+Pirimicarb+Carbendazim (n=3)	1. Paraxantine+Nicotine+Valsartan+ Carbendazim+Naproxen (n=1)

3.3. Relationship between land use, contaminants and other physico-chemical parameters

The results of the Redundancy Analysis (RDA) including the selected physico-chemical parameters, metals and organic contaminants are shown in Figure 6. In this case it is also shown that land use explained a relevant part of the variance. In particular, sites with low anthropogenic impact (i.e., natural) were negatively correlated with nutrients, metals, organic contaminants and some parameters (TSS, DOC, pH) that may alter habitat conditions; while there was no clear separation between indicators of urban pollution (e.g. point-source contaminants) and those deriving from agricultural pollution (e.g. pesticides). The fact that some point-source chemicals showed a strong correlation with agricultural land use (i.e. acetaminophen), or that were equally present in sites with agricultural and urban impact (i.e., paraxantine, valsartan), can be explained by the presence of small urban areas within agriculture-dominating landscapes. In many cases, wastewaters from those small urban areas are discharged to nearby streams with very low dilution potential (as it is the case of the Melgar Stream in Villasequilla, Toledo). On the other hand, it may be taken into account that pesticide emissions may come from intensive agricultural emplacements next to large urban areas (Madrid, Alcalá de Henares) or trough wastewater treatment plant effluents.

3.4. Chemical status and compliance with the Water Framework Directive

The assessment of the chemical status of water bodies according to the WFD evaluates established thresholds for several physico-chemical parameters, as well as for the list of 45 defined priority compounds (EC, 2003). While EQS for priority substances are applicable to surface waters throughout Europe (EC, 2011), the physico-chemical status assessment is performed in relation to reference conditions characteristic of specific water body types. In Spain, reference conditions for pH, dissolved oxygen, ammonium, nitrate and phosphate have been set by the Spanish

Ministry of Agriculture, Food and Environment (RD 817/2015) for different river types. The rivers sampled within this study belong to the following river types: R-T05, R-T11, R-T12, R-13, R-T15 and R-T16. The comparison between measured data and the criteria proposed by the Spanish regulation is shown in Table S6 of the Supporting Information. Conditions corresponding to a "Good" chemical status for all basic parameters, with few marginal exceptions, were determined in sampling sites 1 to 6, 12 and 15. In all other sites parameters such as pH, dissolved oxygen and nutrients, showed deviations from reference conditions. In particular, a parameter of high concern is ammonia, which under high pH and temperature conditions (typical of summer), can be present in its toxic un-dissociated form (NH_3). Water quality criteria for un-dissociated ammonia have been set by various international agencies at 20 μg N-NH_3/L (e.g. US EPA, 2013). In some of our sampling sites, levels were one order of magnitude higher than the proposed criteria (>600 μg N- NH_3/L in sites 9, 14 and 16 in summer). This indicates that unionised ammonia may represent a toxicological threat in our study area.

Some of the metals (Cd, Hg and Pb) and pesticides (chlorpyriphos, diuron, simazine, terbutryn) monitored in this study are included in the list of specific and priority compounds regulated under the WFD. EQS was exceeded by cadmium in site 12 in spring and by mercury in all samples where it was detected above the limit of detection (sites 8, 9, 11, 15, 16 in summer). For this metal, the measured values were not only above the AA-QS (annual average quality standard) but also above the MAC-QS (maximum acceptable concentration quality standard), except for site 15. However, it must be noted that the LOD for mercury in our study (0.058 μg/L) was slightly above the AA-QS (0.05 μg/L). The herbicide simazine never exceeded the WFD EQS, while in the POCIS samples the herbicide diuron exceeded the AA-QS in two sites (8 and 16). The insecticide chlorpyrifos exceeded the AA-QS in five sites (6, 8, 13, 14, and 16) and the MAC-QS in four sites (8, 13, 14, and 16); and terbutryn the AA-QS in 2 sites (8 and 16) (Figure S4 of the Supporting Information). Since these compounds are expected to have discontinuous exposure patterns (spray drift after application or runoff events), the measured values should rather be compared with the MAQ-QS. In a similar monitoring study performed in the lower Tagus river basin, chlorpyrifos was also found to exceed the MAQ-QS in 12 out of the 122 samples that were evaluated (Silva et al. 2015). In conclusion, only sites 1 to 5, which are characterized mainly by a natural land use in the watershed, showed conditions that allow them to be classified as "good" chemical status regarding all parameters measured in this study.

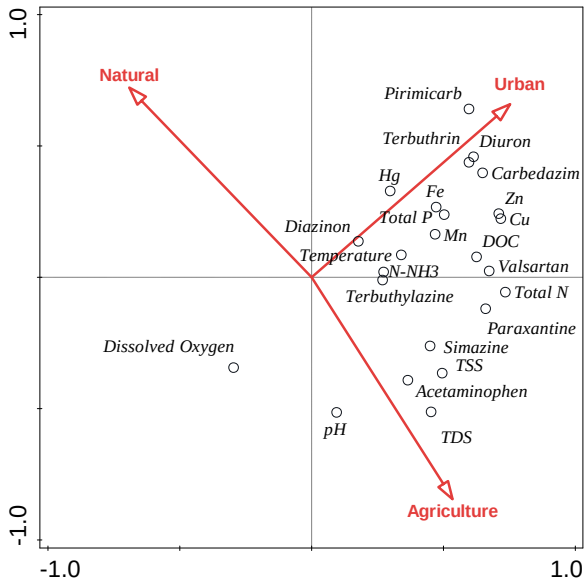

Figure 6. RDA showing the relationship between land use, physico-chemical variables and the selected inorganic and organic contaminants. Land use explains 41% of the variance, of which 79% is represented in the x-axis and 21% in the y-axis (Monte Carlo p-value: 0.002).

4. Conclusions

This study provides the most extensive monitoring of water quality parameters performed so far in the upper Tagus river basin. A complete evaluation of physico-chemical parameters, nutrients and metals was performed, accompanied by a prioritization approach to select pesticides and point-source chemicals that may have a potential ecotoxicological hazard. The results of this study show that the chemical status of the Tagus river tributaries is highly variable and mainly depends on the land use of the different sub-basins. In the largest Tagus tributaries considered in this study (Jarama, Manzanares and Henares) a poor water quality status was identified, with high concentrations of some metals and organic contaminants. Furthermore, we identified alterations of some physico-chemical parameters, such as dissolved oxygen and un-dissociated ammonia, which are indicators of insufficiently treated urban sewage discharges. Clear seasonal variations in water quality parameters were only identified for those parameters less related with human activity and more dependable on hydrological, ecological and climatologic conditions (i.e. physico-chemical parameters). However, a slight seasonal trend was observed for insecticides, with higher concentrations in summer as compared to spring and autumn.

The ecological risk assessment performed in this study indicated that some metals (copper and zinc) may exert acute toxicity to primary producers and invertebrates, primarily in sites influenced by urban activities. The ecological risk assessment preformed on the basis of grab water samples for organic compounds showed limited acute risks; while the assessment performed with the POCIS samples resulted in potential acute risks for primary producers due to diuron exposure, and to invertebrates and fish due to chlorpyrifos contamination. Moreover, we identified imidacloprid as a potential hazardous compound due to its high toxicity to non-standard invertebrate species. This study also identified several chemical mixtures that may result in chronic toxicity for freshwater organisms, which include some additional herbicides (for primary producers), and pesticides and point-source chemicals (for invertebrates and fish). Finally, this study also confirms that contaminant mixtures of concern in the upper Tagus river basin are usually formed by a limited number of

compounds (i.e, 5 or less), and that the composition of such mixtures does not show a marked seasonal variation.

Regarding the regulatory assessment performed as part of the WFD, we conclude that only 5 out of the 16 sites evaluated can be classified as having 'good' chemical status, and identified several EQS exceedances for metals and priority pesticides. This study also demonstrates that the assessment of the ecotoxicological risks for chemicals with discontinuous emission patterns, such as pesticides, may be underestimated by current monitoring programs, which are primarily based on grab samples taken during the spring season. Research is urgently needed to investigate chemical emission hot-spots and to reduce chemical contamination in the Tagus river basin. Particularly, follow-up studies should be dedicated to identify sources of metal contamination and to perform continuous monitoring of pesticides in particular sites of the basin in order to capture worst-case exposure peaks.

Acknowledgments

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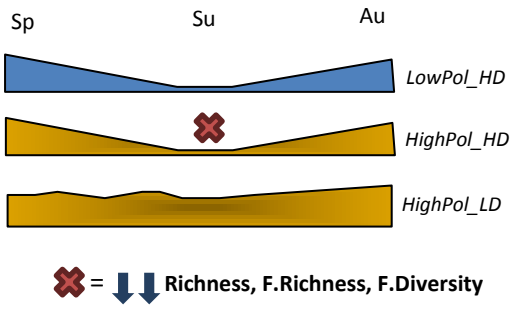
Chapter 5

Drought intensifies the effects of anthropogenic pollution on aquatic invertebrate communities

Alba Arenas-Sánchez, Sylvain Dolédec, Marco Vighi, Andreu Rico



Abstract

This study aims at assessing the combined effects of pollution and drought on macroinvertebrate communities, in terms of taxonomic and functional responses. Twelve sampling sites with different levels of anthropogenic pollution and intrannual hydrological variation related to drought were selected in the upper Tagus river basin (central Spain). Samples were taken in spring, summer and autumn. The sites were classified into three groups: low pollution and high drought, high pollution and high drought, and high pollution and low drought. The daily discharge and the water physico-chemical characteristics were measured at each sampling site, together with concentrations of metals and organic microcontaminants. Significant differences related to toxic pressure and nutrient concentrations were observed between the three groups of sites, whereas seasonal patterns were not that evident. Taxonomic and functional richness were lower in the polluted sites, particularly in summer (i.e. maximum drought period) and autumn (i.e. early ecosystem expansion period). Moreover, richness, functional richness and functional diversity were more severely affected in sites impaired by both pollution and drought stress, leading to simplified communities dominated by generalist taxa. Trait categories such as asexual reproduction, reproduction by clutches, cocoons and plurivoltinism were prevalent in highly polluted sites, whereas reproduction by isolated eggs, semivoltinism or respiration by gills dominated in less polluted sites. Other trait categories showed clearer responses in summer and autumn, and responded to pollution (e.g. interstitial organisms, burrowers, deposit feeders), but also to drought (e.g. aerial dispersal) and to the combined effects of drought and pollution (e.g. diapause). This study shows that drought may exacerbate the impacts of anthropogenic chemical pollution in aquatic ecosystems. Moreover, it highlights macroinvertebrate trait categories that can be used as indicators of these stressors, and that can be used to improve monitoring and risk assessment procedures for aquatic ecosystems in Mediterranean (semi-)arid regions.

Article *in preparation*

1. Introduction

Freshwater ecosystems are affected by a complex mixture of chemical and physical stressors as a result of expanding urban and agricultural pressure and increasing water demands related to human use and consumption, which add up to the impacts of climate change (EEA, 2012). Mediterranean rivers are characterized by marked seasonal hydrological variations, undergoing very low flows or even drying completely during the summer period. In a context of global and climate change, these patterns are currently being altered and are expected to be exacerbated in the future (Gashit and Resh, 1999; IPPC, 2014). Thus, in these regions, diffuse as well as point source chemical pollution, together with increasing water scarcity, have been identified as major sources of impairment for aquatic ecosystems (Ludwig et al., 2011; Manfrin et al., 2013; Perujo et al., 2016, Kuzmanović et al., 2017).

Benthic macroinvertebrates are key components of lotic systems, widely used to evaluate river ecological status and to detect disturbances (Resh and Rosenberg, 1993; Boix et al., 2010; Feio et al., 2015). This group comprises species with different environmental tolerances and preferences, and they are considered good integrative indicators of chemical and physical alterations over mid- to long-term periods (Bonada et al., 2006; Boix et al., 2010). Hydrological variability is known to be an important factor driving the composition of aquatic invertebrate communities in Mediterranean rivers (Bonada et al., 2007a; Bonada and Resh, 2013; Prat et al., 2014). Periods of low flows are associated to reduced habitat availability (Lahr, 1997; Acuña et al., 2005; Verdonschot et al., 2015) and physico-chemical alterations such as increased temperatures, oxygen depletion or high nutrient and suspended solid concentrations, which may act as environmental filters for less tolerant taxa (Stanley et al., 1997; Acuña et al., 2005).

Additionally, pollution coming from different anthropogenic activities can lead to lethal and sub-lethal effects, constituting an additional filter to macroinvertebrate biodiversity (Manfrin et al., 2013; Ortiz et al., 2005; Sabater et al., 2016; Parreira-de Castro et al., 2018). Organic micropollutants exhibit seasonal patterns related with crop-production practices or pest dynamics (i.e. pesticides), and demographic pressure and epidemics (e.g. pharmaceuticals) (López-Doval et al., 2013; Rico et al., 2019). Moreover, the fate and the exposure levels of these contaminants in Mediterranean rivers can also be severely affected by the flow seasonality. In particular, low flow conditions could lessen the dilution capacity of chemical discharges but also alter degradation patterns due to water temperature fluctuations and changes in related physico-chemical parameters (Arenas-Sánchez el al., 2016; López-Doval et al., 2013; Rice and Westerhoff, 2017; Arenas-Sánchez et al., 2019b).

Some studies have found enhanced effects of pollution on community structure, with reduced macroinvertebrate species richness and abundance of sensitive taxa when pollution co-occur with drought conditions (Bollmohr and Schulz, 2009; Kalogianni et al., 2017; Karouzas et al., 2018). However, despite biological responses to these stressors have been investigated separately in ecological and ecotoxicological studies, the number of studies and current knowledge on the combined impact of both groups of stressors on aquatic communities is still limited.

Trait-based approaches have been successfully used to provide information on the mechanistic response of aquatic invertebrate assemblages to environmental constraints (Statzner and Bêche,

2010; Piló et al., 2016, Kuzmanović et al., 2017). Assessing changes in functional structure may contribute to disentangle the impacts of different disturbances on species assemblages (Parreira-de Castro et al., 2018). Moreover, the use of trait data in combination with taxonomic data, has been recommended for future updates in monitoring of the ecological status of surface waters (Baattrup-Pedersen et al., 2017, Berger et al. 2018) and prospective risk assessment procedures (Rubach et al., 2010; Arenas-Sánchez et al., 2016). Nevertheless, the complexity of this type of data relies on the fact that trait categories are known to correlate among each other, forming trait syndromes, which make difficult to separate the effects of multiple stressors on single species attributes (Verberk et al., 2013; Mondy et al., 2016). Statzner and Bêche (2010) suggested that the best way to link trait responses to multiple stressors is to define *a priory* predictions based on the mechanistic understanding on the plausible effects of each stressor. The development of approaches to disentangle trait responses to multiple stressors is still ongoing, with some recent studies providing information on the responses of trait groups to environmental gradients of selected stressors (Mondy et al., 2016) or identifying traits responding to the main stressors in a multiple stressed environmental gradient (Berger et al., 2018). However, the number of studies addressing single trait and trait syndrome responses to the combined effects of drought and pollution is very limited.

In this study, we evaluate the influence of anthropogenic pollution on the taxonomic and functional composition of aquatic invertebrate communities under different hydrological conditions in a Mediterranean basin. Our hypotheses were: 1) both taxonomic and functional diversity should decrease in polluted sites, being the community dominated by tolerant taxa and traits that confer higher resistance and resilience to pollution, and 2) pronounced drought conditions should enhance differences in taxonomic and trait composition between impacted sites and have a detrimental effect on taxonomic and functional diversity. By testing these two hypotheses we also aimed at identifying traits or trait syndromes that are specific of the assessed groups of stressors (i.e. pollution and drought) and their combination. *A priory* predictions for the main functional strategies showing clear mechanistic responses to these groups of stressors were made based on existing literature (see Table 1). For example, small sizes and correlated short life cycles are expected to provide high resilience after pollution and drought stress to organisms (Townsend and Hildrew, 1994; Bonada et al., 2007b), despite small sizes also result in larger surface/volume ratios and a higher exposure of organisms to toxic compounds (Paul and Meyer, 2001). Reproduction by eggs forming clutches increase the surface/volume ratio and increase the resistance of these structures over isolated eggs by reducing the level of external exposure to some chemicals (Díaz et al., 2008). On the other hand, terrestrial reproduction or clutches deposition on vegetation confer organisms high resistance to drought periods with reduced habitat availability and harshened water quality conditions (Bonada et al., 2007b). Aerial active dispersal strategies allow organisms to recolonize less dry sections of the river bed (Bonada et al., 2007b), while aquatic passive dispersion is more related to recolonization of polluted environments in flowing waters (Rico et al., 2015).

Table 1. *A priory* predictions of trait categories influencing the tolerance of organisms to drought and pollution by means of resistance or resilience strategies (+: high tolerance; -: low tolerance; +/-: high or low tolerance could be observed depending on the dominant mechanism of response).

Trait	Category	Pollution		Drought conditions	
Size	Small size <1cm	+/-	Critical large surface/volume ratio, high exposure to some toxicants[1], but better resilience of small sizes after disturbance by organic contamination[2]	+	High resilience capacity to drought cond.[3]
	Large size >2cm	+/-		+/-	Release from action of flow in stagnant pools permits large sizes[3]
Life cycle duration	Short<1year	+	High resilience capacity after global human disturbance[2]	+	High resilience capacity to drought cond.[3]
Reproduction type	Asexual	+	High resilience after pollution disturbance[4,5]	+	High resilience after drought [3,5]
	Ovoviviparity			+	Additional protection to fertilized eggs [6,7]
	Eggs	-		+	Fixed eggs confer additional protection[8]
	Clutches	+	Eggs in clutches are less exposed to pollution by reducing the surface/volume ratio[6]		
	Terrestrial/ Vegetation clutches			+	Resistance against drought[3]
Dispersal	Aerial active			+	Recolonization of less dry sites from dried river beds or other sites[3]
	Aquatic passive	+	Passive recolonization of polluted waters by drift[9]		
Substrate relation	Surface swimmer			+	Flow cessation or stagnant pools permits swimming[3]
	Swimmer			+	
	Crawler			-	Response to fast flows[3]
	Burrowers	+	Benefit from the deposition of om[8]		
	Interstitial	-	Clogging of interstitial mud(om)[8]	+	Resistance in wet spaces [3]
Resistance forms	Diapause			+	Confer resistance to unfavorable drought conditions[3,10]
	Resistant eggs	+	May confer additional resistance under high pollution pressure[11,12]	+	
	Cocoons	+		+	
Respiration	Aerial	+	Oxygen depletion due to high om content favors aerial resp.over gills/tegument[11]	+	Oxygen depletion due to more stagnant flows, high om concentration and high temperatures, requires more specialized strategies (aerial or gills)[3]
	Gills	-		+	
	Tegument	-	Higher exposure to toxicants in gill-bearing or tegument organisms due to higher surface/volume ratios[1,9]	-	
Food/Feeding type	Predators	+/-	Large predators are exposed to toxicants by food ingestion[1], but their size reduce the surface/volume ratio (less external exposure) [9]	+/-	Higher intra-specific competition can lead to dominance of predators, but large sizes have less resilience capacity[3]
	Detritus<1mm	+/-	Benefit from deposition of detritus [13,14], but polluted sediment can have negative effect[13]	+	Deposit of fine detritus under slow flows[15]
	Detritus>1mm	+/-		-	Reduced large detritus input in dry water bodies[3]
	Macrophytes			+	
	Microphytes			+	More abundant macrophyte (shredders) and periphyton biomass (scrappers)[3,16]

1: Paul and Meyer (2001); 2: Townsend and Hildrew (1994); 3: Bonada et al. (2007b); 4: Doledec et al. (2006); 5: Lange et al. (2014); 6: Díaz et al. (2008); 7: Bêche et al. (2006); 8: Usseglio-Polatera et al. (2000); 9: Rico et al. (2015); 10: Williams (2005); 11 : Statzner and Bêche (2010); 12: Mondy et al. (2016); 13: Piló et al. (2016) ; 14: Dolédec and Statzner (2008); 15: Feio and Dolédec (2012); 16: Gashit and Resh (1999).

2. Material and methods

2.1. Study area and site classification

The upper reach of the Tagus river basin (Central Spain) is representative of marked Mediterranean seasonal patterns, with increasing temperatures and pronounced droughts in summer, which affect the majority of surface waters. Twelve sites were selected based on different levels of drought and anthropogenic pollution (Figure 1). Drought levels were established on the basis of daily flow data (Figure S1) measured in flow gauges placed at each sampling site or slightly upstream, which were provided by the Tagus River Basin Authority. Sites

were defined as affected by high drought conditions when presenting more than 15% of the total number of days in the year with flow values below the 20% quantile of the mean annual flow (Figure S1). Pollution levels were established on the basis of anthropogenic land use intensity, determined from a geospatial analysis performed with GIS software (ArcGIS) using land use data from Corine land cover layer (2006). Land uses in the study area were classified as natural (forests, grasslands without human alteration), agricultural and urban (the latter including industrial activities). Sites were defined as having a high anthropogenic impact when their sub-basins presented >75% agricultural land use and/or >1% urban; and vice-versa for low anthropogenic impact sites (Table 2). Thresholds were established from maximum values observed for the range of sites. Based on these classifications, three groups of sites were defined: (1) low pollution and high drought conditions (noted hereafter LowPol_HD); (2) high pollution and low drought conditions (HighPol_LD), in most cases due to influx of tributary waters and the continuous artificial discharge of urban wastewaters; and (3) high pollution and high drought conditions (HighPol_HD) (Table 1). Three sampling campaigns were carried out at each sampling site, in spring (April 11-14), summer (July 11-14) and autumn (November 21-24) of 2016, aiming to cover three representative stages of the hydrological cycle of Mediterranean rivers namely base flow, contraction phase and expansion phase, respectively.

Table 2. Summary data for the classification of the sampling sites regarding their drought level (i.e., total dry days) and pollution level (i.e., land use characteristics). LowPol_HD: low pollution and high drought conditions; HighPol_LD high pollution and low drought conditions; HighPol_HD with high pollution and high drought conditions.

Sampling site [1]	Site class	Tot.dry days	Land use %		
			Urban	Agricultural	Natural
1	LowPol_HD	65	0.03	22.1	77.9
2	LowPol_HD	65	0.11	40.5	59.4
3	LowPol_HD	67	0.02	2.03	97.9
7	LowPol_HD	63	0.14	70.9	28.9
12	HighPol_HD	70	0.73	94.7	4.5
13	HighPol_HD	70	0.73	94.7	4.5
14	HighPol_HD	70	0.73	94.7	4.5
15	HighPol_HD	72	0.4	75.3	24.3
8	HighPol_LD	0	2.05	51.3	46.6
9	HighPol_LD	1	5.2	37.1	57.7
11	HighPol_LD	0	21.99	24.3	53.7
16	HighPol_LD	50	10.06	89	1

[1]Site numbers refer to previous studies in which the physico-chemical characteristics and pollution status have been described (Rico et al., 2019; Arenas-Sanchez et al., 2019a).

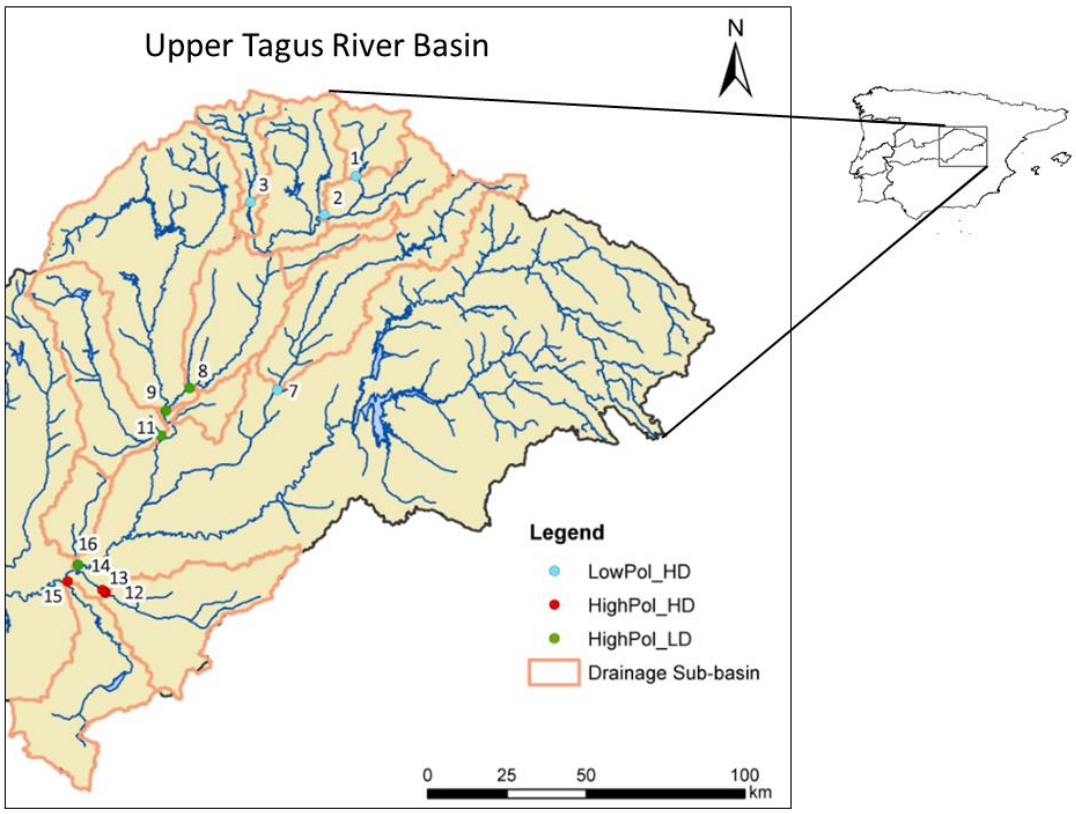


Figure 1. Map of the study area and sampling sites in the upper Tagus river basin selected for the analysis. Sites are marked with different colors regarding their drought and pollution level.

2.2. Sampling and analysis of abiotic parameters

Physico-chemical parameters such as water temperature, dissolved oxygen (DO), conductivity (EC) and total suspended solids (TSS) were measured in the middle section of the river transect with a portable multiparameter probe (HANNA Instruments, USA, model HI98194). At each site and sampling date, flow (m^3/s) values were obtained from the daily series monitored by the Tagus River Basin Authority in the corresponding flow gauges. Substrate composition was recorded as percentage of stones and blocks, gravel and pebbles, sand, clay and fine inorganic material, macrophytes, algae, plant debris and mud (Table S1). PCA performed on substrate proportions indicated a minor influence of substrate among the groups of sites (see Supporting Information Figure S2). As a result, this parameter was not further considered. Water samples were taken for the analysis of nutrients (NO_2^-, NO_3^-, NH_4^+, PO_4^{-3} and total P), dissolved organic carbon (DOC), metals (Mn, Fe, Cu, Zn, Cd, Pb, Hg) and 52 organic contaminants: 20 pesticides and 32 point source contaminants (PSC) including 24 pharmaceuticals (9 of them antibiotics), 4 estrogens and steroids, and 3 alkaloids and other stimulants. The methods used for the sampling and analysis of nutrients and contaminants are described in Rico et al. (2019) and Arenas-Sanchez et al. (2019a).

Sampled metals, pesticides and PSC were assessed in terms of toxicity, by applying the toxic units (TU) approach for invertebrates. These values were calculated as the ratio between the measured environmental concentration and the EC50 for *Daphnia magna* derived from laboratory experiments or from QSARs (Quantitative Structure-Activity Relationships) when

experimental data was not available. Details on the selection of toxicity values and the results of the TU calculations in each sampling site are provided in Arenas-Sanchez et al. (2019a).

2.3. Macroinvertebrate sampling and identification

Three macroinvertebrate samples were collected at each sampling site per sampling campaign. Samples were distributed along the river transect (i.e., trying to cover all available habitats) using a Surber sampler. This sampler consists of two interlocking frames (area=0.1m^2) that support a capture net (mesh size=250µm), with one of them outlining the area of streambed to be sampled. The outlining frame was placed on the river bottom with the net pointing downstream and all substrate within the frame was rubbed or stirred at a depth of 5 to 10 cm for 2 min. Sampled organisms were transferred into a plastic container and preserved with 70% ethanol until further identification in the laboratory.

Identification was performed based on Tachet et al. (2010). Identification at genus level was not possible for some taxa, due to damaged or lost features of preserved organisms. Consequently, taxonomic identification was performed at family level to keep consistency. Chironomidae was one of the most abundant families. To reduce the weight of this family in the analysis, the taxonomic identification was done considering five subfamilies or tribes (Orthocladiinae, Tanypodinae, Diamesinae, Tanitarsini, Chironomini). Since identification for this group was not always possible at the same taxonomic level, a compensative adjustment for the coarser taxonomic resolution was performed according to the method described in the Supporting Information. Macroinvertebrate samples collected at each site were pooled together and abundances were ln(x+1) transformed to reduce the impact of dominant taxa and to contribute to the normality of the data.

2.4. Macroinvertebrate traits

Information on ten biological traits (see Table 6, Table S6 for full set of categories analyzed) for the invertebrate taxa identified in this study were extracted from Tachet et al. (2010), which contains trait information for macroinvertebrates monitored in European surface waters. In this database, the affinity of each taxon to the different trait categories is quantified using a "fuzzy" coding approach (Chevenet et al., 1994). This method gives an affinity score per taxon and trait category ranging from "0" (no affinity) to X (X, the strongest affinity; with X varying from 3 to 5 depending on the trait). The use of fuzzy coding based on the affinity of each taxon for different conditions or habitats or to a given trait has been described elsewhere (e.g. Bournaud et al., 1992; Chevenet et al., 1994). Affinity scores were standardized so that their sum for a given taxon and a given trait equaled one yielding trait category profiles for each taxon (see e.g. Gayraud et al. 2003).

Matching between our invertebrate monitoring dataset and the Tachet et al. (2010) database was done at the family level and trait category profiles were averaged across genera. Some authors have argued that identification at a family level could be sufficient when assessing the responses on functional descriptors along an impact gradient (Gayraud et al., 2003; Sajan et al., 2010). However, since some trait differences are expected between Mediterranean and non-Mediterranean taxa (Bonada et al., 2011), family trait averages were calculated only considering Mediterranean genera identified by Bonada et al. (2011). Each genus was given a weight

proportional to the number of Iberian species recorded in the Freshwater Ecology (https://www.freshwaterecology.info) and the Global Biodiversity Information Facility (GBIF; https://www.gbif.org) databases. When a genus had no identified or recorded species, a minimum value of one was given. For generalist taxa such as Diptera and Oligochaeta, no Mediterranean genera could be identified in most cases, so average values for all the genera included in Tachet et al. (2010) were used.

2.5. Data analyses

2.5.1. *Abiotic variables*

Abiotic variables were individually assessed for normality using the Shapiro-Wilk test. The type of transformation giving the best fit (S-W statistic close to 1, p-value>0.05) for each variable was selected for further analyses. Principal Components Analyses (PCA) were performed on hydrological, physico-chemical and contaminant (i.e., TUs) variables for each season and for all seasons together. Differences between our predefined groups of sites (LowPol_HD, HighPol_HD, HighPol_LD) were tested by means of a between-class PCA using 999 Monte Carlo permutations. Finally, an ANOVA was applied to each variable to account for significant differences between groups of sites, and to test seasonal differences of each variable within each group of sites. Due to the large number of tests performed, a correction for multiple testing was applied in each case (i.e., false discovery rate).

2.5.2. *Structural and functional indexes*

The impacts of pollution and drought conditions on the structural and functional characteristics of the macroinvertebrate community were evaluated in terms of the total number of individuals per sample (abundance) and taxonomic and trait-based richness and diversity indexes. Taxonomic richness was evaluated on the basis of the total number of taxa per sample. Functional richness was calculated as the overall number of trait categories of all taxa in the community (FRic, Villéger et al., 2008). Taxonomic diversity was evaluated using the Simpson index. Functional diversity was assessed using the Rao quadratic entropy (RaoQ, Champely and Chessel, 2002), which sums the trait distances of any pair of taxa weighted by their relative abundance. Finally, the Iberian Biological Monitoring Working Party (IBMWP) index (Alba-Tercedor et al., 2004), which is commonly used for assessing the biological status of surface waters in Spain, was calculated. Significant differences between the three groups of sites for these indexes were tested by ANOVAs followed by a pair-wise t-test. All statistical analyses were performed in the R environment (R Development Core Team, 2016), using the ade4 (Dray and Dufour, 2007), vegan (Oksanen et al., 2016) and FD (Laliberté et al., 2014) packages.

2.5.3. *Taxonomic and trait composition*

A co-inertia analysis was performed on the faunistic dataset for each season to assess the correlation between taxonomic and trait data, and their contribution to the differences between sites. Co-inertia analysis performs the simultaneous ordination of two tables and allows interpreting and testing the relationships between them. The optimizing criterion in co-inertia analysis is that the scores at the row margin of each dataset (i.e. taxa scores in both taxonomic and trait datasets in our case) are the most covariant (Dolédec and Chessel, 1994; Dray et al.,

2003). Before co-inertia analysis, trait profiles of taxa were analyzed using Fuzzy Correspondence Analysis (FCA; Chevenet et al., 1994). In addition, the taxonomic datasets was also analyzed with FCA. In these analyses, taxa were given the same weight (1/*n* being *n* the number of taxa), so that the influence of highly abundant taxa was reduced (see Chevenet et al., 1994). Taxa with less than two organisms in only one sampling site per season were not included in the analysis to reduce bias caused by taxa with sporadic occurrence. We measured the correlation between trait data and taxa distribution by means of the RV coefficient (Robert and Escoufier, 1976), which is an equivalent of the ordinary regressions coefficient for two tables and ranges from 0 to 1. Finally, we assessed the significance of the relationship using 999 random Monte-Carlo permutations of the taxonomy table. The amount of random values higher than our observed RV gave us a simulated p-value. A significant RV coefficient meant that the trait categories did not distribute randomly in communities.

To test differences between groups of sites with different pollution and drought level, an ANOVA followed by a t-test on the site scores of the first-two axes obtained from the co-inertia analysis were performed. Taxa with scores between the maximum absolute values and 50% of the lowest (absolute) value, of the two axes, were selected as those having the largest contribution to the separation of sites. Finally, the assessment of trait-specific responses to pollution, drought or the combined effect of both was performed attending to the frequency of appearance of each trait category in each group of sites over the three sampling seasons. To do that, only trait categories contributing to >90% of the total explained variance along the first-two co-inertia axes (when significant results were obtained from the ANOVA) were considered. Trait categories related to the resistance or resilience capacity of invertebrates to the combined effects of pollution and drought were identified when presenting a higher frequency in the HighPol_HD group during the drought period, as compared to the other two groups of sites. Trait categories related to the resistance or resilience of organisms to pronounced drought conditions were defined when a trait category was more representative (within the 90% of variance explained) during the drought period in the LowPol_HD group. These results were compared with the *a priory* predictions described in Table 1.

3. Results and discussion

3.1. Relationship between groups of sites and abiotic variables

ANOVA analyses performed to assess seasonal differences in abiotic variables within groups of sites showed that, in most cases, the sampling season did not influence the parameter values. Only slight temporal differences were observed for some parameters, as it will be explained in the next paragraph (Table S2, Table S3), but these differences did not influence the overall separation between groups over time (Figure S3). For this reason, PCA results shown here are based on pooled samples from all seasons (Figure 2). The between-class PCA of abiotic variables demonstrated that the three groups of sites were significantly different (simulated p-value: 0.001, R^2: 0.478). The main factors leading to differences along the PCA 1st axis included $TU_{Pesticides}$, Total N, N-NO_2, P-PO_4 and TU_{metals}, being higher in HighPol_LD followed by HighPol_HD (Figure 2, Table 3). Other factors with slight influence on that axis comprised DOC, N-NH_3 and TU_{PSC}, which showed a positive correlation with highly impacted sites and no pronounced

drought (HighPol_LD). Along the 2nd PCA axis, N-NO3, TSS, Conductivity and pH isolated the HighPol_HD group; while flow and TU_{PSC} separated the HighPol_LD group (Figure 2, Table 3).

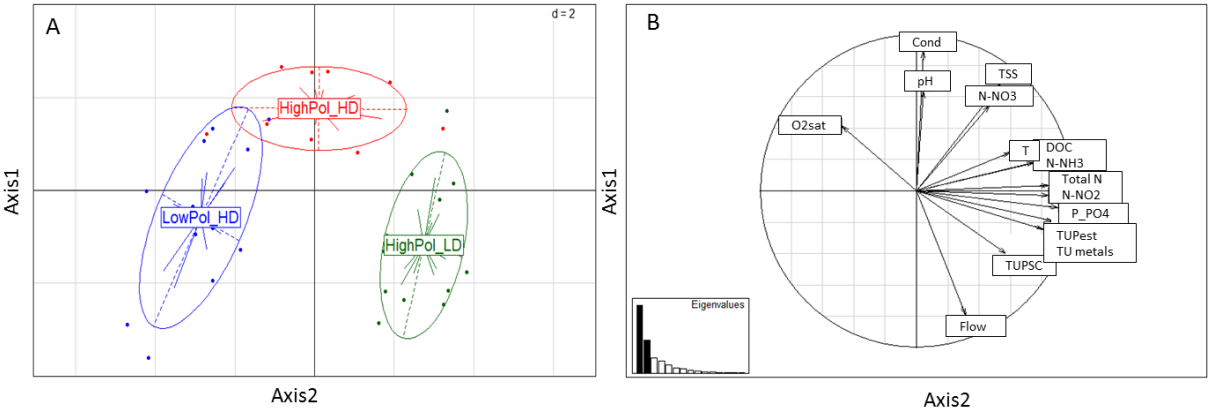


Figure 2. PCA performed using the abiotic variables measured in the 12 sampling sites in spring, summer and autumn. A) Biplot showing the distribution of the sampling sites (dots) and groups of sites (ellipses). B) Correlation circle showing the distribution of the measured abiotic variables. The variance explained by the 1st and 2nd axes is 43% and 21%, respectively.

Table 3. Average values for abiotic variables (n=12) and ANOVA tests for differences between groups of sites (p-value<0.05 denotes a significant influence of the site group on the evaluated parameter).

	LowPol_HD	*HighPol_HD*	*HighPol_LD*	p-value
Flow ($m^3 s^{-1}$)	1.24±0.83	0.15±0.13	8.76±7.18	<0.001
Temperature (°C)	12.0±4.3	13.9±5.1	16.7±5.91	0.055
pH	7.98 ±0.84	8.10±0.53	7.70±0.68	0.321
Conductivity (µS cm^{-1})	1871±1718	5040±287	1383±869	<0.001
TSS (mg L^{-1})	24.2±31.3	113±114	57.2±63.1	<0.001
O_2 saturation (%)	84.3±10.2	81.7±20.6	66.9±13.5	0.022
DOC (mg L^{-1})	2.94±1.42	6.03±1.33	6.76±1.35	<0.001
N_NH_3 (mg L^{-1})	0.01±0.02	0.08±0.18	0.19±0.26	<0.001
N_NO_2 (mg L^{-1})	0.006±0.006	0.05±0.07	0.42±0.46	<0.001
N_NO_3 (mg L^{-1})	1.65±1.30	5.53±2.87	3.61±2.16	<0.001
Total N (mg L^{-1})	1.70±1.32	6.24±2.80	10.2±3.7	<0.001
P_PO_4 (mg L^{-1})	0.005±0.004	0.06±0.07	0.37±0.28	<0.001
TU_{Metals}	0.08±0.05	0.15±0.08	0.51±0.30	<0.001
$TU_{Pesticides}$	1E-05±2E-05	1E-04±1E-04	2E-03±1E-03	<0.001
TU_{PSC}	6E-05±7E-05	3E-04±4E-04	4E-04±3E-04	0.006

Despite no significant overall differences, some trends of seasonal changes were observed for some variables (see Table S2, Table S3). As expected, water flow was noticeably lower in all groups of sites in summer (Table S3). On the other hand, $N\text{-}NO_2$ and $N\text{-}NH_3$ concentrations increased in summer in all groupings, with higher values in polluted sites, especially in the HighPol_LD group (Table S2, Table S3). This could be due to the fact that the majority of

flowing waters close to urban areas (mainly the HighPol_LD group) were most likely sustained by wastewaters effluents (treated or untreated) during drought periods (Rice and Westerhoff, 2017). As expected, water temperature was up to 7˚C higher than in spring in all groups of sites, but was not especially higher in sites with lower flows (Table S3). Overall, oxygen levels were lower in summer and autumn, with levels below 60% of saturation in polluted sites with and without drought. A more evident effect of ecosystems contraction affecting stressors concentrations was observed in highly variable streams less affected by large urban areas (i.e. HighPol_HD group; Table S3). Suspended solids (TSS) showed similar values for the two polluted groups in spring, whereas increased in all groups of sites during summer, becoming slightly higher in HighPol_HD. TU_{PSC} did not show clear seasonal variation, in line with the findings in Arenas-Sánchez et al. (2019a) and Rico et al. (2019), related with the relatively constant emission of PSC and the variability associated to human uses and epidemics. $TU_{Pesticides}$ showed significant seasonal variation in the HighPol_HD group, but this result was most likely related to application patterns, with the highest concentrations found in this study related to herbicides in spring (see Arenas-Sánchez et al., 2019a). Finally, TU_{Metals} showed a clearer seasonality in this group, with higher values measured in summer. In this case, since no variability associated to use or emission patterns is known, the increase in concentration can be interpreted as a result of the ecosystems' contraction.

3.2. Impact of anthropogenic pollution and drought on invertebrate communities

3.2.1. Abundance, richness, diversity and functional diversity

Taxonomic and functional richness, functional diversity and the IBWMP index showed significant (ANOVA p-value<0.05), or marginally significant (ANOVA p-value<0.10), differences between the low (LowPol_HD) and highly polluted sites (HighPol_HD and HighPol_LD), with lower values in highly polluted sites (Figure 3; Table S4). These differences were more evident in summer and autumn for the majority of the indexes. Moreover, in summer (and slightly in autumn) the group of sites with high pollution and pronounced drought (HighPol_HD) showed a higher decrease in richness and functional richness, as compared to the HighPol_LD grouping with no drought conditions (Figure 3, Table S4). Functional richness showed very low values in all groups of sites in spring as compared to summer and autumn (Figure 3), which can be interpreted as a sign of functional redundancy in that season. Functional diversity decreased in the HighPol_HD grouping and tended to increase in HighPol_LD sites in summer and autumn (Figure 3). This suggests that the different trait categories within the HighPol_LD grouping, could be more diverse and have more uniform patterns under stable (polluted) environments, as described by Parreira-de Castro et al. (2017) and Mor et al. (2019). The IBMWP index only showed significant responses to pollution, with overall no signs of drought effects, while other indexes did. This indicates that the current biological quality index for regulatory ecological status assessment of Mediterranean rivers is not sensitive enough to changes in community structure and functionality related to hydrological alteration, which should be taken into account in a context of expanding water scarcity conditions. Total abundance of organisms was not significantly different between the three groups of sites (Table S4), although a trend was observed towards a reduction in the number of individuals in the sites with higher pollution level (Figure S4). These findings were against what has been reported by other authors (Mor et al., 2019; Karouzas et al., 2018), referring to this response as a consequence of higher resource

availability for tolerant taxa in polluted environments. However, our results could be related to the more homogeneous substrate composition (i.e. mud and sand) in some polluted sites (e.g. site 11, 13, see Figure S2, Table S1), which can have a negative correlation with the total number of organisms, as also observed by Mor et al. (2019) in streams with sand-dominated substrates. Taxonomic diversity did not show clear differences among groups (Table S4; Figure S4), which can be related to the non-significant differences in total abundance of organisms and the fact that few taxa showed high abundances in each group of sites (Table S5).

Overall, these results indicate that pollution was an important driver on the taxonomic and functional composition of invertebrate communities, influencing the differences between groups of sites in all seasons. However, hydrology also seemed to play a key role, particularly in summer and in autumn. Other field studies performed along a Mediterranean river assessing the combined effects of pollution and water stress concluded that pollution was the main stressor shaping aquatic invertebrate communities, negatively affecting their taxonomic and functional richness. Still, in all of the studies, low water levels contributed to some additional detrimental effects by increasing toxicity levels due to lowered dilution capacity of water stressed rivers or streams (Kalogianni et al., 2017; Karouzas et al., 2018; Mor et al., 2019). Overall, the results of these studies are comparable to the present study. Nevertheless, it should be considered that the type and level of pollution differ slightly between studies. Pollution sources were associated to WWTP effluents in streams (Mor et al., 2019), as well as agricultural pollution, or oil mill processing wastewaters in larger rivers (Kalogianni et al., 2017; Karouzas et al., 2018). Partial desiccation was assessed in upstream sites in Kalogianni et al. (2017) and Karouzas et al. (2018). Nevertheless, the majority of sites suffered from ecosystems' contraction without complete desiccation as they were maintained by wastewater effluents, especially in small streams. These sites can be compared to our HighPol_HD group and larger reaches to the HighPol_LD, but differences due to the higher level of pollution reached in some sites completely maintained by wastewater effluents, and differences due to the dominant pollution type, should be considered.

Our summer sampling campaign matched with the flow contraction period, mainly in LowPol_HD and HighPol_HD sites, but the minimum flow was generally reached in late summer-early autumn, slightly before our autumn sampling campaign (see Figure S1). This dry period may have had an impact on the sampled autumn community, based on the findings of Karouzas et al (2018), who proved that invertebrate communities show maximum responses to water stress (i.e. larger explained variance related to water stress) based on variables such as discharge or mean duration of low spells simulated 45 days prior to each sampling date. The fact that biotic responses showed more pronounced temporal differences than the measured environmental variables could be related with the high variability of the majority of the parameters measured, as well as the capacity of fauna to integrate responses to stress that cannot always be detected based on in-situ measured parameters. This concept has been described previously (Cid et al., 2016), who focused on the development of a biological assessment tool (i.e. BioAs-tool) based on taxonomic and biological trait composition, to complement monitoring tasks when all environmental conditions could not be monitored.

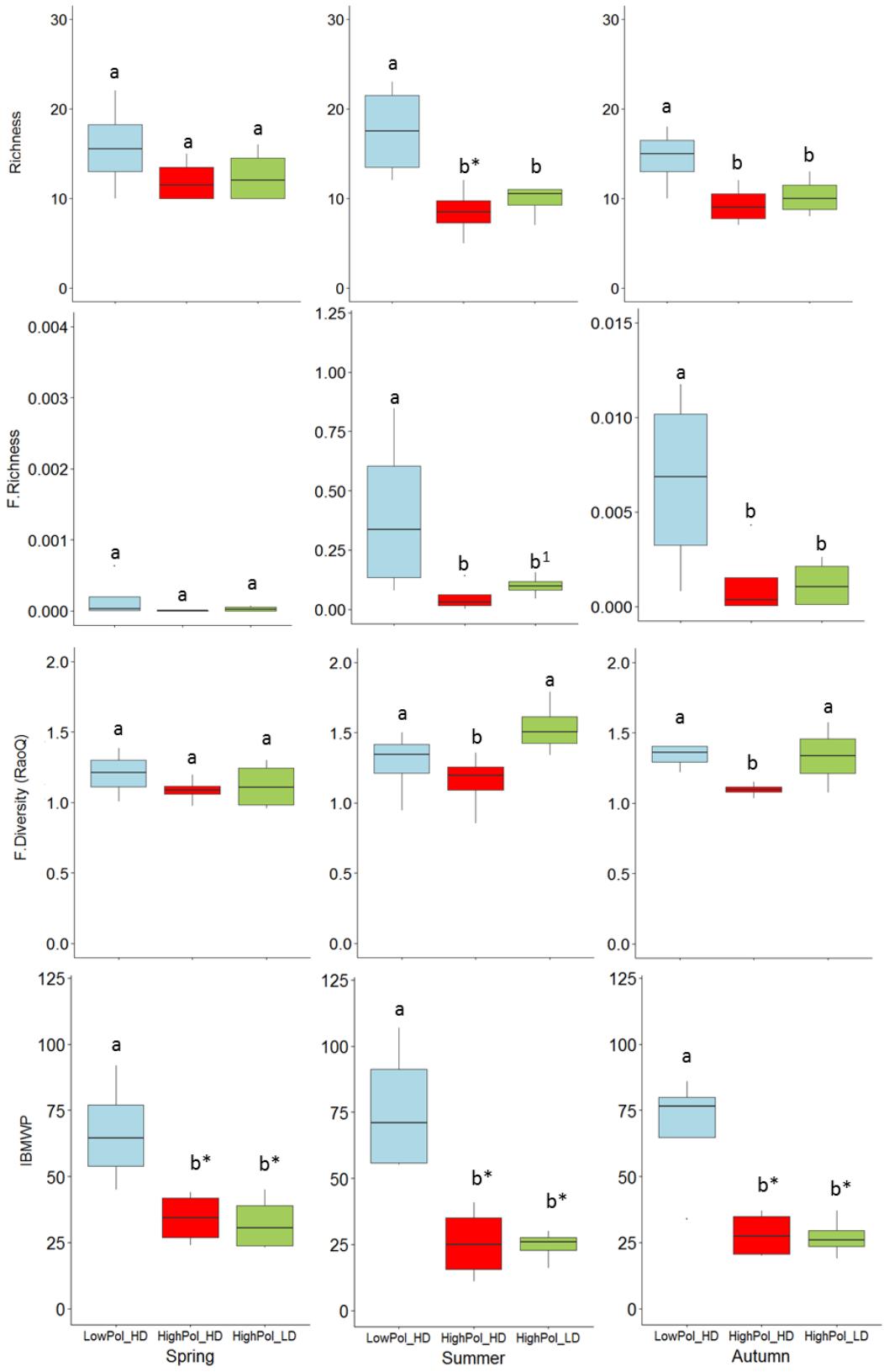


Figure 3. Taxonomic richness, functional richness, functional diversity (RaoQ) and Iberian Biological Monitoring Working Party (IBMWP) indexes in the LowPol_HD, HighPol_HD and HighPol_LD groups of sites in spring, summer and autumn. Different letters indicate significant differences (pairwise t-test, p-value< 0.05) between groups of sites within each season.
*p-value<0.001. [1]0.1>p-value>0.05.

3.2.2. Community composition and trait responses

The co-inertia analysis test showed that the taxonomic and trait compositions were significantly correlated (Table 4). Moreover, both datasets contributed to clear differences in the structural and functional composition of our groups of sites, including variations between seasons (Figure 4). Along the 1st co-inertia axis, highly polluted sites (HighPol_LD and HighPol_HD) were significantly different from lowly polluted sites (LowPol_HD) over the three seasons. Furthermore, on this axis, HighPol_HD sites were significantly distinguished from HighPol_LD sites in summer, with the latter group showing the largest differences with LowPol_HD (Table 5, Figure 4). The percentage of variance explained by the 1st co-inertia axis ranged from 26% to 30%, with the maximum value for spring (Table 5). In autumn, polluted sites (HighPol_HD and HighPol_LD) were significantly separated along the 2nd co-inertia axis. The percentage of variance explained on that axis ranged from 18% to 24%, being maximum in autumn (Table 5).

Table 4. Results of co-inertia analyses performed between taxonomic and trait data. Simulated-*P* indicates the significance (p-value<0.05) of the global trait and taxa correlation. RV is the vectorial correlation coefficient. Rounded percentages of explained variance are given for the 1st and 2nd co-inertia axes (% var.).

	Simulated-*P*	RV	% var. Ax1	% var. Ax2
Spring	0.003	0.40	30	18
Summer	0.015	0.40	29	22
Autumn	0.010	0.36	26	24

Table 5. Results from the ANOVA and pair-wise t-test analyses (p-value) performed on the scores of sites of the 1st and 2nd co-inertia axes (A: LowPol_HD; B: HighPol_HD; C: HighPol_LD)

	Ax1				Ax2			
	ANOVA	Pair-wise t-test			ANOVA	Pair-wise t-test		
		A-B	A-C	B-C		A-B	A-C	B-C
Spring	0.040	0.060	0.020	0.440	0.360	NA	NA	NA
Summer	0.001	0.020	<0.001	0.010	0.300	NA	NA	NA
Autumn	0.006	0.004	0.005	0.860	0.030	0.120	0.150	0.010

NA: not assessed due to the non-significant differences in the ANOVA

Overall, populations of Hirudinea (Glossiphonidae, Erpobdellidae), Gastropoda (Physidae), Oligochaeta (Lumbriculidae, Enchytraeidae, Tubificidae), Diptera (Psychodidae, Tipulidae, Tanitarsini, Chironomini) showed higher relative abundance in sites affected by pollution (HighPol_HD and HighPol_LD); while Plecoptera (Leutricidae, Capniidae), Ephemeroptera (Potamanthidae, or Heptagenidae), Trichoptera (Rhyacophilidae) and Bivalvia (Sphaeriidae) were more abundant in sites with lower degree of pollution (LowPol_HD) (Figure 4B). These results were expected since those taxa have been defined as tolerant or sensitive to pollution in other studies (Sabater et al., 2016; Kalogianni et al., 2017). The number of taxa with a large contribution to the differences between groups of sites on each axis was higher in summer and autumn (i.e. taxa with scores above threshold; Figure 4B). In these two seasons, taxa such as Odonata (Aeshnidae) and Coleoptera (Elmidae) or Diptera (Tipulidae or Athericidae, especially in autumn) were more prominent in low pollution sites and high drought conditions. Several authors have referred to Odonata, Coleoptera and Diptera abundances under water stress

conditions (Williams, 2005; Bonada et al., 2007a; Skoulikidis et al., 2011). Taxa such as Caenidae (Ephemeroptera) were strongly associated to sites in the HighPol_HD condition (Figure 4B). This taxon, together with other taxa such as Lymnaeidae (Gastropoda) or Psychodidae (Diptera) or also Stratiomyidae (Diptera), contributed to the significant differences between polluted groups of sites in summer and autumn (Figure 4B; Table S5). Both Caenidae (i.e. *Caenis luctuosa*) and several Diptera taxa have shown tolerances to the combined effects of pollution and water stress (Sabater et al., 2016; Kalogianni et al., 2017). The traits most likely conferring these taxa high resistance or resilience capacities to pollution and/or drought stress are shown below.

3.2.3. Traits responding to pollution and drought

Overall, 20 out of 28 expected responses from different trait categories with respect to anthropogenic pollution impact were selected based on the established criteria; and 10 of 33 with respect to drought conditions, with 1 of them associated to combined effects (Table 6). This suggests that in this study hydrological variation was a less important driver in shaping community traits as compared to pollution.

The main traits responding to anthropogenic pollution included, by decreasing order of importance, reproduction type, resistance forms, number of cycles per year and respiration, in all sampling seasons (Figure 4C; Table 6). These traits explained together up to 60% of the total explained variance on the 1st co-inertia axis in spring (Table 6, Table S6). In summer and autumn, the role of these traits contributing to differences between groups of sites on the 1st co-inertia axes was slightly reduced and the contribution of other traits such as dispersal strategies, food habits and substrate relation increased (Table 6, Table S6). All these traits generally responded to pollution; but some categories also seemed to respond to drought and the combined effect of pollution and drought in summer and autumn (Figure 4C, Table 6).

Reproduction type

Isolated eggs decreased in frequency in polluted sites, while reproduction by clutches increased (Figure 4C; Table 6), as observed by Mor et al. (2019) and Díaz et al. (2008). This is likely related to the reduced exposure surface of clutches due to low surface/volume ratio. Isolated free eggs showed high frequencies in sites affected by drought (with low and high pollution levels) in autumn (Figure 4C; Table 6), which have no clear mechanistic explanation. Asexual reproduction was more prominent in polluted sites, especially in the HighPol_LD grouping (Figure 4C; Table 6). This is in line with our expectations, as this strategy promotes recovery after disturbance through faster reproduction rates as other authors have found (Dolédec et al., 2006; Lange et al., 2014). Clutches in vegetation or terrestrial clutches showed higher frequencies in low polluted sites in summer and autumn, which match our predictions on the increased resistance to drought conditions of organisms presenting this type of reproduction (Bonada et al., 2007b), and indicate that organisms may benefit from it to resist in dried-up surfaces (Figure 4C; Table 6)

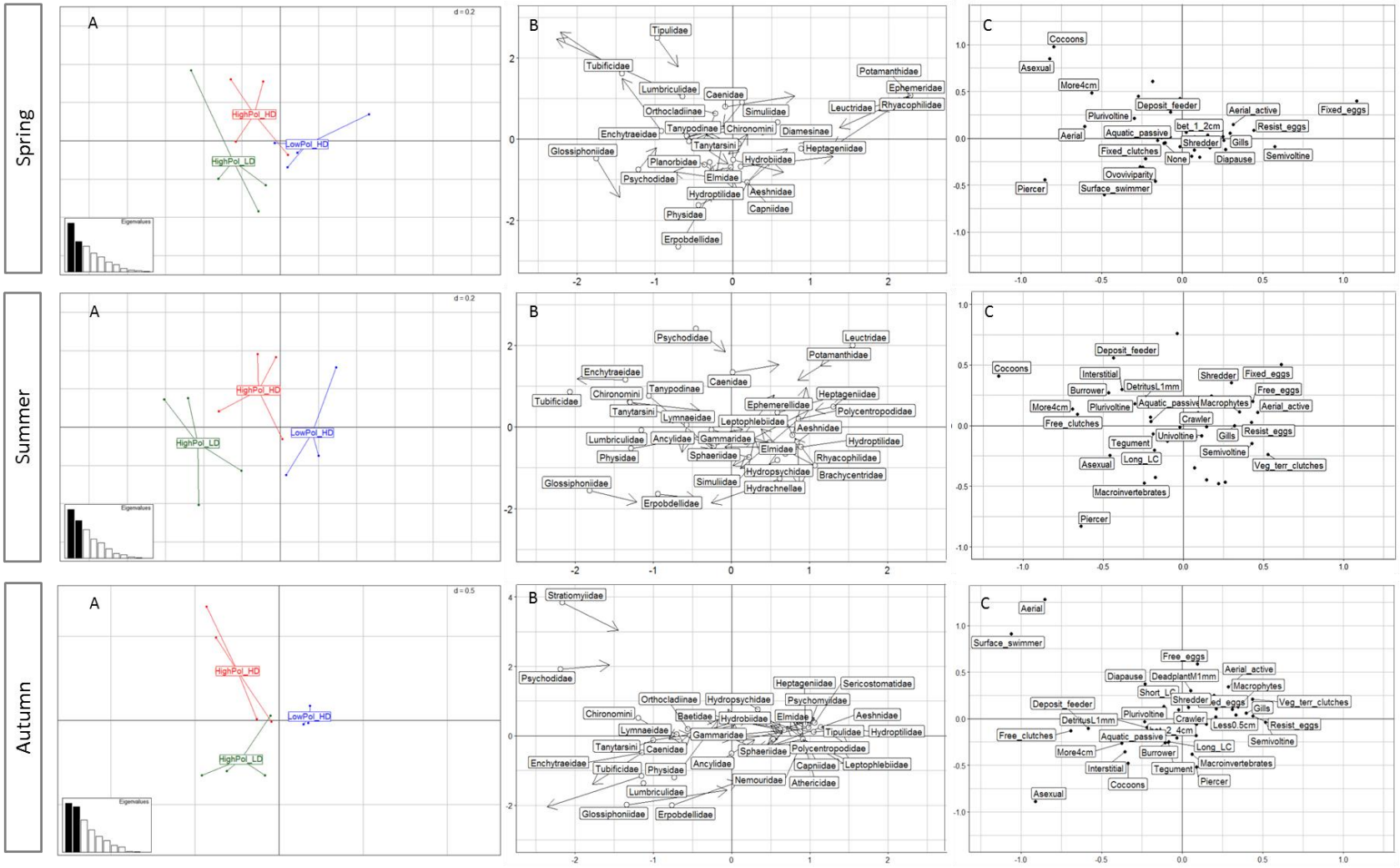


Figure 4. Site, taxonomic and trait positions resulting from the 1st and 2nd co-inertia axes of a co-inertia analysis performed separately on each season. A) Site scores grouped by level of pollution and water stress (see acronyms above in the text). Insert gives the diagram of eigenvalues. B) Scores of taxa contributing to the separation of sites in A). Arrows represent the variability on the trait composition associated to each family. Long arrows represent less robust trait patterns per family. C) Scores of trait categories best associated with the distribution of taxa.

Resistance forms

Cocoons were more frequent in polluted sites, which followed our predictions based on other studies that have referred to this reproduction form conferring higher resistance to unfavorable conditions (Statzner and Bêche, 2010). Resistant eggs were more prevalent in sites less affected by pollution, with no differences between seasons, which makes this result inconclusive based on our criteria with respect to drought. Diapausing forms showed high frequency in the HighPol_HD grouping in autumn (Figure 4C; Table 6). This result should be driven by the increased resistance capacity to unfavorable drought conditions of organisms presenting this adaptation strategy (Bonada et al., 2007b). However, the absence of this category in low polluted sites during the drought period, suggests that diapause could increase the resistance capacity of organisms exposed to the combined effects of both pollution and drought.

Number of life cycles

Shorter life cycles (plurivoltinism) were associated to highly polluted sites as opposed to semivoltinism, more prominent in low pollution sites (Figure 4C; Table 6). This is in line with the expected response related to the high resilience capacity associated to shorter life-cycles after exposure to disturbance (Townsend and Hildrew, 1994). Life cycle duration (expressed as the number of cycles per year) seemed to respond to drought as well (2^{nd} coinertia axis in autumn), with shorter life cycle in drought impacted sites (Figure 4C; Table 6). However, the presence of long life cycles in highly polluted sites (HighPol_LD) in that axis was not according to our expectations, since this strategy is usually associated with favorable conditions (e.g. low pollution levels), where less resilience capacity is needed (Townsend and Hildrew, 1994). The fact that the number of cycles per year showed an opposite response supports the idea that the response could be influenced by a trait syndrome or combination of correlated traits (Poff et al., 2006; Statzner and Bêche, 2010). For example, predators showed high frequencies in HighPol_LD sites as piercers feeding on macroinvertebrates (Figure 4C; Table 6), and these organisms normally present large sizes that are correlated with longer life cycles. Predators can respond positively or negatively to pollution, since their large sizes lead to small surface/volume ratios and lower exposure rates (Rico et al., 2015), but also suffer higher exposure to pollutants through food ingestion (Paul and Meyer, 2001). In this case, the dominance of large predator taxa tolerant to pollution, such as Erpobdellidae, could have influenced this result.

Respiration

Gill respiration was clearly associated to less polluted sites in all seasons (Figure 4C; Table 6), which confirmed our expectations based on the potential negative effect of pollution on the large surface/volume ratio of gill-bearing organisms (Paul and Meyer, 2001). On the other hand, the higher frequency of this category in summer and autumn (Figure 4C; Table 6) could also indicate a response to drought conditions, with more specialized strategies needed under depleted oxygen conditions (Bonada et al., 2007b). Aerial respiration was prominent in polluted sites in spring. However, the dominance of this trait category in autumn was mainly associated to rare taxa (e.g. Stratiomyidae, Psychodidae) present in the HighPol_HD grouping (Figure 4C; Table 6), which cannot be interpreted as a dominant community response to the combined effect of pollution and drought. The dominance of tegument respiration in polluted sites in

summer and autumn (Figure 4C, Table 6), was contrary to our predictions. Despite the response is driven by pollution, this could be understood as an enhanced effect of pollution during drought periods. Tegument breathing organisms might be negatively affected by reduced oxygen levels associated to organic pollution and low flows (Stazner and Bêche, 2010), but also due to aquatic-gas intake of some types of pesticides (Rico et al., 2015). Nevertheless, other authors also found tegument breeding organisms downstream WWTP (Charvet et al., 1998) and argued that cuticular respiration may be sufficient to supply the oxygen needs of relatively inactive organisms (Williams and Feltmate, 1992). This could be the case of dominating taxa in polluted sites such as Ancylidae, Physidae or Erbopdellidae.

Dispersal

Aerial active dispersal was associated to the LowPol_HD group, especially in summer and autumn (Figure 4C; Table 6), in line with the capacity of organisms with that strategy to colonize less dry environments from dried bed or drier sites (Bonada et al., 2007b). Aquatic passive dispersal in polluted sites also confirmed our predictions, being an important dispersal strategy in polluted flowing waters related with drift from upstream waters of tolerant taxa to pollution (Rico et al., 2015).

Substrate relation

Burrowers increased in polluted sites in summer and autumn (Figure 4C; Table 6), benefiting from the deposition of fine material as found by Usseglio-Polatera et al. (2000). The presence of interstitial organisms in polluted waters in summer and autumn (Figure 4C, Table 6) is contrary to our predictions (Table 6). We assumed the clogging of interstitial and accumulating mud normally correlated with water quality gradients, to have a negative effect on interstitial taxa (Usseglio-Polatera et al., 2000). However, the high abundance of tolerant taxa to pollution with high affinity for this category in polluted sites, such as Lumbriculidae, can influence these results. As for aerial respiration, surface swimming was mainly associated to rare taxa in the HighPol_HD group and cannot be interpreted as a representative community response. Contrary to our predictions, crawlers were more frequently found in summer and autumn, which could indicate that the water flow in our 'dry' sampling sites was not as low as to avoid the presence of these organisms. Still, the fact that they appeared in summer and autumn rather than in spring, suggest than other factors such as substrate preference might have played a role.

Food and feeding habits

Despite they could also be exposed to harsh conditions created below the sediment surface, deposit feeders can benefit from the deposition of fine material in polluted sites (Piló et al., 2016), which was the dominant response in this study, especially in summer and autumn (Figure 4C, Table 6). Shredders feeding on dead plant material >1mm (i.e. large detritus) were prominent in lowly polluted sites affected by drought in autumn (Figure 4C; Table S6, Table 6). In sites where complete drought occurs, it is expected that there should be fewer shredders of coarse detritus food (being less abundant and less input of organic material) and more scrapers feeding on periphyton algae (Bonada et al., 2007b; Statzner and Bêche, 2010). Since the proportion of detritus in this group of sites was negligible in our qualitative analysis of substrate (Table S1), the reason explaining this result could be that the amount of detritus might have been underestimated. The presence of macrophyte feeding shredders in summer and autumn

was in line to our predictions of increased plant biomass during drought periods (Bonada et al., 2007b). The fact that scrapers did not show representative differential responses to these conditions (Table S6, Table 6) could be related with the non extreme drought conditions in our sampling sites (i.e. reduced water levels but not reaching desiccation), leading to less optimal conditions for the proliferation of periphyton algae in these type of rivers.

4. Conclusions

In the present study we have shown that high anthropogenic pollution levels drive changes in aquatic macroinvertebrate communities at the taxonomic and functional levels, with the additional result that negative effects were enhanced during the drought period (i.e. summer) and in autumn. In general, pollution resulted in more tolerant and less biodiverse communities, with sites suffering from pronounced drought showing the lowest richness values. Functional diversity indexes showed that pollution decreased the number of functional characteristics in the communities resisting polluted environments. Highly polluted sites with 'low drought' levels showed similar taxonomic composition to other polluted sites, but higher abundances of some tolerant taxa. This can be associated to the presence of more established tolerant communities to pollution in continuously flowing waters. The use of qualitative indexes such as IBMWP in the assessment of the ecological status of Mediterranean rivers should be revised, as they have been proved not to show temporal variability on invertebrate communities' negative responses to pollution and drought, while the majority of other indicators did. The seasonality observed in these responses indicates that the hydrological status influences the response of the biological community to chemical status, and therefore should be jointly taken into account for the ecological status assessment of Mediterranean surface waters.

The main traits showing responses to pollution were reproduction type, resistance forms, number of cycles per year and respiration. Dispersal strategies, substrate relation, food and feeding types showed more prominent responses in summer and autumn, in relation with drought conditions and enhanced pollution levels during the drought period. The majority of traits mainly responded to pollution, but some responses to drought conditions or combined effects of drought and pollution were also observed. Asexual reproduction, reproduction by clutches, cocoons, plurivoltinism, were more prevalent in communities affected by high pollution levels with and without drought; whereas reproduction by eggs, semivoltinism or respiration by gills were more frequent in hydrologically variable environments with low pollution level. Some individual categories were related to drought conditions such as reproduction by clutches in vegetation or terrestrial reproduction and active aerial dispersal strategies. Attention should be paid to the correlation among groups of traits, but these findings shed light in the identification of biological traits of taxa particularly sensitive to pollution, drought and its combined effects. Despite more studies in this direction are needed, these results may be used to develop improved monitoring and risk assessment practices for freshwater ecosystems in Mediterranean regions.

Acknowledgments

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Table 6. Expected responses of trait categories after exposure to pollution and drought (+: high tolerance; -: low tolerance; +/-: high or low tolerance could be observed depending on the dominant mechanism of response. The final observed response is highlighted in bold) and responses obtained in this study in the different seasons (Y: as expected; N: opposite to expected; /: not conclusive results. C: combined effect of drought and pollution). Sp: spring; Su: summer; Au: autumn. Blue highlight: higher frequency on the positive side of the axis. Grey highlight: higher frequency on the negative side of the axis. Percentages of variance shown for different trait categories are those contributing to >90% of the total explained variance in each axis.

Trait	Category	Expected and observed response to pollution		Expected and observed response to drought		% var. Ax1			%var.Ax2
						Spring	Summer	Autumn	Autumn
Size	<0.5cm	+/-	/	+	Y (Au)			3.2	
	0.5-1cm	+/-	/	+	/				
	2-4cm	**+/-**	Y (Au)	+/-	/				1.3[a]
	>4cm	**+/-**	Y (Sp-Su-Au)	+/-	/	4.5	6.2	2.4[a]	1.3[a]
Life cycle duration	<1 year	+	/	+	Y (Au)				1.9
	>1year	-	/	-	/		1.7		3.8
No. life cycles	Semivoltine	-	Y (Sp-Su-Au)	-	/	5.8	2.4	4.6	
	Plurivoltine	+	Y (Sp-Su-Au)	+	/	4.8	4.8	3.5	
Reproduction type	Asexual	+	Y (Sp-Su-Au)	+	/	2.3	1.4	3.3[a]	3.4[a]
	Ovoviviparity			+	/	1.3			
	Free eggs	-	Y (Su)				1.2		5
	Fixed eggs	-	Y (Sp-Su-Au)	+	/	31.4	11.2	1.9	
	Free clutches	+	Y (Su-Au)				6.4	5.7	
	Fixed clutches	+	Y (Sp)			3.5			
	Terrestrial/Vegetation clutches			+	Y (Su-Au)		1.6	2.8	
Dispersal	Aquatic passive	+	Y (Sp-Su-Au)			1.6	2.9	2.2	1.7
	Aerial active			+	Y (Su-Au)	3	5.9	2.8	4.3[c]
Substrate relation	Surface swimmer			+	/	1.3		10.1[b]	8[b]
	Swimmer			+	/				
	Crawler			-	N (Su-Au)		1.7	1.7	
	Burrowers	+	Y (Su-Au)				3.7		1.6[a]
	Interstitial	-	N (Su-Au)	+	/		2.3	2.2[a]	2.4[a]
Resistance forms	Diapause			+	C (Au)	2.2		1.8[b]	5.3[b]
	Resistant eggs	+	N (Sp-Su-Au)	+	/	3.7	3.5	3.7	
	Cocoons	+	Y (Sp-Su-Au)	+	/	4.5[a]	6.6[a]		2[a]
Respiration	Aerial	+	Y (Sp)	+	/	3.7		12.3[b]	29.9[b]
	Gills	-	Y (Sp-Su-Au)	+	Y (Su-Au)	4.6	5.6	9.1	
	Tegument	-	N (Su-Au)	-	/		3.2		6.8[a]
Food	Macroicroinv	**+/-**	Y (Su-Au)	+/-	/		1.2[a]		3.9[a]
	Detritus<1mm	**+/-**	Y (Su-Au)	+	/		2	2	
	Detritus>1mm	+/-	/	-	N (Au)				2.7[c]
	Macrophytes			+	Y (Su-Au)		1.8	1.9	
	Microphytes			+	/				
Feeding type	Predator	+/-	/	+/-	/				
	Deposit feeder	**+/-**	Y (Sp-Su-Au)	+	/	2.3	6	11.8	
	Scraper	+/-	/	-	/				
	Shredder	**+/-**	Y (Sp-Su-Au)	**+/-** +/-	Y (Su-Au) N (Au)	2.3	2.7	1.6	2.8[c]
	Piercer			+	/	4.7[a]	2.7[a]		3.7[a]

[a]:Higher frequency in HighPol_LD; [b]; higher frequency in HighPol_HD; [c]: Higher frequency in LowPol_HD.

Chapter 6

Effects of increased temperature, drought and an insecticide on freshwater zooplankton communities

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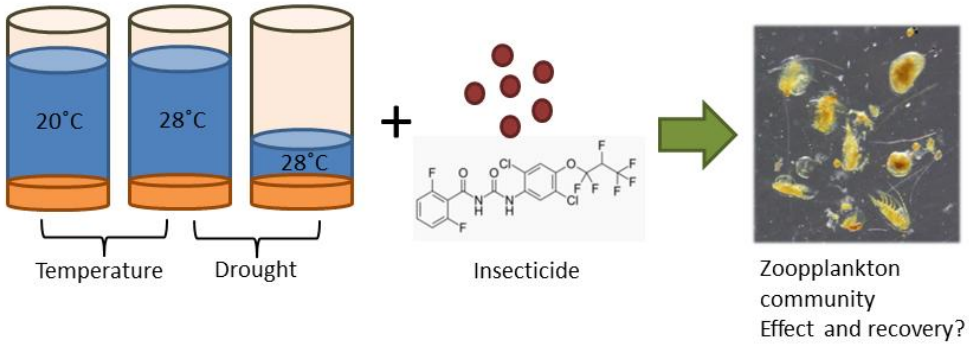

Abstract

In the present study we performed a microcosm experiment to assess the effects of the insecticide lufenuron on zooplankton communities exposed to increased temperature and drought in (semi-)arid regions. The experiment consisted of three environmental scenarios, assessed in two parts. Firstly, we assessed how water temperature (20˚C and 28˚C) affects the sensitivity and resilience of the zooplankton community to lufenuron. Secondly, we investigated the influence of drought on the structure of the zooplankton community at a high water temperature (28˚C) and evaluated its possible interaction with lufenuron. The results show that the community exposed to lufenuron at 28˚C had a faster lufenuron-related response and recovery than the community at 20˚C. The combined effects of lufenuron and temperature resulted in a synergistic effect on some taxa (*Daphnia sp*., Cyclopoida and Copepoda nauplii). The tested zooplankton community had a high resilience to drought, although some particular taxa were severely affected after desiccation (Calanoida). Interactions between drought and lufenuron were not statistically significant. However, rewetting after desiccation contributed to lufenuron remobilization from sediments, and resulted in a slight Cyclopoida population decline at high exposure concentrations. This study shows how environmental conditions related to global change in (semi-)arid regions may influence chemical fate and the vulnerability of zooplankton communities to chemical stress.

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1. Introduction

Climate and socioeconomic pressures associated to global change have been identified as major drivers affecting aquatic ecosystems worldwide, particularly in arid and semiarid regions (Barceló and Sabater, 2010; IPPC, 2012, 2014). The natural hydrological patterns of water bodies in these regions are characterized by partial or complete droughts followed by rewetting and flooding periods (Gasith and Resh, 1999; Lake, 2003). At present, such hydrological patterns are suffering severe alterations due to changes in annual precipitation rates and the occurrence of harsh events, which are expected to become more recurrent and unpredictable in the near future due to climate change (EEA, 2008; Sabater and Tockner, 2010). In addition to that, aquatic ecosystems in (semi-)arid regions are subject to an increasing water abstraction pressure resulting from growing human population demands (Barceló and Sabater, 2010; Petrovic et al., 2011). All these factors have yielded to a condition of water scarcity, defined as a structural and persistent reduction in water availability. Moreover, the marked increase in global mean temperatures specially expected in these regions (Calbó, 2009; IPPC, 2012, 2014) also interferes with water availability. Apart from increasing water evaporation rates, these changing thermal conditions contribute to an alteration of the physico-chemical variables and ecological functions that support aquatic biodiversity (Mantyka-Pringle et al., 2012; Klausmeyer and Shaw, 2009).

The majority of organisms inhabiting aquatic ecosystems in (semi-)arid regions are characterized by adaptive strategies to cope with high water temperatures and droughts, as well as their related habitat and physico-chemical fluctuations (Lahr, 1997; Bonada et al., 2007b; Datry et al., 2016). Some of these strategies include adaptation to low oxygen concentrations, dormancy or production of resistant eggs during drought events and well-developed dispersal abilities to recolonize more favourable environments (Lahr 1997; Storey and Quinn, 2013; Arenas-Sánchez et al., 2016). Although some studies have described taxonomic and functional characteristics of aquatic communities in regions affected by water scarcity (Bonada et al., 2007b), there is still limited information on how such populations and communities may respond to additional stressors.

Climate change and water scarcity are expected to affect use patterns of certain chemical substances (e.g. those used in agricultural production). Additionally, the environmental fate and exposure to these substances are also expected to change through altered degradation rates, lowered dilution capacity and/or sediment resuspension following flood events (Noyes et al., 2009; Daam et al., 2011). A limited number of studies have assessed how climatic and water availability alterations influence chemical exposure and the response of aquatic communities to the combination of both stressors (Arenas-Sánchez et al., 2016). Experiments assessing interactive effects of chemicals and water scarcity on bacteria and invertebrates show inconsistent results (Stampfli et al., 2013; Martin et al., 2014; Corcoll et al., 2015). In theory, one may expect that aquatic communities that are regularly affected by harsh environmental conditions, such as regular desiccation periods, are more specialized and display a lower functional redundancy, thus having lower resilience to chemical stress (Moe et al. 2013). However, some studies suggest that the degree of specialization obtained by adaptation to water scarcity conditions is positively correlated to a higher resilience to short-term chemical exposure (Lahr, 1997; Stampfli et al., 2013). Regulatory procedures used for the

risk assessment of chemicals rely on a very simplistic ecological scenario and usually overlook the possible co-occurrence of chemicals with additional environmental stressors (Rico et al., 2016b). For these reasons, the development of future monitoring and risk assessment strategies for aquatic ecosystems in regions exposed to increasing temperatures and drought stress, such as (semi-)arid regions, requires a better understanding on the interaction between multiple stressors and their impacts on aquatic biodiversity.

The aim of the present study was to evaluate the combined effects of two environmental stressors related to climate change and water scarcity (i.e., increased temperature and drought) and a chemical contaminant on zooplankton communities using freshwater microcosms. The main hypotheses were that increased water temperature and droughts may significantly influence the structure of zooplankton communities, thus altering their sensitivity and resilience (i.e., recovery capacity) to chemical stress. Zooplankton were selected here as focal taxa since they are good indicators of the ecological status of lentic, slow-flow or intermittent ecosystems. Moreover, they exhibit generation times short enough as to show population and community-level responses and recoveries after stress within feasible experimental time frames (Cairns et al., 1993; Shurin et al., 2000; Whitman et al., 2004). The chemical stressor selected was the benzoylurea insecticide lufenuron, commonly used for pest control in agriculture and as veterinary medicine (Brock et al., 2016). It acts by inhibiting chitin synthesis and moulting of invertebrates (McHenery, 2016), and has high toxicity to crustaceans (López-Mancisidor et al., 2008a). Moreover, lufenuron is highly hydrophobic and persistent in freshwater sediments (EFSA, 2008; Brock et al., 2016). Such features allow the evaluation of long-term effects on aquatic organisms and the assessment of chemical remobilization due to wet-dry phases typical of intermittent freshwater ecosystems in (semi)-arid regions.

2. Materials and methods

2.1. Experimental design

The experiment was conducted using a total of 27 indoor microcosms placed into three water baths made of stainless steel. Each microcosm consisted of a glass cylinder (diameter: 20.5 cm, total height: 37 cm) filled with 10 L of unpolluted water (water depth: 30 cm) and 2 kg of natural sediment (sediment depth: 4 cm). In September 2016, just before the experiment started, the water used in the experiment was collected from an outdoor mesocosm facility at the National Institute for Agronomic Research (INIA, Madrid, Spain). The initial planktonic community consisted of a mix of species collected simultaneously from the outdoor mesocosm facilities at INIA and the IMDEA Water Institute (Alcalá de Henares, Spain), which was evenly distributed among the test systems. The zooplankton community was left to establish under the new environmental conditions for a period of two weeks before the insecticide application. To prevent excessive periphyton growth, five snails (*Physella acuta*) were introduced into each microcosm. The sediment was collected from a dried-up pond at the Royal Botanical Garden Juan Carlos I (Alcalá de Henares, Madrid). Organic matter (OM) content of the sediment was measured at the start and at the end of the experiment according to the method described in ASTM (2013). The average OM content was 3%, which corresponds to an approximate Organic Carbon (OC) content of 17.4 g OC/kg dry weight (following Rosell et al., 2001). The microcosms were subject to a light/dark regime of 16:8 h simulated by fluorescent tubes (Osram G13 36W,

Germany) placed about 1 m above the microcosms (light intensity: 34 µE/m^2 s^{-1}). Light aeration was provided in each microcosm by means of a compressed air pump (0.5–1.5 L/min). Nutrients were provided twice a week to reach a target inorganic N and P-PO_4 concentration of 90 and 15 µg/L, respectively (ratio 6:1), using a stock solution formed by $(NH4)_2SO_4$, $NaNO_3$ and KH_2PO_4.

The experimental design (Figure 1) consisted of three lufenuron exposure levels (i.e., Control, C1: low exposure level, and C2: high exposure level), two thermal scenarios without desiccation: T20 (water temperature at 20°C) and T28 (water temperature at 28°C); and a drought scenario at high temperature: T28_Drought (water temperature at 28°C with desiccation). Each chemical treatment was performed in triplicate (n=3) in all scenarios. Lufenuron low (C1) and high (C2) exposure levels consisted of two applications, on day 0 and on day 10. In the first application, intended lufenuron concentrations were 0.1 and 1 µg/L in C1 and C2, respectively, whereas in the second application the concentrations were 2 µg/L in C1 and 8 µg/L in C2. The selected concentrations for the first application were based on existing acute toxicity data referring to an EC50-48h value of 1.1 µg/L (Rufli, 1986) and a NOEC-48h equal to 0.16 µg/L (Schulz and Dark, 2003). These two values were based on water only toxicity tests and yielded too low effects, so higher concentrations were selected for the second pulse. The concentrations used in the second application were one half and twice the 48 hour-EC50 observed in a water-sediment microcosm experiment performed with *Daphnia magna* (Forbis, 1986).

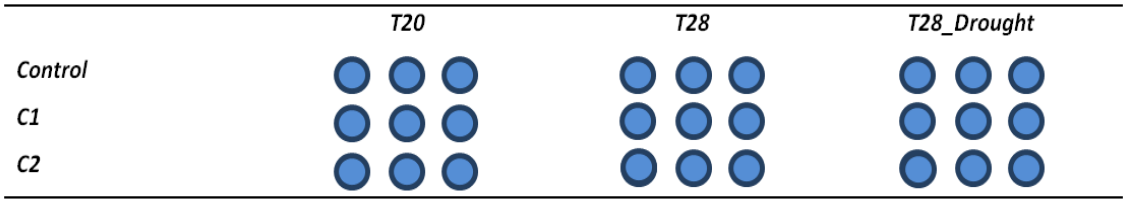

Figure 1. Experimental design. 9 cosms per environmental scenario (T20, T28 and T28_Drought), divided in Control, low (C1) and high (C2) exposure levels for the insecticide lufenuron (n=3, Control; n=3, C1: 0.1 and 2 µg/L; n=3, C2: 1 and 8 µg/L).

The different water temperatures (20 and 28 °C) were achieved by electric heating of the water contained in the water bath surrounding the microcosms. In the test units with no desiccation (T20 and T28), water loses were compensated by refilling the microcosms with distilled water every other day simulating rainfall additions. In the test units affected by desiccation (T28_Drought) water was not refilled since the first lufenuron application. In these systems, the water level decreased up to desiccation (contraction phase), which occurred on day 42. These microcosms were kept for 4 days under extreme drought conditions (desiccation event) and refilled again up to the initial water level with distilled water (rewetting phase). In those systems, lufenuron dosing and nutrient additions were re-calculated based on the water level fluctuations. The experiment had a duration of 73 days from the first lufenuron application.

2.2. Lufenuron dosing, sampling and analysis

Lufenuron (Sigma Aldrich, CAS 10305-07-08) stock solutions were prepared in methanol before each application. Aliquots (1mL) of these stock solutions were evenly distributed over the water surface of the corresponding microcosm and gently stirred to promote homogenous mixing. Additionally, 1 mL of methanol was added to each chemical control according to the requirements specified by OECD (2000). Nominal concentrations were calculated from lufenuron measurements in the corresponding stock solution, the aliquot volume applied and the water volume in the treated microcosms.

Depth-integrated water samples (150 mL) were taken from the microcosms by means of a glass pipette and transferred into glass flasks to measure lufenuron exposure concentrations. Water samples were collected 2 hours and 3 days after the first application and 2 hours, 1, 3 and 7 days after the second lufenuron application. Water samples were also taken in the T28_Drought microcosms on day 46 to measure possible lufenuron remobilization after desiccation and refilling. Samples to determine the lufenuron concentration in sediments were taken on day 4, 14, 21, 46, 60 and 73 after the first lufenuron application. In order to avoid sediment and water disturbance while sampling, sediment samples (60 g) were collected from glass flasks settled in the sediment layer before the start of the experiment. After sampling, water and sediment samples were stored at -20°C until further analysis.

Lufenuron was extracted from water and sediment samples as described in the Supporting Information. Extracts were analysed using an HPLC system (Agilent 1200 Series, Agilent Technologies) equipped with a kinetex F5 column (100 mm x 4.6 mm, 2.6 µm) (Phenomenex), and coupled to an Agilent 6495A triplequad MS/MS (Agilent 6495A, Agilent Technologies). A summary of the optimum parameters for the LC-MS/MS system and the Multiple Reaction Mode (MRM) transitions is available in the Supporting Information (Tables S1 and S2). The concentrations of lufenuron were calculated by external calibration mode using the Quantitative MassHunter Software of Agilent. The average recovery of lufenuron in the water and sediment samples was evaluated at two different concentrations (0.01 µg/L and 1 µg/L for water, and 0.857 µg/kg dw and 857 µg/kg dw for sediment) with calculated recoveries of 106% and 93% for the water, and 96% and 102% for the sediment samples, respectively. The Relative Standard Deviation (RSD) values (n=8) were lower than 10%. The methodological limit of quantification (LOQ) of lufenuron was 10 ng/L in the water samples and 0.3 µg/kg dw in the sediment samples.

The dissipation coefficients (*k*) and half-lives (DT50) of lufenuron in water and sediment of the microcosms were calculated separately for each chemical exposure level in the T20, T28 and T28_Drought scenarios. The dissipation coefficient was calculated by means of linear regression of the ln-transformed concentrations with the software Microsoft Excel version 2010, assuming first-order kinetics. The DT50 values were calculated as: Ln(2)/*k*.

2.3. Water quality measurements

Measurements of dissolved oxygen (DO), electric conductivity (EC), water temperature and pH were performed on a weekly basis using a portable multimeter probe (model HI98194, HANNA Instruments). In addition, water samples (300 mL) were collected in PVC bottles to determine

the concentration of dissolved organic carbon (DOC), nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+), orthophosphate (PO_4^{-3}) and total phosphorus (TP). Nutrient and DOC samples were taken on day -3 (in the pre-exposure period) and on day 14, 28, 46 and 60 after the first lufenuron application. Nutrient analyses were performed according to the methods described in APHA (2005). The DOC concentration was measured on a Shimadzu TOC-$V_{CSH/CSN}$ coupled to an ASI-V autosampler (Shimadzu Corporation).

2.4. Zooplankton sampling and determination

Zooplankton samples were taken on day -3 and on day 4, 14, 21, 28, 46, 53, 60 and 73 relative to the first lufenuron application. Depth integrated water samples (1.5 L) were collected and filtered through a zooplankton net (mesh size, 55 µm; Hydrobios). Subsequently, the filtered water was returned to the original microcosm. The concentrated plankton samples (maximum volume 100 mL) were preserved with Lugol's iodine solution (approximately 4% v/v) and stored in dark conditions. Samples were let to sediment for 24h. Afterwards,the supernatant was carefully removed to obtain a concentrated sample and facilitate further identification and counting. All volumes were properly recorded.

The species composition in the zooplankton samples was determined to the lowest possible taxonomic level. Macro-zooplankton (i.e., Cladocera, adult and copepodite Copepoda, Ostracoda) were identified and counted in the entire sample using an stereoscope (Olympus SZX7; magnification x16-x112, Olympus Life Science Europe GMBH) whereas micro-zooplankton (i.e. Rotifera, Copepoda nauplii) were determined using a sub-sample (1 mL) of the original zooplankton sample and a microscope (Olympus CX4; magnification x100, Olympus Life Science Europe GMBH). The identified zooplankton included Rotifera (13 taxa), Cladocera (5 taxa), Cyclopoida (dominated by *Tropocyclops prasinus, Microcyclops varicans and Diacyclops bisetosus*), Calanoida (dominated by Diaptomidae) and Ostracoda (dominated by *Cypridopsis vidua*). Adult and copepodite stages of Copepoda taxa were counted together.

2.5. Data analyses

To assess separately the impacts of temperature and drought, and their combined effect with lufenuron, the statistical elaboration of the results considered the experimental design as composed by two different parts: 1) T20 vs T28, to evaluate the single and combined effects of lufenuron and temperature, and 2) T28 vs T28_Drought, to evaluate the single and combined effects of lufenuron and drought at high temperature (28°C).

The isolated and combined effects of lufenuron and the tested environmental stressor (temperature and drought) on the measured physico-chemical parameters were assessed by a two-way ANOVA.

In order to assess how temperature and drought may influence the structure of the zooplankton community, a Principal Response Curve (PRC) analysis (Van den Brink and Ter Braak, 1999) was performed using the lufenuron controls of the T20 and T28 scenarios (first part), and the T28 and T28_Drought scenarios (second part), respectively. Additionally, the PRC method was used to assess the effects of lufenuron on the zooplankton community under the different environmental scenarios tested. The PRC analysis produces a diagram showing

the deviations in time of the different chemical treatment levels as compared to the control. The diagram shows the sampling days on the x-axis and the first principal component of the treatment effects expressed as regression coefficient (C_{dt}) on the y-axis. The taxa weights (b_k) shown in the right part of the diagram can be interpreted as the affinity of each taxonomic group with the response shown in the diagram. Taxonomic groups with large positive weights diminish most strongly at higher chemical concentrations, while taxonomic groups with large and negative weights show a positive response with respect to the treatments due to tolerance to the stressor and/or indirect effects. The significance of the PRC diagram in terms of displayed treatment variance was tested by 499 Monte Carlo permutations. The significance of lufenuron exposure per sampling date was calculated by performing single Redundancy Analysis (RDA) permutation tests for each sampling date, using the ln-transformed maximum exposure concentrations as explanatory variable. For those dates that showed significant effects, a Principal Component Analysis (PCA) was performed, and differences to the control were assessed by applying a pairwise t-test to the sample scores of the first PCA axis.

Finally, RDA accompanied by Monte Carlo permutation tests were performed to test whether the zooplankton community was significantly affected by lufenuron (L), temperature (T) and its interaction (LxT), in the first part of the experiment; and by lufenuron (L), drought (D) and its interaction (LxD), in the second part. The influence of the different explanatory variables and their interaction on the zooplankton community was tested for each sampling date as shown in Table 1 (for details see Van Wijngaarden et al., 2006).

Table 1. RDA analysis set-up for assessing the individual and combined effects of temperature and lufenuron in the zooplankton community. L=maximum exposure concentration of lufenuron (ln-transformed); T=temperature; D=drought; LxT and LxD=interactions.

	Parameter(s) tested	**Explanatory variable**	**Covariables**
Experimental Part 1	Lufenuron	L	T, LxT
	Temperature	T	L, LxT
	Interaction	LxT	L, T
Experimental Part 2	Lufenuron	L	D, LxD
	Drought	D	L, LxD
	Interaction	LxD	L, D

All multivariate analyses were performed with the CANOCO software package, version 5 (Ter Braak and Smilauer, 2012). Prior to statistical analyses, the zooplankton density data were ln (Ax+1) transformed, where x stands for the actual density value, and Ax makes 2 when the lowest density value higher than zero is taken for x. This was done to down-weight high density values and to approximate a normal distribution (for rationale see Van den Brink et al., 2000).

In order to assess the single and combined effects of temperature and lufenuron (first part), and drought and lufenuron (second part) on population densities (i.e., Individuals/L), a two-way ANOVA was performed. Such detailed population analyses focused on the taxa that showed, in general, high or low PRC taxa weights (b_k) and that had average density values in the lufenuron controls above 3 individuals/L in >70% of the sampling dates. Taxa showing incidental occurrence in the water samples were not further considered. To assess the toxic effect of the different lufenuron exposure levels as compared to the controls, an ANOVA

followed by a pairwise t-test was conducted. In this last analysis, pooled and un-pooled variances were considered for data with equal and unequal variances, respectively. For these analyses the zooplankton density data were transformed as described above. All ANOVAs and pairwise t-tests were performed using the SPSS software, v.23.0.

Statistical correction for multiple testing in micro- and mesocosm studies is usually not applied since the number of replicates is low and they can have a high variability in population and community responses, with a high risk of getting Type II errors (De Jong et al. 2005). Thus, in the present study we opted for not correcting p-values and drawing conclusions based on significant effects occurring in more than one consecutive sampling date. Isolated significant responses were only considered when there was a plausible mechanism supporting such result.

The geometric mean of the zooplankton taxa densities per sampling date were displayed in density graphs for each environmental scenario. The values in the density graphs were used to describe the combined effects of the chemical and abiotic stressors tested in the experiment, following the approach described by Piggot et al. (2015). In this classification, not statistically significant interactions between the two evaluated stressors were defined as additive effects. On the other hand, statistically significant interactions that were more positive or negative than predicted additively were described as synergistic. Significant interactions that were less positive or less negative than predicted additively were considered antagonistic.

3. Results

3.1. Persistence of lufenuron in water and sediment

Initial measured lufenuron concentrations in water were, on average, 110% (min.-max.: 92-125%) of the nominal concentration (Table S3). In general, lufenuron showed a relatively fast dissipation from the water column, and a much slower dissipation from the sediment compartment (Table 2). Overall, there were no substantial differences between dissipation rates of the different exposure levels within each environmental scenario. Therefore, mean DT50 values (including results of both C1 and C2) were used in all elaborations. Mean water DT50 values were 3.00, 2.54 and 1.67 days in the T20, T28 and T28_Drought scenarios, respectively. In T28_Drought, the refilling process after desiccation resulted in peak exposure concentrations in water of 0.3 and 0.7 µg/L for C1 and C2, respectively. Maximum concentrations in sediment were measured four days after the second application. Mean values for that sampling date were 476, 304 and 314 µg/g OC in the T20, T28 and T28_Drought scenarios for C1; and 2213, 2098, 1452 µg/g OC for C2. The expected lufenuron concentration in sediment for that date, assuming complete sorption from water into the sediment compartment, was comparable to the mean measured concentrations in all the scenarios. Therefore, lufenuron sorption from water into sediment was considered to be the main process affecting lufenuron dissipation from water. Based on our measured concentrations in water and sediment, log K_{oc} values ranged between 2.80 and 3.02 mL/g OC. Sediment dissipation was similar in the T28 and T28_Drought scenarios (DT50: 22.5 and 23.0 days, respectively), and slower in the T20 scenario (DT50: 37.7 days). A graphical description of lufenuron concentrations in water and sediment over time can be observed in Figure 2 and a detailed summary is available in the Supporting Information (Tables S3, S4, S5).

3.2. Water quality parameters

Mean physico-chemical parameter values measured in the microcosms under the different environmental scenarios are shown in Table 3. On average, measured temperatures were very close to the nominal temperatures of the different environmental scenarios. No significant lufenuron-related effects were observed in any of the environmental scenarios (Tables S6 to S9). Exceptionally, lufenuron seemed to influence pH levels, with increasing values at high concentrations (Tables S6 and S7). However, differences were minimal in all scenarios (Figure S1).

The trends of the measured physico-chemical parameters were consistent with temperature differences. DO concentrations and pH values were significantly lower in the microcosms exposed to high temperature in the majority of the sampling dates (Table 3; Table S6). Nitrate concentrations were found to be slightly lower in the T28 microcosms as compared to the T20 ones for the majority of the sampling dates (Table S8; Figure 3)

The concentration of total inorganic N, NO_3^-, DOC and EC were significantly higher in the T28_Drought scenario as compared to T28 during the water contraction phase (Tables S7 and S9). These concentrations decreased immediately after rewetting due to dilution, but reached the levels of the other scenarios as the rewetting phase advanced (see Figure 3 for an example). The high temporal variability for these parameters in T28_Drought is reflected in their large standard deviation values (Table 3).

Table 2. Water and sediment dissipation rate constants (*k*) and calculated half-life (DT50) values for lufenuron at two exposure levels (C1 and C2) under the different environmental scenarios tested.

	Water		**Sediment**	
	k (d^{-1})	DT50 (d)	*k* (d^{-1})	DT50 (d)
T20				
C1	0.23	3.01	0.019	36.1
C2	0.23	2.99	0.018	39.2
T28				
C1	0.29	2.43	0.034	20.4
C2	0.26	2.64	0.028	24.6
T28_Drought				
C1	0.46	1.52	0.031	22.5
C2	0.38	1.82	0.030	23.4

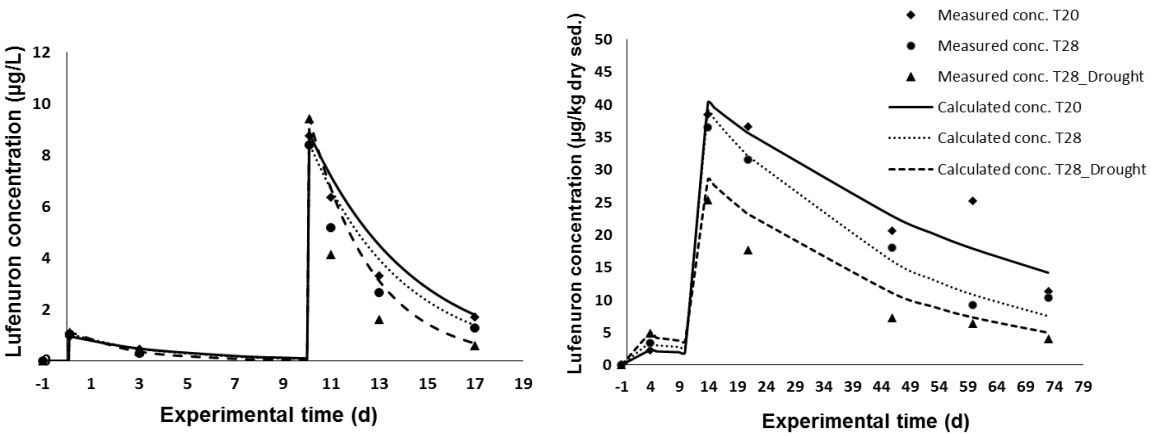

Figure 2. Lufenuron concentration over time in water and sediment at the high chemical exposure level (C2) under different environmental scenarios (T20, T28, T28_Drought). Since DT50 results were comparable, these graphs are also representative of the chemical exposure dynamics at the low exposure level (C1).

Table 3. Water physico-chemical characteristics (mean ± standard deviation) measured in the microcosms of the three lufenuron exposure conditions (Control, C1 and C2) under different environmental scenarios (T20, T28, T28_Drought) during the entire experimental period.

	T20	**T28**	**T28_Drought**
Temp. (°C)	18.7±0.5	27.2±0.5	27.4±0.5
DO (mg/L)	5.63±0.89	4.50±0.60	4.60±0.61
EC (µs/cm)	701±80	721±91	728±293
pH (-)	8.54±0.80	8.06±0.73	8.12±0.76
DOC (mg/L)	17.9±6.9	15.8±2.8	18.0±11.5
N-NH_4^+ (mg /L)	0.030±0.061	0.018±0.042	0.015±0.025
N-NO_2^- (mg/L)	0.005±0.008	0.003±0.003	0.003±0.003
N-NO_3^- (mg /L)	1.45±1.09	1.48±0.84	1.71±1.25
P-PO_4^{-3} (mg /L)	0.007±0.011	0.013±0.021	0.015±0.020
Total inorg. N (mg/L)	1.49±1.15	1.50±0.86	1.73±1.26
Total P (mg/L)	0.035±0.023	0.054±0.065	0.045±0.030

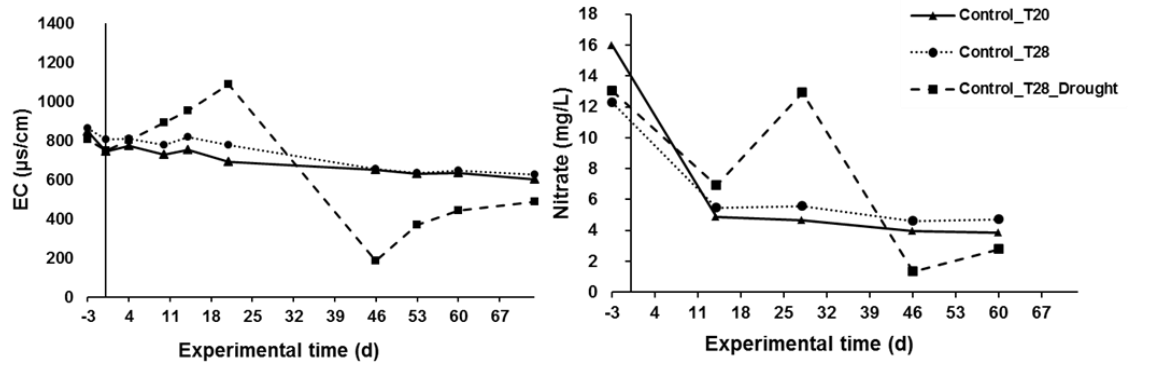

Figure 3. Measured (a) EC and (b) nitrate concentration (mean values; n=3) in microcosm water of controls under different environmental scenarios (T20, T28, T28_Drought). EC values on day 28 were not included due to the high variability found, related with the measurement difficulties in very low water level systems in T28_Drought.

3.3. Effects of temperature on the response of zooplankton to lufenuron

3.3.1. *Community-level responses*

The PRC analyses performed to assess the influence of increased temperature on the zooplankton community (chemical controls comparison) showed only marginal effects (Monte Carlo test, p=0.09; Figure S2). Cyclopoida, *Daphnia* sp., *Alona* sp. and Ostracoda showed lower densities in T28 controls, while *Lecane* sp. and Calanoida showed larger densities.

The PRC analyses performed to evaluate the effects of lufenuron under each thermal scenario showed that the zooplankton community was significantly affected in both of them (Figure 4). In both scenarios, the most negatively affected taxonomic groups (b_k>1) were Copepoda nauplii, Calanoida and *Chydorus* sp. On the other hand, some Rotifera taxa (*Lecane sp., Ascomorpha sp.* and *Cephalodella sp.*) showed a density increase (Figure 4). The results of the pairwise t-test performed to assess the effect of lufenuron under both thermal scenarios show some differences (Figure 4). The maximum effect (degree of change with respect to the control) was similar in the two scenarios, for both chemical exposure levels (C1 and C2). However, in T20 the community exposed to C1 and C2 was not significantly affected until the second lufenuron application; while at T28, the C2 was already affected after the first lufenuron application. On the other hand, the largest community effect under the T20 condition was observed on day 46 after the first application, while the largest effect under the T28 condition occurred right after the second application (day 14). Moreover, at T20, the community exposed to C1 and C2 were significantly affected until the end of the experiment. At T28, the community exposed to C1 was found to recover from day 28 onwards, while the community exposed to C2 recovered on day 73 (i.e., non-significant differences as compared to the control, Figure 4).

The RDA with Monte Carlo permutation tests confirmed lufenuron as the main factor driving the response of the community. Temperature had a significant influence on the zooplankton community up to day 21, with less marked differences on subsequent dates (Table 4). The interaction between lufenuron exposure and temperature was significant on days 4 and 14. Based on the above results, this interaction may be defined as synergistic, with stronger than expected effects on those dates resulting from the combined effects of lufenuron and high temperature (Figure 4; Table 4).

3.3.2. Population-level responses

The following zooplankton taxa were selected for detailed descriptions: 2 Cladocera (*Daphnia* sp. and *Chydorus* sp.), 2 Copepoda (Cyclopoida, Calanoida; and Copepoda nauplii), and 1 Rotifera (*Lecane* sp.), while the rest are presented in the Supporting Information (Table S10; Figure S4). *Daphnia* sp. was significantly influenced by temperature (Table 5), showing lower densities in the T28 scenario (Figure 5). In the T20 scenario, *Daphnia* sp. showed a pronounced lufenuron-related decline in the two exposure levels after the second application; while in the T28, the population decline (although not significant) was already noticeable after the first application (Figure 5; Table 5). Impact of lufenuron was significantly higher in the T28 scenario as compared to the T20, showing a synergistic response on days 14 and 28 (Figure 5, Table 5). *Chydorus* sp. was not affected by temperature and, in both thermal scenarios, the population

showed a similar significant decline in the C2 exposure level after the second application (Figure 5).

Temperature affected the density of Cyclopoida, being significantly lower in the T28 scenario (Figure 5; Table 5). Cyclopoida were synergistically affected by high temperature and lufenuron after the first application (Figure 5, Table 5). Calanoida densities were slightly higher in controls of T28 as compared to T20 (Figure 5), although differences were not statistically significant (Table 5). Lufenuron effects were found to be similar between both scenarios (Figure 5). The density of Copepoda nauplii was significantly higher at T28 as compared to T20 in the first part of the experiment (Table 5; Figure 5). The effect of lufenuron on this group was faster at T28 than at T20 (Figure 5), and a synergistic effect was determined on day 4 (Table 5). However, the population recovery was achieved earlier at T28 than at T20 (Figure 5).
In general, rotifer densities in the chemical controls increased towards the end of the experiment. Such density increases occurred earlier and became slightly larger in the controls of the T28 scenario (Figure S4). In both scenarios, such Rotifera increases were more pronounced in the treatments that had been exposed to C2 (Figure S4). *Lecane* sp. was the taxon showing the clearest response (Figure 5).

3.4. Effects of drought on the response of zooplankton to lufenuron

3.4.1. Community-level responses

The PRC analyses comparing lufenuron controls showed no significant influence of drought on the zooplankton community over the entire experimental period (Monte Carlo test p=0.41). However, as it can be observed in the PRC, the effects of drought on the zooplankton community were more noticeable (but not significant) after desiccation occurred (Figure S3).

The PRC analyses performed to evaluate the effects of lufenuron under the drought and the constant water level scenarios showed a significant effect on the zooplankton community (Figure 4). The taxa most affected by lufenuron under the T28_Drought scenario (i.e., Copepoda nauplii, *Chydorus* sp., *Ceriodaphnia* sp., Calanoida) were found to be similar to those under T28 conditions. The results of the pairwise t-test show a similar response pattern in C1 and C2 of both environmental scenarios, with a full recovery being achieved in C1 between day 28 and 46, and on day 73 for the C2 exposure level (Figure 4).

The RDA with Monte Carlo permutation tests confirm that lufenuron was the main factor driving the response of the community and that drought had only a significant effect towards the end of the experiment, after the desiccation event (days 46-60). The interaction between lufenuron and drought was found to be only marginally significant on day 60 (Monte Carlo test, p-value=0.08; Table 4).

3.4.2. Population-level responses

The population-level analyses focus on the same taxa as in the first part of the experiment, while the rest are presented in Table S11 and Figure S4. *Daphnia sp.* densities were found to be relatively low during the water contraction phase of the T28_Drought scenario, which made the effect of lufenuron less noticeable as compared to T28 (Figure 5; Table 5). Population

dynamics of *Chydorus* sp. were similar in the T28 and in the T28_Drought scenarios, with a marked population decline in C2 occurring after the second lufenuron application (Figure 5). Drought had a significant negative effect on the density of this taxon on day 53, after desiccation and refilling occurred; however, the density increased rapidly afterwards (Table 5, Figure 5).

Cyclopoida showed slightly higher densities in T28_Drought during the water contraction phase (on days 14 and 21) as compared to T28 (Figure 5, Table 5). However, the lufenuron effects and the recovery patterns were very similar among both scenarios, and no significant stressor interactions were identified (Table 5). A sudden population decline was observed on day 60 in the C2 exposure level of the T28_Drought scenario, after refilling occurred. Calanoida densities and its response to lufenuron were found to be similar during the contraction phase in the T28 and T28_Drought scenarios (Figure 5). This taxon was significantly affected by desiccation, showing a population collapse in the controls as well as in all lufenuron concentrations and no recovery within the experimental period (Figure 5; Table 5). Copepoda nauplii were not significantly affected by drought and displayed a very similar response to lufenuron in both scenarios. It must be noted that this group partially recovered from lufenuron exposure during the complete desiccation phase in T28_Drought scenario and no significant treatment-related effects were identified during rewetting (Figure 5; Table 5). However, similarly to Cyclopoida, a density decrease in the C2 exposure level of the T28_Drought scenario was observed on day 60 (Figure 5).

Drought-related effects in Rotifera taxa such as *Lecane sp.* were generally not identified, and the indirect positive population-level effects caused by lufenuron exposure seemed to fairly correspond in the T28 and the T28_Drought scenarios (Figure 5; Table 5; Figure S4; Table S11).

Table 4. Results of the RDA analysis with Monte Carlo permutation tests (p-value) to assess the individual and combined effects of lufenuron and the environmental factors on the zooplankton community[a]

	Experimental part 1: T20 – T28			**Experimental part 2: T28-T28_Drought**		
Time (days)	**Lufenuron (L)**	**Temperature (T)**	**Interaction (LxT)[b]**	**Lufenuron (L)**	**Drought (D)**	**Interaction (LxD)[b]**
4	n.s.	**0.002**	**0.002A**	**0.002**	n.s.	n.s.
14	**0.002**	**0.002**	**0.034A**	**0.002**	n.s.	n.s.
21	**0.008**	**0.012**	n.s.	**0.014**	n.s.	n.s.
28	**0.002**	n.s.[c]	n.s.	**0.006**	n.s.	n.s.
46	**0.002**	n.s.[c]	n.s.	**0.014**	**0.036**	n.s.
53	**0.002**	n.s.	n.s.	**0.002**	n.s.[c]	n.s.
60	**0.006**	n.s.	n.s.	**0.002**	**0.02**	n.s.[c]A
73	**0.002**	n.s.[c]	n.s.[c]B	**0.034**	n.s.	n.s.

[a] Significant p-values are shown in bold. Significant (or marginally significant) interactions are defined as synergistic or antagonistic.

[b] Different letters denote A: synergistic interaction and B: antagonistic interaction.

[c] marginally significant differences 0.05<p-value<0.1.

*p-value<0.05.

n.s.: not significant.

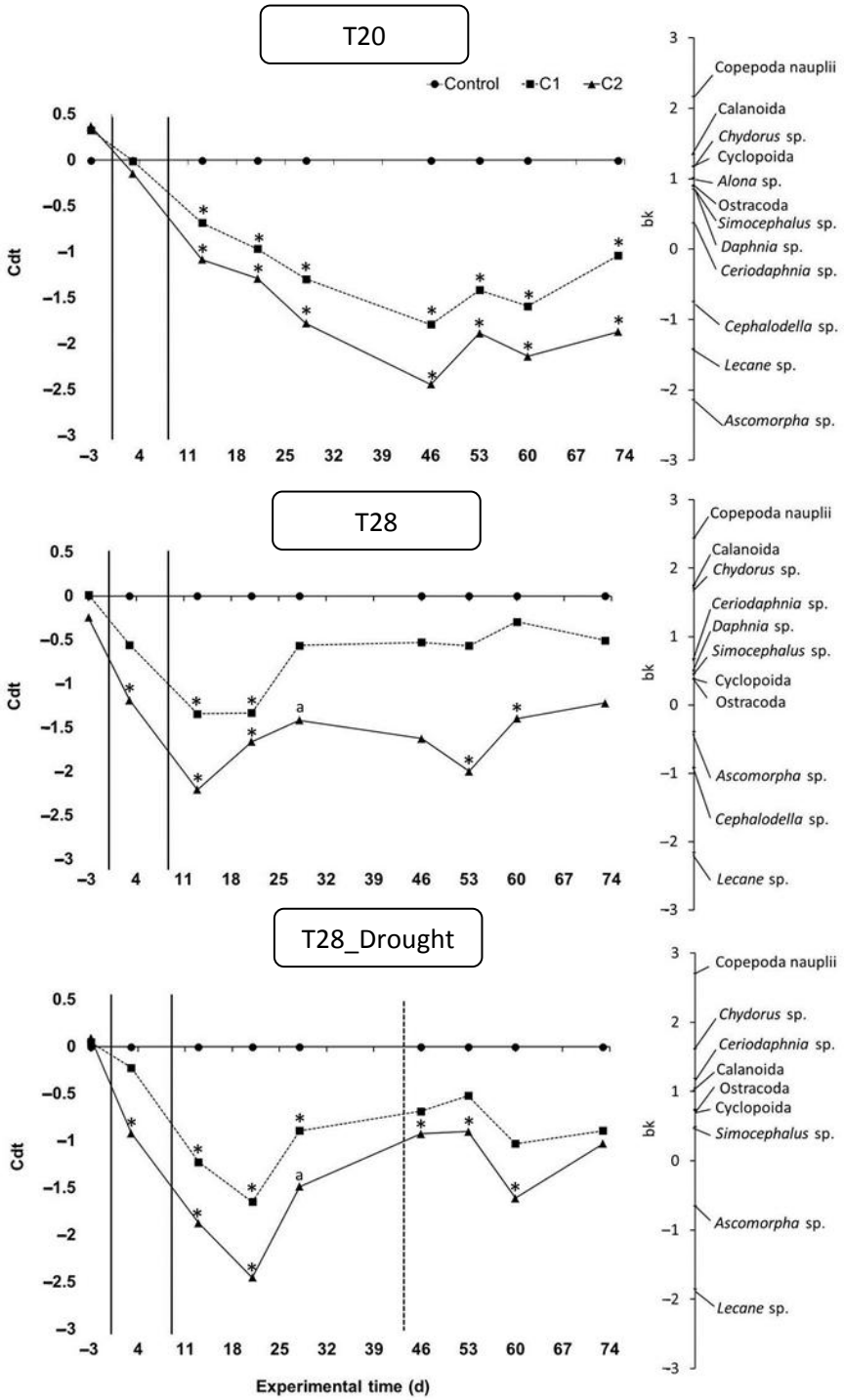

Figure 4. PRCs showing the impact of lufenuron on the zooplankton community under different environmental scenarios (i.e., T20, T28 and T28_Drought). The solid vertical lines indicate the two insecticide applications, while the dashed vertical line indicates the desiccation event. At T20, 39% of all variance could be attributed to the sampling time (displayed on the horizontal axis), while 36% could be attributed to lufenuron (of this, 50% is displayed on the vertical axis). At T28, 33% could be attributed to the sampling time, while 30% could be attributed to lufenuron (of this, 46% is displayed on the vertical axis). At T28_Drought, 30% could be attributed to the sampling time, while 31% could be attributed to lufenuron (of this, 47% is displayed on the vertical axis). In all 3 cases the Monte Carlo permutation tests showed a significant effect of lufenuron on the zooplankton community (p-value<0.05).*: significant differences with controls as result of the pairwise t-test, p-value<0.05. [a]: marginally significant differences 0.05<p-value<0.1. Taxa with calculated taxa weights (b_k) between 0.4 and -0.4 are not displayed.

Table 5. Results of the two-way ANOVA test (p-value) performed to assess the individual and combined effects of lufenuron and the tested environmental factors[a]

		Experiment 1: T20 - T28			Experiment 2: T28 - T28_Drought		
Endpoint	**Time (days)**	**Lufenuron (L)**	**Temperature (T)**	**Interaction (LxT)[b]**	**Lufenuron (L)**	**Drought (D)**	**Interaction (LxD)[b]**
Daphnia sp.	4	n.s	**<0.001**	n.s.[c]	n.s.	n.s.	n.s.
	14	**<0.001**	**<0.001**	**0.011A**	**<0.001**	**0.005**	**0.001**[1]
	21	**0.007**	n.s.[c]	n.s.	n.s.	n.s.	n.s.
	28	**0.001**	**0.006**	**0.027A**	**0.013**	n.s.	n.s.
	46	**0.009**	**<0.001**	**0.009**[1]	n.e.	n.e.	n.e.
	53	**<0.001**	**<0.001**	**<0.001**[1]	n.e.	n.e.	n.e.
	60	n.s	n.s.	n.s.	n.e.	n.e.	n.e.
	73	n.s.	n.s	n.s.	n.e.	n.e.	n.e.
Chydorus sp.	4	n.s.	n.s.	n.s.	n.s	n.s	n.s.
	14	**0.008**	**0.033**	n.s.	**0.006**	n.s.	n.s.
	21	**0.023**	n.s.	n.s.	**0.034**	n.s[c]	n.s.
	28	**0.029**	n.s.	n.s.	n.s.[c]	n.s.	n.s.
	46	**0.017**	n.s.	n.s.	n.s.[c]	n.s.	n.s.
	53	**<0.001**	n.s.	n.s.	**0.005**	**0.025**	n.s.
	60	**<0.001**	n.s.	n.s.	**0.009**	n.s.	n.s.
	73	**0.003**	**0.044**	n.s.	**0.007**	n.s.	n.s.
Cyclopoida	4	n.s.[c]	**0.005**	**0.04A**	**0.001**	n.s.	n.s.
	14	**0.001**	**<0.001**	**0.037A**	**<0.001**	**0.005**	n.s.
	21	**0.02**	**<0.001**	**0.046**[1]	**0.022**	**0.011**	n.s.[c]B
	28	n.s.	**0.002**	**0.005**[1]	n.s.	n.s.[c]	n.s.
	46	n.s.	n.s.[c]	n.s.[c]B	n.s.	n.s.	n.s.
	53	n.s.	**0.035**	n.s.	n.s.	n.s.	n.s.
	60	n.s.	n.s.[c]	n.s.	n.s.	n.s.	n.s.
	73	n.s.[c]	n.s.	**0.045B**	n.s.	n.s.	n.s.
Calanoida	4	**0.04**	n.s.	n.s.	**0.03**	n.s.	n.s.
	14	**0.001**	n.s.	n.s.	**<0.001**	n.s.	n.s.
	21	**<0.001**	n.s.	n.s.	**0.004**	n.s.	n.s.
	28	**0.003**	n.s.	n.s.	**<0.001**	n.s.	n.s.
	46	**<0.001**	n.s.	n.s.	**<0.001**	**<0.001**	**<0.001**[1]
	53	**<0.001**	n.s.[c]	**0.028B**	**<0.001**	**<0.001**	**<0.001**[1]
	60	**<0.001**	n.s.	n.s.	**<0.001**	**<0.001**	**<0.001**[1]
	73	**<0.001**	**0.021**	**0.01**[1]	n.s.	n.s.	n.s.
Copepoda	4	**0.001**	**0.019**	**0.005A**	**<0.001**	n.s.	n.s.
nauplii	14	**<0.001**	**0.019**	n.s.	**<0.001**	n.s.	n.s.
	21	**<0.001**	**0.044**	n.s.	**<0.001**	n.s.	n.s.
	28	**<0.001**	**0.001**	n.s.[c]B	**0.003**	n.s.[c]	n.s.
	46	n.s.	n.s.[c]	**0.019B**	n.s.	n.s.[c]	n.s.
	53	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	60	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	73	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Lecane sp.	4	n.e	n.e.	n.e	n.s.	n.s.	n.s.
	14	**0.024**	**<0.001**	**0.024A**	n.s.[c]	n.s.	n.s.
	21	n.s.	**<0.001**	n.s.	n.s.[c]	n.s.	n.s.
	28	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	46	**0.001**	n.s.	n.s.	**0.012**	n.s.	n.s.
	53	n.s.	n.s.	n.s.	**0.005**	n.s.	n.s.
	60	n.s[c]	n.s.	n.s.	**0.021**	n.s.	n.s.
	73	**0.005**	**0.005**	n.s.	**0.045**	n.s.	n.s.

[a] Significant p-values are shown in bold. Significant (or marginally significant) interactions are defined as synergistic or antagonistic.

[b] Different letters denote A: synergistic interaction and B: antagonistic interaction.

[c] marginally significant differences 0.05<p-value<0.1. (Significant p-values [p<0.05)

[1] Density declines in controls due to environmental factors (T or D) do not allow evaluating toxic effects.

n.s.: not significant. n.e.: not evaluated due to the absence of individuals.

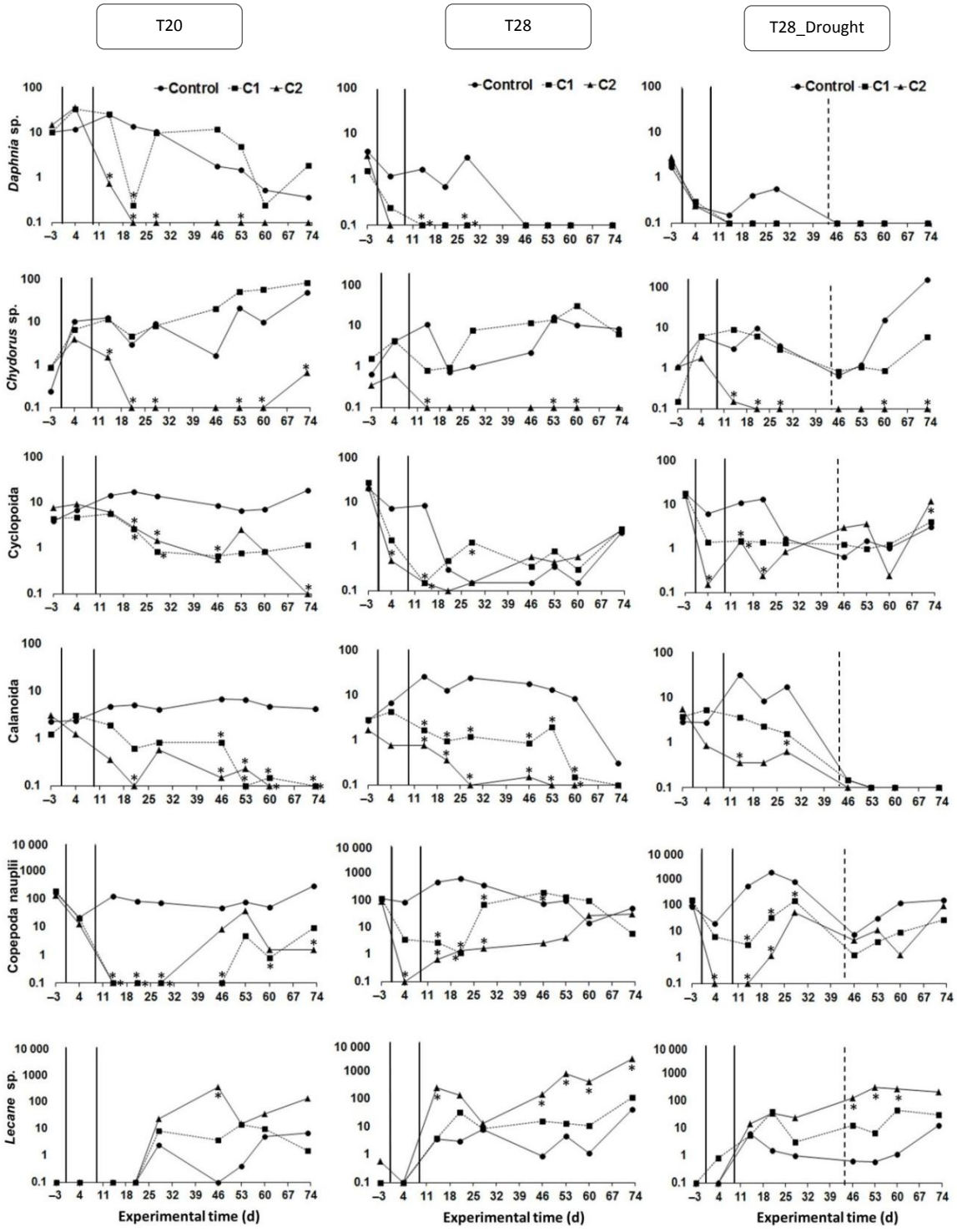

Figure 5. Density dynamics of the selected zooplankton taxa (individuals/L). Dots show the geometric mean of the densities in the three microcosm belonging to the Control, low (C1) and high (C2) lufenuron exposure conditions in the T20, T28 and T28_Drought scenarios. Solid vertical lines indicate the two insecticide applications, while the dashed vertical line indicates the desiccation event. *: significant responses to lufenuron treatment (pair wise t-test, p-value<0.05). T20=water temperature at 20˚C; T28=water temperature at 28 ˚C; T28_Drought=water temperature at 28 ˚C with desiccation.

4. Discussion

4.1. Effects of temperature and drought on water quality and lufenuron fate

The fast disappearance of lufenuron from water (Table 1; Figure 2) and the accumulation of this compound in the sediment were expected due to the hydrophobic characteristics of this compound (log Kow=5.12 at 25°C; EFSA, 2008). Our calculated $logK_{oc}$ values (2.80-3.02 mL/g) are close to the average $logK_{oc}$ value of 4.62 mL/g described in the literature (EFSA, 2008; McHenery, 2016).

López-Mancisidor et al. (2008a) found mean water DT50 values of 2 days in an outdoor experiment conducted in spring in the Netherlands. These values are comparable to the water DT50 values obtained in our study (1.8 to 3.0 days). The sediment DT50 values that we calculated (23 to 38 days) are also comparable to the range of average values reported by González-Valero (1994) for microcosms with sediments sourced from the rivers Po and Rhine (37.9 and 172 days, respectively), and the range reported by EFSA (2008) for two laboratory studies performed at 20°C under dark conditions (34 to 188 days). The faster disappearance of lufenuron from the sediment at high temperature observed in our study may be related to an increase in microbial metabolic activity with increased temperatures. In line with that, Vidali (2001) refers to doubled biochemical reaction rates with 10°C rise in temperature when describing the different factors affecting microbial degradation of contaminants.

We observed a clear remobilization of lufenuron from the microcosm sediment after the rewetting event. Metal mobilization after sediment air-drying and rewetting has been described in several studies (Caille et al., 2003; Vasile et al., 2010). Similarly, pesticide desorption from re-suspended sediments after flood events has been experimentally shown by Smit et al. (2008), suggesting that wet-dry phases of intermittent water bodies are key factors affecting chemical exposure patterns not only for benthic but also for pelagic organisms.

As expected, the physico-chemical conditions of our microcosms were significantly influenced by the tested environmental conditions (Table 3). Increased temperature influenced the oxygen solubility and affected pH, a pattern that has been observed in many aquatic systems (Carere et al., 2011; Arenas-Sánchez et al., 2016). The oxygen variation was relatively low, so that the values at T28 were not expected to impede the development of zooplankton taxa. Nutrient concentrations in our study were typical of oligo-mesotrophic systems with phosphorus limitation (Carey and Rydin, 2011). As water level decreased in the drought scenario, the systems showed a proportional increase in conductivity and in nutrient concentrations, similar to what has been described in the ecosystem contraction phase of intermittent water bodies elsewhere (Stanley et al., 1997; Caruso, 2002; Lake, 2003).

4.2. Influence of temperature on the zooplankton community

Water temperature has been demonstrated to influence the structure of zooplankton communities by modifying the habitat and serving as environmental filter for aquatic biodiversity (Van Wijngaarden et al., 2005; Knillmann et al., 2013). In our study, the community at T28 tended to be dominated by Rotifera and Calanoida, and showed a marked decrease in the density of Cladocera (*Daphnia* sp., *Alona* sp.) and other crustacea (Ostracoda, Cyclopoida). Such community differences may be principally related to differences in the thermal tolerance

range of the different taxa. Heugens et al. (2003) and Boeckman and Bindwell (2006) referred to the sub-optimal conditions for *Daphnia magna* and *Daphnia pulex* in ecosystems above 26°C, which may explain the lower density of *Daphnia sp.* at T28 in the present study. The Cyclopoida species in our study (*Tropocyclos prasinus, Diacyclops bisetosus, Microcyclops varicans*) were apparently less resistant to high temperatures, despite the reported capacity of this group to survive under warm or thermally variable conditions (Peacock and Smyly, 1983; Lopes et al., 2001). Calanoida (including a large variety of genera within Diaptomidae), is generally described to tolerate high temperatures and extreme environments (Cooney et al., 1983; Cooney and Gehrs, 1985; Beaugrand et al., 2002), which could explain the high densities observed in the T28 scenario. In relation to that, it could also be expected that at T28 the majority of Copepoda nauplii were Calanoida. Moreover, apart from having higher tolerance to high temperatures, Calanoida adults and nauplii, as well as *Lecane* sp. and other Rotifera, may have benefited from the predation and competition release resulting from the density decrease of Cyclopoida and *Daphnia sp.* (Soto and Hurlbert, 1991).

4.3. Zooplankton responses to lufenuron under different temperatures

The overall sensitivity of the zooplankton community to C1 and C2 was found to be similar under both thermal scenarios, with Copepoda and Cladocera being the most sensitive taxa. The high sensitivity of these zooplankton taxa to lufenuron is in line with the study by López-Mancisidor et al. (2008a), who observed similar short-term declines in freshwater mesocosms at average concentrations of 3 µg/L. Van Wijngaarden et al. (2005) also found similar sensitivity levels when testing the effects of the insecticide chlorpyrifos on planktonic communities under temperate and Mediterranean-like conditions. Other studies comparing insecticide threshold levels for invertebrate communities did not find marked sensitivity differences among invertebrate communities from different climatic zones (e.g. tropical vs temperate, Mediterranean vs Central Europe) (Daam et al., 2008; López-Mancisidor et al., 2008b). Based on the results of the present study and other model ecosystem experiments, we can conclude that temperature increases related to climate change, altough they may modify the community structure and the response of some taxa, are not expected to modify sensitivity thresholds for aquatic communities.

Despite the overall community sensitivities in the present experiment being similar, the onset time of toxic effects was considerably shorter at high temperature. This resulted in a synergistic community response during the first weeks after the insecticide application. Such response was mainly driven by the interaction between stressors on key zooplankton taxa (i.e., *Daphnia* sp., Cyclopoida and Copepoda nauplii). This is in line with a large body of literature associating high temperatures with increasing chemical toxicity to aquatic organisms (Cooney and Gehrs, 1985; Boechman and Bidwell, 2006; Knillmann et al., 2013; Camp and Buchwalter, 2016), mainly due to increasing metabolic rates and increasing uptake and body distribution of chemicals (Howe et al., 1994; Dyer et al., 1997). Such variation in the timing of the effects was the main driver contributing to the earlier density increase of the less sensitive Rotifera taxa, demonstrating that temperature may also influence the timing and magnitude of indirect effects in aquatic invertebrate communities.

The present study shows that the community exposed to lufenuron at T28 had a higher resilience (i.e., recovery capacity) than the community exposed at T20, at least in the low exposure level. Conversely, other studies evaluating the recovery of invertebrate communities to the insecticide chlorpyrifos under Mediterranean conditions showed delayed recovery times (López-Mancisidor et al., 2008b; Van Wijngaarden et al., 2005). However, such observations were associated with the proliferation of algal blooms at high temperatures, which was not the case in our oligo-mesotrophic systems. The possible reasons for the greater recovery capacity of the zooplankton community at high temperature in our study are multiple. First, as previously discussed, dissipation of lufenuron from the system was slightly faster in the high temperature scenario. Second, temperature enhances metabolism and reproduction of aquatic invertebrates, contributing to a higher population growth after exposure cessation (Bonada et al., 2007b; Daam et al., 2011), which may have contributed to increased Copepoda nauplii densities at T28 during the second half of the experiment. Finally, temperature is known to affect recovery of populations to toxicants by altering species interactions (Knillmann et al., 2013). In our experiment, temperature contributed to a density reduction or extinction of some dominant taxa such as Cyclopoida or *Daphnia* sp., which may have resulted in a lower degree of interspecific competition and a higher survival for other sensitive taxa (e.g. Calanoida).

4.4. Influence of drought on the zooplankton community

The zooplankton community did not display large differences between the systems with and without drought at high temperature, and showed a high resilience to the desiccation event. Zooplankton densities were in the majority of the cases maintained despite desiccation or increased rapidly after rewetting, indicating that the tested community was adapted to water scarcity conditions. Intuitively, it would have been expected that taxa densities increase due to lower water levels during the contraction phase, and decrease after desiccation. However, this was not always the case. On the contrary, the lowered water levels seemed to modulate the carrying capacity of the system and resulted in rapid adaptation of the zooplankton community, most likely due to intra- and interspecific competition mechanisms, as has been hypothesized by some authors (Lake, 2011; Datry et al., 2016). Although in the present study simulated drought resulted in significant changes in some physico-chemical variables, the zooplankton community did not display large differences related to that. The only taxon that showed a population decrease during the beginning of water contraction phase was *Daphnia sp.* This is in line with the results shown by Stampfli et al. (2013), who demonstrated a population decrease of *Daphnia sp.* in outdoor microcosms affected by recurrent water level fluctuations. The decrease in *Daphnia sp.* observed in our study can explain the slight increase in Cyclopoida density in the subsequent sampling days due to reduced food competition. Based on these results, it can be concluded that the impacts of the water contraction phase were generally mild and relatively short in time.

Some of the key taxa that recovered quickly after the desiccation event (e.g. *Chydorus sp.*) or that seemed to be rather unaltered by it (Cyclopoida, Copepoda nauplii) base their adaptation strategy on the production of resistance eggs and rapid hatching and population growth following rewetting (Arnott and Yan, 2002; Wyngaard et al., 1991; Zokan and Drake, 2015). In the present study, desiccation resulted in the complete disappearance of Calanoida. This was

rather unexpected since Calanoida are known to survive dry periods in temporary waters through the production of subitaneous or resting eggs (Williams, 2005). However, as indicated by Hairston and Van Brunt (1994), emergence times after dormancy can vary markedly across species and environmental conditions. In that study, the authors discuss how environmental requirements of different species may induce diapause and describe delayed emergence times of up to two years related with optimal environmental conditions or coexistence of competitor species. In this context, the fact that Calanoida did not recover after desiccation may have two possible reasons. First, physiological processes of adults might have been affected by the last part of the water contraction phase, not reaching optimal metabolic conditions as to lay resting eggs. Second, emergence of resting eggs under such experimental conditions may take longer than four weeks so that population increase after desiccation could not be observed within the experimental period. Further studies should be performed to evaluate the resilience of this taxonomic group to desiccation and to decide on its potential to be used as indicator of stress caused by water scarcity.

4.5. Zooplankton responses to lufenuron under drought conditions

The effects of lufenuron and the recovery capacity of the zooplankton community in the microcosms that were affected by drought were, in general terms, very similar to that with constant water level at high temperature. Thus, a synergistic or antagonistic effect of drought and lufenuron at the community-level was not identified. In line with our study, Stampfli et al. (2013) noted that the interactive effects of water level decreases and the insecticide esfenvalerate were additive, but found higher community sensitivity to the insecticide in the systems that were affected by the hydrological alteration just after the insecticide application. In their study, however, the hydrological alteration had a stronger effect on the community than in our study since it was not based on natural ecosystem contraction due to evaporation, but resulted from direct water extraction followed by zooplankton concentration through a net and return of organisms into the corresponding microcosm. Such practice may have contributed to an additional stress to some sensitive taxa and to an 'artificial' situation as regards to intra- and interspecific competition dynamics. Martin et al. (2014) evaluated the single and combined impacts of drought in mesocosms affected by evapotranspiration and a fire retardant compound in a complex zooplankton community, and demonstrated synergistic responses in diversity. However, the authors also concluded that zooplankton responses are context specific and difficult to predict in an environment with variable hydrology resembling natural conditions.

The interactive effects of drought and lufenuron on the zooplankton community were marginally significant on day 60. Such response is mainly explained by the density decrease of copepods (Cyclopoida and Copepoda nauplii) in the high exposure level of the drought scenario. The density decrease in this taxonomic group can be related to the lufenuron remobilization from the sediment compartment after refilling, during the rewetting phase. In the high exposure level the measured concentrations after remobilization (0.7 μg/L) were very close to the sub-lethal doses that cause moulting impairment to Cyclopoida nauplii (López-Mancisidor et al., 2008a; Macken et al., 2015), and therefore are expected to be the reason for the observed delayed density decline.

The drought condition evaluated in the present study must be considered as a ramp disturbance with marked hydrological phases (i.e., water contraction, desiccation, rewetting), which had different impacts on the zooplankton community. As shown by the statistical analysis, desiccation was the most stressful moment for some taxa. In our study, the complete desiccation event occurred after some dominant groups (e.g. Cyclopoida and/or Calanoida at naupliar stage) had partially or completely recovered from lufenuron exposure. This indicates that although both stressors were applied in the same experiment, the most stressful condition related to each of them (i.e., the peak exposure and the desiccation event) may have acted independently. As discussed by Moe et al. (2013), the magnitude of the interaction between two stressors largely depends on the timing of stressors with respect to the life-stages of sensitive organisms. Therefore, further studies should consider testing worst-case scenarios including pesticide applications at the moment of maximal contraction, pesticide drift depositions over dry sediments (containing resistant zooplankton stages) or drift depositions just after rewetting (containing early development stages). Further research should also consider testing the interaction among both stressors on macroinvertebrate communities using larger, outdoor experimental mesocosms.

5. Conclusions

This study describes the single and combined impact of environmental stress factors related to climate change and water scarcity (i.e., increased temperature and drought) and an insecticide on zooplankton populations and communities. The study shows that the tested environmental stress factors did not influence the overall sensitivity of the zooplankton community to the insecticide. However, increased temperature affected the timing of the response to the chemical and enhanced the recovery capacity of the zooplankton community. Such differences were principally related to the influence of temperature on chemical fate and on the metabolism and reproductive rates of sensitive taxa.

The zooplankton community exhibited a high resilience to the hydrological phases related to water scarcity (i.e., water contraction, desiccation). Chemical pollution resulted in similar direct and indirect effects in the zooplankton community regardless of the tested hydrological alterations. However such results may be influenced by the limited impact of drought and the time lag between the exposure peaks and the desiccation event. This study also shows that remobilization of chemicals adsorbed to dry sediments after rewetting may be a factor contributing to delayed population effects in intermittent water bodies.

Overall, this study evidences that temperature is an important factor to be taken into account in future risk assessment scenarios in (semi-)arid regions. It also highlights taxa that may be considered vulnerable to chemical pollution in scenarios dominated by increasing water temperatures and droughts. Finally, it shows the need for assessing multiple stressor combinations, paying special attention to the adaptation capacity of the affected aquatic organisms and the timing of stress in relation to their life cycle.

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Final discussion and conclusions

1. State of the art: 3 years later.

Overall, this thesis has contributed to a better understanding of the combined effects of water scarcity and pollution on aquatic communities by means of field monitoring studies (Chapter 3-5) and controlled studies at a laboratory scale (model-ecosystem or microcosm, Chapter 6). The results obtained have allowed an improved knowledge on several 'weak points' or knowledge gaps identified in Chapter 2. Results from other recent studies have also contributed to this. Still, some questions remain unsolved and suggestions for addressing them will be provided in this section.

Firstly, in Chapters 3 and 4, the concentration of a wide range of metals, pesticides and point source chemicals (e.g. pharmaceuticals, cosmetics, industrial compounds) were detected and quantified in the Tagus river basin. The influence of land use and time (seasonal variation) on their concentrations and other pysicho-chemical parameters were also analyzed. Results showed a major influence of land use and slight seasonal differences. Seasonal differences were associated to pronounced changes in pysicho-chemical parameters according to the contraction phase (i.e. summer) and to concentrations of some insecticides being higher in summer related to low flows, but also to application patterns. Mandaric et al. (2018) also assessed the concentration of pharmaceuticals in Mediterranean streams of the Llobregat river basin, impacted by WWTP or direct wastewater effluent and seasonal flow variation. They concluded that low flows during drought periods in small streams resulted in higher concentrations of pharmaceuticals after wastewater discharges (treated or untreated) due to the reduced dilution capacity of those systems, especially in effluent-dominated streams with almost absent upstream flow. In a more recent study, Mandaric et al. (2019) also detected higher concentrations of pharmaceuticals during drought periods (partial desiccation) in the Evrotas River (Greece); despite higher degradation efficiencies at the WWTP were also measured in this period. These studies provide new information on the influence of hydrological variability on the fate and exposure of organic micropollutants and on other water quality parameters in Mediterranean aquatic ecosystems. It should be taken into account that the monitoring study performed in this thesis only showed slight seasonal variation, but a larger number of sites per impact type (to cope with the variability within groups of sites), or a more frequent monitoring in fewer representative sites following an impact gradient (e.g. only considering edge-of-field water bodies affected by pesticides; or few upstream and downstream sites along a large river with sources of pollution specifically identified), as well as the use of passive sampling methods to detect peaks of pesticide pollution in different seasons; would improve the statistical robustness of the time variability assessment.

Mor et al. (2019) evaluated the response of macroinvertebrate communities to urban wastewater exposure in hydrologically variable Mediterranean streams of the Llobregat river basin, considering intra-annual flow variation with pronounced drought periods (some streams reaching complete desiccation and being effluent dominated). This study is similar to the field study performed in Chapter 5, despite in this chapter metals and pesticides were also assessed as chemical pollution, apart from nutrients and point source chemicals; while Mor et al (2019) focused on pharmaceuticals and nutrient pollution. Kalogianni et al. (2017) and Karouzas et al.

(2018), assessed the response of macroinvertebrate and fish communities -as well as diatoms, macrophytes, algae and bacteria- at different levels of water stress and pollution (agricultural, WWTP and oil mill processing wastewaters) in the Evrotas River, considering drought levels in summer for two consecutive years. Although these studies covered different types of pollution and ranges of water stress (e.g. intermittency or complete desiccation not considered in Chapter 5); overall results showed reduced taxonomic and functional richness levels of macroinvertebrates assemblages as a response to pollution, which was enhanced by drought or water scarcity periods. Pollution and drought had also a detrimental effect on functional diversity, but polluted waters with more heterogeneous substrate and higher flows seemed to benefit the functional structure of invertebrate communities tolerant to pollution (Chapter 5, Mor et al., 2019). Kalogianni et al. (2017) and Karouzas et al. (2018) also found that drought periods influenced migration of fish species with preference for fast flowing waters upstream. The cumulative effect of pollution and water scarcity in sites affected by wastewater pollution and drought enhanced the deleterious effects in abundance and richness on fish assemblages. Additionally, Karouzas et al. (2018) found that diatoms were negatively affected by pollution and water scarcity; only pollution had a negative effect on macrophytes; and filamentous bacteria and algae increased with high pollution, but when the water scarcity period occurred, only bacteria continued growing. Kalogianni et al. (2017) and Karouzas et al. (2018) considered land use of the drainage area as a descriptive variable, used as a proxy of anthropogenic pollution; while in Chapter 5 and in the study by Mor et al. (2019) biological responses were assessed simultaneously to the influence of hydrological variation on pysicho-chemical parameters and toxicity data of a wide range of contaminants (Chapter 3 and 4, and Mandaric et al. 2018, respectively). Apart from that, all these studies contribute to a better understanding of changes in taxonomic and functional composition on Mediterranean aquatic communities as a response to pollution under water stressed scenarios. This contributes to increase the scientific knowledge on the research field of multiple stressors in Mediterranean regions, despite further studies on stressors prioritization should be performed (Schinegger et al., 2018).

The model-ecosystem study performed in Chapter 6 allowed interpreting the interaction between individual factors (chemical: insecticide, and physical: increased temperature and drought conditions up to desiccation) in aquatic populations and communities. In this study, Mediterranean zooplankton communities responded faster to insecticidal stress under warm conditions, while drought did not result on differential responses with respect to high temperatures. Some taxa showed synergistic responses to combined thermal and chemical stress (e.g. *Daphnia* sp., Cyclopoida), slight delayed effects most likely due to the insecticide remobilization after rewetting (i.e. Cyclopoida), or were drastically affected by physical stress alone (e.g. Calanoida after complete desiccation). Still, the community showed a high recovery capacity to chemical stress at high temperatures and under drought conditions. This study contributes to the need of invertebrate experimental studies on the combined effects of water scarcity and pesticide pollution in edge-of-field water bodies, and provides information on the recovery capacity of zooplankton populations and communities under those circumstances. Another indoor microcosm study performed by Romero et al. (2018) assessed the individual and combined effects of physical (increased temperatures, desiccation) and chemical (herbicide: diuron; antibiotic: erythromycin) on biofilm communities, including the assessment of their recovery capacity (as recommended for short-term responses in Chapter 2). Sediments were

not included in these model-ecosystems. They found that physical stress (temperature and desiccation) was the main driver of biofilm community functions alteration, with desiccation having a negative effect on photosynthetic capacity and high temperature promoting an increase in photosynthetic efficiency. When chemicals were added to the interaction, most of the significant interactions were antagonistic, especially when temperature was involved. Temperature seemed to mitigate individual effects of other stressors. However, results also showed a slight proportion of synergistic interactions, which can be highlighted in the context of climate change and the risk of communities becoming more sensitive to pollutants. The recovery of the biofilm communities based on those endpoints was not reached within the assessed recovery period (i.e. 40 h).

All new studies may contribute to the development of site-specific approaches (i.e. based on specific conditions of ecosystems or regions), as required by the scientific community (Ippolito et al., 2012; EC, 2013) and by the WFD (Directive 2000/60/EC). These approaches aim at identifying more realistic scenarios of ecosystems' vulnerability to stressors, to be used in improved models or management measures. Finally, all of the studies were performed at a community level, and some at population level as well (Chapter 6), as recommended for the development of risk assessment approaches (Ippolito et al., 2010). However, the complete understanding of those responses is complex and remains challenging. A better understanding of food web interactions could help disentangling community responses by identifying direct and indirect responses to stress of specific taxa (or their associated traits). However, in complex communities this is difficult to distinguish. A further step could be performing experiments with simplified communities including few taxa with different sensitivities and tolerances (at least to individual stressors). Still, the most representative degree of complexity of these simulations is still under investigation (Rico et al., 2016b). Sensitivity analyses of models and particular food webs scenarios can be used to identify scenarios with fundamental repercussion for ecosystems (De Laender et al., 2015). Finally, Chapter 5 and Mor et al. (2019) have provided valuable information on functional characteristics of aquatic communities, adding knowledge on the mechanistic and ecological processes driving community responses to pollution under different drought conditions. This can contribute to more effective and efficient management measures and the development of ecological models for these multiple stressed Mediterranean aquatic ecosystems, identifying key vulnerable or resistant and resilient taxa in these variable and highly impacted ecosystems (see section 7.3).

2. Potential risk of pollutants in Mediterranean basins.

The WFD requirement of more site-specific approaches on the evaluation of ecological status of surface waters includes the determination of specific substances that pose high toxicity risk at a more regional level (Directive 2000/60/EC; Directive 2013/39/UE). In that sense, taking into account the influence of hydrological variability on the concentration of pollutants in Mediterranean rivers (López-Doval et al., 2013) is essential for identifying potentially hazardous substances in these highly variable aquatic ecosystems. Moreover, several authors have referred to the need of assessing not only established priority compounds by the WFD, but also other regular and emergent compounds whose toxicity might be underestimated or unknown (Silva et al., 2015; Blasco et al., 2016). Chapter 3 and 4 of this thesis focused on the identification of a wide range of compounds posing a potential toxic risk for aquatic ecosystems

in the Tagus river basin and the assessment of their spatio-temporal distribution. However, most of the existing studies identifying potentially toxic compounds (regulated and emergent) in different Mediterranean basins (Table 1) do not consider variation over time as a factor to be assessed, and just provide a snap-shot of contamination over the emission continuum. Mor et al. (2019), based on data from Mandaric et al (2018), assessed the level of toxicity in each sampling site over time; however, the contribution of each compound to the total toxicity of the mixture was not described. A suggestion resulting from this thesis is that once compounds with high potential toxicity risk have been identified, the variation of risk over time should be also taken into account, particularly for diffuse pollution compounds (i.e. pesticides).

Comparing the findings in Chapter 4 with other studies performed in different Mediterranean basins (Table 1) allows evaluating whether the climatic region might have an influence on some patterns of chemical use, emission and ecological risks associated to them. These studies based the selection of compounds on their ecological risk or ecotoxicity to standard test species, and assessed the detection frequency to cover as much as possible the risk of chronic toxicity (e.g. Kuzmanović et al., 2015), or alternatively considered assessment factors related to chronic risk assessment (Ccanccapa et al., 2016; Chapter 4). Tsaboula et al. (2016) performed a more exhaustive toxicity analysis, evaluating not only standard test species but all available toxicity data for aquatic organisms (algae, invertebrate and fish) compiled in two databases, selecting the lowest toxicity value (covering three trophic levels) per compound. Apart from the frequency of detection and exceedance of the toxicity threshold; they also took into account the fate of pesticides to perform an acute or chronic risk assessment, and their persistence, their spatial distribution, bioaccumulation and endocrine disruption potential, as factors considered to calculate a final 'level of risk for the environment' by means of a scoring system.

Within the most potentially toxic compounds, in Table 1, compounds with high frequency of spatial and temporal detection have been highlighted. The comparison of these studies provides some interesting results. First, the majority of the Mediterranean basins studied so far are Iberian basins, with only one study performed in Greece. Silva et al. (2015) assessed the levels of pollution for regulated priority compounds in three Portuguese basins and prioritized compounds on the basis of frequency of exceedance of existing or derived Environmental Quality Standards (EQS). Nevertheless, no similar studies were found for other (semi-)arid regions around the globe. Second, it can be concluded that (when evaluated), metals were responsible for most of the toxicity to all biotic endpoints (López-Doval et al., 2012, Chapter 4), especially Zn and Cu. Apart from that, pesticides were the most toxic and frequently detected compounds in all studied basins, with some similitude between basins that are described below.

Primary producers were highly sensitive to herbicides and some fungicides, with some comparable results between basins and/or studies. Diuron was identified as a highly hazardous compound in the Tagus river basin; while in others such as the Llobregat or the Guadalquivir river basins, this compound had lower occurrence than others, but was ranked within the ten compounds with higher risk for algae (Kuzmanović et al., 2015). The fungicide prochloraz seemed to pose a high risk to algae in the Jucar river basin in two studies monitoring contaminant levels in that basin between 2010 and 2013 (Kuzmanović et al., 2015; Ccanccapa et al., 2016). The Pinios river basin presented different compounds negatively affecting primary producers (Tsaboula et al., 2016), in comparison with the chemicals detected in Iberian river

basins. However, the majority of those were also herbicides and some fungicides. From all highlighted compounds in Table 1, only Hg, diuron, terbutryn and alachlor are included in the list of WFD priority compounds. Zn, Cu, terbuthylazine and metolachlor are regulated in the specific list at national level in Spain and linuron in Greece. It is also remarkable that the use of terbutryn and metolachlor with agricultural purposes is not approved in the EU and in any Member State (Regulation EC 1107/2009; PPDB database, https://sitem.herts.ac.uk/aeru/ppdb/en/index.htm).

In the case of invertebrates, insecticides were the most toxic compounds after metals. Chlorpyrifos seems to be a highly toxic and frequently detected compound in almost all basins (Table 1). Carbendazim was identified as potentially hazardous substance in the Tagus, Jucar (2010-2013) and in the Pinios river basins. However, in that last case, carbendazim had lower environmental risk than other compounds due to its low frequency and level of exceedance, as well as specific spatial distribution. Diazinon seems to be also a potentially hazardous compound, being present in most of the basins with intermediate to high risk (especially in the Jucar river basin), despite its frequency of detection was slightly lower. In the Pinios river basin, chlorpyrifos and diazion were also identified as potentially highly toxic compounds with high frequency of detection (Tsaboula et al., 2016). However, they are not included in Table 1 since in that study the number of identified compounds is large, and these two compounds were included in the second group of potentially toxic compounds based on analytical inadequacies (the first group was selected for this analysis). From the highlighted compounds, only Hg, chlorpyrifos and chlorfenvinphos are included in the list of WFD priority compounds, and Zn and Cu at a national level in Spain. Moreover, the insecticides chlorfenvinphos, ethion, diazinon and dichlofention are not approved for agricultural use neither at a European nor at a national level (Regulation EC 1107/2009; PPDB database). Other interesting result is that using non-standard test species highlight the risk of some pesticides, which might be underestimated by applying standard procedures, as it is the case of the neocotinoid imidacloprid. This compound was identified as highly toxic in the Pinios river basin based on Diptera toxicity data (*Chironomus tentants*), with a PNEC equal to 0.17 µg/L and a maximum concentrations of 0.3 µg/L, which is comparable to the maximum concentrations measured in Chapter 3 in several polluted sites. However, in Chapter 4 imidacloprid could not be identified as a potentially toxic compound due to the use of standard toxicity data for *Daphnia* sp. Moreover, in a mesocosm study performed in our group (Rico et al. 2018a), ecological thresholds for this compound under Mediterranean conditions is suggested to be lower than the above PNEC, based on the high toxicity of this compound to mayfly larvae and Chironomids.

Fish were potentially affected by insecticides, fungicides and herbicides. The most remarkable result is that chlorpyrifos also resulted in high potential ecotoxicological risks for these organisms in most of the studied basins. Also carbendazim was identified as potentially toxic for those organisms, apart from invertebrates, in the Jucar river basin (Ccanccappa et al., 2016). From the list of highlighted compounds, only chlorpyrifos and trifluralin are included in the list of WFD priority compounds. Dichlofention, as well as trifluralin, are not approved for agricultural use at a European level and in any Member State (Regulation EC 1107/2009; PPDB database).

Despite less acutely toxic, some point source contaminants (i.e. pharmaceuticals such as valsartan, or life-style compounds such as caffeine or its metabolite paraxantine) were also identified as potentially hazardous compounds for some groups of organisms. Caffeine was one of the compounds with potentially higher risk for algae based on the risk index elaborated by Kuzmanović et al. (2015). This was mainly due to its high frequency of occurrence (between 84 to 100% of the samples) rather than to its acute toxicity (EC50 of 760 µg/L for algae, 46000 µg/L for *Daphnia* sp. and fish; with maximum concentrations of 3.2 µg/L). In Chapter 4, measured concentrations reached maximum levels of 5.8 µg/L and 15 µg/L, depending on the sampling method (grab and POCIS samples, respectively). However, caffeine was not identified as a high risk compound, which can be related to the fact that this study only considered toxicity and not frequency of detection. The caffeine's metabolite, paraxantine, was quantified at higher concentrations than caffeine in that chapter, resulting in potential toxic chronic effects for fish. Due to the high concentrations that these compounds need to reach to produce an acute toxic effect, chronic or behavioral effects are rather expected (Valcarcel et al., 2011, Rodríguez-Gil et al., 2018). Finally, due to their nature (biologically active compounds), it is more likely that pharmaceuticals could have long-term effects on reproductive, endocrine or developmental dysfunctions due to chronic exposure, rather than direct acute toxic effects. As stated in Chapter 4, the evaluation of the chronic risk of these compounds estimated from acute toxicity data (TU) should be interpreted with caution. Further development of ecotoxicological studies for this type of chemicals are needed, taking into account their specific mode-of-action and sub-lethal effects on appropriate biological endpoints.

The concentrations of some priority compounds frequently exceed the EQS (MAC-QS) established by the WFD (EC, 2011; Directive 2013/39/EU). The concentrations considered were the maximum annual concentrations, or the derived as a time weighted average from passive samplers in summer, in the case of pesticides in Chapter 3 (Tagus river basin). For specific substances regulated in Spain (e.g. metolachlor), the assessment was performed as indicated in the corresponding Spanish national regulatory document (RD 817/2015). None of the specific substances measured exceeded the regulatory threshold. Nevertheless, the measured concentration of Hg and chlorpyrifos in Chapter 3, were frequently above the regulatory threshold in summer (i.e. drought period). Silva et al. (2015) also identified chlorpyrifos as one of the priority compounds more frequently exceeding this threshold in the Portuguese section of the Tagus river basin. Other studies (López-Doval et al., 2012; Tsaboula et al., 2016) did not provide information on the frequency of exceedance. However, some compounds exceeded the limits in at least one occasion. Chlorpyrifos was once more a compound detected over the threshold in the Ebro and Jucar river basins, and in the Pinios river basin. Hg seemed also to rank as priority compound in the Ebro and Guadalquivir river basins. Other compounds quantified above the limit were Ni and nonylphenol in the Ebro river basin; trifluralin in the Llobregat river basin; and Cd and simazine in the Guadalquivir river basin. In the Pinios river basin, high maximum values were also found for alachlor, atrazine, terbutryn, trifluralin, endosulfan or cypermetrin. The priority compounds measured in Ccanccapa et al. (2016) never exceeded the EQS due to the very low concentrations found, which can be a result of the sampling performed out of the application period, and the use of grab instead of passive sampling methods.

The inclusion in specific management plans at a basin level of potentially hazardous substances identified in each basin may be considered. Previously to that, the high estimated risk of those substances should be validated. To do that, monitoring schemes could be designed as a support and validation tool of expected chemical use and emissions, considering agricultural practices, climatic and hydromorphological characteristics of the basin. This would help defining the more cost-effective scheme covering worst-case exposure scenarios. For substances such as chlorpyrifos or Hg, frequently detected above the threshold, urgent management measures should be put in place, especially for the periods were risk could be higher (i.e. drought periods). Some recommendations are: (1) to revise current procedures for good agricultural practices related to chlorpyrifos in the context of a Mediterranean climate, (2) to identify sources of Hg pollution or physico-chemical conditions favoring the mobilization of this metal, (3) to revise possible uncontrolled metal emissions, or (4) to apply contention measures to avoid pollution by run-off in detected high risk areas. The sources of pollution for those pesticides not approved for agricultural use should be investigated. Some compounds could be used with non-agricultural purposes (e.g. terbutryn used for aquatic algae control) or veterinary purposes such as diazion or chlorfenvinphos, and others could appear as transformation products of other pesticides (e.g. ethion). Attention should be paid to these findings, especially for those highly toxic and whose use is supposedly forbidden for years, such as dichlofenthion (EC, 2002). Finally, it should be taken into account that these compounds do not appear alone in the environment, and its interactions with other compounds present in the mixture should assessed. The studies explained in this section that considered the TU approach, applied a simplistic approach to get an estimation of this additive toxicity of compounds, which is one of the few available methods to assess mixture toxicity on freshwater organisms. Still, when this toxicity has been detected, and the main substances contributing to it have been identified (no more than 5, as proved in Chapter 4 and López-Doval et al., 2012), combined toxicity assessments should be performed to evaluate possible synergistic (or antagonistic) responses.

3. Biological responses to chemical stress under drought conditions. Is this properly considered in regulatory chemical risk assessment?

Retrospective ecological risk assessment of surface waters (WFD)

In light of the requirements of the Water Framework Directive (WFD), which aim for a good chemical, hydromorphological and biological status of all water bodies in Europe by 2027, interpreting possible interactions between chemicals and other kind of abiotic stressors is a must (Schinegger et al., 2016; Carvalho et al., 2019). This section evaluates whether the influence of variable hydrological conditions in Mediterranean aquatic ecosystems is properly covered in current assessment procedures to determine the ecological status of surface waters.

The studies described above (Chapter 5; Kalogianni et al., 2017; Karouzas et al., 2018; Mor et al., 2019) have repeatedly shown that macroinvertebrate richness and diversity at a taxonomic and at a functional level were reduced as a response to the combined effects of pollution and drought or water scarcity periods. However, this was not the case for the IBMWP index.

Table 1: Overview of studies performing risk prioritization of contaminants in Mediterranean regions.

Basin	Sampling time	Compounds monitored	Risk assessment method	Potentially toxic compounds			Reference
				Primary producers	Invertebrates	Fish	
Tagus (Central Spain)	Spring, summer, autumn 2016	7 metals and 52 organic compounds (pesticides and point source)	TUs[a]	**Zn, Cu,** Hg **Diuron, terbutryn,** simazine[1], terbuthylazyne	**Cu, Zn,** Hg **Pirimicarb, carbendazim, valsartan,** chlorpyrifos[2], diazinon, acetaminophen	**Cu, Zn** **Paraxantine,** chlorpyrifos[2]	Chapter 4
Jucar (East Spain)	Autumn 2010-2013 15 days sampling in simultaneous years, in Jucar and Turia Rivers	50 pesticides	RQ[b] (EC/PNEC*)	**Metolachlor, prochloraz, imazalil,** hexythiazox	**Chlorfenvinphos, carbendazim,chlorpyrifos, ethion, diazinon, imazalil, prochloraz**, azinphos methyl, dichlofenthion, fenitrothion, hexythiazox, malathion, methiocarb, pyriproxyfen, thiabendazole	**Chlorpyrifos, carbendazim, imazalil, thiabendazole,** azimphos methyl, carbofuran, chlorfenvinphos, dichlofenthion, ethion, metholachlor, prochloraz, pyriproxyfen	Ccancacapa et al. (2016)
Llobregat (North-East Spain)	2008-2010. Available monthly data	45 WFD priority compounds	TUs[c]		**Zn, Cr, Cu**, Se		López-Doval et al. (2012)
Ebro (North-East Spain)					**Zn, Cu,**Cr, Ni, Pb, chlorpyrifos, desethylatrazyne, diuron, N-Methylalanine		
Jucar (East Spain)					**Zn,** Cr, Cu, **chlorpyrifos**, diuron		
Guadalquivir (South Spain)					**Zn,** Cu, Cr, benz(a)thracene, diuron, As, Fe, diazinon, endosulfan		
Llobregat (North-East Spain)	Autumn 2010-2011	200 organic compounds (pesticides and point source)	TUs[d]	Diuron, caffeine, triclosan, sertraline	**Chlorpyrifos**, diazinon, carbofuran, octylphenol, azinphos ethyl, nonylphenol, NP1EC, NP2EO	Chlorpyrifos, NP1EC, nonylphenol, NP2EO	Kuzmanovic et al. (2015)
Ebro (North-East Spain)				Phrochloraz, caffeine, pyriproxyfen, sertraline, terbutryn	**Chlorfenvinphos, chlorpyrifos, dichlofenthion**, diazinon, NP1EC	Dichlofenthion, chlorpyrifos, pyriproxyfen	
Jucar (East Spain)				**Prochloraz**, pyriproxyfen, caffeine, sertraline	**Chlorfenvinphos, chlorpyrifos, dichlofenthion**, diazinon, **ethion**, parathion-ethyl, octylphenol, pyriproxyfen, malathion	**Dichlofenthion**, chlorpyrifos, pyriproxyfen, imazalil	
Guadalquivir (South Spain)				Diuron, caffeine	**Chlorpyrifos**, diazinon, chlorfenvinphos, malathion, NP2EO, nonylphenol	Chlorpyrifos	
Pinios (Central Greece)	August to December 2012 periodical monitoring	302 pesticides	RQ[e] (EC/PNEC†)	**Prometryn, linuron, alachlor, acetochlor, etridiazole, fluorochloridone, s-metholachlor, terbuthylazine, fluometuron,** atrazine, terbutryn, pendimethalin	Thiacloprid, methomyl, carbendazim, imidachloprid	**Trifluralin**, chlorpropylat	Tsaboula et al. (2016)

[1] Mainly in spring, dominating toxicity alone; [2] Only detected in summer in passive samplers (POCIS).

*PNEC calculated for chronic toxicity; † PNEC for chronic or acute toxicity as a function of compounds' DT50.

[a] Pollutants explaining on average >90% of the total toxicity of the mixture, considering samples with TU>0.001 (i.e. chronic toxicity). Highlighted compounds present in >2 sampling campaigns, in >60%of most polluted sites.

[b] RQ>1 for mean and/or maximum environmental concentration (EC) in any sampling campaign. Highlighted compounds with RQ>1 for mean or maximum concentration in >3 sampling campaigns.

[c] Pollutants explaining >95% of the total acute toxicity (TU) of the mixture in different sites identified as the most polluted per basin in 2008. Maximum environmental concentration (EC) per year selected for TU calculations. Highlighted compounds present in >2 sampling campaigns, in >60% of most polluted selected sites.

[d] Pollutants contributing in >5% to the total Risk Index. High % indicates that a compound was frequently found at high TU. Highlighted compounds with RI>20%.

[e] RQ>1 for maximum environmental concentration or EC over the monitoring campaign. Highlighted compounds with high Level of Environmental Risk (>50% of the maximum score), calculated as a proportion of frequency of exceedance, extent of exceedance, spatial distribution and fate and behavior in the environment.

The IBMWP is a qualitative index based on presence/absence of different macroinvertebrate families with scores based on higher (maximum 10) or lower tolerance (minimum 0) to pollution (Alba-Tercedor et al., 2004), which may not be sufficient to show temporal variation associated to hydrology or other community dynamics. However, the temporal differences observed when other biotic indexes were assessed, suggest that their inclusion in the vulnerability assessment of invertebrate communities could be useful to detect temporal changes in community responses due to varying hydrological conditions. Biological monitoring is preferably performed in spring as recommended by the national environmental regulatory agency, being considered as the most optimal sampling period due to maximum diversity levels reached (MAGRAMA, 2013). Nevertheless, since intensified detrimental effects of pollution on invertebrate communities were observed during drought periods, a more protective approach covering the combined risks of drought and high pollution levels, would be performing biological quality assessments in late summer or early autumn (before drying).

The physico-chemical (excluding specific chemicals' thresholds, EQS) and biological status assessments are performed in relation to established reference conditions for different river types, defined according to the WFD guidelines (Annex II). In Spain, reference conditions for a set of Mediterranean like rivers with different substrates and altitude conditions are established (RD 817/2015). Sánchez-Montoya et al. (2009a) performed a study to validate reference sites in Mediterranean rivers with biological quality assessment (IBMWP) performed in spring, summer and autumn, and all values being above the established standard threshold of 100. Moreover, Sanchez-Montoya et al. (2009b) concluded that seasonal variation of macroinvertebrate communities and the metrics used for their evaluation were very low (<15% coefficient of variation), with the commonly used IBMWP showing one of the lowest variation values. That 15% of variation would not transform a site with very good quality status into a moderate status, based on currently established IBMWP reference conditions. This could justify that biological measurements performed in spring should be representative enough for the assessment of the ecological status of Mediterranean surface waters. Nevertheless, on the basis of the above results (i.e. other indexes responding to changes in hydrological conditions) and an expanding climate change and water scarcity scenario (Sabater and Tockner, 2010; IPPC, 2014), this concept should be reevaluated. The study by Sanchez-Montoya et al. (2009b) was performed between 2003 and 2005 and it is likely that some changes in Mediterranean community responses could be observed after a 15 years period. Before making any conclusions, these potential changes could be evaluated by assessing available historical biological and hydrological data on Mediterranean reference sites defined by Sanchez-Montoya et al. (2009a), as well as on reference sites for other river types observed to be affected by drought and water scarcity conditions in this study, as it is the case of RT-05 (Ríos Manchegos). The assessment of biological responses should be rather based on the taxonomic or functional indexes described above that responded to hydrological variation. This would help confirming whether the above results on the combined effect of pollution and water stress were related to an increased pollution impact with no actual need to modify reference condition values, but that would require the establishment of more restrictive water quality thresholds during drought periods. Otherwise, the update of reference conditions for drought periods would require the revision of class thresholds for biological indexes as a function of an impact gradient, once the degree of influence of drought and pollution is better identified.

Hydrological conditions are daily monitored and regulated by the corresponding management agencies based on basin specific Hydrological Management Plans, as required by the WFD (RD 907/2007). These Management Plans regulate hydrodynamic patterns of surface waters and their connectivity with groundwater, attending at differences between permanent, intermittent and temporary rivers. They should also define ecological flows which are the minimum flow levels to be respected to maintain the functionality and structure of aquatic ecosystems. The parameters measured to determine these ecological flows depend on the management agency in charge. In the Tagus river basin, the determination is based on accurate hydrological models and at least the influence of those flows in fish populations and riparian vegetation (RD 1/2016). Based on the importance that temporal variability seems to have on the measured pysicho-chemical and biological parameters, determining ecological flows for different hydrological periods (i.e. base flow, contraction and expansion), considering comparable quality elements (e.g. including invertebrates in the biological evaluation, chemical status) as in the determination of ecological status for different river types and sections, would be recommendable to define more ecologically realistic flows in Mediterranean rivers.

Prospective ecological risk assessment of edge-of-field pesticides (ERA)

Currently, prospective risk assessment procedures are based on a combination of effect and exposure assessment tiered studies (from more conservative to more realistic), whose results are combined in the final assessment, but are performed separately. The exposure assessment of pesticides in European surface water bodies is based on a series of exposure scenarios for several climatic regions, crops and water bodies (i.e. ditches, streams, ponds), that were developed by the Forum for the Coordination of Pesticide Fate Models and Their Use (FOCUS) Surface Water Group (FOCUS, 2001a, 2001b). These FOCUS scenarios have been developed and used in risk assessment in the EU (with several updates and developments) for more than 10 years (FOCUS, 2001a, 2001b). Nevertheless, some suggestions for further developments can be discussed. The first 2 steps of the exposure risk assessment are highly conservative. Step 1 is the worst-case scenario simulating a single loading (sum of individual applications) that will enter a static water body of 30 cm depth. Step 2 refine the procedure by simulating sequential applications in which a first drift to the water body occurs, followed by runoff/erosion/drainage input four days after the last application, differentiating between the region of use (Northern or Southern Europe), season of application, and the crop interception. At step 3, 10 FOCUS scenarios are described considering all relevant entry routes, appropriate target crops, representative surface water body types, topography, climate, soil type and agricultural management practices. The scenario that applies to Mediterranean conditions is the D6 Thiva (Greece) scenario, which covers areas with warm Mediterranean climate and moderate precipitation and select field ditches as representative water body type. At this step, hydrological dynamics are considered through the TOXWA model, which simulates water balances considering incoming and outgoing fluxes over time. The incoming fluxes are based on upstream discharges (base flow component plus runoff or drainage component), the runoff or drainage fluxes from the neighboring field; and, as appropriate, the precipitation and upward seepage through the sediment. The outgoing fluxes are composed of the outgoing discharge of the water body and, if considered, a downward leakage through the sediment. However, this simulated temporal variation never reach levels below 30 cm, as all scenarios try to match as

much as possible the scenarios defined for the effect assessment of pesticides (see below). The TOXWA model also considers different degradation pathways (hydrolysis, photolysis and biodegradation as a function of temperature) and dissipation processes into organic surfaces (sediment, suspended matter, macrophytes); but the remobilization of compounds from the sediment after flooding events, characteristic of temporary waters or highly variable Mediterranean water bodies, are currently absent. Step 4 consist on exposure simulations based on Step 3 scenarios, including mitigation measures, refined fate input parameters, or more local/regional landscape and input parameters. Still, at this high-tier step, desiccation or extremely variable water flows, as well as remobilization, are not considered. In relation to that, exposure scenarios including desiccation and hydrological variation over time in the receiving water body should be better developed at higher-tiers (step 3 and 4).

Model-ecosystem studies reproducing more ecologically realistic scenarios are required for higher-tier effect assessment (EFSA, 2013). Current assessment guidelines (EFSA, 2013) recognize the importance of variable flows on pesticide fate processes and effects on aquatic organisms; however, this document also refers to the lack of procedures to assess the effect of these pollutants under extreme drought conditions, with a minimum simulated depth of 30 cm. In this regard, the study performed in Chapter 6 is one of the most novel studies in high-tier environmental risk assessment, simulating chemical risk in Mediterranean temporary water bodies.

Apart from the need of more realistic scenarios in high-tier risk assessment with model-ecosystems, one of the biggest challenges is the development of ecological models assessing the magnitude of effects in different spatio-temporal conditions, the recovery potential of exposed populations, and the potential indirect effects (Brock et al., 2010; Brock, 2013; EFSA, 2014). For the development of ecological scenarios to be included in those models, representative focal taxa need to be identified within key driver taxonomic groups (algae, macrophytes, invertebrates and vertebrates) and the habitats we intend to protect, under the environmental conditions that represent realistic worst-case scenario. In line with that, Chapter 6 contributed finding sensitive and resistant taxa to lufenuron (insecticide) exposure under Mediterranean conditions. Some of those sensitive taxa were *Daphnia* sp. or Cyclopoida, which showed synergistic responses to the combined effects of high temperature and lufenuron; but, in general, Cyclopoida and Copepoda nauplii showed high recovery capacity to those conditions. Other taxa such as Calanoida showed tolerance to high temperatures, but there was a drastic negative effect of desiccation on that population (with no recovery); while most other taxa showed a high resilience to drought conditions. However, since toxicity data cannot be obtained for all species and chemicals in the environment, information on sensitivity-related traits (Rubach et al., 2010; Rico and Van den Brink, 2015) can be used to perform preliminary sensitivity rankings on different pesticide classes separated by mode-of-action (Rico and Van den Brink, 2015). Traits responding to pollution and drought (not complete desiccation) were assessed in Chapter 5. Some traits such as asexual reproduction, reproduction by clutches, cocoons and plurivoltinism, were more prevalent in highly polluted sites, whereas reproduction by isolated eggs, semivoltinism or respiration by gills were traits dominating in less polluted sites. Aerial active dispersal or terrestrial reproduction were associated with drought conditions. Other traits related with locomotion and attachment to substrate, food and feeding types showed clearer responses to drought and pollution during drought periods. The traits identified

in this study can be considered a step forward in the development of ecological scenarios and the identification of focal taxa under Mediterranean conditions. Nevertheless, the number of studies on that direction is still reduced and more studies covering different levels of water stress and pollution are needed. Moreover, this type of field studies need to be complemented with experimental studies similar to Chapter 6, or at a larger scale (i.e. mesocosm level), to disentangle responses to specific stressors related with pollution and water scarcity, and their potential interactions. The development of worst-case ecological scenarios should include the assessment of a varied range of water scarcity pressures, considering the timing of co-occurrence of stressors and chemicals with different mode-of-action or lower hydrophobicity (i.e. persistence in sediment) than the tested chemical in Chapter 6. Sensitive life-stages under those conditions should be also identified.

Finally, the importance of improving the link between both the exposure and the effect assessment needs to be highlighted (Brock et al., 2010; Rico et al., 2016b). The challenge now is to identify environmental parameters that represent the worst-case scenario for both exposure and effect risk assessment, and try to link them together. One of the approaches suggested by Rico et al. (2016b) was to perform combined exposure and effect simulations to identify realistic worst-case scenario under an ecological perspective. The findings in Chapter 6 contributed to that point, with the exposure assessment related with thermal and hydrological conditions, i.e. faster dissipation under high temperatures and remobilization from sediment after rewetting in the desiccated cosms. The physico-chemical conditions in each scenario were also controlled in that experiment. This is one of the few studies assessing the combined exposure and effect of an insecticide under Mediterranean conditions (i.e. high temperatures and drought up to desiccation) on local taxa. The final challenge here would be developing advanced modelling tools that integrate biological and pesticide-related parameters, which would need a stronger communication between the fate and ecological modelers.

4. Concluding remarks and recommendations

The assessment of multiple stressors related to chemical and hydrological stress in (semi-)arid regions is a rather unexplored field. Chapter 2 showed that most available studies were conducted with biofilms and algae and that the amount of experimental studies assessing the effects of pesticides in water bodies affected by water scarcity was much reduced. More studies on the post-stress recovery capacity of aquatic communities were also recommended in that chapter. Thus, this thesis has contributed to fill the gap on the experimental assessment of the combined effects of pesticide pollution and water scarcity on invertebrate (zooplankton) populations and communities and their recovery capacity (Chapter 6). Overall, the impact of hydrological variability on water quality and biological responses in a more site-specific/regional context (i.e. Mediterranean regions) has been assessed combining field and experimental studies (Chapter 3-6), as recommended for future scientific and regulatory updates. Moreover, invertebrate (macroinvertebrates and zooplankton) responses evaluated in Chapter 5 and 6 have contributed to the identification of taxa and biological traits responding to the combined effect of stressors in Mediterranean regions, which was also recommended for the development of future regulatory risk assessment tools, such as ecological models and scenarios. Still, more studies focusing on the identification of sensitive life-stages, and better understanding of community responses and food web interactions, are needed.

Results from Chapter 4 showed that key contaminant mixtures were usually formed by a reduced number of compounds (i.e. 5 or less), in most cases potentially exerting a chronic risk for aquatic ecosystems, despite some metals (Cu, Zn) and pesticides (chlorpyrifos, diuron) could also exert acute risks in some cases. In this chapter the influence of land use and temporal patterns in the presence and concentration (i.e. toxicity) of substances was also highlighted, with temporal variability mainly associated to diffuse pollution (i.e. pesticides). Despite sampling designs and methods (i.e. passive sampling for diffuse pollution) should be revised to get more robust results, a recommendation from this thesis is that once compounds with high potential toxicity have been identified, the risk associated to temporal patterns of exposure and the hydrological conditions in which they take place, as well we their potential interactive effects on biota, should be assessed.

Metals (Cu, Zn) and some pesticides (chlorpyrifos, diuron, carbendazim, diazinon or dichlofenthion) were detected as the most potentially toxic compounds in the Tagus river basin and other Mediterranean basins. Still, the potential ecological risk of point source chemicals should be evaluated carefully, attending to their specific mode-of-action and sub-lethal effects (e.g. growth, behavioral effects) on appropriate biological endpoints (e.g. bacteria, vertebrates). The inclusion of potentially hazardous substances at a basin level should be considered in specific management plans, after proper cost-effective validation through monitoring. Sources of pesticides with high toxicity potential found in several basins (e.g. diazinon, dichlofenthion) not approved for agricultural use in European waters should be evaluated. For substances frequently detected above the regulatory threshold, especially chlorpyrifos or Hg, urgent management measures should be put in place, attending to the sources of pollution and the hydrological and physico-chemical conditions that might influence their temporal patterns of exposure.

The assessment of macroinvertebrate responses to chemical pollution under hydrological stress (Chapter 5) showed enhanced negative effects of pollution during drought or water scarcity periods in terms of species richness, functional richness and functional diversity, despite pollution was the main driver of responses. The seasonality observed in these responses suggests that current regulatory procedures for the assessment of ecological status of Mediterranean water bodies (at least at a national level) may need to be adapted, covering periods with the highest ecological disturbance (i.e. drought periods). In relation to this temporal variability, an in the context of expanding climate change conditions, it is also recommendable to revise current reference conditions in Mediterranean rivers and streams, attending to the seasonal variation of natural communities. This would help establishing more realistic thresholds, according to the degree of influence of hydrological variation and anthropogenic pollution.

Trait-based approaches seem to be helpful on the identification of sensitive or tolerant taxa to pollution and water stress. In Chapter 5, traits responding to pollution, drought or the combined effect of drought and pollution were identified. From a list of *a priory* expected responses, more than half could be confirmed for pollution stress, such as asexual reproduction, reproduction by clutches, cocoons and plurivoltinism, associated with high pollution levels and reproduction by eggs or semivoltinism with less polluted sites, among others. Responses to drought were confirmed for a lower number of trait categories, which may be related to the dominant effect

of pollution over drought. Still, more field and laboratory studies in this direction are needed, considering different drought and pollution levels, and attending to possible trait correlations (syndromes).

The model-ecosystem study performed in Chapter 6 showed that environmental conditions related to water scarcity may influence chemical fate and the vulnerability of zooplankton communities to chemical stress. Temperature modulated the response of zooplankton to the chitin-inhibitor insecticide tested, with faster response time but higher recovery potential of the community. The exposure assessment showed faster dissipation under high temperatures and remobilization from sediment after rewetting in the desiccated microcosms. The community tested also showed a high resilience capacity to the ecosystem‘s contraction and desiccation. Drought conditions did not interact with chemical stress, with similar effects as in the high temperature scenario, with the exception of slight delayed effects after rewetting in the lufenuron treated microcosms at higher concentrations. However, this response can be related to the time lag between the exposure peak and the high contraction and desiccation events. At a population level, some taxa (*Daphnia* sp., Cyclopoida) were synergistically affected by the combination of high temperature and chemical stress, and a slight decline in Cyclopoida was observed after rewetting. Drought alone had only drastic negative effects on some taxa such as Calanoida.

This model-ecosystem study is one of the most novel high-tier studies simulating an ecological scenario under Mediterranean conditions covering complete desiccation and rewetting, which can be used in the development of ecological models for prospective risk assessment in Mediterranean regions. Since exposure and effect were assessed simultaneously under more realistic environmental conditions, it also gives a step forward on the required link between effect and exposure assessment in ecological risk assessment models. Still, to determine realistic worst-case scenarios, more experimental studies attending to the impact of pesticides (different mode-of-action and persistence) under different drought levels and timing of stressors are needed. Meanwhile ecological models combining effect and exposure assessment are developed, exposure scenarios considering desiccation and flow variability over time, as well as related physico-chemical processes such as compounds remobilization or altered degradation rates, should be better developed at higher-tiers (step 3 and 4).

Table S1. Summary of selected experimental studies (laboratory, micro- and meso-cosms studies) dealing with the combined effects of water scarcity and chemical exposure in aquatic ecosystems.

Hydrological stressor	Chemical stressor	Experimental design	Taxonomic group	Biological endpoint	Stressors' interaction[a]	Major findings	Reference
Flow intermittency	Fungicide (tebuconazole)	40 days artificial streams	Fungi and Bacteria	Biomass	AD (Fungi) AD (Bacteria)	Flow intermittency increased microbial biomass, changed microbial community structure, reduced leaf litter decomposition, enzymatic activity and *Gammarus* feeding rates.	(Pesce et al., 2016)
				Community structure	N/A		
				Leaf litter decomposition	AD		
				Enzymatic activity	AD	Tebuconazole effects were not significant for any endpoint except for fungal community structure.	
		2 × 4 days *Gammarus* feeding assays	Macroinvertebrates	*Gammarus fossarium* feeding rate	AD	Combined stress slightly increased the effects caused by flow intermittency, but were not statistically significant.	
Flow intermittency	Pharmaceuticals (1 psychiatric drug, 2 antibiotics, 2 β-blockers, 1 anti-inflammatory, 1 lipid regulator, 1 diuretic)	42 days artificial streams	Biofilms (Algae + Bacteria)	Total biomass	AD	Flow intermittency decreased algal biomass, algal taxa richness, diatom abundance, NPP and CR. It increased PA and green algae and cyanobacteria abundance. The slight decrease in bacteria taxa richness was not significant.	(Corcoll et al., 2015)
				Net Primary Production (NPP)	-A		
				Community Respiration (CR)	AD		
				Algae			
				Biomass	AD		
				Photosynthetic activity (PA)	-A	Pharmaceuticals had comparable effects, but decreased cyanobacteria abundance and bacteria OTUs richness.	
				Community structure	N/A		
				Algal taxa richness	AD		
				Bacteria		Bacterial density was not significantly affected in any of the isolated treatments.	
				Bacterial density	AD		
				Bacterial Operational Taxonomic Unit (OTUs) richness	AD	Combined stress significantly increased green algae abundance, PA and primary productivity with respect to flow intermittency. Bacterial community previously exposed to flow intermittency was not significantly affected after acute exposure, but algae showed significantly higher sensitivity.	
		2 × 24 h acute toxicity test		Photosynthetic activity (PA)	-S		
				Bacterial enzymatic activity	AD		

Table S1 (cont.)

Hydrological stressor	Chemical stressor	Experimental design	Taxonomic group	Biological endpoint	Stressors' interaction[a]	Major findings	
Flow intermittency	Bactericide (triclosan)	47 days artificial streams	Biofilms (Algae + Bacteria)	Total biomass Enzymatic activity Phosphorus (P) uptake rate *Algae* Community structure Live-to-dead ratio Photosynthetic activity (PA) *Bacteria* Live-to-dead ratio	N/E AD -S N/A -A AD -S	Flow reduction and flow intermittency decreased significantly bacterial and diatom live-to-dead ratios, diatom abundance and PA. A significant increase in enzymatic activity and green algae abundance was observed. Triclosan increased significantly biofilm enzymatic activity and decreased PA and bacterial live-to-dead ratio. Combined stress showed significant stronger decrease in bacterial live-to-dead ratio, and delayed decrease in diatom abundance. Intermittency has a significantly stronger negative effect in diatom live-to-dead ratio and P uptake than the combined effect, despite effects were also significantly negative compared to controls. Recovery based on P uptake rates was slower for the combined effect than for triclosan treatment. Recovery was not achieved due to intermittency or the combined effect of both stressors for the diatom live-to-dead ratio.	(Proia et al., 2013)
Low flow velocity	Cu^{2+}	7 days artificial streams	Algae (in biofilms)	Biomass Community structure Shannon-Wiener biodiversity index Photosynthetic activity (PA)	-A N/A N/E -A	Lower flow velocity decreased algae biomass and PA. Cu^{2+} caused a significant decrease in biomass and PA. Combined effect at lower velocities needed longer time to show significant effects on biomass and PA. Community structure changed significantly after 7 days after Cu^{2+} exposure at high flow velocities, with a decrease in *Synedra ulna* abundance and an increase of *Achnanthes minutissima* and *Stigeoclonium tenue*. Effects on biodiversity were not significant in any treatment.	(Sabater et al., 2002)

Table S1 (cont.)

Hydrological stressor	Chemical stressor	Experimental design	Taxonomic group	Biological endpoint	Stressors' interaction[a]	Major findings	Reference
Decreasing water depth	Fire-retardant (>90% ammonium polyphosphates and <1% yellow prussiate of soda)	1 year mesocosm	Zooplankton	Species richness Pielou´s evenness index Simpson diversity index Total density Community structure	N/E -S -S -A N/A	Decreasing water depth alone had no significant effect on any selected endpoint. Fire-retardant alone impacted community structure by reducing diversity and total density at higher concentrations. Combined stress had a significant stronger negative effect on community diversity and a positive effect on total density. The responses to decreasing water depth and contamination at community level resulted from complex ecological interactions that could be observed at the population level.	(Martin et al., 2014)
Desiccation	Fire-retardant (>90% ammonium polyphosphates and <1% yellow prussiate of soda)	4.5 months indoor microcosms (3 months dry phase and chemical treatment, 1.5 months wet phase)	Zooplankton	Community structure Shannon-Wiener biodiversity index Evenness index	N/A N/E N/E	Desiccation alone allowed emerging species to recover within a period of 3 weeks. Ostracods and Cladocerans species were the most abundant taxons. Fire-retardant combined with desiccation resulted in a significant decrease in species diversity and abundance compared to desiccation alone. Bdelloid rotifers were significantly more abundant than the rest of species at lower fire-retardant concentrations. Higher concentrations resulted in an almost complete disappearance of zooplankton species.	(Angeler et al., 2005)

The acronyms refer to the five types of interactions between stressors described in this study. Depending on the direction of individual stressor effects and the direction the cumulative effect, the interactions can be: additive (AD), positive synergistic (+S, more positive than predicted additively), negative synergistic (-S, more negative than predicted additively), positive antagonistic (+A; less positive than predicted additively) and negative antagonistic (-A; less negative than predicted additively). N/A: not applicable classification since it is not possible to define interactive effects' direction and magnitude based on the indicated endpoint (i.e., community structure). N/E: not evaluated due to the absence of statistical effects between none of the tested stressors and the evaluated endpoint.

[a] Classification based on Piggot et al. 2015.

Table S2. Summary of selected field monitoring studies in which the combined impact of water scarcity and chemical exposure have been evaluated in aquatic ecosystems.

Hydrological stressor	Chemical stressor	Taxonomic group	Biological endpoint	Experimental design	Location	Major findings	Reference
Inter-annual flow variation	157 organic micropollutants (urban, industrial and agricultural sources)	Biofilms (Algae + Bacteria)	*Algae* Biomass Community structure *Bacteria* Enzymatic activity	19 sampling points in 4 rivers, pollution gradient, 2 sampling periods: end of summer on 2 consecutive years (wet-dry years)	North-East, East and South Spain	Pollutants had lower impact on biofilm and macroinvertebrate community structure than flow variation and other correlated physicochemical variables. Considering the impact of both groups of stressors and land use together, gave better correlation values with change in community structure. Less diverse communities, dominated by more tolerant species, were associated with increase in impairment (increase in pollution and flow variability). Enzymatic activity was also inversely correlated with pollution. Impairment generally occurred in an upstream-downstream gradient.	(Sabater et al., 2016)
		Macroinvertebrates	Community structure				
Inter-annual flow variation	157 organic micropollutants (urban, industrial and agricultural sources)	Biofilms	Total density *Algae* Biomass Photosynthetic capacity (PC) Tolerance to excess light (NPQ) Community structure *Bacteria* Density Enzymatic activity	19 sampling points in 4 rivers, pollution gradient, 2 sampling periods: summer-autumn on 2 consecutive years (wet-dry years)	North-East, East and South Spain	Industrial organic compounds, herbicides, pharmaceuticals, Dissolved Organic Carbon (DOC), Dissolved Inorganic Nitrogen (DIN) and hydrological variation, were the most important variables, in that order. Pollutants explained the majority of the variance. However, the combined analysis of the six variables gave better correlation values with systems variability. Sensitive diatom taxa, NPQ, bacterial density and enzymatic activity were negatively related with these variables. Normally found in upstream sites. Tolerant taxa, algae biomass and PC were positively related with those variables. Normally found in downstream sites.	(Ponsatí et al., 2016)

Table S2 (cont.)

Hydrological stressor	Chemical stressor	Taxonomic group	Biological endpoint	Experimental design	Location	Major findings	Reference
Intra-annual water level variation	Insecticide, larvicide (*Bti*: *Bacillus thuringiensis* var. *israelensis* serotype H14)	Algae	Total density Community structure	3 sampling sites in shallow Mediterranean temporary wetlands, 5 years monitoring, sampling after *Bti* application related with flooding	France	Total density and community diversity showed large temporal fluctuations. No significant increase in density was observed following *Bti* application. Phytoplankton community variability is driven by natural fluctuations in environmental conditions related to flooding and drought events.	(Fayolle et al., 2015)
Intra-annual flow variation	73 pharmaceuticals	Biofilms (Algae + Bacteria)	*Algae* Biomass Photosynthetic activity (PA) *Bacteria* Enzymatic activity Live-to-dead ratio	2 sampling sites in one river, 2 sampling periods: winter-spring and spring-summer	North-East Spain	Pharmaceuticals concentration had a significant inverse relationship with flow. Algae biomass decreased from low to high polluted site during the low flow period. Stable but low flows showed higher algae biomass and bacterial enzymatic activity than high but variable flows, even with higher concentrations. Flood had a negative effect on biofilm biomass, structure, and recovery. Antibiotics showed a significant negative effect on bacterial survival, independently of the hydrological conditions.	(Osorio et al., 2014)

Table S2 (cont.)

Hydrological stressor	Chemical stressor	Taxonomic group	Biological endpoint	Experimental design	Location	Major findings	Reference
Intra-annual flow variation	6 insecticides (azinphos-methyl, chlorpyrifos, endosulfan, fenvalerate, cypermethrin and malathion)	Macroinvertebrates	Community structure Population dynamics	3 sampling sites in one river, 2 sampling periods: winter-spring and spring-summer	South Africa	Flow decrease resulted in a significant increase in Ephemeroptera abundance. Insectides were the only significant variable negatively affecting community structure when the combined effect was assessed. Azinphos-methyl and chlorpyrifos were the organophosphate chemicals detected at quantifiable amounts in the 2 polluted sites. Concentrations were significantly higher during the low flow period at site 3. Ephemeroptera and Tricoptera populations decreased due to the combined effect of low flow and high insecticide concentration at site 3. However, only the increase in insecticide concentration showed a statistically significant effect. *Demoreptus* sp. and *Castanophlebia* sp. were significantly the most sensitive Ephemeroptera species to chemical pollution during the low flow period. *Baetis* sp. density increased from in low flow periods at no or less polluted sites and decreased at site 3. However, these differences were not significant.	(Bollmohr and Schulz, 2009)
Inter- and Intra-annual flow variation	9 endocrine disruptors (Alkylphenolic compounds - APCs-)	Diatoms (in biofilms) Macroinvertebrates	Community structure Population densities	7 sampling sites in 2 rivers, pollution gradient, 4 sampling periods: late spring and Autumn on 2 consecutive years	North-East Spain	The main stressors in the macroinvertebrate community were conductivity, temperature and soluble reactive phosphorus. APCs had also an effect. The main stressors in the diatom community were APCs, but combined analysis of APC exposure and physicochemical variables gave better correlation values with systems variability. Flow variability was not directly included in the analysis as part of the physical variables, but some parameters associated to its variation.	(Brix et al., 2012)

Table S2 (cont.)

Hydrological stressor	Chemical stressor	Taxonomic group	Biological endpoint	Experimental design	Location	Major findings	Reference
Inter- and Intra-annual flow variation	22 pesticides (18 herbicides and 4 insecticides)	Diatoms (in biofilms)	Biomass Community structure Photosynthetic activity (PA) Extracellular polysaccharide content (EPS) Green algae/cyanobacteria ratio (F1/F3) Enzymatic activity	7 sampling sites in 2 rivers, pollution gradient, 4 sampling periods: late spring and autumn on 2 consecutive years	North-East Spain	Temperature (T), conductivity and NO_3^- influenced invertebrate community structure. Pesticides did not influence this endpoint. Herbicides influenced diatom community structure, biomass and PA. T and SO_4^{2-} influenced mainly enzymatic activities; EPS and F1/F3 were influenced by the three variables. The potential contribution of each separated group on biofilms was not statistically significant. However, covariance analyses showed significant shared effects on biofilm responses. Flow variability was not included in the analysis as part of the physical variables.	(Ricart et al., 2010)
		Macroinvertebrates	Community structure				
Inter- and Intra-annual flow variation	Insecticides, larvicides (permethrin and organophosphates)	Macroinvertebrates	Community structure	12 year monitoring program in 2 rivers (several applications and several samplings per year)	West Africa	Hydrological seasonal patterns influence significantly invertebrate community structure, with shifts in relative abundances. Permethrin did not show significant influence on this natural variation. A combined effect could be observed on gathering collectors at the end of the dry season, but recovery was observed when flow was reestablished. Seasonal variation on fish endpoints could only be attributed to hydrological changes.	(Crosa et al., 2001)
		Fish	Species number Total weight per individual				

Table S2 (cont.)

Hydrological stressor	Chemical stressor	Taxonomic group	Biological endpoint	Experimental design	Location	Major findings	Reference
Inter- and Intra-annual flow variation	4 insecticides (fenitrothion, diflubenzuron, deltamethrin and bendiocarb)	Zooplankton Macroinvertebrates	Community structure Population densities	16 sampling points in temporary ponds, 4 sampling periods: 4 consecutive years alternating treatment and non-treatment years, covering wet and dry period each year.	West Africa	Cladocerans, fairy shrimps (*Streptocephalus spp.*) and backswimmers (*Anisops spp.*) were the most sensitive species. *Anisops* and cladocerans showed fast recovery. *Streptocephalus spp.*did not recover as resting eggs could not hatch during rainy season of application. No direct analysis of hydrological changes impact on community structure and response to chemicals, but monitoring included several cyclic hydrological periods and community successions during that period.	(Lahr et al., 2000)

Inter-annual: several sampling periods on consecutive years, with at least one sampling time per year. Comparison of different flow conditions among years, at the same sampling period.
Intra-annual: several sampling periods covering wet and dry cycles along the year

Table S1. Operational conditions for the analysis of organic contaminants by LC-QTOF.

LC-QTOF parameters	
Ionization mode	Positive
ESI temperature	550 °C
Curtain gas pressure	30 psi
Ion spray voltage floating	5500 V
Declustering potential	80 V
Ion source gas 1 and 2	55 psi
CE	30 ± 15 V
Chromatographic conditions	
	Point source chemicals and pesticides
Chromatographic column	Kinetex Biphenyl, 50 x 3 mm x 2.7 µm (Phenomenex)
Mobile phases	A: 0.1% formic acid in water
	B: 0.1% formic acid in methanol
Elution mode	Initial mobile phase composition (5% B) constant for 1 min, followed by a linear gradient to 100% B up to 30 min, and kept for 3 min at 100% B
Flow rate	0.6 mL/min
Column temperature	40 °C
Injection volume	20 µL

Table S2. Operational conditions for the analysis of target compounds by LC-MS/MS.

Triple Quadrupole (MS/MS) parameters			
Ionization mode	Positive/Negative		
Sheath gas temperature	350 °C		
Sheath gas flow	11 L/min		
Drying gas temperature	250 °C		
Drying gas flow	13 L/min		
Capillary voltage	4000 V		
Nozzle voltage	500 V		
Δ EMV	400 V		
Chromatographic conditions			
	Point source chemicals		**Pesticides**
Ionization mode	Positive	Negative	Positive
Chromatographic column	Kinetex Biphenyl, 50 x 3 mm x 2.7 µm (Phenomenex)	Poroshell 120 EC-C18, 50 x 3 mm x 2.7 µm (Agilent Technologies)	ACE C18 PFP, 50 x 2.1 mm x 3 µm (Symta)
Mobile phases	A: 0.1% formic acid in water	A: 1 mM ammonium fluoride in water	A: 0.1% formic acid in water
	B: 0.1% formic acid in methanol	B: methanol (65%) + acetonitrile (35%)	B: 0.1% formic acid in methanol (65 %) + acetonitrile (35%)
Elution mode	Initial mobile phase composition (2% B) constant for 1 min, followed by a linear gradient to 100% B up to 30 min, and kept for 5 min at 100% B	Initial mobile phase composition (5% B) constant for 1 min, followed by a linear gradient to 100% B up to 12 min, and kept for 5 min at 100% B	Initial mobile phase composition (5% B) constant for 1 min, followed by a linear gradient, at 0.8 ml/min, to 56% B up to 16 min, and kept for 1 min. Then, apply a linear gradient, at 0.4 ml/min, to 100%B up to 32 min, and kept for 1 min
Flow rate	0.6 mL/min	0.6 mL/min	0.8 mL/min (0min–17min), 0.4 mL/min (17min–32min)
Column temperature	40 °C	40 °C	40 °C
Injection volume	20 µL	20 µL	20 µL

Table S3. Retention times (t_R), collision energies (CE), precursors and product ions (Q; quantifier, and q; qualifier) selected for the analysis of target compounds in Multiple Reaction Mode (MRM).

Compound	Formula	Pollutant family	t_R (min)	Precursor $[M+H]^+$ (m/z)	Product ion (m/z)	CE (V)	MRM transition	Abundance (q/Qx 100) (%)
Carbedazim	$C_9H_9N_3O_2$	Pesticide	2.29	192	160	16	Q	
					132	30	q_1	18
Carbofuran	$C_{12}H_{15}NO_3$	Pesticide	8.45	222.1	165	8	Q	
					123	22	q_1	89
					77.1	40	q_2	11
Chlortoluron	$C_{10}H_{13}ClN_2O$	Pesticide	10.23	213	72.1	24	Q	
					140	16	q_1	5
					168	26	q_2	2
Chlorpyrifos ethyl	$C_9H_{11}Cl_3NO_3PS$	Pesticide	23.52	349.9	197.8	16	Q	
					124.9	20	q_1	47
Diazinon	$C_{12}H_{21}N2O_3PS$	Pesticide	16.79	305	169.2	26	Q	
					153.2	26	q_1	56
Dimethoate	$C_5H_{12}NO_3PS_2$	Pesticide	4.44	229.9	198.8	6	Q	
					125	14	q_1	74
					171	16	q_2	37
Diuron	$C_9H_{10}Cl_2N_2O$	Pesticide	11.59	233	72	22	Q	
					160	26	q_1	4
Imidachloprid	$C_9H_{10}ClN_5O_2$	Pesticide	5.11	256	209	14	Q	
					175	12	q_1	71
Kresoxim methyl	$C_{18}H_{19}NO_4$	Pesticide	16.97	314.1	221.9	16	Q	
					234.8	16	q_1	48
					116	24	q_2	19
Malathion	$C_{10}H_{19}O_6PS_2$	Pesticide	15.35	331	127	10	Q	
					99	22	q_1	79
					125	26	q_2	17
Metolcarb	$C_9H_{11}NO_2$	Pesticide	6.68	166	109	8	Q	
					94	30	q_1	18
Metribuzin	$C_8H_{14}N_4OS$	Pesticide	6.93	215.1	187	20	Q	
					84.3	22	q_1	33
Pirimicarb	$C_{11}H_{18}N_4O_2$	Pesticide	5.81	239.1	72.1	16	Q	
					182	14	q_1	63
					194.9	20	q_2	41
Propiconazole	$C_{15}H_{17}Cl_2N_3O_2$	Pesticide	16.23	342	158.8	30	Q	
					69.2	22	q_1	95
Simazine	$C_7H_{12}ClN_5$	Pesticide	7.47	202.1	124	18	Q	
					131.9	22	q_1	80
Spinosyn-A	$C_{41}H_{65}NO_{10}$	Pesticide	21.74	732.2	141.9	32	Q	
					98	44	q_1	9
Spiroxamine	$C_{18}H_{35}NO_2$	Pesticide	14.92	298	144.2	22	Q	
					100.1	36	q_1	56
Tebuconazole	$C_{16}H_{22}ClN_3O$	Pesticide	16.57	308	70.3	48	Q	
					124.9	40	q_1	11
Terbuthryn	$C_{10}H_{19}N_5S$	Pesticide	11.02	241.9	186	18	Q	
					91.2	28	q_1	10
					158	28	q_2	5
Terbuthylazine	$C_{17}H_8Cl_2F_8N_2O_3$	Pesticide	12.70	229.9	173.9	14	Q	
					132	28	q_1	12
Acetaminophen	$C_{10}H_9NO_2$	Pharmaceutical	2.00	152	109.9	20	Q	
					93	24	q_1	28
Amoxicillin	$C_{16}H_{19}N_3O_5S$	Pharmaceutical	2.71	365.9	113.8	18	Q	
					133.8	26	q_1	21
Amphetamine	$C_9H_{13}N$	Stimulant	4.85	136.1	91	20	Q	
					119	6	q_1	65
Atenolol	$C_{14}H_{22}N_2O_3$	Pharmaceutical	4.98	266.8	189.9	19	Q	
					145.1	31	q_1	133
Azithromycin	$C_{38}H_{72}N_2O_{12}$	Pharmaceutical	14.55	749.4	591.2	38	Q	
					158	40	q_1	62
Caffeine	$C_8H_{10}N_4O_2$	Stimulant	9.47	195	138	19	Q	
					110	23	q_1	22
Carbamazepine	$C_{15}H_{12}N_2O$	Pharmaceutical	17.69	237	193.8	21	Q	
					192.8	40	q_1	22
Ciprofloxacin	$C_{17}H_{18}FN_3O_3$	Pharmaceutical	10.94	332.1	314	18	Q	
					288	18	q_1	8

Table S3 (cont.)

Compound	Formula	Pollutant family	t_R (min)	Precursor $[M+H]^+$ (m/z)	Product ion (m/z)	CE (V)	MRM transition	Abundance (q/Qx 100) (%)
Citalopram	$C_{20}H_{21}FN_2O$	Pharmaceutical	16.09	325	108.9	20	Q	
					261.9	19	q_1	89
Diclofenac	$C_{14}H_{11}Cl_2NO_2$	Pharmaceutical	22.56	295.8	214.7	20	Q	
					249.9	12	q_1	72
Erythromycin	$C_{37}H_{67}NO_{13}$	Pharmaceutical	17.92	734.4	157.9	38	Q	
					576.3	20	q_1	101
Ketoprofen	$C_{16}H_{14}O_3$	Pharmaceutical	20.57	255	105	24	Q	
					209	12	q_1	172
Lincomycin	$C_{18}H_{34}N_2O_6S$	Pharmaceutical	6.66	407.2	126.1	32	Q	
					359	18	q_1	7
Loratadine	$C_{22}H_{23}N_2O_2Cl$	Pharmaceutical	23.34	382.9	336.9	26	Q	
					266.8	40	q_1	45
Metronidazole	$C_6H_9N_3O_3$	Pharmaceutical	3.57	172	128	10	Q	
					82	30	q_1	47
Naproxen	$C_{14}H_{14}O_3$	Pharmaceutical	20.39	230.9	185	11	Q	
					170	30	q_1	23
Nicotine	$C_{10}H_{14}N_2$	Life-style compound	1.00	163.1	130	30	Q	
					131.9	16	q_1	100
Omeprazole	$C_{17}H_{19}N3O_3S$	Pharmaceutical	15.93	346.1	198	8	Q	
					136	36	q_1	38
Paraxanthine	$C_7H_8N_4O_2$	Life-style compound (metabolite)	6.45	181	124.1	22	Q	
					96.1	30	q_1	6
Progesterone	$C_{21}H_{30}O_2$	Steroid	26.20	315.1	97	30	Q	
					108.9	26	q_1	108
Salbutamol	$C_{13}H_{21}NO_3$	Pharmaceutical	3.35	240.1	148.2	18	Q	
					166.1	10	q_1	31
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	Pharmaceutical	11.06	253.9	92	31	Q	
					155.8	14	q_1	88
Tributyl-phosphate	$C_{12}H_{27}O_4P$	Industrial chemical	23.27	267.1	99	26	Q	
					81.1	56	q_1	32
Testosterone	$C_{19}H_{28}O_2$	Steroid	22.64	289	97	30	Q	
					108.9	25	q_1	76
Trimethoprim	$C_{14}H_{18}N_4O_3$	Pharmaceutical	8.96	291.2	230	25	Q	
					123	24	q_1	56
Tylosin	$C_{46}H_{77}NO_{17}$	Pharmaceutical	20.15	916.3	173.9	32	Q	
					772.2	34	q_1	51
Valsartan	$C_{24}H_{29}N_5O_3$	Pharmaceutical	22.64	436	235	20	Q	
					291	18	q_1	87
Venlafaxine	$C_{17}H_{27}NO_2$	Pharmaceutical	13.85	278.2	58	18	Q	
					260	8	q_1	52
Estrone	$C_{18}H_{22}O_2$	Estrogen	10.11	269.1	145	50	Q	
					143	46	q_1	17
Estradiol,17-beta-(E2)	$C_{18}H_{24}O_2$	Estrogen	9.81	271	145	50	Q	
					183	45	q_1	89
Gemfibrocil	$C_{15}H_{22}O_3$	Pharmaceutical	11.08	249	121	10	Q	
					127	8	q_1	7
Ibuprofen	$C_{13}H_{18}O_2$	Pharmaceutical	9.46	205.1	161.1	4	Q	

q: selected product ion for qualification. Q: selected product ion for quantification.

Table S4. Results of the Screening analysis. Positive responses of the qualitative screening analysis are reported for all the chemicals considered in all sampling sites and times. Site code: numbers indicate the codes of the sampling sites reported in Figure 1 of the main text. Sampling time: Sp= Spring; Su= Summer; Au= Autumn. Sampling type: G= grab; P= passive sample (POCIS). Ph= pharmaceuticals; P= pesticides; D: drugs and life style chemicals; V= various.

Site code		1				2				3				4			5				6				7				8				9			10			11			12				13				14				15				16				Total positive data New				
sampling time		Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su	Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su	Au	Sp	Su	Au	Sp	Su	Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	spring	summer		autumn	
sampling type		G	G	P	G	G	G	P	G	G	G	P	G	G	G	G	G	G	P	G	G	G	P	G	G	G	P	G	G	G	P	G	G	G	G	G	G	G	G	G	G	G	G	P	G	G	G	P	G	G	G	P	G	G	G	P	G	G	G	P	G	grab	grab	POCIS	grab	total
Positive data in the column		14	15	29	13	24	23	36	31	10	11	17	13	10	14	13	15	23	32	20	30	40	81	27	21	36	42	34	74	74	94	77	58	79	63	54	63	62	60	71	78	19	19	35	23	28	47	56	41	28	54	65	49	12	23	33	24	60	69	85	78	517	661	605	646	2429
4-AA (4-Aminoantipyrine)	Ph																						1						1	1	1	1	1	1	1	1	1	1	1	1	1					1	1	1	1	1	1	1	1					1		1	1	7	6	5	7	25
4-AAA (4-Acetamidoantipyrine)	Ph	1				1	1	1	1		1						1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		1	1	1	1	1	1		1	1		1	1	1	1	13	13	10	12	48
4-FAA	Ph					1	1	1	1									1		1	1	1	1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		1	1	1	1	1	1		1	1		1	1	1	1	11	12	8	12	43
8-hydroxyquinoline	Ph		1	1			1	1	1		1	1	1		1	1		1	1	1		1	1	1		1	1	1		1	1	1		1	1		1	1		1	1			1	1		1	1	1		1	1	1		1	1	1		1	1	1	0	15	12	15	42
Acephate	P																																		1																											1	0	0	0	1
Acetaminophen	Ph					1			1	1				1			1				1	1	1	1	1			1					1			1											1	1	1	1	1	1	1	1					1		1	10	4	3	6	23
Acetamiprid	p																					1												1						1																						0	3	0	0	3
Adenosine	Ph		1	1	1		1	1	1		1	1	1		1	1		1	1	1		1	1	1		1	1	1		1	1	1		1	1		1	1		1	1		1	1	1		1	1	1		1	1	1		1	1	1			1	1	0	15	12	16	43
Aldicarb	P			1																																					1																				1	0	1	0	2
Aldicarb-sulfone	P									1																															1											1										1	0	1	1	3
Amidotrizoic acid	Ph																													1	1	1																														0	1	1	1	3
Aminocarb	P			1								1							1			1	1				1		1		1	1																									1	1					1	1	7	2	11
Amoxicillin	Ph																													1				1									1									1	1									0	3	1	1	5
Amphetamine	D							1				1											1				1		1				1		1						1							1													1	2	0	5	3	10
Ampicillin	Ph								1											1				1				1	1			1			1																											1	0	0	6	7
Antipyrine	Ph					1	1																							1																												1	1			2	3	0	0	5
Asulam	P																																									1		1				1						1				1			0	3	0	2	5
Atenolol	Ph					1	1		1									1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			1		1	1	1	1	1	1	1	1		1			1	1	1	1	10	12	8	10	40
Atenolol acid	Ph					1															1	1	1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1						1			1	1				1			1	1	1	1	9	10	3	7	29
Atrazine	P																													1	1																									1			1	1		0	2	3	0	5
Atrazine-desethyl	P				1														1	1								1																				1						1			1		1	1	1	1	1	3	5	10
Atropine	Ph																													1																															1	0	1	0	1	2
Azinphos methyl	P					1											1																																													2	0	0	0	2
Azithromicin	Ph		1												1						1			1		1			1	1	1	1																			1		1						1			2	6	1	3	12
Azoxystobin	P																						1						1		1						1																				1				1		2	0	4	0	6
Benalaxil	P																																	1																											0	0	0	1	1
Bendro flumethiazide	Ph																																																										1		0	0	1	0	1
benfuracarb	P				1																										1																															0	0	1	1	2
Bensulfuron methyl	P																														1	1												1																		0	0	2	1	3
Bensulide	P																																																										1		0	0	1	0	1
Bentazone	P		1																										1																																	1	1	0	0	2
Benzalkonium chloride-C10	V																						1																																							0	0	1	0	1
Benzalkonium chloride-C12	V			1				1															1				1				1		1											1			1	1				1	1			1	1			1		1	1	10	2	14
Benzalkonium chloride-C14	V			1				1															1				1				1						1						1	1				1				1				1				1		0	2	10	0	12
Benzalkonium chloride-C16	V	1		1										1			1						1		1						1															1				1		1								1		6	0	5	0	11
Benzoylecgonine	D			1			1	1	1										1		1	1	1		1	1	1	1	1	1	1	1	1	1	1	1		1	1	1	1	1	1			1	1		1		1	1	1			1		1	1	1	1	9	10	9	9	37
Bezafibrate	Ph			1																															1					1	1						1	1	1		1	1	1							1	1	0	3	4	5	12
Boscalid	P																																																		1										0	0	1	0	1
Butoxycarboxim	P																																							1																					0	0	0	1	1
Buturon	P					1																																																						1	0	0	0	1
C3-Pentafluoropropionic-acid-fragment	V														1																																												0	1	0	0	1
Caffeine	D			1				1	1		1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			1	1	1	1	1	1	1	1	1	1		1		1	1	1	1	1	11	13	10	15	49
Cannabidiol	D																																											1							1										0	0	1	1	2
Carbamazepine	Ph							1							1				1	1	1	1	1	1		1	1	1		1	1		1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		1	1	1	1	8	12	10	10	40
Carbamazepine hepoxyde	Ph																													1	1		1	1					1	1	1						1				1									1	1	2	5	2	2	11
Carbendazim	P				1														1				1				1		1		1					1				1			1	1				1				1			1					1		2	3	8	1	14
Carbofuran	P																					1				1				1		1		1		1	1			1							1				1			1	1		1		1	1		2	10	1	2	15

Table S4 (cont.)

Site code		1				2				3				4			5				6				7				8				9			10			11			12				13				14				15				16				Total positive data New				
sampling time		Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su	Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su	Au	Sp	Su	Au	Sp	Su	Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	spring	summer		autumn	
sampling type		G	G	P	G	G	G	P	G	G	G	P	G	G	G	G	G	G	P	G	G	G	P	G	G	G	P	G	G	G	P	G	G	G	G	G	G	G	G	G	G	G	G	P	G	G	G	P	G	G	G	P	G	G	G	P	G	G	G	P	G	grab	grab	POCIS	grab	total
Positive data in the column		14	15	29	13	24	23	36	31	10	11	17	13	10	14	13	15	23	32	20	30	40	81	27	21	36	42	34	74	74	94	77	58	79	63	54	63	62	60	71	78	19	19	35	23	28	47	56	41	28	54	65	49	12	23	33	24	60	69	85	78	517	661	605	646	2429
Cefalexin	Ph																						1						1		1	1			1	1	1	1	1		1						1	1			1							1			1	4	3	3	5	15
Chloramphenicol	Ph																												1								1	1		1	1		1				1								1			1			1		2	5	1	2	10
Chlordimeform	P									1																																																				1	0	0	0	1
Chlorfenvinfos	P																																																										1			0	1	0	0	1
Chlorothiazide	Ph																												1			1							1	1	1																				1	2	1	0	3	6
Chloroxuron	P																			1															1							1				1	1		1		1											3	2	0	2	7
chlorpyrifos	P																														1																					1									1		0	0	3	0	3
Chlorsulfuron	P	1																																																						1						1	0	1	0	2
Chlortetracycline	Ph																		1										1																																	1	0	1	0	2
Chlortoluron	P																												1		1								1	1																		1		1	1	3	1	2	1	7
Cinosulfuron	P											1																												1																			1		0	0	1	2	3
Ciprofloxacin	Ph																												1	1	1	1		1																	1	1						1		1	1	2	3	3	2	10
Citalopram	Ph	1							1												1								1		1									1								1			1						1	1	1			4	3	2	2	11
Clarithromycin	Ph																										1				1				1		1	1			1	1																	1	1		1	2	3	3	9
Clenbuterol	Ph								1																								1												1																	1	0	0	2	3
Clofibric acid	P																																	1																												0	1	0	0	1
Cloxacillin	Ph																																	1	1					1																				1		0	2	1	1	4
Cocaine	D																														1																															0	0	1	0	1
Codeine	D																					1	1					1	1	1	1	1	1	1	1	1	1	1	1	1	1						1	1	1	1	1	1	1			1		1	1	1	1	6	8	6	8	28
Compactin	Ph					1																																																								1	0	0	0	1
Cotinine	D				1		1		1									1		1	1	1	1	1		1	1	1	1		1	1	1	1	1	1			1							1	1	1	1	1	1	1	1							1	1	7	7	6	10	30
cyanazine	P												1																						1										1				1													0	0	0	4	4
Cyclophosphamide	Ph																																																											1		0	0	1	0	1
Cypermethrin	P																																	1																												0	1	0	0	1
Cyromazine	P																					1																																								0	1	0	0	1
DEET	Ph	1		1			1	1		1		1		1	1		1	1	1	1	1	1	1	1	1	1	1		1	1	1	1	1	1	1		1	1	1	1	1	1		1		1	1	1		1	1	1	1	1				1	1	1	1	14	12	11	8	45
DEHP	V								1																																																					0	0	0	1	1
Demeclocycline	Ph																																																												1	0	0	0	1	1
Diafentiuron	P				1			1	1																		1		1																																	1	0	2	2	5
Diatrizoic acid	Ph																												1								1			1																						1	2	0	0	3
Diazepham	Ph									1																									1				1		1																					2	0	0	2	4
Diazinon	P			1					1		1	1							1			1	1							1	1	1						1										1		1							1						0	4	6	4	14
Diclofenac	Ph								1													1	1						1	1	1	1	1	1	1	1	1	1	1	1	1						1	1		1	1	1						1	1	1	1	6	8	5	6	25
Dicrotofos	P																																															1														0	0	1	0	1
Dietofencarb	P																	1	1									1										1						1	1			1				1					1						0	1	5	3	9
Difenacoum	P																										1			1																																0	1	1	0	2
Diflubenzuron	P																															1		1						1	1																	1			1	1	2	0	3	6
Diflufenican	P																						1								1																															0	0	2	0	2
Digoxigenin	Ph																																	1														1					1										0	1	2	0	3
Diltiazem	Ph															1				1																			1	1	1										1								1			1	3	0	3	7
Dimethoate	P							1															1											1					1	1	1							1				1					1		1	1	1	1	2	3	6	2	13
Dimethylaniline 2,6	Ph					1		1				1							1				1				1			1		1	1			1			1														1			1		1		1		5	1	7	2	15
Diuron	P																		1				1				1					1		1		1	1	1			1							1				1						1	1		1	2	3	5	4	14
EDDP	D																																								1				1																2	0	0	0	2
Edifenphos	P																															1																													1	0	0	0	1
Enalapril	Ph															1																1				1																										1	0	0	2	3
Enoxacin	Ph																																						1																						0	1	0	0	1
Ephedrine	Ph			1			1	1	1	1						1	1	1	1	1	1		1		1	1	1		1	1	1	1	1	1	1		1	1	1	1	1	1	1	1	1			1	1			1	1		1	1	1		1	1		8	10	11	11	40
EPN C14H14NO4PS	P				1																						1																																		0	0	1	1	2
Erythromycin	Ph																												1				1			1	1	1			1																		1			3	2	0	2	7
Estradiol 17-acetate	Ph																													1	1	1		1		1		1		1												1							1	1		1	2	4	2	3	11
Estriol	Ph												1											1						1			1	1			1			1		1					1				1			1							1		3	6	1	2	12
Estrone 3-sulfate	Ph	1					1														1		1					1			1						1															1	1						1	1	1	1	3	3	4	3	13
Ethiofencarb	P																													1				1			1																								0	3	0	0	3
Ethion	P																									1																																				0	1	0	0	1
Ethoprophos	P				1																		1											1		1	1		1																							2	2	1	1	6
Ethoxyquin	P																																				1	1	1														1				1				1	1	1	0	4	6
Ethylmorphine	D																																					1																							0	0	0	1	1
Etrimphos	P			1																											1																															0	0	2	0	2

Table S4 (cont.)

Site code		1				2				3				4			5				6				7				8				9			10			11			12				13				14				15				16				Total positive data New				
sampling time		Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su	Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su	Au	Sp	Su	Au	Sp	Su	Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	spring	summer		autumn	
sampling type		G	G	P	G	G	G	P	G	G	G	P	G	G	G	G	G	G	P	G	G	G	P	G	G	G	P	G	G	G	P	G	G	G	G	G	G	G	G	G	G	G	G	P	G	G	G	P	G	G	G	P	G	G	G	P	G	G	G	P	G	grab	grab	POCIS	grab	total
Positive data in the column		14	15	29	13	24	23	36	31	10	11	17	13	10	14	13	15	23	32	20	30	40	81	27	21	36	42	34	74	74	94	77	58	79	63	54	63	62	60	71	78	19	19	35	23	28	47	56	41	28	54	65	49	12	23	33	24	60	69	85	78	517	661	605	646	2429
Famotidine	Ph																						1						1	1	1	1									1																					1	1	2	2	6
Fenazaquin	P																											1					1						1																			1				3	0	0	1	4
Fenitrothion	P																																1																													1	0	0	0	1
Fenobucarb	P							1																							1					1	1	1						1			1														1		1	2	4	1	8
Fenofibrate	Ph																						1								1																															0	0	2	0	2
Fenoxycarb	P																													1																																0	1	0	0	1
Fenpropidin	P																1																																												0	1	0	0	1
Fenpropimorph	P																																	1												1							1						1	1		1	2	1	1	5
Fensusfothion	P											1											1																																							0	0	0	2	2
Fenthion	P							1						1													1										1													1								1				2	1	0	3	6
Fenuron	P																																1				1		1																			1	1		0	3	1	1	5
Fluazifop buthyl	P							1													1	1			1	1										1							1																			0	3	4	0	7
Flufenamic acid	Ph																						1						1		1	1	1	1	1	1	1	1	1	1						1	1			1	1						1		1	1	4	6	4	5	19
Fluometuron	P	1				1				1	1			1	1		1				1				1	1																																			7	3	0	0	10
Fluoxetine	Ph																																														1															0	0	1	0	1
Flutriafol	P																																										1				1													0	0	0	2	2
Fosthiazate	P																																		1				1	1						1													1		1	2	1	1	5
Fuberidazole	P																																														1															0	0	1	0	1
Furmeciclox	Ph																											1		1	1	1	1				1		1												1							1	1	1	2	3	3	3	11
Gemfibrozil	Ph								1														1						1	1	1	1	1	1	1	1		1	1	1	1						1	1	1	1	1	1	1					1	1	1	1	6	6	5	8	25
Griseofulvin	Ph	1	1	1	1	1	1	1	1								1	1	1	1	1	1	1	1	1	1	1	1			1						1	1				1	1	1	1	1	1	1	1		1	1		1	1	1	1	1	1	1		9	11	11	9	40
Hexathyazox	P							1	1																	1																															1					0	1	1	2	4
Hexazinone	P					1												1					1		1				1					1								1				1						1	1	1				1	1	1		7	3	3	1	14
Hydrochlorotiazide	Ph																						1						1	1			1	1	1	1	1	1	1	1	1																	1		1	1	5	4	2	4	15
Hydroxybiphenyl	P																																																												1	0	0	0	1	1
Ibuprofen	Ph		1				1								1								1									1																														0	3	1	1	5
Imazalil	P		1																										1			1		1	1	1				1	1																		1	1	1	2	4	1	4	11
Imazalil-metabolite	P																						1						1	1	1	1	1					1	1	1	1																	1	1	1	3	3	3	4	13
Imazametabenz methyl	P			1			1				1		1			1						1		1		1		1				1	1	1	1			1			1				1												1					1	5	1	10	17
Imazapyr	P																					1				1			1	1		1	1	1	1	1	1	1	1	1	1					1			1	1			1					1	1	1	1	7	7	1	7	22
Imazaquin	P																						1								1				1																										0	0	2	1	3
Imidachloprid	P										1										1	1	1				1		1	1	1	1		1	1	1	1	1	1	1							1	1		1		1						1	1	1	1	6	7	7	4	24
Indomethacin	Ph																														1	1	1	1																											1	1	1	1	4
Iohexol	Ph																												1	1	1	1	1	1	1		1	1	1	1	1																	1	1	1	1	4	5	2	5	16
Iomeprol	Ph																																1							1										1											1	2	1	0	1	4
Iopamidol	Ph																												1	1	1	1	1	1	1	1	1	1	1	1	1							1										1	1	1		5	5	3	4	17
Iopromide	Ph																												1	1	1	1	1	1	1					1	1		1		1			1	1		1	1	1					1	1	1	1	3	6	4	7	20
Iprodione	P																						1								1																															0	0	2	0	2
Isoprocarb	P								1														1							1	1			1			1								1			1	1		1	1	1					1	1	1	1	1	5	5	5	16
isoproturon	P							1																												1							1																		0	1	2	0	3
Josamycin	Ph																						1				1					1				1										1							1					1				3	0	2	2	7
Ketamine	Ph																													1				1			1			1	1																		1		1	0	5	0	2	7
Ketoprofen	Ph										1								1			1	1	1					1	1	1	1		1	1		1	1	1	1	1						1	1	1		1	1	1			1					1	2	8	6	8	24
Kresoxim methyl	P																						1							1	1		1	1	1	1	1	1	1	1	1			1				1										1	1	1	1	4	5	5	4	18
Lenacil	P								1																						1																															0	0	1	1	2
Lincomycin	Ph																																	1	1					1													1			1			1	1		0	3	2	2	7
Linuron	P																																							1																					0	0	0	1	1
Loratadine	Ph																						1					1	1	1	1						1				1								1						1	1					1	1	3	3	4	11
Lorazepam	Ph																						1									1	1	1	1	1	1	1	1	1	1									1		1							1	1	1	4	4	3	5	16
Malachite green	Ph	1																			1																																								2	0	0	0	2
Malaoxon	P																														1	1				1	1																									1	1	1	1	4
Malathion	P				1	1												1								1			1		1	1													1		1														1		2	3	2	3	10
MDA/3,4-Methylenedioxyamphetamine	D																						1						1		1															1															1	1	2	0	4
MDMA (3,4-Methylenedioxy-N-methyla	D																																	1											1						1											1	1	1	0	3
MDMA-Ethyl-3,4-N-methylenedioxyam	D																												1			1																													1	0	0	1	2
Mecarbam	P																												1	1	1																														1	1	1	0	3
Mefenacet	P																														1																														0	0	1	0	1
Mefenamic acid	Ph																						1							1	1	1																												1	0	1	2	2	5
Metalaxil	P			1				1											1			1	1				1		1	1	1	1	1	1	1	1	1	1	1	1	1																1	1	1	1	1	5	6	7	6	24
Metamitron	P																																							1																					0	0	0	1	1

Table S4 (cont.)

Site code		1				2				3				4			5				6				7				8				9			10			11			12				13				14				15				16				Total positive data New				
sampling time		Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su	Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su	Au	Sp	Su	Au	Sp	Su	Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	spring	summer		autumn	
sampling type		G	G	P	G	G	G	P	G	G	G	P	G	G	G	G	G	G	P	G	G	G	P	G	G	G	P	G	G	G	P	G	G	G	G	G	G	G	G	G	G	G	G	P	G	G	G	P	G	G	G	P	G	G	G	P	G	G	G	P	G	grab	grab	POCIS	grab	total
Positive data in the column		14	15	29	13	24	23	36	31	10	11	17	13	10	14	13	15	23	32	20	30	40	81	27	21	36	42	34	74	74	94	77	58	79	63	54	63	62	60	71	78	19	19	35	23	28	47	56	41	28	54	65	49	12	23	33	24	60	69	85	78	517	661	605	646	2429
Metformin	Ph					1							1									1		1				1	1	1		1		1	1	1		1	1	1	1				1				1				1					1	1		1	5	5	0	11	21
Methabenzthiazuron	P																																				1																									0	1	0	0	1
Methadone	D																														1																															0	0	1	0	1
Methamfetamine	D											1																						1				1	1		1																	1				2	1	1	2	6
Metolcarb	P		1	1	1	1	1	1	1		1	1			1	1			1	1		1	1	1		1	1	1		1	1	1		1	1		1			1	1		1	1	1		1	1	1		1	1	1		1	1	1		1	1	1	1	15	12	14	42
Metoprolol	Ph																					1	1						1	1	1	1		1	1	1	1	1	1	1	1										1				1	1		1	1	1	1	4	8	4	5	21
Metoxuron	P	1						1									1																																													2	0	1	0	3
Metribuzin	P																				1		1								1																															1	0	2	0	3
Metronidazole	Ph																												1	1		1		1	1			1	1	1	1							1	1	1	1	1	1					1	1			4	5	2	6	17
Mevinphos	P																																																											1		0	0	1	0	1
Molinate	P					1			1																																																					1	0	0	1	2
Monocrotofos	P																																	1													1															0	2	0	0	2
Monolinuron	P	1								1				1			1				1																																									5	0	0	0	5
Morphine	D							1															1			1	1		1			1	1				1				1							1	1		1	1	1								1	2	3	5	5	15
Myclobutanil	P																						1													1	1				1			1																		1	1	2	1	5
Nalidixic acid	Ph	1												1	1										1																																					3	1	0	0	4
Naproxen	Ph																				1	1	1		1	1	1	1	1	1	1	1	1	1	1	1			1	1	1					1	1	1	1	1	1	1	1					1	1		1	9	8	5	7	29
Nicotine*	D		1	1				1										1	1		1	1	1		1		1		1	1	1	1	1	1		1	1	1	1	1	1			1		1	1	1	1		1	1	1		1	1		1	1	1	1	8	11	11	6	36
Nitempyram	P											1								1																								1																		0	0	2	1	3
N-nitroso diethylamine	V																						1					1																1							1	1	1			1						0	1	4	2	7
N-nitrosodi-n-dibuthylamine	V																																														1		1		1		1									0	2	0	2	4
Norfloxacin	Ph																															1														1																1	0	0	1	2
Nuarimol	P																																					1																								0	0	0	1	1
Ofloxacin	Ph																												1	1		1									1						1															1	2	0	2	5
Omeprazole	Ph																				1		1		1	1			1	1	1	1	1	1	1	1			1	1	1					1				1			1							1	1	8	4	3	5	20
Oxadiazon	P																														1			1																												0	1	1	0	2
Oxadixyl	P																																																				1									0	0	0	1	1
Oxfendazole	Ph																										1			1	1																															0	1	2	0	3
Parathion methyl	P																1																																													1	0	0	0	1
Paraxanthine*	D			1				1			1	1									1	1	1	1	1	1	1	1			1	1	1	1	1	1	1		1	1	1	1				1	1	1	1	1	1	1	1						1	1	1	8	9	9	8	34
Penconazole	P																						1																																							0	0	1	0	1
Pencycuron	P							1										1						1													1					1		1						1						1						2	2	3	1	8
Penicillin benzyl	Ph																															1																				1										0	0	1	1	2
Perfluoro optanoic acid (PFOA)	V																																															1			1											0	1	1	0	2
Perfluoropentanoic acid	V																												1																																	1	0	0	0	1
Phenacetin	Ph	1										1										1							1					1										1		1		1			1	1			1							3	4	4	0	11
Phenazone	Ph						1														1									1	1	1				1			1	1	1							1			1								1	1	1	3	5	3	3	14
phenethylamin	D			1																																																1										0	0	2	0	2
Phenprobamate	Ph																													1																																0	1	0	0	1
phenylbutazone	Ph																									1				1	1	1		1			1															1						1	1			1	5	2	1	9
Phenylephrine	Ph																						1						1	1	1	1																														1	1	2	1	5
Phenytoin	Ph						1															1		1	1			1	1	1	1		1	1	1		1	1	1		1		1		1		1	1	1		1		1		1			1		1		5	9	3	8	25
Picolinafen	P																														1																															0	0	1	0	1
Pindolol	Ph						1																																			1																1				2	1	0	0	3
Pipemidic acid	Ph			1															1				1					1																1								1										0	0	5	1	6
Pirimicarb	P			1				1			1	1						1	1				1			1	1	1	1		1				1			1			1			1				1	1		1	1	1	1		1		1	1	1	1	3	5	12	7	27
Pirimiphos methyl	P																																																	1												1	0	0	0	1
Pravastatin	Ph												1							1																																										0	0	0	2	2
Progesterone	Ph													1			1				1																		1					1					1													4	0	1	1	6
Promecarb	P			1		1																									1			1						1	1						1											1	1	1	1	2	4	3	2	11
Propamocarb	P																	1	1																																											0	1	1	0	2
Propiconazole	P																												1	1	1								1		1																				1	2	1	1	2	6
Propisochlor	P																		1																																					1			1	1		0	1	3	0	4
Propoxur	P																						1																							1				1												2	0	1	0	3
Propranolol	Ph																														1	1					1		1	1	1												1								1	1	2	1	4	8
propyzamide	P																		1																																											0	0	1	0	1
proquinazid	P											1																						1										1								1										0	1	3	0	4
prosulfocarb	P																																																				1								1	0	0	0	2	2
prosulfuron	P																		1																																						1		1	1		0	1	2	1	4
Pymetrozine	P			1				1																																																1						0	0	3	0	3

Table S4 (cont.)

Site code		1				2				3				4			5				6				7				8				9			10			11			12				13				14				15				16				Total positive data New				
sampling time		Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su	Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su	Au	Sp	Su	Au	Sp	Su	Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	Sp	Su		Au	spring	summer		autumn	
sampling type		G	G	P	G	G	G	P	G	G	G	P	G	G	G	G	G	G	P	G	G	G	P	G	G	G	P	G	G	G	P	G	G	G	G	G	G	G	G	G	G	G	G	P	G	G	G	P	G	G	G	P	G	G	G	P	G	G	G	P	G	grab	grab	POCIS	grab	total
Positive data in the column		14	15	29	13	24	23	36	31	10	11	17	13	10	14	13	15	23	32	20	30	40	81	27	21	36	42	34	74	74	94	77	58	79	63	54	63	62	60	71	78	19	19	35	23	28	47	56	41	28	54	65	49	12	23	33	24	60	69	85	78	517	661	605	646	2429
Pyranocoumarin	Ph		1	1																			1			1			1	1		1	1	1		1				1								1						1							1	4	5	3	2	14
Pyridaben	P							1																															1																							1	0	1	0	2
Pyroquilon	P			1			1	1	1									1	1				1			1	1		1	1	1	1	1	1	1	1	1	1	1	1	1										1	1				1		1	1		1	5	9	8	6	28
Quinalphos	P																														1																															0	0	1	0	1
Quinmerac	P						1																											1			1				1																			1		0	3	1	1	5
Quizalofop-P-ethyl	P					1																																																								1	0	0	0	1
Ranitidine	Ph																												1		1	1	1		1	1		1	1	1	1						1	1														4	2	2	4	12
rotenone	P																						1				1								1							1																			1	1	0	2	2	5
Salbutamol	Ph		1						1							1				1	1	1	1	1	1	1		1	1	1		1	1	1	1	1		1	1	1	1					1	1	1	1		1	1	1					1	1		1	8	9	3	12	32
Simazine	P					1	1	1										1	1				1			1	1			1	1	1		1								1	1	1	1	1		1		1				1	1	1	1		1	1	1	5	8	9	4	26
Sotalol	Ph																																1							1																		1				2	1	0	0	3
Spinosin-A	P														1														1				1			1					1																	1				4	1	0	1	6
Spiramycin	Ph																										1			1					1																	1										0	1	2	1	4
Spiroxamine	P		1			1	1		1	1			1	1		1	1			1	1	1		1	1	1		1				1			1			1			1				1		1		1				1				1	1		1	1	7	5	1	15	28
Sulfadimethoxyne	Ph																																			1												1				1			1							1	1	2	0	4
Sulfamethazine	Ph		1		1				1							1				1										1			1										1								1				1							1	5	0	4	10
Sulfamethizole	Ph																					1							1				1				1		1	1						1																4	3	0	0	7
Sulfamethoxazole	Ph					1	1	1	1												1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		1	1		1	1	1	1	1	1	1	1					1	1	1	1	9	11	8	10	38
Sulfanilamide	Ph					1																																																								1	0	0	0	1
Sulfapiridine	Ph																												1		1				1				1		1							1			1									1	1	2	1	3	3	9
TBP	V	1				1			1	1		1	1	1	1	1	1			1	1	1	1	1	1	1		1	1				1	1	1	1		1	1												1						1	1				12	5	2	9	28
TCPP	V		1	1			1	1	1				1			1		1		1		1	1	1		1	1	1		1	1	1	1	1	1		1	1		1	1		1	1	1		1	1	1		1	1			1				1	1	1	1	14	9	13	37
Tebuconazole	P																		1				1								1					1	1	1						1																		1	1	4	1	7
TEP	V																												1			1	1			1			1											1								1				6	0	0	1	7
Terbuthylazine	P																	1	1											1	1													1										1		1	1		1	1		1	3	5	1	10
Terbuthylazine-desethyl	P		1															1					1							1		1															1												1			0	5	1	1	7
Terbutryn	P																						1				1		1	1	1	1	1	1	1		1	1	1	1	1			1												1			1	1	1	3	5	6	5	19
Testosterone	Ph	1				1								1								1	1																1								1															4	2	1	0	7
THC - tetrahydrocannabinol	D							1																											1			1						1							1	1						1	1	1	1	1	2	4	3	10
Theobromine	D							1			1						1										1		1									1				1				1	1		1	1	1	1					1	1		1	1	6	3	4	4	17
Theofilline	Ph																																																				1				1				1	0	0	0	3	3
Thiabendazole	P			1				1															1				1		1	1	1		1	1	1	1	1	1	1	1	1											1						1		1		5	4	7	3	19
Thiamethoxam	P																										1										1																							1		0	1	2	0	3
Thymopentin	Ph				1													1										1							1								1						1		1		1				1				1	0	3	0	7	10
Trietazine	P																																					1																								0	0	0	1	1
Trimethoprim	Ph																						1						1	1	1	1	1	1	1	1	1	1	1	1	1							1			1	1	1					1	1	1	1	5	6	5	6	22
Trinexapac ethyl	P																		1																																						1					0	0	1	1	2
Tylosine	Ph																														1	1	1											1								1	1						1			1	1	3	2	7
Valsartan	Ph			1			1	1	1									1	1	1		1	1	1		1	1	1		1	1	1		1	1		1	1		1	1		1	1	1		1	1	1		1	1	1						1	1	1	0	12	10	12	34
Venlafaxine	Ph		1			1	1	1	1		1			1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		1	1	1	1	1	1	1				1	1	1	1	1	12	15	9	14	50
Warfarin	Ph							1											1																			1						1			1	1				1	1									0	1	5	2	8
Xantine 3-methyl	D						1																																																							0	1	0	0	1

Table S5. Physical-chemical properties of selected compounds. Water solubility and logKow: data for pesticides were taken from Tomlin (2003); data for other chermicals were taken from the PubChem database (https//pubchem.ncbi.nlm.nih.gov/compound). Rs, sampling rate for POCIS calculations: references are listed; est= estimated values (see text below). Chemical classes: I: insecticides; H: herbicides; F: fungicides; Ph= pharmaceuticals; A: antibiotics; E: estrogens and steroids; P= pesticides; D: drugs and life style chemicals; Pl= plasticizers**.**

	Chemical class	Details of uses	CAS	MW	WS mg/L	logKow	Rs	
Pesticides							L/d	Ref.
Carbofuran	I		1563-66-2	221	350	2.37	0.18	1
Chlorpyrifos ethyl	I		2921-88-2	351	0.73	3	0.05	1
Diazinon	I		333-41-5	304	60	3.4	0.4	1
Dimethoate	I		60-51-5	229	25000	0.8	0.22	2
Imidacloprid	I		138261-41-3	256	610	0.6	0.18	1
Malathion	I		121-75-5	330	145	2.57	0.2	est
Metolcarb	I		1129-41-5	165	2600	1.7	0.2	est
Pirimicarb	I		23103-98-2	238	2700	3	0.3	1-2
Spinosin-A	I		168316-95-8	370	89	3	0.2	est
Chlorturon	H		15545-48-9	244	70	2.3	0.2	est
Diuron	H		330-54-1	233	40	2.5	0.1	1-2
Metribuzine	H		21087-64-9	214	1050	2	0.168	1-2
Simazine	H		122-34-9	202	5	2.2	0.22	1-2
Terbuthrin	H		886-50-0	241	25	3.7	0.25	1
Terbuthylazine	H		5915-41-3	235	9	2.9	0.28	1-2
Carbedazim	F		10605-21-7	191	8	1.5	0.22	1
Kresoxim methyl	F		143390-89-0	313	2	4.1	0.3	est
Propiconazole	F		60207-90-1	342	110	3.7	0.3	2
Spiroxaminie	F		118134-30-8	297	405	4.2	0.25	1
Tebuconazole	F		107534-96-3	308	32	3.7	0.24	2
Pharmaceuticals								
Acetaminophen (paracetamol)	Ph	Analgesic/anti inflammatory	103-90-2	151	14000	0.46	0.14	2
Atenolol	Ph	β blocker	29122-68-7	266	13000	0.16	0.025	3
Carbamazepine	Ph	antiepylectic	298-46-4	236	18	2.5	0.16	4
Citalopram	Ph	antidepressant	59729-33-8	324	6	3.5	0.17	4
Diclofenac	Ph	Analgesic/anti inflammatory	15307-86-5	296	2.35	4.7	0.09	8
Gemfibrozil	Ph	hypolipidemic	25812-30-0	250	10	4.8	0.089	5
Ibuprofen	Ph	Analgesic/anti inflammatory	15687-27-1	206	21	3.9	0.118	3
Ketoprofen	Ph	Analgesic/anti inflammatory	22071-15-4	254	50	3.1	0.083	5
Loratadine	Ph	antiistaminic	79794-75-5	383	0.011	5.2	0.2	3
Naproxen	Ph	Analgesic/anti inflammatory	22204-53-1	230	16	3.2	0.072	4
Omeprazole	Ph	gastroprotector	73590-58-6	345	35	2.2	0.03	6
Salbutamol	Ph	antiasthmatic	18559-94-9	239	14000	0.3	0.09	est

Table S5 (cont.)

	Chemical class	Details of uses	CAS	MW	WS mg/L	logKow	Rs	
Pharmaceuticals							L/d	Ref.
Valsartan	Ph	anti hypertension	137862-53-4	435	1.5	5.8	0.18	est
Venlafaxine	Ph	antidepressant	93413-69-5	277	570	3.2	0.14	4
Antibiotics								
Amoxicillin	A	antibiotic	26787-78-0	365	3400	0.87	0.10	est
Azithromicin	A	antibiotic	83905-01-5	749	500	3	0.06	2
Ciprofloxacin	A	antibiotic	85721-33-1	331	30000	-1.1	0.07	est
Erythromycin	A	antibiotic	114-07-8	734	2000	2.5	0.18	5
Lincomycin	A	antibiotic	154-21-2	407	927	0.2	0.09	est
Metronidazole	A	antibiotic	443-48-1	171	11000	-0.02	0.09	est
Sulfamethoxazole	A	antibiotic	723-46-6	253	610	0.9	0.03	3
Trimethoprim	A	antibiotic	738-70-5	290	400	0.91	0.08	4
Tylosine	A	antibiotic	1401-69-0	916	5	1.63	0.11	est
Estrogens and steroids								
Estradiol	E	estrogen	50-28-2	272	3.6	4	0.074	4
Estrone	E	estrogen	481-97-0	350	0.04	2.5	0.12	2
Progesterone	E	steroid	57-83-0	314	9	3.9	0.35	3
Testosterone	E	steroid	58-22-0	288	23	3.3	0.28	3
Others								
Amphetamine	D	nervous stimulant	300-62-9	135	28000	1.8	0.27	2
Caffeine	D	nervous stimulant	58-08-2	194	20000	-0.07	0.1	7
Nicotine	D	alcaloid	54-11-5	162	100000	1.2	0.11	est
Paraxanthine	D	nervous stimulant	611-59-6	180	1000	-0.2	0.09	est
Industrial chemicals								
TBP - Tributyl-phosphate	Pl	plasticizer	126-73-9	266	280	3	0.18	4

References:

1. Ahrens et al. (2015)

2. Morin et al. (2012)

3. Morin et al. (2013)

4. Bayen et al. (2014)

5. Alvarez et al. (2004)

6. Li et al. (2018)

7. Li et al. (2010)

Selection of R_s values

Values of the sampling rate coefficients (R_s) for the calculation of water concentrations from POCIS data were taken from the literature for most compounds (Table S5). If literature data were not available, approximated values were estimated according with the following procedures.

Pesticides. A precise relationship between R_s and other properties (e.g. log Kow) was not found. However, some rules were observed. For chemicals with logKow between 0.5 and 3, R_s values are in the range 0.15 to 0.25. An approximated values of 0.2 was assumed if literature data were not available. For chemicals with logKow between 3 and 5, R_s values are in the range 0.25 to 0.35. In this case, an approximated value of 0.3 was assumed for the unique chemical in this class (kresoxim- methyl).

Point Source Chemicals. For these chemicals the variability range of properties is higher. Excluding three outliers with very high values (progesterone, testosterone and amphetamine) the following relationship between R_s and log Kow was found and used for the calculation of unknown values:

$R_s = 0.08 + 0.02 \log Kow \qquad (R^2 = 0.6)$

Pesticide scoring for the selection of the chemicals for the quantitative analysis. Selected chemicals are in bold. The reported EC50 represents the acute toxicity for the most sensitive aquatic organism. The uses in the watershed indicate the number of crops treated with the compound (maximum 10). The procedures for calculating the different scores (SSc , STox, SCrop, STotal) is described in the main text. Other information: NRS: not registered in Spain for agricultural uses. This additional information was searched only for the first thirty chemicals in the list.

Table S6. Pesticide scoring for the selection of the chemicals for the quantitative analysis. Selected chemicals are in bold. The reported EC50 represents the acute toxicity for the most sensitive aquatic organism. The uses in the watershed indicate the number of crops treated with the compound (maximum 10). The procedures for calculating the different scores (SSc, STox, SCrop, STotal) is described in the main text. Other information: NRS: not registered in Spain for agricultural uses. This additional information was searched only for the first thirty chemicals in the list.

Chemicals	Screening results		Toxicity		Agricultural use		S_{Total}	Other information
	Positive samples	S_{Sc}	log EC50 mg/L	S_{Tox}	Treated crops	S_{Crop}		
Pirimicarb	**27**	**6.43**	**-4.00**	**10.00**	**3**	**3.33**	**26.19**	
Metolcarb	**42**	**10.00**	**-0.02**	**3.36**	**0**	**0.00**	**23.36**	
Chlorpyrifos	**3**	**0.71**	**-3.70**	**9.50**	**9**	**10.00**	**20.93**	
Spiroxamine	**28**	**6.67**	**-2.22**	**7.04**	**0**	**0.00**	**20.37**	
Cypermethrin	1	0.24	-2.52	7.54	9	10.00	18.01	
Simazine	**26**	**6.19**	**-1.22**	**5.37**	**0**	**0.00**	**17.75**	**NRS**
Propiconazole	**6**	**1.43**	**-1.70**	**6.16**	**6**	**6.67**	**15.69**	
Imidacloprid	**24**	**5.71**	**1.23**	**1.28**	**3**	**3.33**	**16.04**	
Terbutryn	**19**	**4.52**	**-2.10**	**6.83**	**0**	**0.00**	**15.88**	**NRS**
Kresoxim methyl	**18**	**4.29**	**-0.82**	**4.71**	**2**	**2.22**	**15.50**	
Diazinon	**14**	**3.33**	**-3.00**	**8.33**	**0**	**0.00**	**15.00**	**NRS**
Chlortoluron	**7**	**1.67**	**-1.49**	**5.82**	**5**	**5.56**	**14.71**	
Dimethoate	**13**	**3.10**	**-0.70**	**4.50**	**3**	**3.33**	**14.02**	
Metalaxil	24	5.71	0.48	2.54	0	0.00	13.97	NRS
Pyroquilon	28	6.67	1.60	0.66	0	0.00	14.00	NRS
Tebuconazole	**7**	**1.67**	**0.60**	**2.33**	**7**	**7.78**	**13.44**	
Diuron	**14**	**3.33**	**-2.15**	**6.92**	**0**	**0.00**	**13.59**	**NRS**
Isoprocarb	16	3.81	-1.40	5.66	0	0.00	13.28	NRS
Imazamethabenz-methyl	17	4.05	-1.00	5.00	0	0.00	13.10	NRS
Carbofuran	**15**	**3.57**	**-1.40**	**5.66**	**0**	**0.00**	**12.81**	**NRS**
Thiabendazole	19	4.52	-0.30	3.84	0	0.00	12.88	NRS
Spinosin-A	**6**	**1.43**	**-0.88**	**4.80**	**4**	**4.44**	**12.10**	
Terbuthylazine	**10**	**2.38**	**-1.70**	**6.16**	**1**	**1.11**	**12.04**	
Metribuzin	**3**	**0.71**	**-1.40**	**5.66**	**4**	**4.44**	**11.53**	
Carbendazim	**14**	**3.33**	**-1.05**	**5.08**	**0**	**0.00**	**11.74**	**NRS**
Malathion	**10**	**2.38**	**-2.10**	**6.83**	**0**	**0.00**	**11.59**	
Imazapyr	22	5.24	1.18	1.37	0	0.00	11.84	
Hexazinone	14	3.33	-0.70	4.50	0	0.00	11.16	
Chloroxuron	7	1.67	-2.52	7.54	0	0.00	10.87	
Azinphos methyl	2	0.48	-3.70	9.50	0	0.00	10.45	NRS
Fenitrothion	1	0.24	-3.70	9.50	0	0.00	9.97	
Picolinafen	1	0.24	-3.70	9.50	0	0.00	9.97	
Fenthion	6	1.43	-2.22	7.04	0	0.00	9.89	
Edifenphos	1	0.24	-3.52	9.20	0	0.00	9.68	
Pirimiphos methyl	1	0.24	-3.52	9.20	0	0.00	9.68	
Diflubenzuron	6	1.43	-2.15	6.92	0	0.00	9.78	
Prosulfuron	4	0.95	-2.00	6.67	1	1.11	9.68	
Ethoprophos	6	1.43	-1.30	5.50	1	1.11	9.47	
Promecarb	11	2.62	-0.52	4.20	0	0.00	9.44	
Diafentiuron	5	1.19	-2.15	6.92	0	0.00	9.31	
Fenobucarb	8	1.90	-1.30	5.50	0	0.00	9.31	
Isoproturon	3	0.71	-1.33	5.55	2	2.22	9.20	
Quinalphos	1	0.24	-3.15	8.59	0	0.00	9.07	
Chlorfenvinphos	1	0.24	-3.15	8.58	0	0.00	9.06	

Table S6 (cont.)

Chemicals	Screening results		Toxicity		Agricultural use		S_{Total}	Other information
	Positive samples	S_{Sc}	log EC50 mg/L	S_{Tox}	Treated crops	S_{Crop}		
Fenazaquin	4	0.95	-2.30	7.17	0	0.00	9.07	
Chlorsulfuron	1	0.24	-1.00	5.00	3	3.33	8.81	
Pyridaben	2	0.48	-2.70	7.83	0	0.00	8.78	
Mecarbam	3	0.71	-2.40	7.33	0	0.00	8.76	
Fluometuron	10	2.38	-0.46	4.09	0	0.00	8.86	
Rotenone	6	1.43	-1.52	5.87	0	0.00	8.73	
Aminocarb	11	2.62	-0.15	3.59	0	0.00	8.83	
Myclobutanil	5	1.19	-1.10	5.16	1	1.11	8.65	
Furmeciclox	11	2.62	0.17	3.05	0	0.00	8.29	
Imazalil	11	2.62	0.18	3.04	0	0.00	8.28	
Fluazifop buthyl	9	2.14	-0.30	3.84	0	0.00	8.12	
Propisochlor	3	0.71	-1.92	6.53	0	0.00	7.96	
Atrazine-desethyl	10	2.38	0.00	3.33	0	0.00	8.10	
Parathion methyl	1	0.24	-2.40	7.33	0	0.00	7.81	
Pencycuron	7	1.67	-0.72	4.54	0	0.00	7.87	
Hexathyazox	5	1.19	0.08	3.20	2	2.22	7.80	
Cyanazine	4	0.95	-1.52	5.87	0	0.00	7.78	
Lenacil	2	0.48	-2.00	6.67	0	0.00	7.62	
Difenacoum	3	0.71	-1.68	6.14	0	0.00	7.56	
Fenpropidin	1	0.24	-2.22	7.04	0	0.00	7.51	
Atrazine	4	0.95	-1.40	5.66	0	0.00	7.57	
Fenpropimorph	5	1.19	-1.10	5.16	0	0.00	7.54	
Monocrotofos	2	0.48	-1.82	6.37	0	0.00	7.33	
Azoxystobin	5	1.19	-1.00	5.00	0	0.00	7.38	
Etrimphos	2	0.48	-1.77	6.28	0	0.00	7.23	
Dietofencarb	8	1.90	0.57	2.39	1	1.11	7.31	
Bentazone	2	0.48	1.00	1.67	4	4.44	7.06	
Propyzamide	1	0.24	0.74	2.10	4	4.44	7.02	
Mevinphos	1	0.24	-1.92	6.53	0	0.00	7.01	
Acetamiprid	3	0.71	0.04	3.26	2	2.22	6.92	
Monolinuron	5	1.19	-0.68	4.46	0	0.00	6.84	
Fenoxycarb	1	0.24	-0.40	4.00	2	2.22	6.69	
Dicrotofos	1	0.24	-1.70	6.16	0	0.00	6.64	
Imazalil-metabolite	13	3.10	1.58	0.70	0	0.00	6.89	
Linuron	1	0.24	-1.66	6.10	0	0.00	6.57	
Fosthiazate	5	1.19	-0.55	4.25	0	0.00	6.64	
Prosulfocarb	2	0.48	-1.30	5.50	0	0.00	6.45	
Propoxur	3	0.71	-1.00	5.00	0	0.00	6.43	
Metoxuron	3	0.71	-0.96	4.93	0	0.00	6.36	
Oxadiazon	2	0.48	-1.22	5.37	0	0.00	6.32	
Terbuthylazine-desethyl	9	2.14	0.73	2.11	0	0.00	6.40	
Aldicarb	2	0.48	-1.15	5.26	0	0.00	6.21	
Fensusfothion	2	0.48	-1.15	5.26	0	0.00	6.21	
Methabenzthiazuron	1	0.24	-1.40	5.66	0	0.00	6.14	
Proquinazid	4	0.95	-0.52	4.20	0	0.00	6.11	
Fenuron	5	1.19	-0.15	3.59	0	0.00	5.97	
Aldicarb-sulfone	3	0.71	-0.70	4.50	0	0.00	5.93	
Mefenacet	1	0.24	-1.22	5.37	0	0.00	5.85	

Table S6 (cont.)

Chemicals	Screening results		Toxicity		Agricultural use		S_{Total}	Other information
	Positive samples	S_{Sc}	log EC50 mg/L	S_{Tox}	Treated crops	S_{Crop}		
EPN - C14H14NO4PS	3	0.71	-0.52	4.20	0	0.00	5.63	
Ethoxyquin	6	1.43	0.30	2.83	0	0.00	5.69	
Ethion	2	0.48	-0.70	4.50	0	0.00	5.45	
Molinate	2	0.48	-0.70	4.50	0	0.00	5.45	
Trietazine	1	0.24	-0.89	4.81	0	0.00	5.29	
Iprodione	2	0.48	-0.52	4.20	0	0.00	5.16	
Buturon	1	0.24	-0.74	4.57	0	0.00	5.05	
Metamitron	2	0.48	-0.40	4.00	0	0.00	4.95	
Flutriafol	2	0.48	1.08	1.53	2	2.22	4.71	
Benfuracarb	2	0.48	-0.22	3.70	0	0.00	4.66	
Benalaxil	1	0.24	-0.22	3.70	0	0.00	4.18	
Bensulide	1	0.24	-0.22	3.70	0	0.00	4.18	
Ethiofencarb	3	0.71	0.34	2.76	0	0.00	4.19	
Thiamethoxam	4	0.95	>2	0.00	2	2.22	4.13	
Penconazole	1	0.24	-0.10	3.49	0	0.00	3.97	
Hydroxybiphenyl	1	0.24	-0.07	3.45	0	0.00	3.93	
Fuberidazole	1	0.24	-0.05	3.41	0	0.00	3.89	
Quizalofop-P-ethyl	1	0.24	0.04	3.26	0	0.00	3.74	
Cinosulfuron	3	0.71	0.68	2.20	0	0.00	3.63	
Nuarimol	1	0.24	0.40	2.67	0	0.00	3.15	
Asulam	5	1.19	1.51	0.82	0	0.00	3.21	
Boscalid	1	0.24	0.43	2.61	0	0.00	3.09	
Nitenpyram	3	0.71	1.00	1.67	0	0.00	3.10	
Imazaquin	3	0.71	1.11	1.48	0	0.00	2.91	
Quinmerac	5	1.19	1.68	0.53	0	0.00	2.91	
Trinexapac ethyl	2	0.48	0.97	1.71	0	0.00	2.66	
Pymetrozine	3	0.71	1.34	1.10	0	0.00	2.52	
Diflufenican	2	0.48	1.37	1.06	0	0.00	2.01	
Chlordimeform	1	0.24	1.11	1.48	0	0.00	1.95	
Malaoxon	4	0.95	>2	0.00	0	0.00	1.90	
Bensulfuron methyl	3	0.71	>2	0.00	0	0.00	1.43	
Clofibric acid	1	0.24	1.50	0.83	0	0.00	1.31	
Oxadixyl	1	0.24	1.66	0.57	0	0.00	1.04	
Propamocarb	2	0.48	>2	0.00	0	0.00	0.95	
Acephate	1	0.24	1.83	0.29	0	0.00	0.77	
Butoxycarboxim	1	0.24	>2	0.00	0	0.00	0.48	
Cyromazine	1	0.24	>2	0.00	0	0.00	0.48	

Table S7. Pharmaceuticals scoring and ranking. Pharmaceutical scoring for the selection of the chemicals for the quantitative analysis. Selected chemicals are in bold. The reported EC50 represents the acute toxicity for the most sensitive aquatic organism. The procedures for calculating the different scores (SSc, STox, STotal) is described in the main text.

Chemicals	Type	Screening results		Toxicity		S_{Total}
		Positive samples	S_{Sc}	log EC50 mg/L	S_{Tox}	
Venlafaxine	**antidepressant**	**50**	**10.00**	**1.00**	**3.97**	**23.97**
4-AAA (4-Acetamidoantipyrine)	metabolite	48	9.60	>2	0.00	19.20
Carbamazepine	**antiepylectic**	**40**	**8.00**	**1.30**	**2.78**	**18.78**
DEET - diethyltoluamide	insect repellent	45	9.00	>2	0.00	18.00
Valsartan	**anti hypertension**	**34**	**6.80**	**0.90**	**4.37**	**17.97**
Adenosine	nucleoside	44	8.80	>2	0.00	17.60
4-FAA	metabolite	43	8.60	>2	0.00	17.20
8-hydroxyquinoline	antiseptic	42	8.40	1.93	0.27	17.07
Atenolol	**b blocker**	**40**	**8.00**	**>2**	**0.00**	**16.00**
Griseofulvin	antimicotic	40	8.00	>2	0.00	16.00
Gemfibrozil	**hypolipidemic**	**25**	**5.00**	**0.77**	**4.88**	**14.88**
Naproxen	**anti inflammatory**	**29**	**5.80**	**1.27**	**2.90**	**14.50**
Ketoprofen	**anti inflammatory**	**24**	**4.80**	**1.07**	**3.67**	**13.27**
Loratadine	**antiistaminic**	**11**	**2.20**	**-0.15**	**8.53**	**12.93**
Salbutamol	**antiasthmatic**	**32**	**6.40**	**>2**	**0.00**	**12.80**
Acetaminophen/paracetamol	**analgesic**	**23**	**4.60**	**1.20**	**3.16**	**12.36**
Ibuprofen	**antiflammatory**	**5**	**1.00**	**-0.52**	**10.00**	**12.00**
Diclofenac	**antiflammatory**	**25**	**5.00**	**1.50**	**1.98**	**11.98**
Atenolol acid	metabolite	29	5.80	>2	0.00	11.60
Omeprazole	**gastroprotector**	**20**	**4.00**	**1.10**	**3.57**	**11.57**
Citalopram	**antidepressant**	**11**	**2.20**	**0.30**	**6.74**	**11.14**
Phenytoin	antiepilectic	24	4.80	1.70	1.18	10.78
4-AA (4-Aminoantipyrine)	metabolite	25	5.00	>2	0.00	10.00
Dimethylaniline 2,6	Metabolite	15	3.00	1.00	3.97	9.97
Fenofibrate	hypolipidemic	2	0.40	-0.27	9.01	9.81
Indomethacin	anti inflammatory	4	0.80	0.02	7.84	9.44
Mefenamic acid	analgesic	5	1.00	0.15	7.33	9.33
Propranolol	b blocker	8	1.60	0.68	5.23	8.43
Metformin	antidiabetic	21	4.20	>2	0.00	8.40
Metoprolol	b blocker	21	4.20	>2	0.00	8.40
Iopromide	contrasting agent	20	4.00	>2	0.00	8.00
Flufenamic acid	anti inflammatory	19	3.80	>2	0.00	7.60
Phenylbutazone	anti inflammatory	9	1.80	1.06	3.74	7.34
Fluoxetine	antidepressant	1	0.20	0.26	6.90	7.30

Table S7 (cont.)

Chemicals	**Type**	**Screening results**		**Toxicity**		**S_{Total}**
		Positive samples	**S_{Sc}**	**log EC50 mg/L**	**S_{Tox}**	
Lorazepam	ansiolitic	16	3.20	1.78	0.88	7.28
Iopamidol	contrasting agent	17	3.40	>2	0.00	6.80
Bezafibrate	control hyperlipidaemia	12	2.40	1.53	1.87	6.67
Iohexol	contrasting agent	16	3.20	>2	0.00	6.40
Hydrochlorotiazide	diuretic	15	3.00	>2	0.00	6.00
Phenazone	anti inflammatory	14	2.80	>2	0.00	5.60
Dilthiazem	anti angina	7	1.40	1.30	2.77	5.57
Diazepham	ansiolitic	4	0.80	1.04	3.80	5.40
Ranitidine	gastroprotector	12	2.40	>2	0.00	4.80
Warfarin	anticoagulant	8	1.60	1.66	1.35	4.55
Carbamazepine hepoxyde	metabolite	11	2.20	>2	0.00	4.40
Phenacetin	anti inflammatory	11	2.20	>2	0.00	4.40
Thymopentin	immunostimulant	10	2.00	>2	0.00	4.00
Compactin	hypolipidemic	1	0.20	1.16	3.33	3.73
Ketamine	anestethic	7	1.40	1.95	0.21	3.01
Chlorothiazide	diuretic	6	1.20	>2	0.00	2.40
Famotidine	gastroprotector	6	1.20	>2	0.00	2.40
Antipyrine	analgesic	5	1.00	>2	0.00	2.00
Phenylephrine	vasopressor	5	1.00	>2	0.00	2.00
Iomeprol	contrasting agent	4	0.80	>2	0.00	1.60
Oxfendazole	antielmintic	3	0.60	1.97	0.12	1.32
Amidotrizoic acid	contrasting agent	3	0.60	>2	0.00	1.20
Clenbuterol	bronchodilator	3	0.60	>2	0.00	1.20
Diatrizoic acid	contrasting agent	3	0.60	>2	0.00	1.20
Enalapril	anti hypertension	3	0.60	>2	0.00	1.20
Pindolol	b blocker	3	0.60	>2	0.00	1.20
Sotalol	b blocker	3	0.60	>2	0.00	1.20
Theofilline	antiasthmatic	3	0.60	>2	0.00	1.20
Atropine	nervous control	2	0.40	>2	0.00	0.80
Pravastatin	hypolipidemic	2	0.40	>2	0.00	0.80
Cyclophosphamide	chemioterapic agent	1	0.20	>2	0.00	0.40
Phenprobamate	sedative, anticonvulsant	1	0.20	>2	0.00	0.40
Bendro flumethiazide	diuretic	1	0.20	>2	0.00	0.00

Table S8. Antibiotics scoring and ranking. Antibiotics scoring for the selection of the chemicals for the quantitative analysis. Selected chemicals are in bold. The reported EC50 represents the acute toxicity for the most sensitive aquatic organism. The procedures for calculating the different scores (SSc, STox, STotal) is described in the main text.

	Screening results		Toxicity		
	Positive samples	S_{Sc}	log EC50 mg/L	S_{Tox}	S_{Total}
Sulfamethoxazole	**38**	**10.00**	**>2**	**0.00**	**20.00**
Erythromycin	**7**	**1.84**	**-1.22**	**10.22**	**13.91**
Lincomycin	**7**	**1.84**	**-1.15**	**10.02**	**13.70**
Azithromicin	**12**	**3.16**	**0.00**	**6.35**	**12.66**
Trimethoprim	**22**	**5.79**	**>2**	**0.00**	**11.58**
Amoxicillin	**5**	**1.32**	**-0.25**	**7.14**	**9.77**
Tylosine	**7**	**1.84**	**0.23**	**5.62**	**9.30**
Metronidazole	**15**	**3.95**	**1.60**	**1.27**	**9.16**
Malachite green	2	0.53	-0.52	8.01	9.06
Ciprofloxacin	**10**	**2.63**	**0.83**	**3.73**	**8.99**
Sulfapiridine	9	2.37	0.72	4.05	8.79
Clarithromycin	6	1.58	0.30	5.40	8.55
Cefalexin	15	3.95	>2	0.00	7.89
Chloramphenicol	10	2.63	1.30	2.22	7.49
Sulfamethazine	10	2.63	1.30	2.22	7.49
Ampicillin	7	1.84	1.08	2.92	6.61
Spiramycin	4	1.05	0.60	4.44	6.55
Sulfamethizole	7	1.84	1.40	1.91	5.60
Ofloxacin	5	1.32	1.08	2.92	5.55
Josamycin	7	1.84	1.79	0.67	4.36
Pipemidic acid	6	1.58	>2	0.00	3.16
Norfloxacin	2	0.53	1.56	1.40	2.45
Cloxacillin	4	1.05	>2	0.00	2.11
Nalidixic acid	4	1.05	>2	0.00	2.11
Sulfadimethoxyne	4	1.05	>2	0.00	2.11
Chlortetracycline	2	0.53	1.90	0.32	1.38
Penicillin benzyl	2	0.53	>2	0.00	1.05
Demeclocycline	1	0.26	>2	0.00	0.53
Enoxacin	1	0.26	>2	0.00	0.53
Sulfanilamide	1	0.26	>2	0.00	0.53

Table S9. Estrogens and steroids scoring and ranking. Scoring for the selection of estrogens and steroids for the quantitative analysis. Selected chemicals are in bold. The reported EC50 represents the acute toxicity for the most sensitive aquatic organism. The procedures for calculating the different scores (SSc , STox, STotal) is described in the main text.

Chemicals	Type	Screening results		Toxicity		S_{Total}
		Positive samples	S_{Sc}	log EC50 mg/L	S_{Tox}	
Pyranocoumarin	anti androgen	14	10.00	0.80	7.06	27.06
Estradiol	**estrogen**	**11**	**7.86**	**0.40**	**9.41**	**25.13**
Estrone 3-sulfate	**estrogen**	**13**	**9.29**	**1.81**	**1.09**	**19.67**
Estriol	estrogen	12	8.57	1.73	1.59	18.73
Progesterone	**steroid**	**6**	**4.29**	**0.30**	**10.00**	**18.57**
Testosterone	**steroid**	**7**	**5.00**	**0.93**	**6.30**	**16.30**
Digoxigenin	steroid	3	2.14	>2	0.00	4.29

Table S10. Life-style chemicals socring and ranking. Scoring for the selection of life-style chemicals for the quantitative analysis. Selected chemicals are in bold. The reported EC50 represents the acute toxicity for the most sensitive aquatic organism. The procedures for calculating the different scores (SSc, STox, STotal) is described in the main text.

Chemicals	Type	Screening results		Toxicity		S_{Total}
		Positive samples	S_{Sc}	log EC50 mg/L	S_{Tox}	
Nicotine	**alcaloid**	**36**	**7.35**	**0.60**	**8.08**	**22.77**
Caffeine	**nervous stimulant**	**49**	**10.00**	**>2**	**0.00**	**20.00**
Ephedrine	**nervous stimulant**	**40**	**8.16**	**>2**	**0.00**	**16.33**
Benzoylecgonine	metabolite (cocaine)	37	7.55	>2	0.00	15.10
Paraxanthine	**metabolite (caffeine)**	**34**	**6.94**	**>2**	**0.00**	**13.88**
Cotinine	alcaloid	30	6.12	>2	0.00	12.24
Codeine	analgesic-opiate	28	5.71	>2	0.00	11.43
Cannabidiol	metabolite (cannabis)	2	0.41	0.27	10.00	10.82
Methadone	synthetic opiate	1	0.20	0.36	9.48	9.89
THC - tetrahydrocannabinol	psychoactive drug	10	2.04	1.27	4.22	8.30
Theobromine	alcaloid (chocolate)	17	3.47	>2	0.00	6.94
Morphine	opiate	15	3.06	>2	0.00	6.12
Amphetamine	**nervous stimulant**	**10**	**2.04**	**1.9**	**0.58**	**4.66**
MDMA - 3,4-Methylenedioxy-N-methylamphetamine	psychoactive drug	3	0.61	1.56	2.57	3.79
Methamphetamine	psychoactive drug	6	1.22	1.84	0.91	3.36
MDEA -N-Ethyl-3,4-methylenedioxyamphetamine	psychoactive drug	2	0.41	1.59	2.39	3.21
MDA - Methylenedioxyamphetamine	psychoactive drug	4	0.82	>2	0.00	1.63
EDDP	methadone metabolite	2	0.41	>2	0.00	0.82
phenethylamin	alcaloid	2	0.41	>2	0.00	0.82
Cocaine	alcaloid	1	0.20	1.95	0.28	0.69
Ethylmorphine	opioid analgesic	1	0.20	>2	0.00	0.41
Xantine 3-methyl	caffeine and theofilline metabolite	1	0.20	>2	0.00	0.41

Table S11. Industrial chemicals scoring and ranking. Scoring for the selection of industrial chemicals for the quantitative analysis. Selected chemicals are in bold. The reported EC50 represents the acute toxicity for the most sensitive aquatic organism. The procedures for calculating the different scores (SSc , STox, STotal) is described in the main text.

Chemicals	Type	Screening results		Toxicity		S_{Total}
		Positive samples	S_{Sc}	log EC50 mg/L	S_{Tox}	
TCPP - tris(1-chloropropan-2-yl) phosphate	plasticizer	37	10.00	1.69	0.52	20.52
TBP - Tributyl-phosphate	**plasticizer**	**28**	**7.57**	**0.25**	**2.92**	**18.05**
Benzalkonium chloride-C12	cationic surfactant	14	3.78	-0.85	4.76	12.32
Benzalkonium chloride-C14	cationic surfactant	12	3.24	-0.85	4.76	11.24
Benzalkonium chloride C16	cationic surfactant	11	2.97	-0.85	4.76	10.70
DEHP-di eyhylhexyl phtalate	plasticizer	1	0.27	-3.24	8.73	9.27
Benzalkonium chloride-C10	cationic surfactant	1	0.27	-0.85	4.76	5.30
N-nitroso diethylamine	nitrosamine	7	1.89	>2	0.00	3.78
Perfluoro optanoic acid (PFOA)	perfluorinated compound	2	0.54	0.26	2.91	3.99
TEP - Triethyl-phosphate	plasticizer	7	1.89	>2	0.00	3.78
N-nitrosodibuthylamine	nitrosamine	4	1.08	1.37	1.05	3.21
Perfluoropentanoic acid	perfluorinated compound	1	0.36	>2	0.00	0.71
C3-Pentafluoropropionic-acid-fragment-1	perfluorinated compound	1	0.27	>2	0.00	0.54

Table S12. Concentrations (ng/L) of the selected pesticides in spring water samples (April 11-14, 2016). The station codes correspond to the numbers of the sampling sites in Figure 1. (I: insecticides; H: herbicides; F: fungicides; <LOD: below the limit of detection; <LOQ: below the limit of quantification. Detection and quantification limits are reported in Table 1). Green cells represent the samples with positive results in the screening analysis. In the last columns, the comparison between quantitative data and screening results is reported. Tot P: number of the samples positively quantified; Tot N: number of the negative samples in the quantitative analysis (<LOD or <LOQ); P corr: number of the positive samples detected in the screening; N corr number of the negative samples not detected in the screening; FP: false positive; FN: false negative; FN>10*LOD: number of false negative with a concentration higher than 10*LOD; FN%, FN%>10*LOD, FP%: percentages of false negative and false positive.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Tot P	Tot N	P corr	N corr	F P	F N	F N >10*LOD	F N %	F N %> 10*LOD	F P %
Carbofuran	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	16	0	14	2	0	0	0	0	12.5
Chlorpyrifos	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	16	0	16	0	0	0	0	0	0.0
Diazinon	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.13	0.05	0.11	0.14	<LOQ	0.03	0.03	<LOQ	0.15	7	9	0	9	0	7	0	100	0.0	0.0
Dimethoate	<LOD	0.28	0.94	<LOD	0.41	<LOD	0.31	1.27	2.69	2.18	21.2	0.05	0.14	0.06	<LOD	4.22	12	4	2	4	0	10	8	83.3	66.7	0.0
Imidacloprid	<LOQ	0.50	0.03	<LOQ	0.21	5.26	1.47	7.77	11.1	31.2	31.8	0.52	1.19	7.12	0.10	25.0	14	2	6	2	0	8	4	57.1	28.6	0.0
Malathion	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	16	0	14	2	0	0	0	0	12.5
Metolcarb	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	16	0	15	1	0	0	0	0	6.3
Pirimicarb	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.02	0.03	0.02	0.26	<LOD	<LOD	<LOD	<LOD	0.04	5	11	2	10	1	3	1	60	20.0	9.1
Spinosin-A	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	0	16	0	12	4	0	0	0	0	25.0
Chlorturon	0.17	0.10	<LOD	<LOQ	0.17	<LOQ	<LOD	12.4	3.69	0.24	1.37	<LOD	<LOD	<LOQ	<LOD	16.6	8	8	3	8	0	5	2	62.5	25.0	0.0
Diuron	<LOD	0.16	<LOQ	<LOQ	0.14	<LOQ	0.37	18.8	27.8	33.7	66.3	<LOQ	2.54	<LOQ	<LOD	19.4	9	7	2	7	0	7	5	77.8	55.6	0.0
Metribuzine	<LOD	0.06	<LOD	<LOD	1.69	15.3	<LOD	0.87	<LOQ	0.44	0.51	<LOD	<LOD	<LOD	<LOD	0.54	7	9	1	9	0	6	4	85.7	57.1	0.0
Simazine	<LOQ	0.14	0.09	<LOQ	0.14	<LOQ	0.21	3.43	0.23	8.38	4.36	261	237	176	0.53	4.56	13	3	4	3	0	9	6	69.2	46.2	0.0
Terbuthrin	<LOQ	0.08	<LOQ	0.07	0.09	2.65	0.22	7.69	7.78	7.63	24.9	0.09	0.16	1.92	0.12	5.88	14	2	3	2	0	11	4	78.6	28.6	0.0
Terbuthylazine	<LOQ	0.30	<LOQ	<LOQ	0.87	<LOQ	0.17	2.48	0.64	0.63	3.14	0.27	0.31	0.40	2.66	8.93	12	4	1	4	0	11	6	91.7	50.0	0.0
Carbendazim	<LOD	0.17	<LOQ	<LOQ	0.16	2.94	0.73	20.4	22.2	21.4	67.0	0.45	1.73	1.62	<LOD	18.3	12	4	2	4	0	10	7	83.3	58.3	0.0
Kresoxim methyl	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	16	0	12	4	0	0	0	0	25.0
Propiconazole	<LOQ	<LOQ	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	13.3	4.18	5.82	10.1	<LOQ	<LOQ	<LOQ	<LOQ	3.92	5	11	2	11	0	3	3	60.0	60.0	0.0
Spiroxamine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0	16	0	9	7	0	0	0	0	43.8
Tebuconazole	0.20	0.16	<LOQ	<LOQ	0.24	0.25	0.24	3.76	2.90	200	3.31	4.44	3.34	3.97	0.22	2.02	14	2	1	2	0	13	7	92.9	50.0	0.0
																Total	**132**	**188**	**29**	**167**	**21**	**103**	**57**	**78.0**	**43.2**	**11.2**

Table S13. Concentrations (ng/L) of the selected pesticides in summer water samples (July 11-14, 2016). The station codes correspond to the numbers of the sampling sites in Figure 1. (I: insecticides; H: herbicides; F: fungicides; <LOD.: below the limit of detection; <LOQ: below the limit of quantification. Detection and quantification limits are reported in Table 1). Green cells represent the samples with positive results in the screening analysis. In the last columns, the comparison between quantitative data and screening results is reported. Tot P: number of the samples positively quantified; Tot N: number of the negative samples in the quantitative analysis (<LOD or <LOQ); P corr: number of the positive samples detected in the screening; N corr number of the negative samples not detected in the screening; FP: false positive; FN: false negative; FN>10*LOD: number of false negative with a concentration higher than 10*LOD; FN%, FN%>10*LOD, FP%: percentages of false negative and false positive.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Tot P	Tot N	P corr	N corr	F P	F N	F N >10*LOD	F N %	F N % >10*LOD	F P %
Carbofuran	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	16	0	6	10	0	0	0	0	62.5
Chlorpyrifos	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	0	16	0	16	0	0	0	0	0	0.0
Diazinon	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.02	<LOQ	0.35	0.06	0.29	0.11	<LOQ	0.02	0.03	<LOQ	0.05	8	8	3	7	1	5	1	62.5	12.5	12.5
Dimethoate	<LOD	0.59	<LOD	<LOD	0.09	0.20	0.03	1.51	13.8	1.80	4.80	0.04	0.18	0.24	<LOQ	4.78	12	4	3	4	0	9	3	75.0	25.0	0.0
Imidacloprid	0.44	1.04	3.51	2.15	1.81	24.0	2.53	67.3	33.6	97.0	82.1	1.66	7.82	9.89	2.20	26.7	16	0	7	0	0	9	8	56.3	50.0	-
Malathion	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	16	0	13	3	0	0	0	0	18.8
Metolcarb	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	16	0	1	15	0	0	0	0	93.8
Pirimicarb	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ	0.07	0.08	0.06	0.35	<LOD	<LOQ	<LOQ	<LOQ	0.12	5	11	2	7	4	3	1	60.0	20.0	36.4
Spinosin-A	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	0	16	0	15	1	0	0	0	0	6.3
Chlorturon	<LOD	<LOD	<LOD	<LOD	0.30	<LOD	<LOD	1.63	0.65	<LOQ	9.61	<LOD	0.18	<LOQ	<LOD	2.67	6	10	1	10	0	5	3	83.3	50.0	0.0
Diuron	<LOD	0.23	<LOQ	<LOD	<LOQ	<LOQ	1.33	38.1	28.5	109	61.6	<LOQ	0.42	<LOQ	<LOQ	22.9	8	8	3	8	0	5	4	62.5	50.0	0.0
Metribuzine	<LOD	<LOD	<LOD	<LOD	<LOD	3.32	0.15	0.71	0.56	0.80	0.80	<LOD	0.20	<LOD	<LOD	<LOQ	7	9	0	9	0	7	4	100	57.1	0.0
Simazine	0.38	0.27	1.37	0.20	0.34	0.27	0.38	15.7	6.33	3.17	4.35	5.63	5.13	1.97	22.1	7.42	16	0	8	0	0	8	5	50.0	31.3	-
Terbuthrin	0.07	0.11	<LOQ	<LOQ	0.07	0.26	0.71	45.4	16.6	24.3	26.4	0.28	0.50	0.71	0.95	6.53	14	2	5	2	0	9	3	64.3	21.4	0.0
Terbuthylazine	0.23	0.16	0.07	<LOQ	2.12	0.04	0.17	15.6	0.32	0.19	0.26	0.32	0.30	0.31	2.17	16.1	15	1	3	0	1	12	2	80.0	13.3	100.0
Carbedazim	0.44	0.40	0.99	0.20	2.24	3.32	1.32	115	39.6	61.2	118	5.20	8.68	2.75	4.02	27.1	16	0	3	0	0	13	9	81.3	56.3	-
Kresoxim methyl	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	16	0	5	11	0	0	0	0	68.8
Propiconazole	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5.10	2.50	5.24	4.51	<LOQ	<LOQ	0.36	<LOQ	1.34	6	10	2	10	0	4	2	66.7	33.3	0.0
Spiroxamine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0	16	0	11	5	0	0	0	0	31.3
Tebuconazole	0.35	0.26	<LOQ	<LOQ	0.19	0.94	0.44	6.60	1.33	447	2.95	1.67	2.10	1.67	1.84	3.41	14	2	1	2	0	13	8	92.9	57.1	0.0
																Total	**143**	**177**	**41**	**126**	**51**	**102**	**53**	**71.3**	**37.1**	**28.8**

Table S14. Concentrations (ng/L) of the selected pesticides in autumn water samples (November 22-24, 2016). The station codes correspond to the numbers of the sampling sites in Figure 1. (I: insecticides; H: herbicides; F: fungicides; <LOD: below the limit of detection; <LOQ: below the limit of quantification. Detection and quantification limits are reported in Table 1). Green cells represent the samples with positive results in the screening analysis. In the last columns, the comparison between quantitative data and screening results is reported. Tot P: number of the samples positively quantified; Tot N: number of the negative samples in the quantitative analysis (<LOD or <LOQ); P corr: number of the positive samples detected in the screening; N corr number of the negative samples not detected in the screening; FP: false positive; FN: false negative; FN>10*LOD: number of false negative with a concentration higher than 10*LOD; FN%, FN%>10*LOD, FP%: percentages of false negative and false positive.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Tot P	Tot N	P corr	N corr	F P	F N	F N >10*LOD	F N %	F N % >10*LOD	F P %
Carbofuran	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	16	0	14	2	0	0	0	0	13
Chlorpyrifos	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	16	0	16	0	0	0	0	0	0.0
Diazinon	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.02	2.11	0.07	0.44	0.21	<LOQ	<LOQ	<LOQ	<LOQ	0.12	6	10	2	7	2	4	3	67	50	20
Dimethoate	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOD	0.14	1.04	0.09	8.40	<LOD	<LOQ	1.60	<LOD	5.32	6	10	2	10	0	4	2	67	33	0
Imidacloprid	<LOQ	0.40	0.33	0.06	0.36	2.83	0.90	15.7	10.8	15.2	26.5	0.34	0.93	1.58	<LOQ	12.0	14	2	4	2	0	10	4	71	29	0
Malathion	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	0	16	0	13	3	0	0	0	0	19
Metolcarb	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	16	0	2	14	0	0	0	0	88
Pirimicarb	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	0.03	0.03	<LOQ	0.09	<LOQ	<LOD	<LOD	<LOD	0.06	4	12	3	8	4	1	0	25	0	33
Spinosin-A	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3.35	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOD	1	15	0	14	1	1	0	100	0	7
Chlorturon	0.28	<LOQ	0.77	<LOQ	0.40	0.69	0.33	6.61	2.99	1.60	10.3	<LOQ	<LOQ	<LOQ	<LOQ	20.0	10	6	1	6	0	9	6	90	60	0
Diuron	<LOQ	<LOQ	<LOQ	<LOD	0.80	1.39	1.97	46.1	30.3	56.5	84.6	<LOQ	<LOQ	<LOQ	<LOD	71.9	8	8	4	8	0	4	5	50	63	0
Metribuzine	<LOD	<LOD	<LOD	<LOD	0.14	0.53	0.07	0.18	0.19	0.28	0.44	<LOD	<LOD	<LOD	<LOD	0.38	8	8	0	8	0	8	5	100	63	0
Simazine	0.25	1.16	0.57	0.43	1.85	1.28	19.2	0.73	1.10	0.57	5.84	4.08	2.84	2.12	1.11	11.7	16	0	4	0	0	12	8	75	50	0
Terbuthrin	<LOQ	0.12	<LOQ	<LOQ	0.25	0.85	0.32	21.3	14.1	11.7	25.6	0.13	0.16	0.33	0.15	11.5	13	3	5	3	0	8	1	62	8	0
Terbuthylazine	0.18	0.13	0.49	0.09	1.19	0.39	0.14	1.67	0.52	0.14	1.87	0.11	0.12	0.12	5.15	6.75	16	0	2	0	0	14	5	88	31	0
Carbedazim	0.58	1.44	0.32	0.89	2.03	1.77	1.70	38.4	21.8	22.1	68.9	0.56	0.56	0.55	0.11	37.2	16	0	1	0	0	15	13	94	81	0
Kresoxim methyl	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	16	0	12	4	0	0	0	0	25
Propiconazole	<LOQ	<LOQ	<LOD	<LOQ	<LOQ	0.36	<LOQ	21.8	4.96	6.37	12.4	<LOQ	<LOQ	<LOQ	<LOQ	4.06	6	10	2	10	0	4	3	67	50	0
Spiroxamine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3.76	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1	15	1	1	14	0	0	0	0	93
Tebuconazole	0.62	0.42	<LOQ	<LOQ	3.64	1.39	0.69	3.71	2.81	53.0	3.79	0.67	0.90	1.01	<LOQ	4.46	13	3	1	3	0	12	7	92	54	0
																Total	**138**	**182**	**32**	**137**	**44**	**106**	**62**	**77**	**45**	**24**

Table S15. Time weighted averages of the concentrations (ng/L) of the selected pesticides in the two weeks of POCIS exposure in summer (July, 2016) samples. The station codes correspond to the numbers of the sampling sites in Figure 1. (I: insecticides; H: herbicides; F: fungicides; <LOD.: below the limit of detection; <LOQ: below the limit of quantification. Detection and quantification limits are reported in Table 1). Green cells represent the samples with positive results in the screening analysis. In the last columns, the comparison between quantitative data and screening results is reported. Tot P: number of the samples positively quantified; Tot N: number of the negative samples in the quantitative analysis (<LOD or <LOQ); P corr: number of the positive samples detected in the screening; N corr number of the negative samples not detected in the screening; FP: false positive; FN: false negative; FN>10*LOD: number of false negative with a concentration higher than 10*LOD; FN%, FN%>10*LOD, FP%: percentages of false negative and false positive.

	1	2	3	5	6	7	8	12	13	14	15	16	Tot P	Tot N	P corr	N corr	F P	F N	F N >10*LOD	F N %	F N % >10*LOD	F P %
Carbofuran	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	0	12	0	11	1	0	0	0	0	8
Chlorpyrifos	<LOD	1.16	<LOQ	2.20	34.2	2.95	244	<LOD	396	217	<LOD	329	8	4	3	4	0	5	6	63	75	0
Diazinon	0.02	0.03	<LOQ	0.34	1.47	0.03	9.45	0.03	0.11	0.12	0.15	0.93	11	1	5	0	1	6	5	55	45	100
Dimethoate	<LOD	2.08	<LOQ	2.50	13.2	0.43	6.95	0.13	10.8	22.7	4.54	351	10	2	6	2	0	4	3	40	30	0
Imidacloprid	0.85	3.92	35.0	6.33	174	29.1	127	1.77	55.3	57.7	11.2	342	12	0	7	0	0	5	6	42	50	0
Malathion	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	0	12	0	10	2	0	0	0	0	17
Metolcarb	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	0	12	0	0	12	0	0	0	0	100
Pirimicarb	<LOD	<LOQ	<LOQ	<LOD	2.70	<LOD	1.03	<LOD	<LOQ	<LOD	<LOQ	3.69	3	9	3	0	9	0	1	0	33	100
Spinosin-A	<LOQ	<LOD	<LOQ	<LOD	3.54	<LOD	105	<LOD	<LOD	1.42	<LOD	1.30	4	8	0	8	0	4	4	100	100	0
Chlortoluron	0.94	0.24	<LOQ	6.89	9.01	<LOQ	75.9	<LOQ	<LOQ	<LOQ	<LOQ	98.0	6	6	2	6	0	4	2	67	33	0
Diuron	<LOQ	4.73	<LOQ	20.2	140	59.1	555	2.10	42.5	41.2	<LOQ	995	9	3	5	3	0	4	6	44	67	0
Metribuzine	<LOD	<LOQ	<LOD	15.3	439	0.75	52.3	<LOD	<LOQ	<LOD	15.1	15.9	6	6	2	6	0	4	4	67	67	0
Simazine	0.26	1.20	0.13	1.52	7.55	4.22	15.4	10.5	8.87	5.92	60.6	159	12	0	9	0	0	3	1	25	8	0
Terbuthrin	0.28	1.12	<LOQ	2.59	21.3	6.44	66.0	1.20	1.68	3.92	8.98	77.5	11	1	6	1	0	5	5	45	45	0
Terbuthylazine	0.51	0.53	0.25	77.1	7.33	0.92	32.4	1.87	0.38	1.27	121	60.2	12	0	5	0	0	7	6	58	50	0
Carbendazim	0.78	3.11	2.03	12.0	89.2	8.77	146	1.37	14.6	15.3	7.04	273	12	0	8	0	0	4	2	33	17	0
Kresoxim methyl	<LOD	<LOD	N,D	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOD	0	12	0	7	5	0	0	0	0	42
Propiconazole	<LOQ	0.51	<LOQ	10.6	4.44	0.59	27.5	<LOQ	0.77	0.79	<LOQ	24.5	10	2	2	5	-3	8	8	80	80	-150
Spiroxamine	<LOQ	<LOQ	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOQ	0	12	0	11	1	0	1	0	0	8
Tebuconazole	2.71	2.27	0.21	26.5	77.1	1.85	53.2	8.03	13.7	12.6	14.9	30.7	12	0	4	0	0	8	10	67	83	0
												Total	**119**	**85**	**59**	**59**	**26**	**60**	**59**	**50**	**50**	**31**

Table S16. Concentrations (ng/L) of the selected point source chemicals in spring water samples (April 11-14, 2016). The station codes correspond to the numbers of the sampling sites in Figure 1. (I: insecticides; H: herbicides; F: fungicides; <LOD: not detected; <LOQ: below the limit of quantification. Detection and quantification limits are reported in Table 1). Green cells represent the samples with positive results in the screening analysis. In the last columns, the comparison between quantitative data and screening results is reported. Tot P: number of the sampbelow the limit of detectionles positively quantified; Tot N: number of the negative samples in the quantitative analysis (<LOD or <LOQ); P corr: number of the positive samples detected in the screening; N corr number of the negative samples not detected in the screening; FP: false positive; FN: false negative; FN>10*LOD: number of false negative with a concentration higher than 10*LOD; FN%, FN%>10*LOD, FP%: percentages of false negative and false positive.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Tot P	Tot N	P corr	N corr	F P	F N	F N >10*LOD	F N %	F N % >10*LOD	F P %
Acetaminophen	1.08	6.46	5.27	2.40	10.24	596	86.11	39.79	55.64	598	20.39	5.96	3501	3542	6.16	10.11	16	0	10	0	0	6	3	37.5	18.8	-
Atenolol	0.31	2.53	0.53	<LOQ	1.59	61.30	4.41	305	91.72	161	223	1.68	26.40	52.43	1.64	86.84	15	1	9	1	0	6	1	40.0	6.7	0.0
Carbamazepine	0.18	1.34	0.44	0.06	0.82	4.12	2.98	17.79	342	12.78	127	7.28	9.24	10.27	2.65	31.40	16	0	8	0	0	8	2	50.0	12.5	-
Ciprofloxacin	2.10	1.82	<LOD	<LOD	4.73	3.84	5.86	394	8.82	13.29	16.52	20.45	3.98	8.03	4.57	9.78	14	2	2	2	0	12	0	85.7	0.0	0.0
Citalopram	25.21	0.99	<LOD	0.11	8.31	2.57	2.75	17.58	8.25	4.71	12.65	3.92	5.84	7.29	2.39	25.71	15	1	4	1	0	11	5	73.3	33.3	0.0
Diclofenac	<LOD	2.56	<LOD	<LOD	<LOD	3.13	5.14	102	73.81	103	373	1.70	15.50	26.85	<LOD	81	11	5	6	5	0	5	1	45.5	9.1	0.0
Gemfibrozil	<LOQ	4.70	<LOQ	<LOQ	1.01	3.24	9.01	441	202	308	798	0.98	13.00	72.46	0.10	218	13	3	6	3	0	7	5	53.8	38.5	0.0
Ibuprofen	2.46	5.28	2.91	3.26	6.37	121	22.50	231	907	364	127	1.47	326	844	1.61	14.35	16	0	0	0	0	16	8	100.0	50.0	-
Ketoprofen	<LOD	<LOD	<LOD	<LOD	<LOD	3.06	<LOD	96.11	13.18	24.54	178	<LOQ	3.58	3.75	<LOD	2.69	8	8	2	8	0	6	1	75.0	12.5	0.0
Loratadine	<LOQ	<LOD	<LOQ	<LOQ	<LOQ	<LOD	<LOD	6.44	<LOQ	<LOQ	<LOQ	2.45	<LOQ	<LOD	<LOD	<LOD	2	14	1	14	0	1	1	50.0	50.0	0.0
Naproxen	<LOD	2.13	<LOD	<LOD	<LOD	68.58	39.85	507	276	<LOQ	562	<LOQ	<LOQ	476	<LOD	<LOD	7	9	6	6	3	1	0	14.3	0.0	33.3
Omeprazole	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	179	0.93	1.77	4.64	0.75	<LOQ	0.21	<LOQ	<LOQ	6	10	5	7	3	1	1	16.7	16.7	30.0
Paraxantine	6.67	330	2.81	2.37	17.46	2450	893	4283	2621	8176	4043	382	2067	6581	14.83	1588	16	0	8	0	0	8	5	50.0	31.3	-
Salbutamol	<LOD	0.06	0.10	<LOD	<LOQ	0.07	0.14	3.82	2.82	3.55	8.91	<LOQ	6.40	0.39	<LOD	4.12	10	6	8	6	0	2	2	20.0	20.0	0.0
Trimethoprim	0.16	0.34	0.09	0.54	0.38	0.15	0.26	47.49	20.91	21.89	87.11	0.40	2.39	4.42	0.57	13.25	16	0	6	0	0	10	4	62.5	25.0	-
Valsartan	2.93	30.44	<LOQ	<LOQ	13.31	25.51	74.83	1445	697	832	2251	26.25	399	562	<LOQ	652	13	3	0	3	0	13	11	100.0	84.6	0.0
Venlafaxine	<LOQ	3.62	<LOQ	<LOQ	5.04	<LOQ	4.12	614	70.07	37.37	161	10.56	4.30	19.85	<LOQ	23.95	11	5	9	3	2	2	1	18.2	9.1	40.0
Amoxicillin	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3.62	0.79	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2	14	0	14	0	2	0	100.0	0.0	0.0
Azithromycin	5.64	4.35	<LOQ	<LOQ	31.84	6.33	3.47	99.61	<LOD	3.45	3.81	14.94	20.52	6.81	2.76	3.92	13	3	2	3	0	11	3	84.6	23.1	0.0
Erythromycin	<LOQ	0.20	<LOD	<LOD	0.20	0.08	0.19	4.92	5.18	2.82	16.92	0.80	0.09	0.48	<LOQ	0.93	12	4	3	4	0	9	1	75.0	8.3	0.0
Lincomycin	0.04	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.96	3.59	1.74	4.17	0.21	0.25	0.25	<LOQ	1.48	9	7	0	7	0	9	3	100.0	33.3	0.0

Table S16 (cont.)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Tot P	Tot N	P corr	N corr	F P	F N	F N >10*LOD	F N %	F N % >10*LOD	F P %
Metronidazole	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	50.70	28.59	26.33	121	<LOD	2.71	87.71	<LOD	4.85	6	10	4	10	0	2	0	33.3	0.0	0.0
Sulfamethoxazole	0.58	2.07	<LOQ	<LOQ	0.76	17.06	4.59	145	82.30	37.21	189	4.05	5.70	10.74	0.29	36.49	14	2	9	2	0	5	0	35.7	0.0	0.0
Tylosin	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	16	0	15	1	0	0	-	-	6.3
Estradiol, 17-beta-(E2)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	0.24	<LOD	<LOQ	0.40	<LOD	<LOQ	0.41	<LOQ	<LOQ	3	13	1	11	2	2	0	66.7	0.0	15.4
Estrone	0.10	0.24	0.86	<LOQ	<LOQ	0.20	0.30	5.27	5.21	3.24	5.59	0.61	1.33	2.91	0.20	0.44	14	2	3	2	0	11	5	78.6	35.7	0.0
Progesterone	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOD	0	16	0	12	4	0	0	-	-	25.0
Testosterone	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOD	<LOD	0	16	0	12	4	0	0	-	-	25.0
Amphetamine	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	0	16	0	14	2	0	0	-	-	12.5
Caffeine	20.60	48.14	11.64	6.38	29.59	293	136	424	353	937	452	114	351	706	5.96	117	16	0	11	0	0	5	3	31.3	18.8	-
Nicotine	56.65	221	39.17	13.28	61.99	107	49.39	66.55	77.52	103	107	1.66	300	323	2.63	7.44	16	0	8	0	0	8	5	50.0	31.3	-
Tributyl-phosphate	889	199	445	511.81	1075	102	242	25.15	453	77.13	135	23.18	25.40	6.53	11.86	30.35	16	0	12	0	0	4	4	25.0	25.0	-
																Total	**205**	**67**	**90**	**59**	**8**	**115**	**51**	**56.1**	**24.9**	**11.9**

Table S17. Concentrations (ng/L) of the selected point source chemicals in summer water samples (July 11-14, 2016). The station codes correspond to the numbers of the sampling sites in Figure 1. (I: insecticides; H: herbicides; F: fungicides; <LOD: below the limit of detection; <LOQ: below the limit of quantification. Detection and quantification limits are reported in Table 1). Green cells represent the samples with positive results in the screening analysis. In the last columns, the comparison between quantitative data and screening results is reported. Tot P: number of the samples positively quantified; Tot N: number of the negative samples in the quantitative analysis (<LOD or <LOQ); P corr: number of the positive samples detected in the screening; N corr number of the negative samples not detected in the screening; FP: false positive; FN: false negative; FN>10*LOD: number of false negative with a concentration higher than 10*LOD; FN%, FN%>10*LOD, FP%: percentages of false negative and false positive.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Tot P	Tot N	P corr	N corr	F P	F N	F N >10*LOD	F N %	F N % >10*LOD	F P %
Acetaminophen	<LOD	<LOQ	3.12	<LOD	5.60	225	10.78	3.06	9.49	6.27	21.61	2.08	6710	5661	0.68	3.13	13	3	7	3	0	6	5	46.2	38.5	0.0
Atenolol	<LOQ	2.73	<LOQ	<LOQ	2.13	29.47	5.88	530	102	74.38	439.2	0.79	85.68	92.75	<LOQ	54.51	12	4	11	3	1	1	0	8.3	0.0	25.0
Carbamazepine	0.51	1.35	0.27	8.84	0.72	25.32	5.32	49.80	76.89	119.45	174.21	9.27	22.25	23.28	13.38	45.12	16	0	12	0	0	4	3	25.0	18.8	-
Ciprofloxacin	5.43	4.35	<LOQ	2.86	<LOQ	<LOQ	1.82	456	<LOQ	12.00	21.77	<LOD	182.4	79.89	1.90	8.59	11	5	2	4	1	9	1	81.8	9.1	20.0
Citalopram	0.56	0.50	0.08	0.27	0.84	0.44	1.09	18.24	4.71	4.31	13.03	1.03	6.45	1.90	0.71	2.31	16	0	3	0	0	13	9	81.3	56.3	-
Diclofenac	<LOD	2.61	<LOD	<LOD	<LOQ	28.41	3.55	<LOQ	93.30	301	428	2.12	64.65	76.65	<LOD	38.36	10	6	7	5	1	3	0	30.0	0.0	16.7
Gemfibrozil	<LOQ	3.54	<LOQ	<LOQ	1.01	21.33	2.16	457	447	47.46	789	<LOQ	104	124	0.15	117.66	12	4	6	4	0	6	5	50.0	41.7	0.0
Ibuprofen	0.41	0.66	0.97	0.93	1.42	241	5.18	204	977	6.45	2761	1.37	670	1126	0.52	44.08	16	0	3	0	0	13	7	81.3	43.8	-
Ketoprofen	<LOD	<LOD	<LOD	<LOD	<LOD	8.35	<LOD	277	8.56	31.44	115.45	<LOD	39.87	13.79	<LOD	<LOQ	7	9	7	8	1	0	0	0.0	0.0	11.1
Loratadine	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOD	<LOD	26.20	2.20	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOD	<LOD	2	14	1	12	2	1	0	50.0	0.0	14.3
Naproxen	<LOD	<LOD	<LOD	<LOD	<LOD	236.6	39.0	605	181	<LOQ	491	<LOQ	645	1064	<LOD	111	8	8	8	8	0	0	0	0.0	0.0	0.0
Omeprazole	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	44.33	0.30	2.18	3.36	<LOD	1.31	0.39	<LOQ	<LOQ	6	10	3	9	1	3	0	50.0	0.0	10.0
Paraxantine	5.87	695	29.26	2.39	25.46	2484	594	954	2076	506	4916	57.90	6679	7722	219	843	16	0	9	0	0	7	4	43.8	25.0	-
Salbutamol	<LOD	0.05	<LOD	<LOQ	<LOQ	0.20	0.15	9.20	5.28	8.21	10.22	<LOQ	0.23	0.62	<LOD	4.10	10	6	8	5	1	2	1	20.0	10.0	16.7
Trimethoprim	<LOD	<LOQ	<LOQ	0.07	<LOQ	0.47	0.18	323	25.10	51.16	115.71	0.10	15.10	29.75	<LOQ	10.17	11	5	7	5	0	4	0	36.4	0.0	0.0
Valsartan	2.31	37.94	4.07	<LOQ	19.44	153.8	95.23	1669	727.2	286	1455	48.55	3337	2170	6.82	554	15	1	12	1	0	3	0	20.0	0.0	0.0
Venlafaxine	2.23	4.36	2.68	2.76	1.77	5.36	4.95	250	76.44	60.59	212	1.82	31.97	33.76	<LOQ	29.11	15	1	15	1	0	0	0	0.0	0.0	0.0
Amoxicillin	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.63	<LOD	<LOD	0.29	<LOD	<LOD	<LOD	<LOD	0.43	3	13	1	13	0	2	0	66.7	0.0	0.0
Azithromycin	14.03	5.46	<LOQ	7.62	0.47	2.73	3.15	96.38	<LOD	2.75	3.05	3.41	11.15	2.29	0.91	5.06	14	2	6	2	0	8	1	57.1	7.1	0.0
Erythromycin	<LOQ	0.10	0.07	<LOQ	0.05	0.07	0.11	4.36	3.84	4.47	10.65	0.18	0.19	0.21	0.08	2.13	14	2	2	2	0	12	0	85.7	0.0	0.0
Lincomycin	0.09	<LOQ	<LOQ	<LOD	<LOQ	<LOQ	<LOQ	2.21	4.11	2.37	7.10	<LOQ	<LOQ	0.14	0.11	1.71	8	8	3	8	0	5	1	62.5	12.5	0.0

Table S17 (cont.)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Tot P	Tot N	P corr	N corr	F P	F N	F N >10*LOD	F N %	F N % >10*LOD	F P %
Metronidazole	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	24.73	22.59	9.56	123.9	<LOD	2.88	2.27	<LOD	3.36	7	9	5	9	0	2	0	28.6	0.0	0.0
Sulfamethoxazole	0.58	4.23	<LOD	<LOD	0.74	78.24	4.84	113	34.68	499	124	5.50	29.38	47.51	1.22	78.37	14	2	10	2	0	4	0	28.6	0.0	0.0
Tylosin	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	16	0	16	1	0	0	-	-	6.3
Estradiol, 17-beta-(E2)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ	0.17	<LOQ	0.29	<LOD	<LOQ	0.60	<LOQ	<LOQ	3	13	2	11	2	1	0	33.3	0.0	15.4
Estrone	0.29	<LOQ	<LOQ	<LOQ	<LOQ	0.25	<LOQ	2.91	3.82	0.59	7.40	0.37	3.21	4.59	<LOQ	0.48	10	6	2	5	1	8	5	80.0	50.0	16.7
Progesterone	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOD	0	16	0	16	0	0	0	-	-	0.0
Testosterone	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOQ	<LOQ	4.15	3.68	<LOD	<LOD	2	14	2	13	1	0	0	0.0	0.0	7.1
Amphetamine	<LOQ	<LOQ	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0	16	0	16	0	0	0	-	-	0.0
Caffeine	17.06	99.50	54.05	5.95	43.60	460.7	93.9	46.5	302.4	42.4	496.6	19.4	2212	2409	66.0	71.0	16	0	13	0	0	3	4	18.8	25.0	-
Nicotine	3.47	6.90	17.10	3.61	7.88	212.2	26.64	44.55	39.36	34.86	20.76	1.46	411	599	7.13	9.24	16	0	11	0	0	5	2	31.3	12.5	-
Tributyl-phosphate	5.27	11.28	81.61	109.71	8.14	24.54	43.41	11.40	49.42	14.92	21.93	3.35	3.80	<LOQ	5.41	9.21	15	1	4	0	1	11	2	73.3	13.3	100.0
																Total	**318**	**194**	**182**	**181**	**14**	**136**	**50**	**42.8**	**15.7**	**7.2**

Table S18. Concentrations (ng/L) of the selected point source chemicals in autumn water samples (November 22-24, 2016). The station codes correspond to the numbers of the sampling sites in Figure 1. (I: insecticides; H: herbicides; F: fungicides; <LOD: below the limit of detection; <LOQ: below the limit of quantification. Detection and quantification limits are reported in Table 1). Green cells represent the samples with positive results in the screening analysis. In the last columns, the comparison between quantitative data and screening results is reported. Tot P: number of the samples positively quantified; Tot N: number of the negative samples in the quantitative analysis (<LOD or <LOQ); P corr: number of the positive samples detected in the screening; N corr number of the negative samples not detected in the screening; FP: false positive; FN: false negative; FN>10*LOD: number of false negative with a concentration higher than 10*LOD; FN%, FN%>10*LOD, FP%: percentages of false negative and false positive.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Tot P	Tot N	P corr	N corr	F P	F N	F N >10*LOD	F N %	F N % >10*LOD	F P %
Acetaminophen	2.63	27.13	1.65	0.74	6.45	104.8	118.32	75.04	87.57	18.69	51.48	4.85	5838	9825	3.67	25.78	16	0	6	0	0	10	5	62.5	31.3	-
Atenolol	4.40	3.25	0.98	<LOQ	1.41	12.99	27.31	673	136.9	99.19	397	0.71	29.16	52.93	0.45	167	15	1	10	1	0	5	1	33.3	6.7	0.0
Carbamazepine	0.40	2.30	0.23	0.12	1.24	5.08	7.99	23.60	57.76	33.25	125.01	8.33	11.86	17.83	1.57	44.40	16	0	10	0	0	6	1	37.5	6.3	-
Ciprofloxacin	6.92	3.71	<LOQ	<LOQ	<LOQ	2.28	5.24	786	14.08	22.38	41.85	4.24	87.37	241	13.44	12.36	13	3	2	3	0	11	1	84.6	7.7	0.0
Citalopram	4.42	4.40	<LOD	0.66	0.43	1.04	0.97	12.10	7.48	4.02	20.05	5.06	5.26	7.93	2.60	2.30	15	1	2	1	0	13	8	86.7	53.3	0.0
Diclofenac	<LOD	7.81	<LOQ	<LOD	<LOQ	5.09	9.83	235	105	115	440	3.38	12.54	32.35	<LOD	227	11	5	6	5	0	5	1	45.5	9.1	0.0
Gemfibrozil	<LOQ	21.03	0.56	<LOQ	0.97	7.52	8.40	342	231	110	551	0.51	40.03	98.75	0.11	280	14	2	8	2	0	6	3	42.9	21.4	0.0
Ibuprofen	0.52	5.32	0.93	1.15	1.49	78.41	53.09	1521	828.01	8.11	113.72	2.49	553	1028	0.80	303	16	0	1	0	0	15	9	93.8	56.3	-
Ketoprofen	<LOD	4.32	<LOD	<LOD	<LOD	12.11	3.18	356	152	41.94	171.19	<LOQ	31.95	64.49	<LOD	32.65	10	6	8	6	0	2	0	20.0	0.0	0.0
Loratadine	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOD	6.31	4.45	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOD	<LOD	2	14	0	10	4	2	2	100.0	100.0	28.6
Naproxen	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	97.55	1404	308	<LOQ	556	<LOQ	201	370	<LOD	809	7	9	7	9	0	0	0	0.0	0.0	0.0
Omeprazole	0.55	<LOQ	0.25	0.22	0.24	0.80	1.06	392	2.50	<LOQ	5.39	<LOQ	<LOQ	0.28	<LOQ	1.37	11	5	5	5	0	6	0	54.5	0.0	0.0
Paraxantine	6.22	575.16	3.35	2.20	16.72	1246	1205	57587	7880	810	5591	134	2719	6779	56.90	11250	16	0	8	0	0	8	4	50.0	25.0	-
Salbutamol	<LOQ	0.12	<LOD	<LOD	<LOQ	0.07	0.29	6.12	3.11	5.38	7.99	<LOD	0.10	0.32	<LOD	7.99	10	6	9	6	0	1	0	10.0	0.0	0.0
Trimethoprim	<LOQ	0.22	<LOQ	<LOQ	0.40	<LOQ	0.30	1288	22.47	25.81	99.57	0.07	4.90	8.49	0.14	18.84	12	4	6	4	0	6	0	50.0	0.0	0.0
Valsartan	3.23	96.65	3.41	2.31	21.34	76.75	114.51	2410	732	311	1850	27.69	586	1424	<LOQ	1222	15	1	12	1	0	3	0	20.0	0.0	0.0
Venlafaxine	3.59	13.90	<LOQ	2.63	3.29	2.67	6.30	342	38.64	44.35	168.8	3.04	5.44	9.98	8.15	48.50	15	1	14	0	1	1	0	6.7	0.0	100.0
Amoxicillin	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	4.35	<LOD	<LOD	0.71	<LOQ	<LOD	15.08	<LOD	<LOD	3	13	1	13	0	2	0	66.7	0.0	0.0
Azithromycin	23.18	1.27	1.57	6.48	4.44	6.02	1.89	1032	4.73	3.27	16.62	33.23	9.78	204	5.36	1.44	16	0	3	0	0	13	6	81.3	37.5	-

Table S18 (cont.)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Tot P	Tot N	P corr	N corr	F P	F N	F N >10*LOD	F N %	F N % >10*LOD	F P %
Erythromycin	<LOD	0.29	<LOD	<LOQ	0.44	0.08	0.12	1.22	5.20	6.30	17.78	0.27	1.62	1.45	0.24	1.81	13	3	2	3	0	11	2	84.6	15.4	0.0
Lincomycin	0.05	0.09	<LOQ	<LOQ	0.05	<LOQ	<LOQ	0.94	11.06	1.04	5.12	0.12	0.14	0.15	<LOQ	3.79	11	5	2	5	0	9	4	81.8	36.4	0.0
Metronidazole	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.60	21.08	56.13	30.50	130.9	<LOQ	0.67	1.69	<LOD	12.26	8	8	5	8	0	3	0	37.5	0.0	0.0
Sulfamethoxazole	1.04	4.59	<LOD	<LOD	1.76	0.38	3.96	5963	49.06	104.18	165.3	2.21	8.10	13.36	0.28	89.77	14	2	10	2	0	4	1	28.6	7.1	0.0
Tylosin	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	0	16	0	14	2	0	0	-	-	12.5
Estradiol, 17-beta-(E2)	<LOQ	<LOQ	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.83	<LOD	<LOQ	0.64	<LOQ	<LOQ	2	14	0	11	3	2	0	100.0	0.0	21.4
Estrone	0.17	<LOQ	<LOQ	<LOQ	0.09	0.51	0.36	3.20	4.60	1.03	17.25	0.65	1.57	3.95	0.14	1.13	13	3	3	3	0	10	4	76.9	30.8	0.0
Progesterone	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOD	0	16	0	15	1	0	0	-	-	6.3
Testosterone	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOQ	<LOQ	<LOQ	2.30	<LOD	<LOD	1	15	0	15	0	1	0	100.0	0.0	0.0
Amphetamine	<LOQ	<LOQ	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0	16	0	13	3	0	0	-	-	18.8
Caffeine	16.67	43.14	11.38	8.58	33.16	177.4	204	5870	1070	134	407	23.59	322	817	12.35	624	16	0	15	0	0	1	1	6.3	6.3	-
Nicotine	6.05	28.28	15.72	6.21	12.48	42.44	40.72	497	137.2	34.72	85.01	14.84	190.1	554	5.86	48.48	16	0	6	0	0	10	7	62.5	43.8	-
Tributyl-phosphate	<LOD	18.01	249	139	304	42.76	12.69	9.94	323	41.00	50.80	6.46	4.17	6.30	5.30	15.02	15	1	9	1	0	6	2	40.0	13.3	0.0
																Total	**342**	**170**	**170**	**156**	**14**	**172**	**62**	**50.3**	**18.1**	**8.2**

Table S19. Time weighted averages of the concentrations (ng/L) of the selected point source chemicals in the two weeks of POCIS exposure in summer (July, 2016) samples. The station codes correspond to the numbers of the sampling sites in Figure 1. (I: insecticides; H: herbicides; F: fungicides; <LOD: below the limit of detection; <LOQ: below the limit of quantification. Detection and quantification limits are reported in Table 1). Green cells represent the samples with positive results in the screening analysis. In the last columns, the comparison between quantitative data and screening results is reported. Tot P: number of the samples positively quantified; Tot N: number of the negative samples in the quantitative analysis (<LOD or <LOQ); P corr: number of the positive samples detected in the screening; N corr number of the negative samples not detected in the screening; FP: false positive; FN: false negative; FN>10*LOD: number of false negative with a concentration higher than 10*LOD; FN%, FN%>10*LOD, FP%: percentages of false negative and false positive.

	1	2	3	5	6	7	8	12	13	14	15	16	Tot P	Tot N	P corr	N corr	F P	F N	F N >10*LOD	F N %	F N % >10*LOD	F P %
Acetaminophen	172	<LOQ	<LOQ	<LOQ	3862	131	434	3.02	5606	3833	<LOQ	<LOQ	7	5	3	5	0	4	4	57.1	57.1	0.0
Atenolol	1.32	21.0	<LOQ	12.23	722	43.9	833	14.99	216	105	3.25	482	11	1	8	1	0	3	2	27.3	18.2	0.0
Carbamazepine	5.02	42.3	3.52	13.9	1512	93.8	851	63.19	302	292	161	2880	12	0	10	0	0	2	1	16.7	8.3	-
Citalopram	<LOQ	9.80	1.23	8.5	85.24	34.8	442	0.68	260	15.9	<LOD	143	10	2	2	2	0	8	6	80.0	60.0	0.0
Diclofenac	<LOD	37.6	<LOD	17.0	621	40.7	1121	<LOQ	887	503	<LOQ	2667	8	4	5	4	0	3	3	37.5	37.5	0.0
Gemfibrozil	<LOQ	166	<LOQ	55.3	2953	113.92	6434	<LOQ	3417	2262	93.16	9093	9	3	5	3	0	4	3	44.4	33.3	0.0
Ibuprofen	<LOD	125	3.20	14.8	21384	396	5034	<LOD	8861	9621	<LOD	5543	9	3	1	3	0	8	6	88.9	66.7	0.0
Ketoprofen	<LOD	<LOD	<LOD	<LOD	1153	<LOQ	2149	<LOD	486	277	<LOD	143	5	7	4	4	3	1	1	20.0	20.0	42.9
Loratadine	<LOQ	<LOQ	<LOD	<LOQ	243	<LOQ	2977	<LOD	<LOD	<LOQ	<LOQ	18.9	3	9	2	8	1	1	3	33.3	100.0	11.1
Naproxen	<LOD	<LOD	<LOD	<LOD	7961	724.53	3275	<LOD	1449	<LOD	<LOD	<LOD	4	8	4	7	1	0	0	0.0	0.0	12.5
Omeprazole	<LOQ	<LOQ	<LOD	<LOQ	240	<LOQ	1537	<LOQ	<LOQ	4.12	<LOQ	1.16	4	8	1	8	0	3	0	75.0	0.0	0.0
Paraxantine	10446	3055	2387	1028	17560	16153	5062	<LOQ	14485	15145	<LOQ	7146	10	2	9	2	0	1	1	10.0	10.0	0.0
Salbutamol	<LOD	0.28	<LOD	<LOQ	5.18	<LOD	10.63	<LOQ	1.28	1.42	<LOD	28.4	6	6	3	6	0	3	2	50.0	33.3	0.0
Trimethoprim	<LOQ	1.47	0.18	0.48	293	3.07	2283	<LOQ	239	197	0.80	390	10	2	5	2	0	5	0	50.0	0.0	0.0
Valsartan	24.9	789	7.95	487	7261	1021.26	17789	137	14626	10853	40.4	22940	12	0	10	0	0	2	1	16.7	8.3	-
Venlafaxine	<LOQ	26.9	<LOQ	30.3	357	57.59	1407	<LOQ	<LOQ	7.88	<LOQ	281	7	5	7	3	2	0	0	0.0	0.0	40.0
Amoxicillin	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	15.97	<LOD	<LOD	<LOD	<LOD	<LOD	1	11	0	10	1	1	1	100.0	100.0	9.1
Azithromycin	2.42	5.19	<LOQ	6.60	<LOD	10.7	73058	9.54	8.23	7.87	9.45	<LOQ	9	3	1	3	0	8	7	88.9	77.8	0.0
Ciprofloxacin	<LOD	<LOD	<LOD	<LOQ	509	12.7	1026	<LOQ	<LOQ	17.7	<LOQ	<LOQ	4	8	2	7	1	2	1	50.0	25.0	12.5

Table S19 (cont.)

	1	2	3	5	6	7	8	12	13	14	15	16	Tot P	Tot N	P corr	N corr	F P	F N	F N >10*LOD	F N %	F N % >10*LOD	F P %
Erythromycin	0.25	<LOQ	<LOD	<LOQ	0.77	0.93	139	<LOQ	<LOD	<LOD	<LOD	177	5	7	0	7	0	5	5	100.0	100.0	0.0
Lincomycin	0.62	0.37	<LOQ	<LOQ	4.37	0.23	13.1	0.44	0.73	0.80	0.96	60.3	10	2	2	2	0	8	7	80.0	70.0	0.0
Metronidazole	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	19.9	12.21	<LOD	<LOQ	<LOD	7.98	3	9	0	7	2	3	1	100.0	33.3	22.2
Sulfamethoxazole	1.64	27.5	<LOD	4.10	827	12.3	3043	3.89	122	144	4.28	1221	11	1	8	1	0	3	1	27.3	9.1	0.0
Tylosin	<LOD	<LOD	<LOD	<LOQ	5.90	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	1	11	0	8	3	1	0	100.0	0.0	27.3
Estradiol, 17-beta-(E2)	<LOD	<LOQ	<LOD	<LOQ	4.08	<LOQ	5.56	<LOD	8.13	9.97	<LOD	<LOQ	4	8	2	8	0	2	2	50.0	50.0	0.0
Estrone	31.5	27.1	4.21	54.9	155	11.75	276	23.8	269	172	34.6	273	12	0	4	0	0	8	9	66.7	75.0	-
Progesterone	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	12.4	<LOQ	<LOD	<LOD	1	11	0	10	1	1	1	100.0	100.0	9.1
Testosterone	<LOD	<LOD	<LOD	<LOD	15.7	8.86	<LOQ	<LOD	34.6	18.7	7.36	<LOD	5	7	1	7	0	4	2	80.0	40.0	0.0
Amphetamine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0	12	0	7	5	0	0	-	-	41.7
Caffeine	1577	673	458	431	14533	1989	2859	330	5139	4719	566	2337	12	0	10	0	0	2	1	16.7	8.3	-
Nicotine	714	591	56.65	507	5785	660	1367	406	1903	2063	566	663	12	0	11	0	0	1	1	8.3	8.3	-
Tributyl-phosphate	68.9	70.8	45.5	59.7	84.6	28.3	121	51.0	39.7	16.1	64.7	708	12	0	2	0	0	10	9	83.3	75.0	-
												Total	**127**	**65**	**79**	**58**	**7**	**48**	**33**	**37.8**	**26.0**	**10.8**

Table S20. Synthesis of the comparison between the screening and the quantitative analysis for pesticides (I= Insecticides; H= Herbicides; F= Fungicides) in the water samples of the 16 sampling sites in the three sampling campaigns. Tot P: number of the samples positively quantified; Tot N: number of the negative samples in the quantitative analysis (<LOD or <LOQ); P corr: number of the positive samples detected in the screening; N corr number of the negative samples not detected in the screening; FP: false positive; FN: false negative; FN>10*LOD: number of false negative with a concentration higher than 10*LOD; FN%, FN%>10*LOD, FP%: percentages of false negative and false positive.

	Tot P	Tot N	P corr	N corr	F P	F N	F N >10*LOD	F N %	F N % >10*LOD	F P %
Carbofuran	0	48	0	34	14	0	0	-	-	29.2
Chlorpyrifos ethyl	0	48	0	48	0	0	0	-	-	0.0
Diazinon	21	27	5	23	4	16	4	76.2	19.0	14.8
Dimethoate	30	18	7	18	0	23	13	76.7	43.3	0.0
Imidacloprid	44	4	17	4	0	27	16	61.4	36.4	0.0
Malathion	0	48	0	40	8	0	0	-	-	16.7
Metolcarb	0	48	0	18	30	0	0	-	-	62.5
Pirimicarb	14	34	7	25	9	7	2	50.0	14.3	26.5
Spinosin-A	1	47	0	41	6	1	0	100.0	0.0	12.8
Chlorturon	24	24	5	24	0	19	11	79.2	45.8	0.0
Diuron	25	23	9	23	0	16	14	64.0	56.0	0.0
Metribuzine	22	26	1	26	0	21	13	95.5	59.1	0.0
Simazine	45	3	16	3	0	29	19	64.4	42.2	0.0
Terbuthrin	41	7	13	7	0	28	8	68.3	19.5	0.0
Terbuthylazine	43	5	6	4	1	37	13	86.0	30.2	20.0
Carbedazim	44	4	6	4	0	38	29	86.4	65.9	0.0
Kresoxim methyl	0	48	0	29	19	0	0	-	-	39.6
Propiconazole	17	31	6	31	0	11	8	64.7	47.1	0.0
Spiroxamine	1	47	1	21	26	0	0	0.0	0.0	55.3
Tebuconazole	41	7	3	7	0	38	22	92.7	53.7	0.0
	413	**547**	**102**	**430**	**117**	**311**	**172**	**75.3**	**41.6**	**21.4**

Table S21. Synthesis of the comparison between the screening and the quantitative analysis for pharmaceuticals and other point source compounds in the water samples of the 16 sampling sites in the three sampling campaigns (Ph= pharmaceuticals excluding antibiotics; St= steroids; A= antibiotics; Ls= stimulants and life-style compounds; Pl= Plasticisers) . Tot P: number of the samples positively quantified; Tot N: number of the negative samples in the quantitative analysis (<LOD or <LOQ); Pcorr: number of the positive samples detected in the screening; Ncorr: number of the negative samples not detected in the screening; FP: false positive; FN: false negative; FN>10*LOD: number of false negative with a concentration higher than 10*LOD; FN%, FN%>10*LOD, FP%: percentages of false negative and false positive.

	Tot P	Tot N	P corr	N corr	F P	F N	F N>10*LOD	F N %	F N %>10*LOD	F P %
Acetaminophen	45	3	23	3	0	22	13	48.9	28.9	0.0
Atenolol	42	6	30	5	1	12	2	28.6	4.8	16.7
Carbamazepine	48	0	30	0	0	18	6	37.5	12.5	-
Citalopram	46	2	9	2	0	37	22	80.4	47.8	0.0
Diclofenac	32	16	19	15	1	13	2	40.6	6.3	6.3
Gemfibrozil	39	9	20	9	0	19	13	48.7	33.3	0.0
Ibuprofen	48	0	4	0	0	44	24	91.7	50.0	-
Ketoprofen	25	23	17	22	1	8	1	32.0	4.0	4.3
Loratadine	6	42	2	36	6	4	3	66.7	50.0	14.3
Naproxen	22	26	21	23	3	1	0	4.5	0.0	11.5
Omeprazole	23	25	13	21	4	10	1	43.5	4.3	16.0
Paraxantine	48	0	25	0	0	23	13	47.9	27.1	-
Salbutamol	30	18	25	17	1	5	3	16.7	10.0	5.6
Trimethoprim	39	9	19	9	0	20	4	51.3	10.3	0.0
Valsartan	43	5	24	5	0	19	11	44.2	25.6	0.0
Venlafaxine	41	7	38	4	3	3	1	7.3	2.4	42.9
Amoxicillin	8	40	2	40	0	6	0	75.0	0.0	0.0
Azithromycin	43	5	11	5	0	32	10	74.4	23.3	0.0
Ciprofloxacin	38	10	6	9	1	32	2	84.2	5.3	10.0
Erythromycin	39	9	10	9	0	29	3	74.4	7.7	0.0
Lincomycin	28	20	5	20	0	23	8	82.1	28.6	0.0
Metronidazole	21	27	14	27	0	7	0	33.3	0.0	0.0
Sulfamethoxazole	42	6	29	6	0	13	1	31.0	2.4	0.0
Tylosin	0	48	0	45	4	0	0	-	-	8.3
Estradiol, 17-beta-(E2)	8	40	3	33	7	5	0	62.5	0.0	17.5
Estrone	37	11	8	10	1	29	14	78.4	37.8	9.1
Progesterone	0	48	0	43	5	0	0	-	-	10.4
Testosterone	3	45	2	40	5	1	0	33.3	0.0	11.1
Amphetamine	0	48	0	43	5	0	0	-	-	10.4
Caffeine	48	0	39	0	0	9	8	18.8	16.7	-
Nicotine	48	0	25	0	0	23	14	47.9	29.2	-
Tributyl-phosphate	46	2	25	1	1	21	8	45.7	17.4	50.0
	986	**550**	**498**	**502**	**49**	**258**	**119**	**42.1**	**20.2**	**7.6**

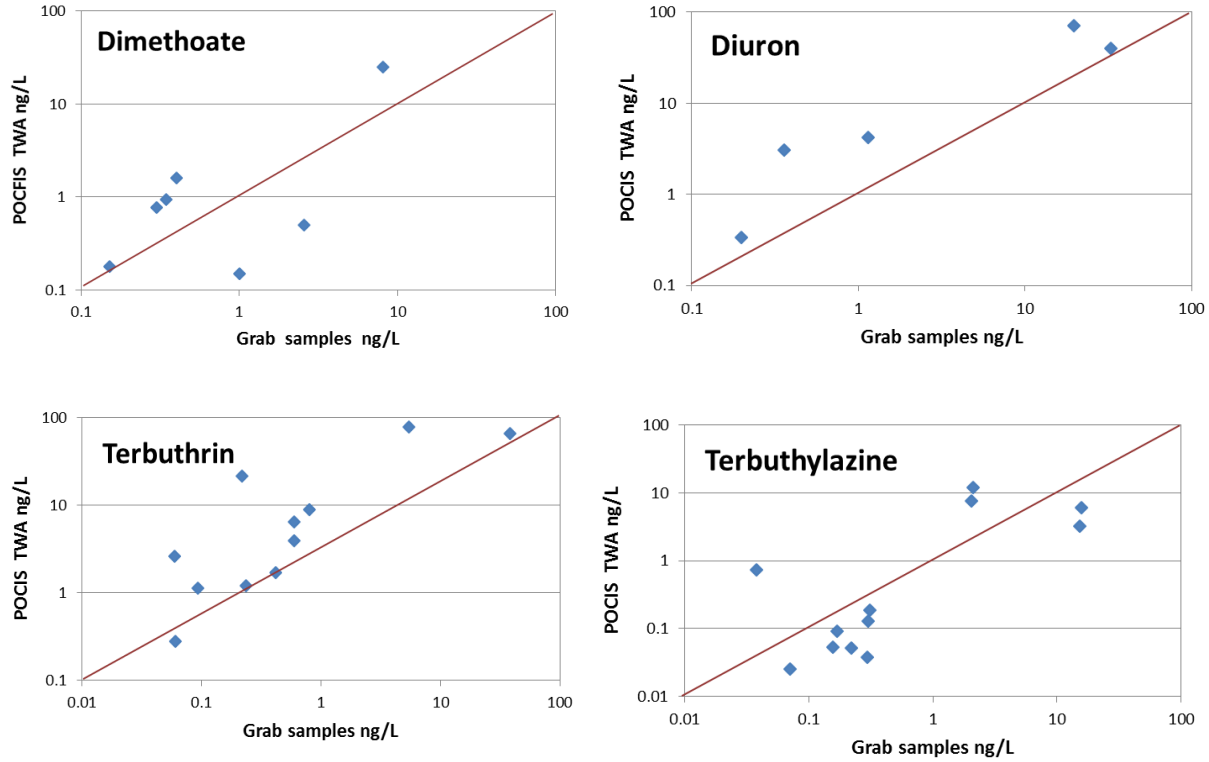


Figure S1. Comparison for pesticides between concentrations in water measured in grab samples and time weighted averages (TWA) calculated in POCIS samples. The line represents the 1/1 correspondence between water and POCIS concentrations. The comparison is reported only for the compounds with more than three positive POCIS data higher than 0.1 ng/L (see Table 3 in the main text). Imidacloprid, simazine, carbendazim and tebuconazole are shown in the main text (Figure 5).

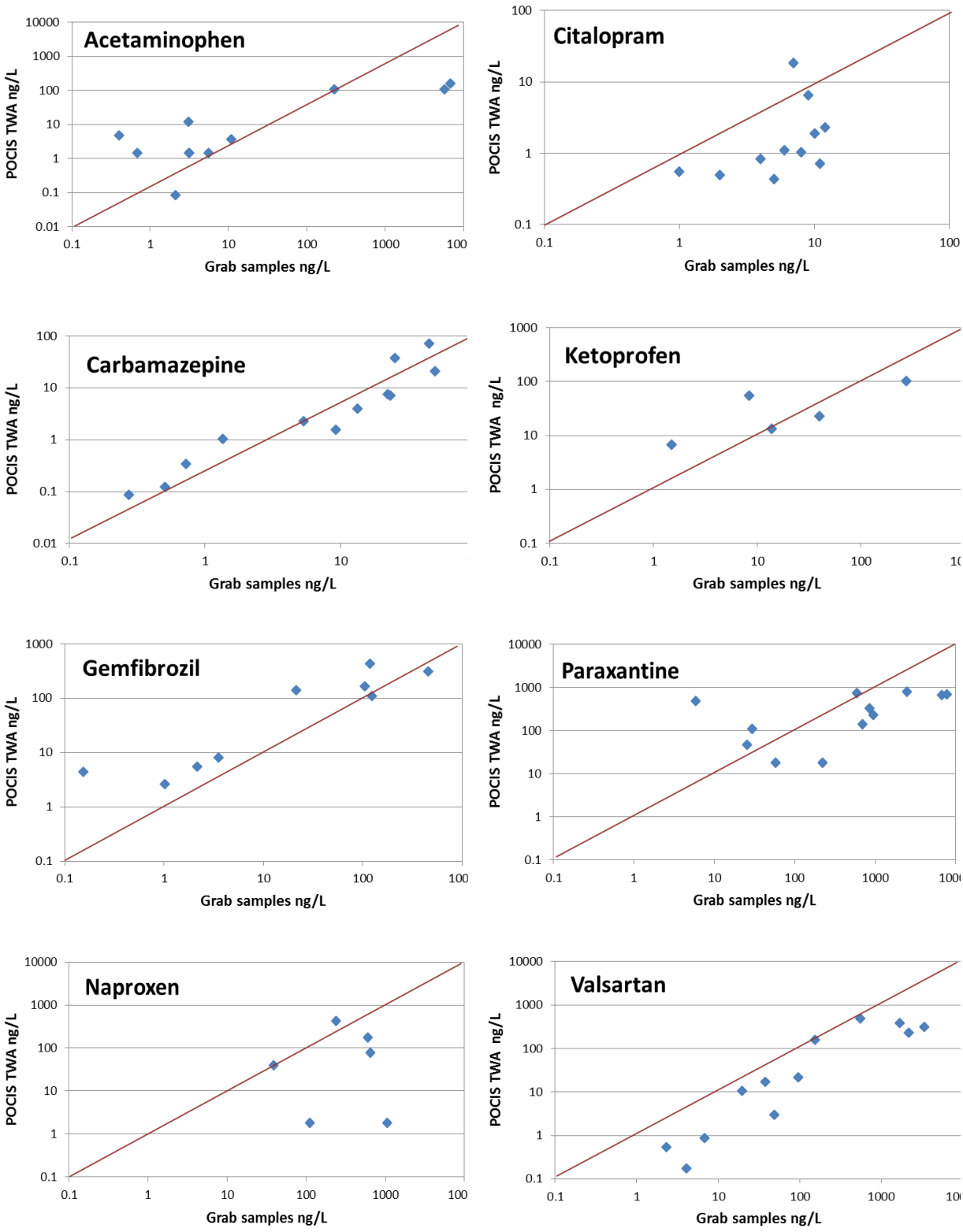
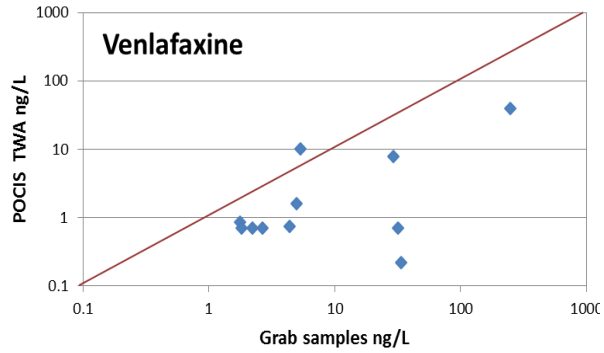
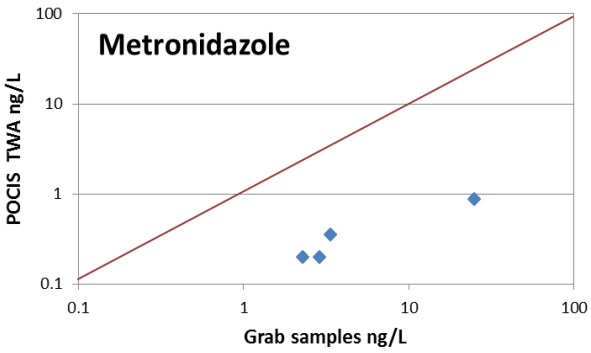
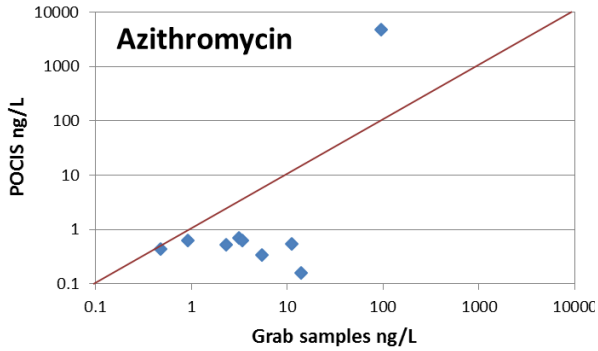
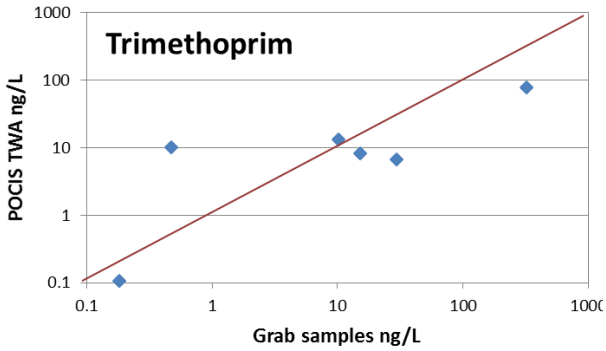


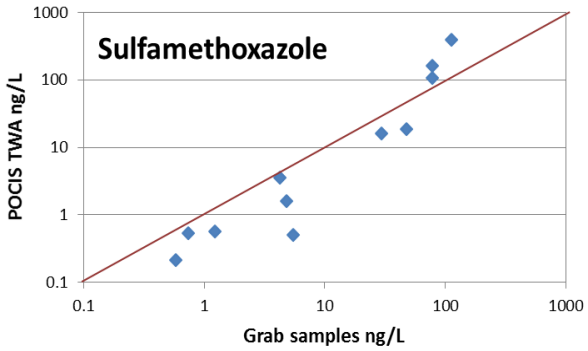
Figure S2. Comparison for point source contaminants between concentrations in water measured in grab samples and those calculated in POCIS samples. The line represents the 1/1 correspondence between water and POCIS concentrations. The comparison is reported only for the compounds with more than three positive POCIS data higher than 0.1 ng/L (see Table 3 in the main text). Atenolol, diclofenac, ibuprofen and caffeine are shown in the main text (Figure 6).

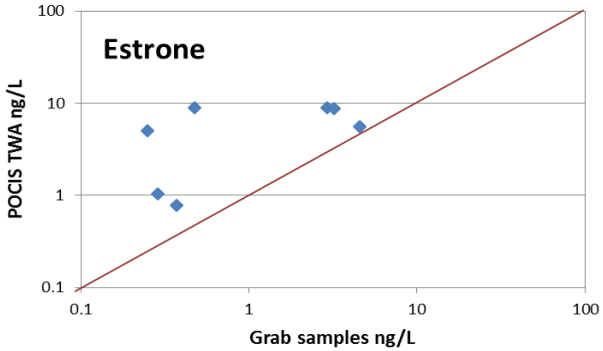


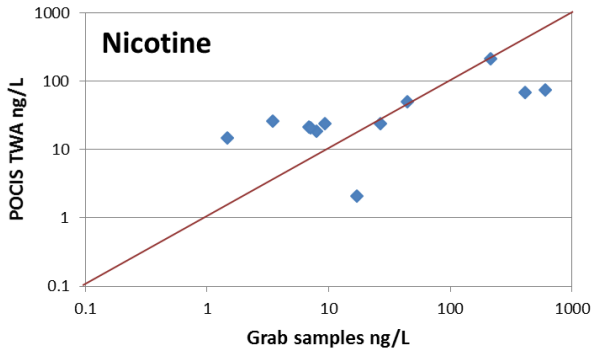












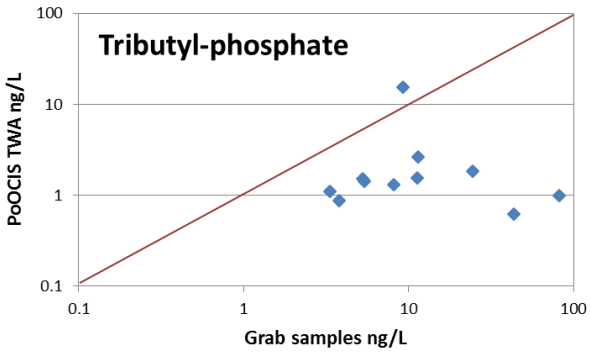


Figure S2 (cont.)

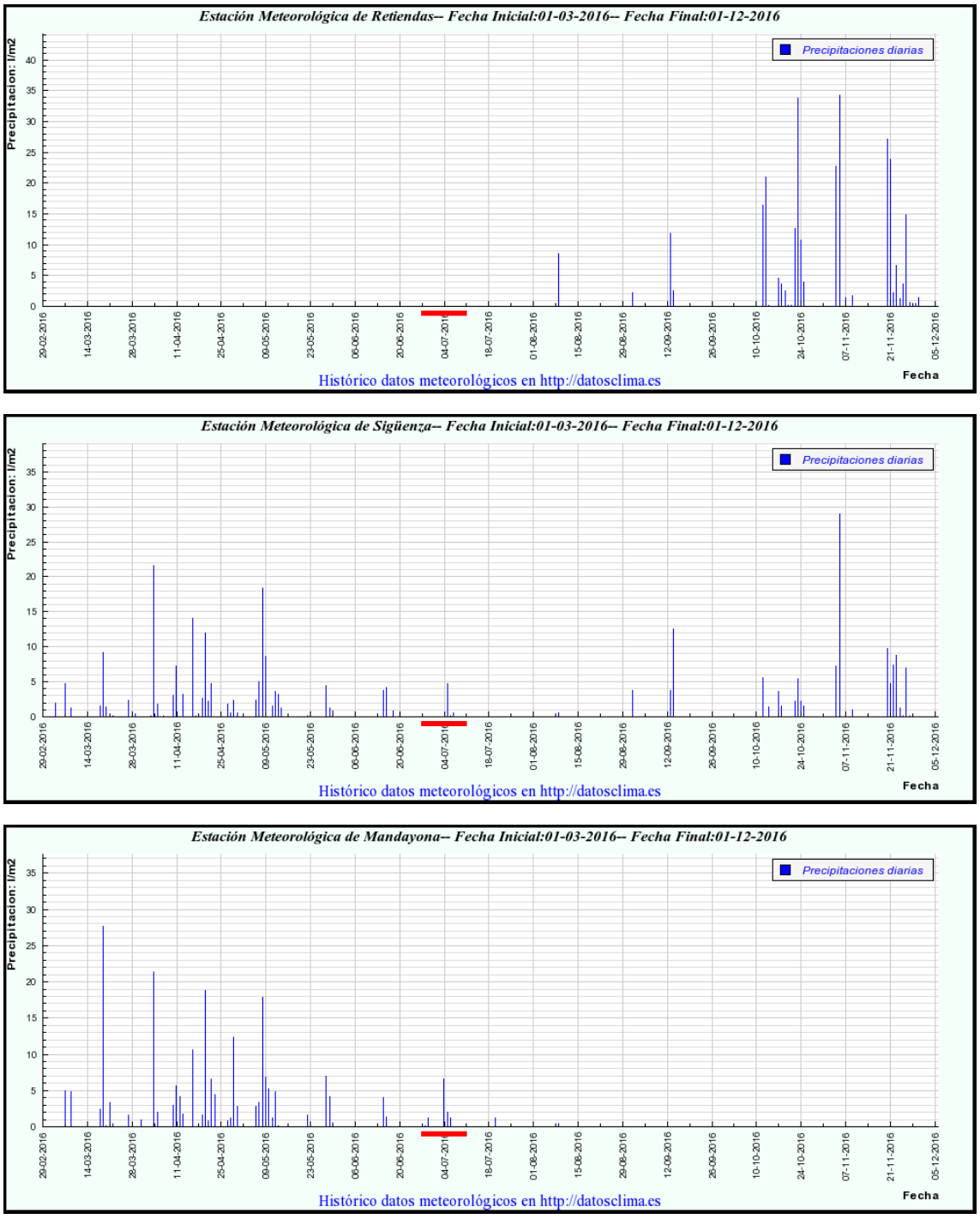
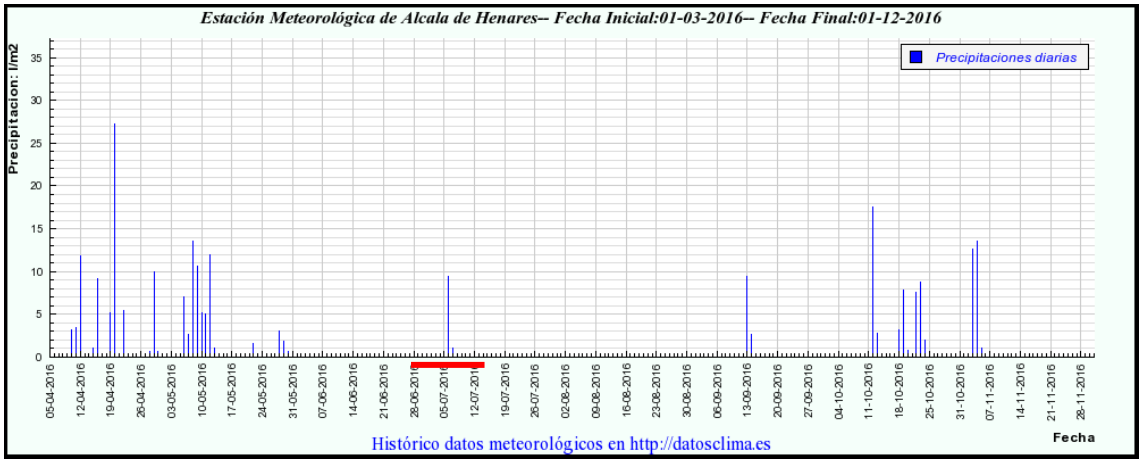
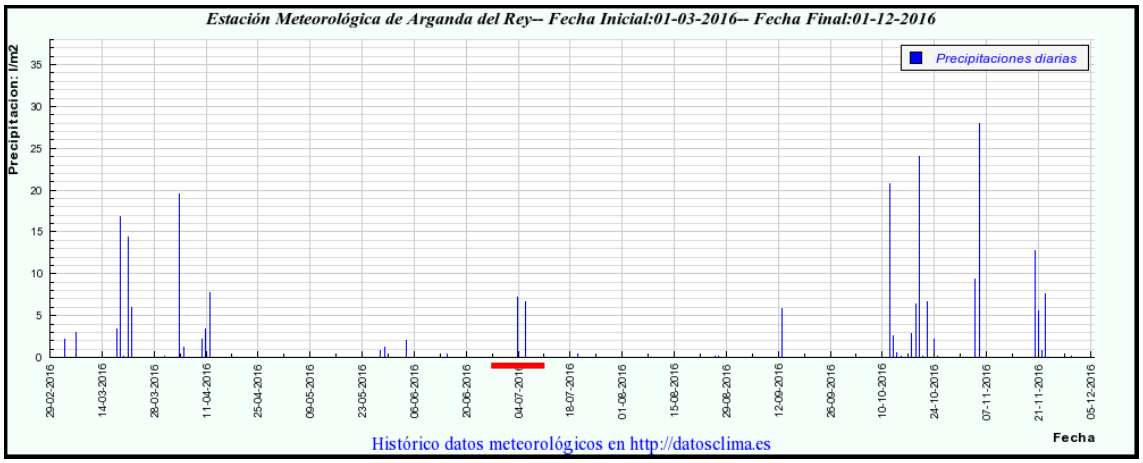


Figure S3. Rainfall measured in some meteorological stations located in the proximity of selected sampling sites. The meteorological stations of Retiendas, Siguenza and Mandayona correspond to sampling sites 5 and 6; the meteorological stations of Alcalá de Henares and Arganda del Rey correspond to sampling site 8; the meteorological stations of Aranjuez, Ocaña and Tembleque correspond to sampling sites 13, 14 and 16. The red line indicates the two weeks of POCIS exposure.





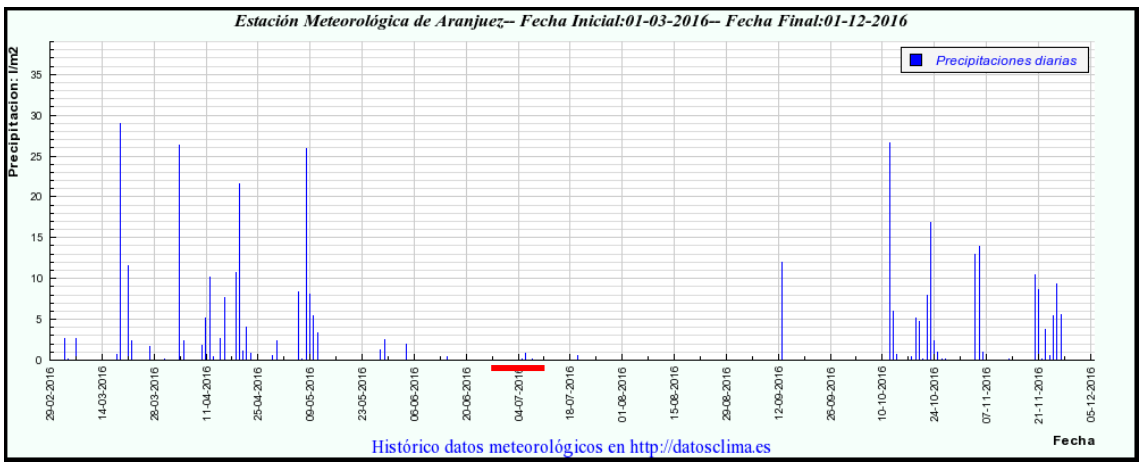


Figure S3 (cont.)

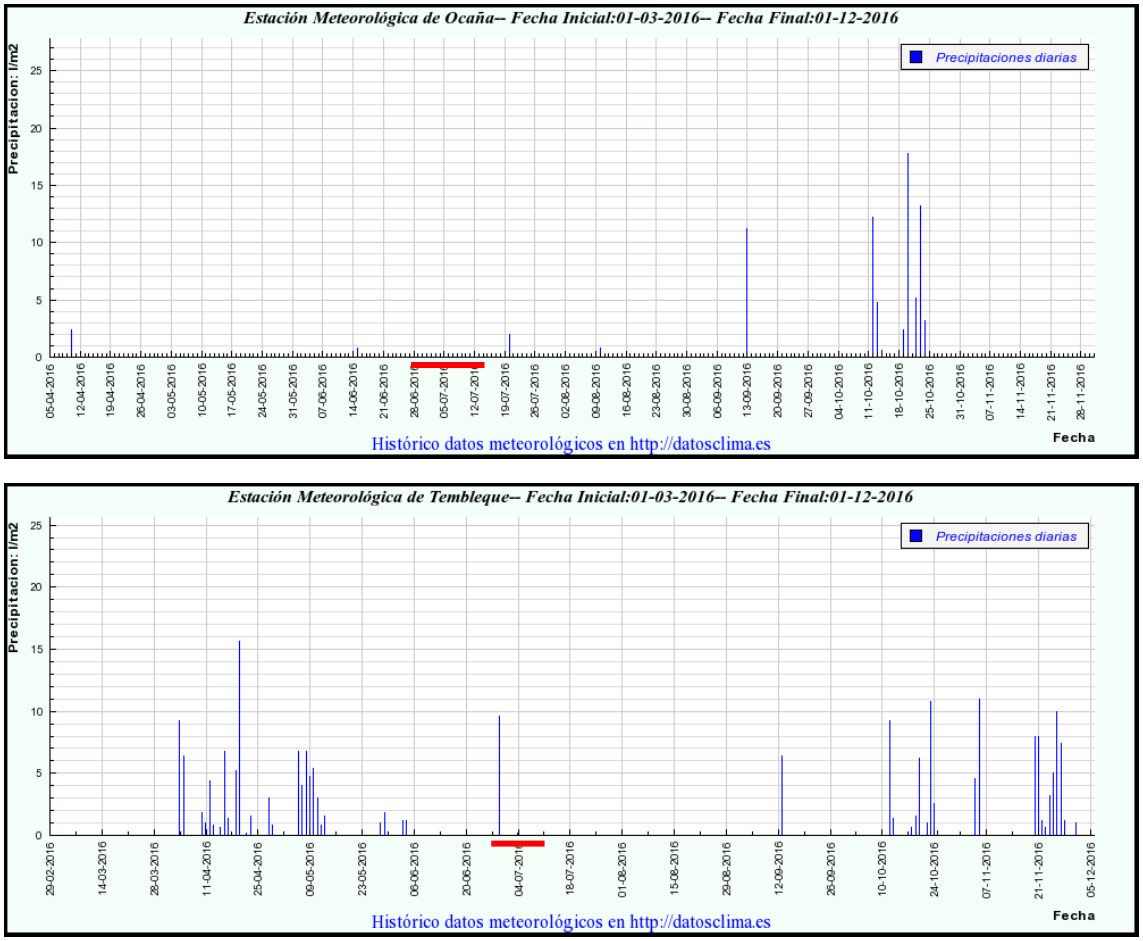


Figure S3 (cont.)

Procedures for selecting or estimating toxicity data and for calculating TUs

The toxicity data were taken from the literature. The main source of data was the database ECOTOX. If needed, other relevant data sources were used.

For the toxicity data of the base set (algae, crustaceans, fish) the following procedure was followed to determine the final selected EC50 for the calculation of TUs:

- Algae: the preferred species was *Pseudokirkneriella subcapitata*, if not available, other green algae species (*Clorella, Scenedesmus*) were selected. Selected values were short term (72 to 96 hours) EC50 for growth inhibition. If more reliable data were available the geometric mean was calculated.
- Crustaceans: the preferred species was *Daphnia magna*, if not available, other crustacean species (e.g. *Ceriodaphnia*) were selected. Selected values were 48 hours EC50 for immobilisation. If more reliable data were available the geometric mean was calculated.
- Fish: the preferred species was *Oncorhinchus mikiss*, if not available, other species accepted for standard fish tests (e.g. *Poecilia, Danio, Pimephales*) were selected. Selected values were 96 hours LC50 for mortality. If more than one reliable value was available the geometric mean was calculated.

In case of absence of suitable data, toxicity was calculated using QSARS with the following equations:

Algae (EC, 2003):

$$\log 1/96hEC50 = 1 \times \log Kow - 1.71$$

Daphnia (EC, 2003):

$$\log 1/48hEC50 = 0.95 \times \log Kow - 1.68$$

Fish (EC, 2003):

$$\log 1/96hLC50 = 0.73 \times \log Kow - 1.61$$

where all values of EC/LC50 are in μmols/L.

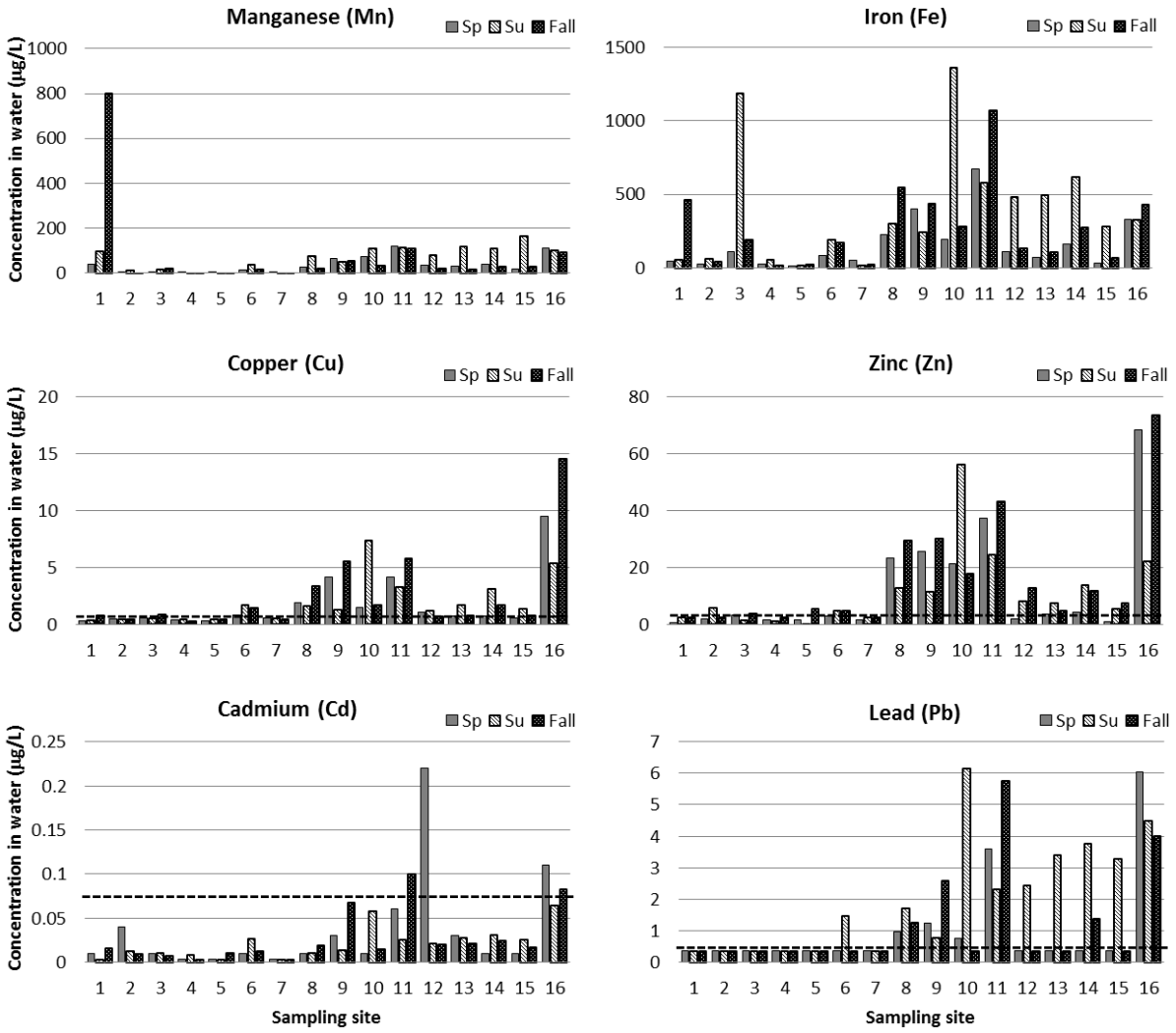


Figure S1. Concentrations of metals in the 16 sampling stations and in the three seasonal sampling dates. Dotted lines represent approximated background levels according to Crommentuijn et al. (1997). Mercury data are not shown because only five values were above LOD (see Table S3).

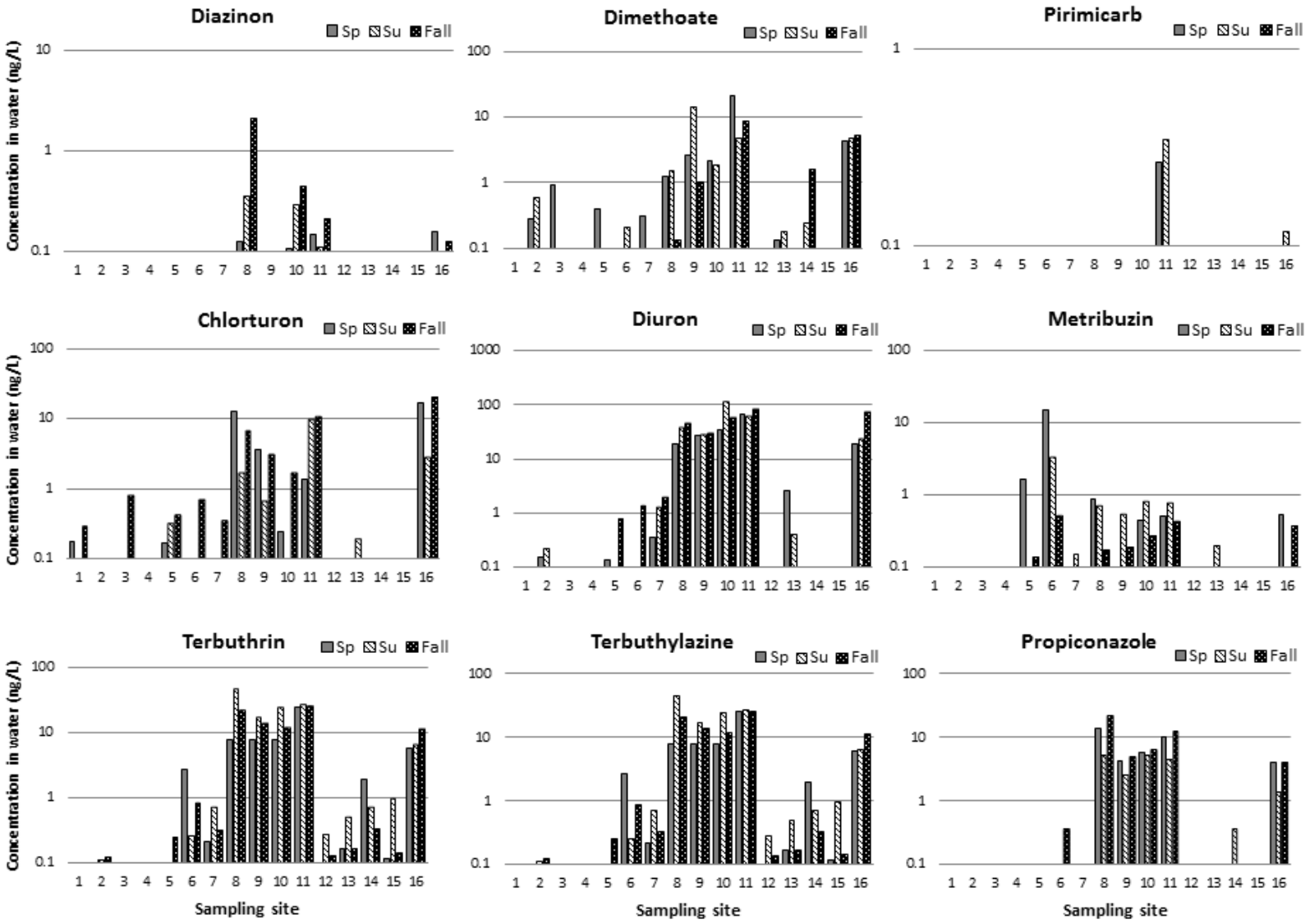


Figure S2. Concentration of pesticides in the grab samples of the 16 sampling stations and in the three seasonal sampling dates (Sp: spring; Su: summer).

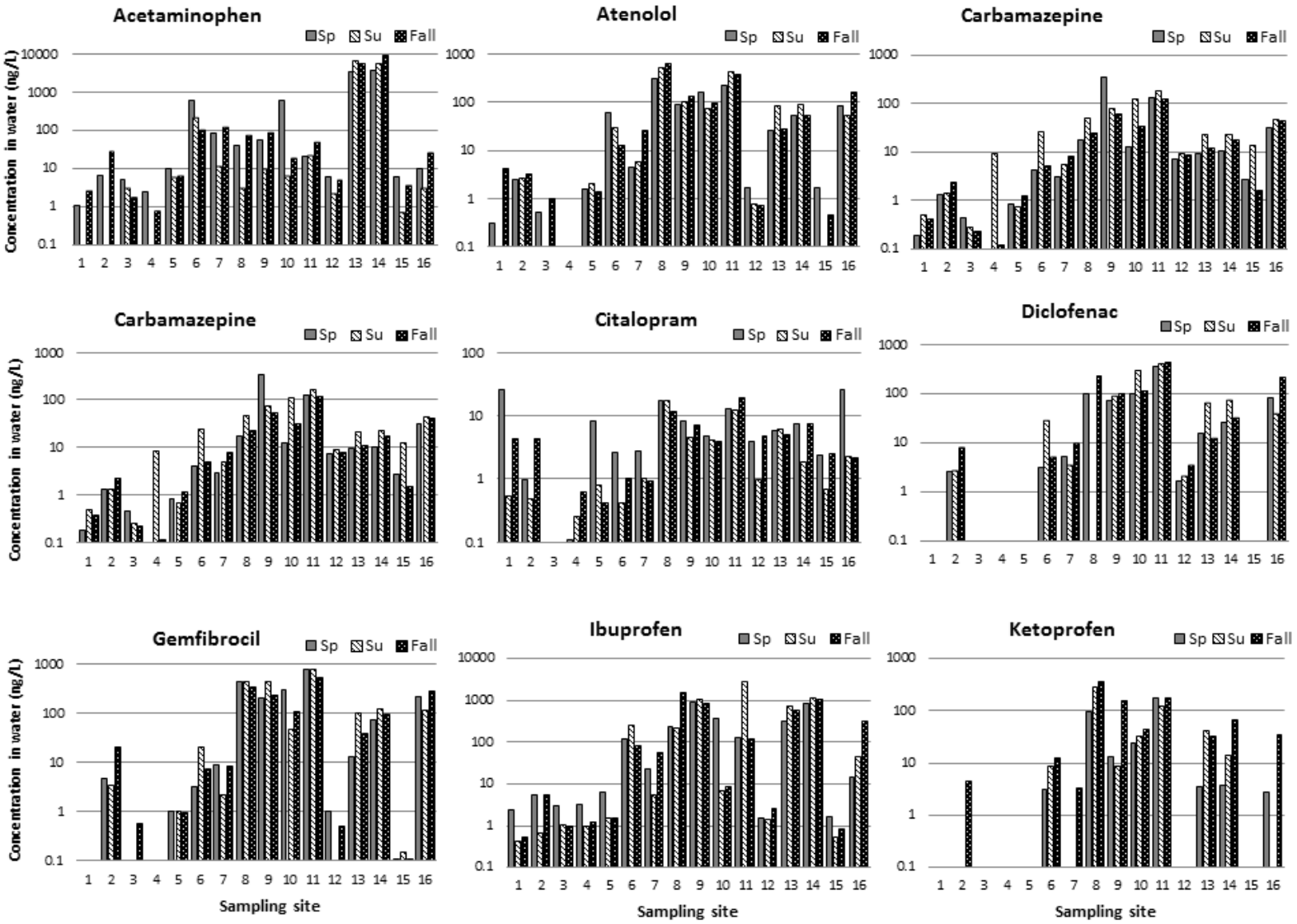


Figure S3. Concentrations and time trends of point source chemicals in the 16 sampling stations and in the three seasonal samples.

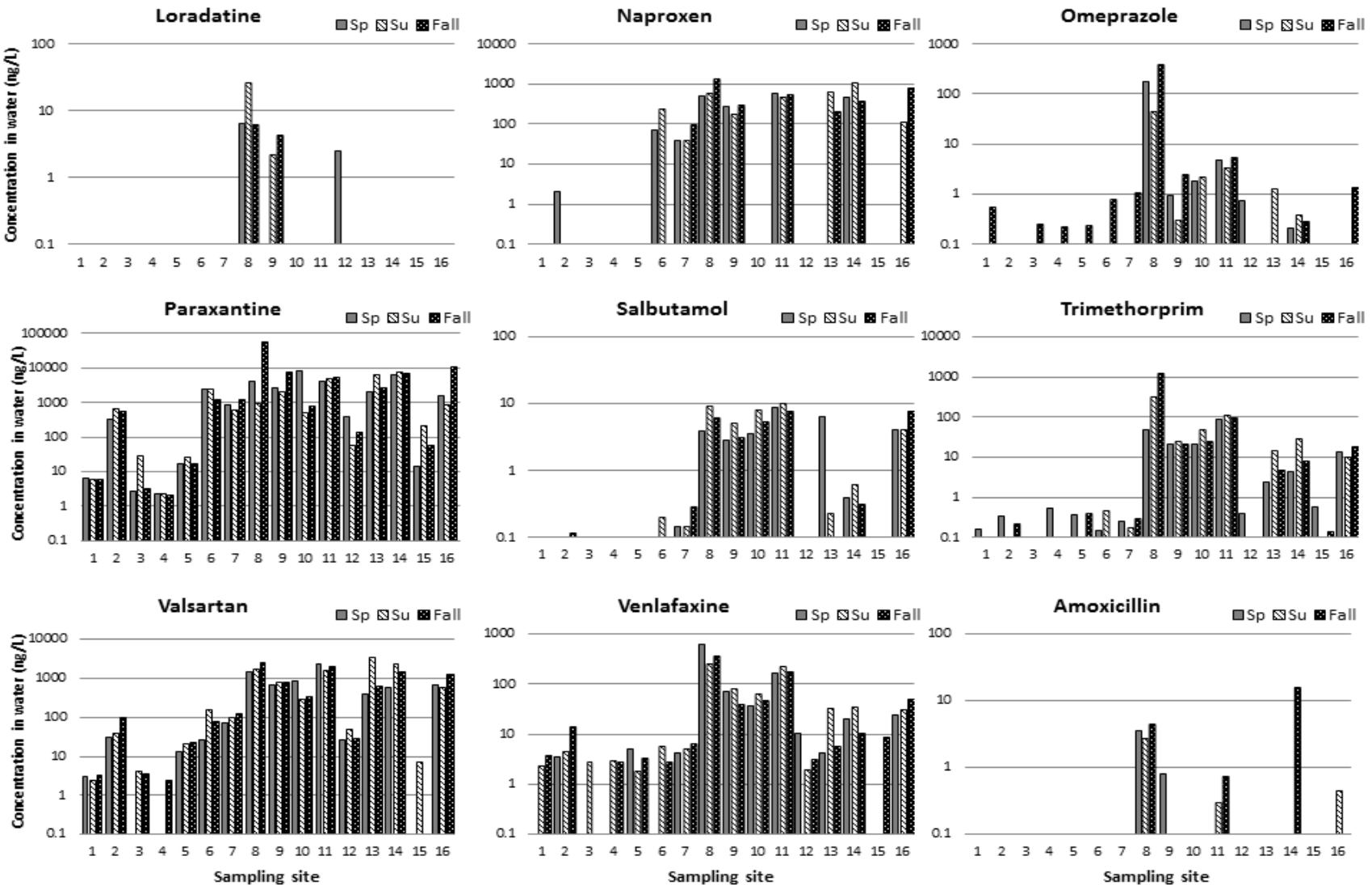


Figure S3 (cont.). Concentrations and time trends of point source chemicals in the 16 sampling stations and in the three seasonal samples.

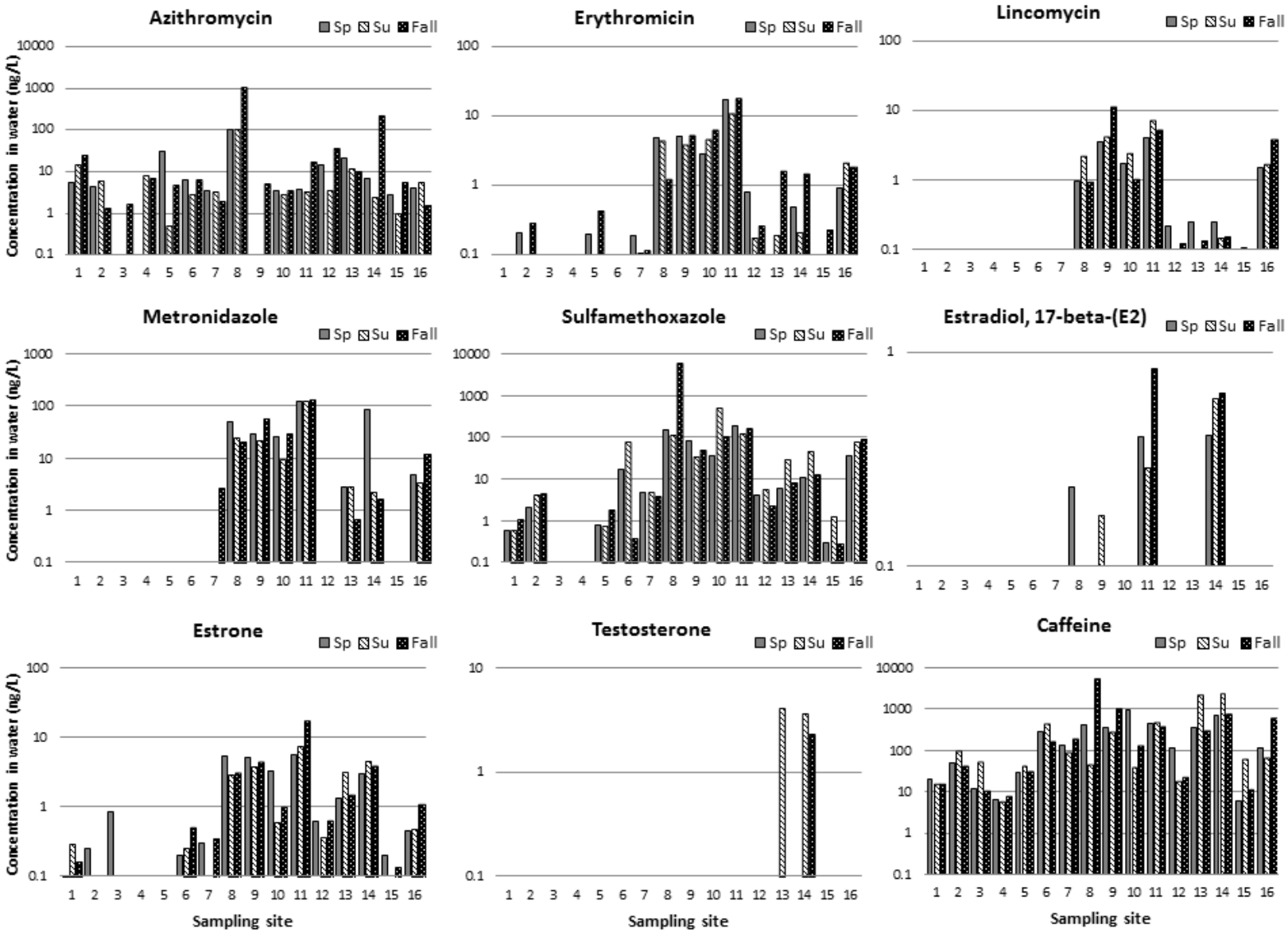


Figure S3 (cont.). Concentrations and time trends of point source chemicals in the 16 sampling stations and in the three seasonal samples.

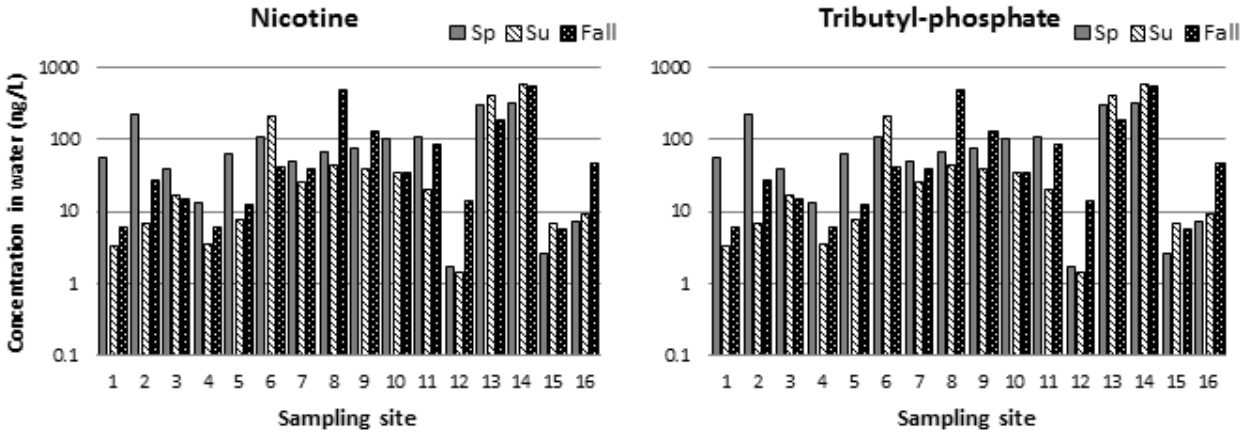


Figure S3 (cont.). Concentrations and time trends of point source chemicals in the 16 sampling stations and in the three seasonal samples.

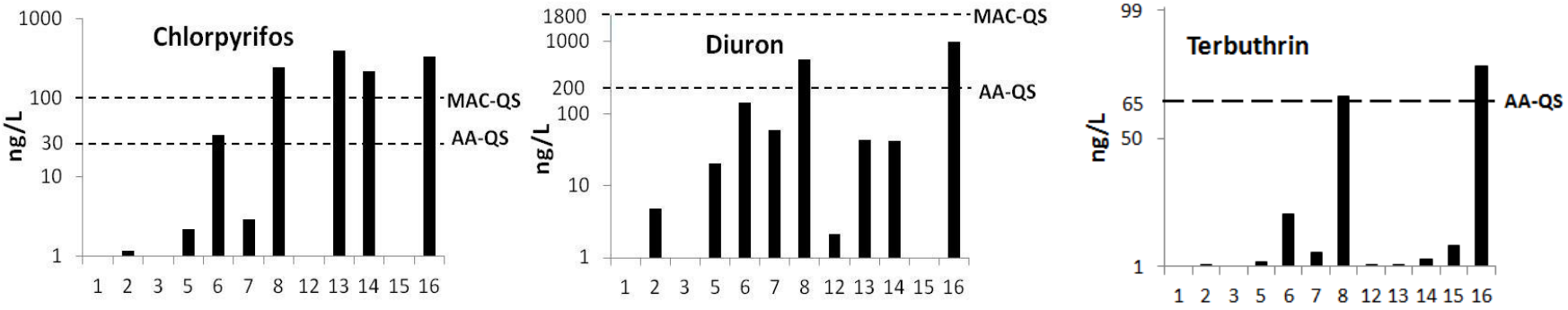


Figure S4. Concentrations of chlorpyrifos, diuron and terbuthrin in summer POCIS samples compared with the AA-QS and the MAC-QS proposed for priority substances in the European WFD.

Table S1. Main physico-chemical and ecotoxicological properties of the evaluated compounds. Water solubility and log Kow: data for pesticides were taken from Tomlin (2003); data for other chermicals were taken from the PubChem database (https//pubchem.ncbi.nlm.nih.gov/compound). Toxicity data: were taken from the ECOTOX database (https://cfpub.epa.gov/ecotox/) or from other references listed in the table. If not available, toxicity data were calculated using QSAR equations. E(L)C50 values higher than 100 mg/l are considered as low toxicity and not precisely quantified (inicated as >100). The procedures for the selection of toxicity data, for QSAR calculation are described above. Chemical classes: I: insecticides; H: herbicides; F: fungicides; Ph= pharmaceuticals; A: antibiotics; E: estrogens and steroids; P= pesticides; D: drugs and life style chemicals; Pl= plasticizers

	Chemical class	Details of uses	CAS	MW	WS mg/L	logKow	E(L)C50 mg/L				References			
							Bacteria/ Cianobacteria	Algae	Daphnia	Fish	Bacteria/ Cianobacteria	Algae	Daphnia	Fish
Pesticides														
Carbofuran	I		1563-66-2	221	350	2.37	>100	19	0.04	0.6	QSAR	7	7	7
Chlorpyrifos	I		2921-88-2	351	0.73	3	54	21	0.0004	0.003	1	QSAR	2	8
Diazinon	I		333-41-5	304	60	3.4	79	10	0.001	0.4	QSAR	5	2	5
Dimethoate	I		60-51-5	229	25000	0.8	>100	>100	0.2	6	QSAR	5	2	5
Imidacloprid	I		138261-41-3	256	610	0.6	>100	>100	17	>100	QSAR		5	5
Malathion	I		121-75-5	330	145	2.57	>100	52	0.008	0.1	QSAR	QSAR	2	5
Metolcarb	I		1129-41-5	165	2600	1.7	>100	>100	>100	12	QSAR	QSAR	QSAR	5
Pirimicarb	I		23103-98-2	238	2700	3	>100	140	0.0001	29	QSAR	8	8	8
Spinosin-A	I		168316-95-8	370	89	3	>100	48	9.1	4	QSAR	10	10	10
Chlorturon	H		15545-48-9	244	70	2.3	95	0.032	67	35	1	4	8	8
Diuron	H		330-54-1	233	40	2.5	54	0.007	18	5.6	1	4	5	5
Metribuzine	H		21087-64-9	214	1050	2	1.4	0.04	4.5	76	5	5	8	5
Simazine	H		122-34-9	202	5	2.2	50	0.057	1	>100	1	3	11	9
Terbutryn	H		886-50-0	241	25	3.7	33	0.008	2.7	2	QSAR	3	8	5
Terbuthylazine	H		5915-41-3	235	9	2.9	42	0.016	21	4	1	3	8	5
Carbendazim	F		10605-21-7	191	8	1.5	>100	1.3	0.09	0.4	QSAR	8	5	5
Kresoxim methyl	F		143390-89-0	313	2	4.1	0.25	0.33	0.18	0.19	5	6	12	5
Propiconazole	F		60207-90-1	342	110	3.7	46	0.02	8.7	5.3	QSAR	8	5	5
Spiroxamine	F		118134-30-8	297	405	4.2	0.99	0.006	1.0	17	5	5	QSAR	5
Tebuconazole	F		107534-96-3	308	32	3.7	83	2.8	11	4.4	5	5	8	5

Table S1 (cont.)

	Chemical class	Details of uses	CAS	MW	WS mg/L	logKow	E(L)C50 mg/L				References			
							Bacteria/ Cianobacteria	Algae	Daphnia	Fish	Bacteria/ Cianobacteria	Algae	Daphnia	Fish
Pharmaceuticals														
Acetaminophen (paracetamol)	Ph	Analgesic/anti inflammatory	103-90-2	151	14000	0.46	>100	>100	16	>100	QSAR	13	5	5
Atenolol	Ph	b blocker	29122-68-7	266	13000	0.16	>100	>100	>100	>100	QSAR	5		5
Carbamazepine	Ph	antiepylectic	298-46-4	236	18	2.5	>100	>100	>100	20	QSAR	5	5	5
Citalopram	Ph	antidepressant	59729-33-8	324	6	3.5	>100	2	4	7		5	5	5
Diclofenac	Ph	Analgesic/anti inflammatory	15307-86-5	296	2.35	4.7	38	186	87	71		5	5	5
Gemfibrozil	Ph	hypolipidemic	25812-30-0	250	10	4.8	65	5.9	7	13	QSAR	QSAR	QSAR	QSAR
Ibuprofen	Ph	Analgesic/anti inflammatory	15687-27-1	206	21	3.9	18	>100	>100	>100	QSAR	5	5	5
Ketoprofen	Ph	Analgesic/anti inflammatory	22071-15-4	254	50	3.1	>100	>100	>100	>100	QSAR	QSAR	QSAR	QSAR
Loratadine	Ph	antiistaminic	79794-75-5	383	0.011	5.2	18	0.7	1	2		QSAR	QSAR	QSAR
Naproxen	Ph	Analgesic/anti inflammatory	22204-53-1	230	16	3.2	96	>100	82	19	QSAR	5	5	QSAR
Omeprazole	Ph	gastroprotector	73590-58-6	345	35	2.2	>100	>100	>100	31	QSAR	QSAR	QSAR	5
Salbutamol	Ph	antiasthmatic	18559-94-9	239	14000	0.3	>100	>100	>100	>100	QSAR	QSAR	QSAR	QSAR
Valsartan	Ph	anti hypertension	137862-53-4	435	1.5	5.8	31	8	10	19	QSAR	QSAR	QSAR	QSAR
Venlafaxine	Ph	antidepressant	93413-69-5	277	570	3.2	>100	12	10	16	QSAR			
Antibiotics														
Amoxicillin	A	antibiotic	26787-78-0	365	3400	0.87	56	>100	>100	>100	5	5		5
Azithromicin	A	antibiotic	83905-01-5	749	500	3	>100	36	51	47	QSAR		QSAR	
Ciprofloxacin	A	antibiotic	85721-33-1	331	30000	-1.1	>100	6.7	>100	>100	QSAR		QSAR	QSAR
Erythromycin	A	antibiotic	114-07-8	734	2000	2.5	0.2	0.06	>100	>100	5	5	QSAR	5
Lincomycin	A	antibiotic	154-21-2	407	927	0.2	>100	0.07	7.2	>1000	QSAR	5	5	5
Metronidazole	A	antibiotic	443-48-1	171	11000	-0.02	>100	40	>100	>100	QSAR	5	5	5
Sulfamethoxazole	A	antibiotic	723-46-6	253	610	0.9	>100	>100	>100	>100			5	5
Trimethoprim	A	antibiotic	738-70-5	290	400	0.91	>100	>100	>100	>100	5	5	5	5
Tylosine	A	antibiotic	1401-69-0	916	5	1.63	>100	>100	>100	>100	QSAR	QSAR	QSAR	QSAR

Table S1 (cont.)

	Chemical class	Details of uses	CAS	MW	WS mg/L	logKow	E(L)C50 mg/L				References			
							Bacteria/ Cianobacteria	Algae	Daphnia	Fish	Bacteria/ Cianobacteria	Algae	Daphnia	Fish
Estrogens and steroids														
Estradiol	E	estrogen	50-28-2	272	3.6	4	19	2.5	2.9	3.5	QSAR	5	5	5
Estrone	E	estrogen	481-97-0	350	0.04	2.5	>100	65	71	>100	QSAR	QSAR	QSAR	QSAR
Progesterone	E	steroid	57-83-0	314	9	3.9	28	2	3	6	QSAR	QSAR	QSAR	QSAR
Testosterone	E	steroid	58-22-0	288	23	3.3	93	8	10	18	QSAR	QSAR	QSAR	QSAR
Others														
Amphetamine	D	nervous stimulant	300-62-9	135	28000	1.8	>100	>100	>100	>100	QSAR	QSAR	QSAR	QSAR
Caffeine	D	nervous stimulant	58-08-2	194	20000	-0.07	>100	>100	>100	>100	QSAR	5	5	5
Nicotine	D	alcaloid	54-11-5	162	100000	1.2	>100	>100	>100	4	QSAR	QSAR	QSAR	5
Paraxanthine	D	nervous stimulant	611-59-6	180	1000	-0.2	>100	>100	>100	>100	QSAR	QSAR	QSAR	QSAR
Industrial chemicals														
TBP - Tributyl-phosphate	Pl	plasticizer	126-73-8	266	280	3	164	1.8	3.7	8	QSAR	5	5	5

1: Villa et al. (2012); 2: Vighi et al. (1991); 3: Faust et al. (2001); 4: Backhaus et al. (2004); 5: USEPA - ECOTOX Database; 6: Faust et al. (2003); 7: EFSA (2009); 8: Tomlin (2003); 9: Mayer and Ellersieck (1986); 10: EC (2006); 11: Verschueren (1996); 12: University of Hertfordshire-Pesticide Properties Database (https://sitem.herts.ac.uk/aeru/ppdb/en/index.htm); 13: Cunningham et al. (2006).

	Toxicity on aquatic organisms				References			
Metals	Algae	Daphnia	Fish	WQO	Algae	Daphnia	Fish	**WQO**
	E(L)C50 mg/L			mg/L				
Mn	11.5	28	15.2		1	1	1	
Fe	no data	7.2	71			1	1	
Cu	0.078	0.014	0.071	1.5	1	1	1	3
Zn	0.077	0.55	0.41	9.4	1	1	1	3
Cd	0.021	0.033	0.335	0.2	2	2	2	2
Pb	0.22	1.00	1.8	7.2	1	1	1	2
Hg	0.009	0.003	0.087	0.05	2	2	1	2

1: US EPA - ECOTOX ; 2: EC (2005); 3: Crommentuijn et al. (1997).

Table S2. Main physico-chemical characteristics of the sampling sites in the three seasonal sampling periods (S2a: spring; S2b: summer; S2c: autumn).

S2a - Spring

	Physico-chemical parameters									Nutrients								*Metals*						
Sampling site	Temp. (˚C)	pH	Conductivity (µS/cm)	Alkalinity (mg CaCO3/L)	TDS (mg/L)	TSS (mg/L)	Dissolved oxygen		DOC (mg/L)	N-NH4+ N-NH3 (mg/L)	N-NH3 (mg/L)	N-NO2 (mg/L)	N-NO3 (mg/L)	N-Inorg. Tot (mg/L)	P-PO4 (mg/L)	Total P (mg/L)	N/P	Mn (µg/L)	Fe (µg/L)	Cu (µg/L)	Zn (µg/L)	Cd (µg/L)	Pb (µg/L)	Hg (µg/L)
							mg/L	% sat																
1	9.2	8.2	3524	225	1772	1.6	10.1	89	5.60	<0.001	<0.001	<0.001	1.10	1.10	<0.003	0.005	>367	40.5	43.1	0.35	0.70	0.01	<0.73	<0.058
2	11.2	8.4	1772	261	886	3.3	10.3	95	2.90	<0.001	<0.001	<0.001	2.94	2.94	0.010	0.020	298	6.39	25.4	0.46	1.90	0.04	<0.73	<0.058
3	7.3	7.3	45.5	20.2	22.5	2.4	10.6	88	1.70	<0.001	<0.001	<0.001	0.090	0.09	<0.003	0.040	>30	6.72	110	0.56	3.32	0.01	<0.73	<0.058
4	8.6	8.2	318	53.0	159	6.7	11.0	95	2.90	<0.001	<0.001	0.001	0.25	0.25	<0.003	0.050	>83	3.00	24.5	0.39	1.62	<0.005	<0.73	<0.058
5	10.6	8.5	1319	213	660	4.7	11.2	102	2.60	<0.001	<0.001	<0.001	2.40	2.40	<0.003	0.009	>800	5.60	17.0	0.35	1.66	<0.005	<0.73	<0.058
6	10.4	8.4	1029	314	514	25.4	10.0	90	3.40	0.078	0.004	0.008	3.34	3.43	0.011	0.090	323	14.0	84.4	0.85	2.80	0.01	<0.73	<0.058
7	10.3	8.4	983	229	492	1.6	9.9	89	2.30	0.002	<0.001	<0.001	2.76	2.76	0.008	0.020	345	6.20	50.6	0.55	1.48	<0.005	<0.73	<0.058
8	13.7	7.9	1157	197	572	9.5	8.9	84	6.40	12.3	0.215	0.017	2.15	14.50	0.170	0.200	86	27.3	225	1.90	23.2	0.01	0.97	<0.058
9	13.5	8.0	605	107	303	24.2	6.8	63	5.00	5.33	0.114	0.134	1.12	6.59	0.114	0.310	58	63.7	404	4.18	25.6	0.03	1.25	<0.058
10	12.5	8.4	2163	153	1081	39.4	9.8	93	5.20	0.54	0.029	0.006	2.35	2.89	0.293	0.120	9.9	72.6	197	1.45	21.4	0.01	0.75	<0.058
11	15.3	7.7	993	127	696	5.0	5.1	50	7.80	15.3	0.200	0.226	1.65	17.16	0.127	0.310	135	118	676	4.16	37.2	0.06	3.59	<0.058
12	13.8	8.6	5290	248	2644	40.3	10.4	99	5.80	0.05	0.004	0.001	4.05	4.09	0.013	0.040	314	37.1	108	1.04	1.86	0.22	<0.73	<0.058
13	13.5	8.5	5315	243	2656	32.6	10.6	100	7.50	0.61	0.045	0.001	4.23	4.84	0.009	0.050	562	30.4	69.2	0.74	3.68	0.03	<0.73	<0.058
14	13.4	8.5	5288	247	2644	61.5	9.9	95	7.50	0.84	0.063	0.003	3.98	4.82	0.075	0.110	64	39.7	161	0.76	4.16	0.01	<0.73	<0.058
15	11.6	7.9	5173	358	2587	22.3	9.7	88	6.00	<0.001	<0.001	0.038	13.4	13.4	0.006	0.009	2089	20.2	31.1	0.60	0.93	0.01	<0.73	<0.058
16	16.3	8.1	2479	230	1239	167	7.3	73	7.90	1.42	0.054	0.016	2.96	4.40	0.652	0.002	6.7	111	331	9.48	68.3	0.11	6.05	<0.058

Table S2 (cont.)

S2b - Summer

	Physico-chemical parameters									Nutrients							*Metals*							
Sampling site	Temp. (˚C)	pH	Conductivity (µS/cm)	Alkalinity (mg CaCO3/L)	TDS (mg/L)	TSS (mg/L)	Dissolved oxygen		DOC (mg/L)	N-NH4+ N-NH3 (mg/L)	N-NH3 (mg/L)	N-NO2	N-NO3	N-Inorg. Tot (mg/L)	P-PO4	Total P (mg P/L)	N-In Tot/ P-PO4	Mn (µg/L)	Fe (µg/L)	Cu (µg/L)	Zn (µg/L)	Cd (µg/L)	Pb (µg/L)	Hg (µg/L)
							mg/L	% sat																
1	16.1	8.3	4810	137	2405	59.3	7.9	80	3.80	0.11	0.006	0.006	0.362	0.48	<0.003	0.009	>160	96.3	58.3	0.39	2.47	<0.005	<0.73	<0.058
2	16.7	8.5	2454	188	1217	82.9	8.3	85	1.80	0.25	0.021	0.007	2.06	2.31	0.004	0.033	581	11.8	62.5	0.44	5.74	0.013	<0.73	<0.058
3	17.6	7.3	89.5	39.0	44.5	2.2	8.6	89	2.60	0.03	<0.001	0.011	0.429	0.47	<0.003	0.006	>156	16.5	1188	0.49	1.49	0.011	<0.73	<0.058
4	13.8	8.5	376	58.4	193	0.2	10.2	98	2.70	0.05	0.003	0.001	0.384	0.43	<0.003	0.006	>143	6.07	55.9	0.42	1.20	0.009	<0.73	<0.058
5	18.5	8.8	1088	119	544	47.0	10.6	110	2.40	0.04	0.007	0.011	2.10	2.15	<0.003	0.024	>717	4.72	21.6	0.47	0.71	<0.005	<0.73	<0.058
6	17.4	8.7	924	257	472	264	8.2	85	1.10	0.26	0.034	0.033	4.54	4.84	0.004	0.075	1125	37.0	194	1.72	4.75	0.027	1.47	<0.058
7	19.5	9.6	1064	190	532	55.6	9.7	104	1.60	0.14	0.085	0.015	3.50	3.66	0.011	0.064	340	3.03	20.1	0.49	2.37	<0.005	<0.73	<0.058
8	24.2	7.7	1146	131	572	68.8	7.2	84	7.60	4.35	0.106	1.672	6.17	12.19	0.046	0.250	267	76.3	305	1.59	12.9	0.011	1.72	0.075
9	23.5	8.5	791	125	395	38.2	5.1	57	5.60	5.43	0.749	0.608	1.45	7.49	0.391	0.950	19	51.0	246	1.28	11.5	0.014	0.77	0.098
10	19.5	8.0	2410	198	1204	315	8.2	90	6.10	0.09	0.003	0.026	4.14	4.25	0.235	0.240	18	110	1362	7.41	56.2	0.058	6.17	<0.058
11	21.8	7.1	1143	141	587	20.9	4.6	52	9.20	6.60	0.038	0.638	3.98	11.21	0.300	0.290	37	116	585	3.27	24.6	0.026	2.32	0.144
12	19.8	8.3	5114	221	2553	224	8.0	88	4.20	0.14	0.011	0.040	4.54	4.72	0.007	0.062	658	79.6	486	1.23	8.01	0.022	2.45	<0.058
13	21.3	8.6	5097	261	2542	365	8.5	95	6.10	1.24	0.174	0.088	4.43	5.76	0.130	0.110	44	118	500	1.73	7.55	0.028	3.42	<0.058
14	22.6	8.8	5057	264	2528	216	2.2	25	6.80	2.79	0.614	0.264	4.00	7.06	0.251	0.230	28	112	618	3.09	13.8	0.031	3.78	<0.058
15	16.7	7.6	4226	251	2128	231	7.9	82	7.90	0.13	0.001	0.030	6.83	6.99	0.007	0.010	1072	163	282	1.37	5.32	0.026	3.29	0.06
16	22.5	9.0	3238	281	1619	198	7.6	88	6.40	2.25	0.678	0.578	5.94	8.77	0.619	0.600	14	101	330	5.41	22.0	0.065	4.49	0.089

Table S2 (cont.)

S2c - Autumn

	Physico-chemical parameters									Nutrients							*Metals*							
Sampling site	Temp. (˚C)	pH	Conductivity (µS/cm)	Alkalinity (mg CaCO3/L)	TDS (mg/L)	TSS (mg/L)	Dissolved oxygen		DOC (mg/L)	N-NH4+ N-NH3 (mg/L)	N-NH3 (mg/L)	N-NO2	N-NO3	N-Inorg. Tot (mg/L)	P-PO4	Total P (mg P/L)	N-In Tot/ P-PO4	Mn (µg/L)	Fe (µg/L)	Cu (µg/L)	Zn (µg/L)	Cd (µg/L)	Pb (µg/L)	Hg (µg/L)
							mg/L	% sat																
1	9.5	8.0	4811.5	193	2405	63.9	8.1	70	5.72	0.002	<0.001	0.001	0.463	0.47	<0.003	0.003	>154	801	463	0.74	<4.7	0.016	<0.73	<0.058
2	10.0	7.5	1923.0	276	961.5	14.8	7.9	69	1.83	<0.001	<0.001	0.005	2.55	2.56	0.008	0.017	305	8.25	46.0	0.47	<4.7	0.01	<0.73	<0.058
3	7.4	6.2	75.5	21.1	35	1.25	9.0	75	2.95	<0.001	<0.001	0.001	0.362	0.36	<0.003	<0.003	>120	22.5	196	0.84	3.67	0.008	<0.73	<0.058
4	11.4	7.2	365.0	70.1	182.5	0.4	8.4	75	1.78	<0.001	<0.001	<0.001	0.285	0.28	<0.003	<0.003	>93	0.41	18.3	0.29	<4.7	<0,005	<0.73	<0.058
5	9.2	7.8	1131.0	196	565.5	1.21	9.6	83	1.97	0.004	<0.001	0.005	2.53	2.54	<0.003	0.006	>847	4.61	25.8	0.47	5.43	0.011	<0.73	<0.058
6	8.4	8.0	959.0	305	474.5	39.3	9.2	78	3.21	0.099	0.002	0.020	3.96	4.07	0.026	0.039	157	17.6	176	1.44	4.90	0.013	<0.73	<0.058
7	9.3	8.1	902.0	237	451	2.03	9.1	79	2.42	0.019	<0.001	0.020	3.21	3.25	0.014	0.032	238	2.32	24.1	0.47	<4.7	<0,005	<0.73	<0.058
8	12.6	7.1	1021.5	212	515.5	6.74	6.9	65	5.61	5.93	0.015	0.164	4.05	10.14	0.134	0.188	75	22.2	547	3.33	29.4	0.019	1.268	<0.058
9	10.8	6.7	527.5	119	263.5	25.6	6.7	59	4.78	4.77	0.005	0.108	1.78	6.66	0.259	0.240	26	56.3	443	5.53	30.1	0.068	2.603	<0.058
10	10.6	8.1	2092.0	174	1046	13	8.9	78	4.40	0.054	0.001	0.048	4.23	4.33	0.408	0.258	11	34.8	286	1.69	17.7	0.015	<0.73	<0.058
11	15.1	6.8	955.0	116	477.5	47.5	5.5	53	7.70	7.53	0.012	0.250	4.20	11.99	0.271	0.210	44	108	1074	5.76	43.1	0.1	5.751	<0.058
12	7.7	7.9	5000.5	254	2501.5	27	10.2	86	5.17	0.013	<0.001	0.009	6.49	6.51	0.044	0.017	148	19.2	138.9	0.69	12.7	0.021	<0.73	<0.058
13	8.1	7.7	4981.5	256	2542	37.5	10.0	85	5.70	0.633	0.005	0.015	6.64	7.29	0.072	0.105	101	18.3	110	0.81	4.85	0.022	<0.73	<0.058
14	8.4	7.8	4965.5	260	2483.5	60	7.9	68	6.23	1.47	0.017	0.089	6.12	7.69	0.112	0.148	68	27.6	277	1.72	11.7	0.025	1.381	<0.058
15	11.0	7.0	4973.0	380	2483.5	42.1	7.7	69	3.42	0.009	<0.001	0.051	1.74	1.80	<0.003	<0.003	>600	30.0	70.3	0.74	7.45	0.017	<0.73	<0.058
16	11.4	7.8	2542.5	262	1271	74.7	8.3	75	7.08	3.46	0.049	0.568	7.86	11.89	0.965	0.637	12	91.4	435	14.5	73.7	0.083	4.02	<0.058

Table S3. Toxic Units (TUs) for different taxa of aquatic organisms referred to individual metals and mixtures.

Spring	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Toxic Units (C/EC50) Algae															
Mn	4.E-03	6.E-04	6.E-04	3.E-04	5.E-04	1.E-03	5.E-04	2.E-03	0.0055	0.0063	0.0103	3.E-03	3.E-03	3.E-03	2.E-03	0.01
Fe																
Cu	4.E-03	0.0059	0.0072	0.005	4.E-03	0.0109	0.0071	0.0244	0.0536	0.0186	0.0533	0.0133	0.0095	0.0097	0.0077	0.122
Zn	0.0091	0.0247	0.0431	0.021	0.0216	0.0364	0.0192	0.3013	0.3325	0.2779	0.4831	0.0242	0.0478	0.054	0.0121	0.887
Cd	5E-04	2E-03	5E-04	<1E-4	<1E-4	0.0005	<1E-4	5E-04	1E-03	5E-04	3E-03	0.0105	1E-03	5E-04	5E-04	0.005
Pb	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	0.0043	0.0056	0.0034	0.0161	<0.002	<0.002	<0.002	<0.002	0.027
Hg	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
Total metals	0.0224	0.0379	0.0562	0.0313	0.0315	0.0538	0.0318	0.3361	0.4019	0.3099	0.5689	0.0561	0.0662	0.0726	0.0269	1.054
Summer	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Toxic Units (C/EC50) Algae															
Mn	0.0084	0.001	0.0014	0.0005	0.0004	0.0032	0.0003	0.0066	0.0044	0.0096	0.0101	0.0069	0.0103	0.0097	0.0142	0.009
Fe																
Cu	0.005	0.0056	0.0063	0.0054	0.006	0.0221	0.0063	0.0204	0.0164	0.095	0.0419	0.0158	0.0222	0.0396	0.0176	0.069
Zn	0.0321	0.0745	0.0194	0.0156	0.0092	0.0617	0.0308	0.1675	0.1494	0.7299	0.3195	0.104	0.0981	0.1792	0.0691	0.286
Cd	<1E-4	0.0006	0.0005	0.0004	<1E-4	0.0013	<1E-4	0.0005	0.0007	0.0028	0.0012	0.001	0.0013	0.0015	0.0012	0.003
Pb	<0.002	<0.002	<0.002	<0.002	<0.002	0.0066	<0.002	0.0077	0.0035	0.0277	0.0104	0.011	0.0153	0.017	0.0148	0.02
Hg	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	0.0109	0.0032	0.016	<0.003	<0.003	<0.003	0.0067	0.01
Total metals	0.0504	0.0867	0.0325	0.0268	0.0206	0.0981	0.0423	0.2111	0.1852	0.8681	0.3991	0.142	0.1504	0.2502	0.1235	0.397
Autumn	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Toxic Units (C/EC50) Algae															
Mn	0.0697	0.0007	0.002	4E-05	0.0004	0.0015	0.0002	0.0019	0.0049	0.003	0.0094	0.0017	0.0016	0.0024	0.0026	0.008
Fe																
Cu	0.0095	0.006	0.0107	0.0037	0.006	0.0184	0.006	0.0427	0.0709	0.0216	0.0739	0.0088	0.0103	0.022	0.0095	0.186
Zn	<0.03	<0.03	0.0476	<0.03	0.0705	0.0636	<0.03	0.3812	0.391	0.2304	0.5592	0.1648	0.0629	0.1517	0.0968	0.957
Cd	0.0008	0.0005	0.0004	<1E-4	0.0005	0.0006	<1E-4	0.0009	0.0032	0.0007	0.0048	0.001	0.001	0.0012	0.0008	0.004
Pb	<0.002	<0.002	<0.002	<0.002	<0.002	0.0035	<0.002	0.0057	0.0117	0.0046	0.0258	<0.002	<0.002	0.0062	<0.002	0.018
Hg	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
Total metals	0.1154	0.0425	0.0656	0.0393	0.0823	0.0908	0.0417	0.4356	0.485	0.2636	0.6763	0.1812	0.0808	0.1867	0.1145	1.177

Table S3 (cont.)

Spring	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Toxic Units (C/EC50) Daphnia															
Mn	1E-03	2E-04	2E-04	1E-04	2E-04	5E-04	2E-04	1E-03	2E-03	3E-03	4E-03	1E-03	1E-03	1E-03	7E-04	4E-03
Fe	0.006	4E-03	0.0153	3E-03	2E-03	0.0117	0.007	0.0313	0.0561	0.0274	0.0939	0.015	0.0096	0.0224	4E-03	0.046
Cu	0.025	0.0329	0.04	0.0279	0.025	0.0607	0.0393	0.1357	0.2986	0.1036	0.2971	0.0743	0.0529	0.0543	0.0429	0.6771
Zn	1E-03	3E-03	0.0061	3E-03	3E-03	0.0051	3E-03	0.0425	0.0469	0.0392	0.0681	3E-03	0.0067	0.0076	2E-03	0.1251
Cd	3E-04	1E-03	3E-04	<8E-5	<8E-5	0.0003	<8E-5	3E-04	9E-04	3E-04	2E-03	0.0067	9E-04	3E-04	3E-04	3E-03
Pb	4E-04	4E-04	4E-04	4E-04	4E-04	4E-04	4E-04	1E-03	1E-03	7E-04	4E-03	4E-04	4E-04	4E-04	4E-04	6E-03
Hg	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Total metals	0.044	0.0513	0.0719	0.0444	0.0407	0.0884	0.0594	0.2214	0.4157	0.1834	0.4784	0.1107	0.0812	0.096	0.0599	0.8712
Summer	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Toxic Units (C/EC50) Daphnia															
Mn	3E-03	4E-04	6E-04	2E-04	2E-04	1E-03	1E-04	3E-03	2E-03	4E-03	4E-03	3E-03	4E-03	4E-03	6E-03	4E-03
Fe	0.0081	0.0087	0.165	0.0078	3E-03	0.0269	3E-03	0.0424	0.0342	0.1892	0.0813	0.0675	0.0694	0.0858	0.0392	0.0458
Cu	0.0279	0.0314	0.035	0.03	0.0336	0.1229	0.035	0.1136	0.0914	0.5293	0.2336	0.0879	0.1236	0.2207	0.0979	0.3864
Zn	0.0045	0.0105	0.0027	0.0022	0.0013	0.0087	0.0043	0.0236	0.0211	0.1029	0.0451	0.0147	0.0138	0.0253	0.0097	0.0403
Cd	<8E-5	4E-04	3E-04	3E-04	<8E-5	8E-04	<8E-5	3E-04	4E-04	2E-03	8E-04	7E-04	8E-04	9E-04	8E-04	2E-03
Pb	4E-04	4E-04	4E-04	4E-04	4E-04	1E-03	4E-04	2E-03	8E-04	0.0062	2E-03	2E-03	3E-03	4E-03	3E-03	4E-03
Hg	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.025	0.0327	<0.001	0.048	<0.001	<0.001	<0.001	0.02	0.0297
Total metals	0.054	0.0615	0.2137	0.0505	0.0481	0.1718	0.0523	0.2093	0.1823	0.8429	0.4151	0.1856	0.225	0.3502	0.1767	0.5123
Autumn	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Toxic Units (C/EC50) Daphnia															
Mn	0.0286	0.0003	0.0008	1E-05	0.0002	0.0006	8E-05	0.0008	0.002	0.0012	0.0039	0.0007	0.0007	0.001	0.0011	0.0033
Fe	0.0643	0.0064	0.0273	0.0025	0.0036	0.0245	0.0033	0.0759	0.0615	0.0397	0.1492	0.0193	0.0153	0.0385	0.0098	0.0604
Cu	0.0529	0.0332	0.0599	0.0208	0.0334	0.1026	0.0336	0.2377	0.395	0.1206	0.4116	0.0492	0.0576	0.1227	0.0529	1.0379
Zn	<0.004	<0.004	0.0067	<0.004	0.0099	0.009	<0.004	0.0538	0.0551	0.0325	0.0789	0.0232	0.0089	0.0214	0.0136	0.135
Cd	0.0005	0.0003	0.0002	<8E-5	0.0003	0.0004	<8E-5	0.0006	0.0021	0.0005	0.003	0.0006	0.0007	0.0008	0.0005	0.0025
Pb	0.0004	0.0004	0.0004	0.0004	0.0004	0.0008	0.0004	0.0013	0.0026	0.001	0.0057	0.0004	0.0004	0.0014	0.0004	0.004
Hg	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Total metals	0.1606	0.0545	0.1049	0.0377	0.0575	0.1475	0.0515	0.3797	0.528	0.2051	0.662	0.1031	0.0932	0.1954	0.0879	1.2527

Table S3 (cont.)

Spring	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Toxic Units (C/LC50) Fish															
Mn	0.0027	0.0004	0.0004	0.0002	0.0004	0.0009	0.0004	0.0018	0.0042	0.0048	0.0078	0.0024	0.002	0.0026	0.0013	0.0073
Fe	0.0006	0.0004	0.0015	0.0003	0.0002	0.0012	0.0007	0.0032	0.0057	0.0028	0.0095	0.0015	0.001	0.0023	0.0004	0.0047
Cu	0.0049	0.0065	0.0079	0.0055	0.0049	0.012	0.0077	0.0268	0.0589	0.0204	0.0586	0.0146	0.0104	0.0107	0.0085	0.1335
Zn	0.0017	0.0047	0.0082	0.004	0.0041	0.0069	0.0036	0.0571	0.0631	0.0527	0.0916	0.0046	0.0091	0.0102	0.0023	0.1682
Cd	3E-05	0.0001	3E-05	<7E-6	<7E-6	3E-05	<7E-6	3E-05	9E-05	3E-05	0.0002	0.0007	9E-05	3E-05	3E-05	0.0003
Pb	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0005	0.0007	0.0004	0.002	0.0002	0.0002	0.0002	0.0002	0.0033
Hg	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4
Total metals	0.0105	0.0126	0.0186	0.0106	0.0102	0.0215	0.0131	0.0898	0.1329	0.0815	0.17	0.0244	0.0231	0.0264	0.0131	0.3177
Summer	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Toxic Units (C/LC50) Fish															
Mn	0.0063	8.E-04	1.E-03	4.E-04	3.E-04	2.E-03	2.E-04	0.005	3.E-03	0.0072	0.0076	0.0052	0.0078	0.0074	0.0107	0.0066
Fe	8.E-04	9.E-04	0.0167	8.E-04	3.E-04	3.E-03	3.E-04	4.E-03	3.E-03	0.0192	0.0082	0.0068	0.007	0.0087	4.E-03	5.E-03
Cu	0.0055	0.0062	0.0069	0.0059	0.0066	0.0242	0.0069	0.0224	0.018	0.1044	0.0461	0.0173	0.0244	0.0435	0.0193	0.0762
Zn	0.0061	0.0141	4.E-03	3.E-03	2.E-03	0.0117	0.0058	0.0318	0.0283	0.1384	0.0606	0.0197	0.0186	0.034	0.0131	0.0542
Cd	<7E-6	4.E-05	3.E-05	3.E-05	<7E-6	8E-05	<7E-6	3.E-05	4.E-05	2.E-04	8.E-05	7.E-05	8.E-05	9.E-05	8.E-05	2.E-04
Pb	2.E-04	2.E-04	2.E-04	2.E-04	2.E-04	8.E-04	2.E-04	1.E-03	4.E-04	3.E-03	1.E-03	1.E-03	2.E-03	2.E-03	2.E-03	2.E-03
Hg	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	9.E-04	1.E-03	<3E-4	0.0017	<3E-4	<3E-4	<3E-4	0.0007	0.001
Total metals	0.0193	0.0226	0.029	0.0106	0.0095	0.0423	0.0138	0.0653	0.0548	0.2731	0.1255	0.0509	0.0601	0.0961	0.0497	0.1454
Autumn	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Toxic Units (C/LC50) Fish															
Mn	0.0528	5.E-04	1.E-03	3.E-05	3.E-04	1.E-03	2.E-04	1.E-03	4.E-03	2.E-03	0.0071	1.E-03	1.E-03	2.E-03	2.E-03	0.006
Fe	0.0065	0.0006	0.0028	0.0003	0.0004	0.0025	0.0003	0.0077	0.0062	0.004	0.0151	2.E-03	2.E-03	4.E-03	1.E-03	0.0061
Cu	0.0104	0.0065	0.0118	0.0041	0.0066	0.0202	0.0066	0.0469	0.0779	0.0238	0.0812	0.0097	0.0114	0.0242	0.0104	0.2046
Zn	<0.006	<0.006	0.009	<0.006	0.0134	0.0121	<0.006	0.0723	0.0742	0.0437	0.1061	0.0313	0.0119	0.0288	0.0184	0.1816
Cd	5.E-05	3.E-05	2.E-05	<7E-6	3.E-05	4.E-05	<7E-6	6.E-05	2.E-04	4.E-05	3.E-04	6.E-05	7.E-05	7.E-05	5.E-05	2.E-04
Pb	2.E-04	2.E-04	2.E-04	2.E-04	2.E-04	4.E-04	2.E-04	7.E-04	1.E-03	6.E-04	3.E-03	2.E-04	2.E-04	8.E-04	2.E-04	2.E-03
Hg	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4	<3E-4
Total metals	0.0761	0.0141	0.0256	0.0107	0.0212	0.0367	0.0135	0.1294	0.164	0.0747	0.2133	0.0448	0.0267	0.0599	0.0323	0.4011

Table S4. Toxic Units (TUs) for individual pesticides and mixtures

Spring	Toxic Units (C/EC50) Algae															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Carbofuran	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorpyrifos ethyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diazinon	0	0	0	0	0	0	0	1.26E-08	5.1E-09	1.06E-08	1.45E-08	0	3.24E-09	3.23E-09	0	1.55E-08
Dimethoate	0	2.81E-09	9.37E-09	0	4.09E-09	0	3.14E-09	1.27E-08	2.69E-08	2.18E-08	2.12E-07	4.9E-10	1.36E-09	6.42E-10	0	4.22E-08
Imidacloprid	0	4.99E-09	2.82E-10	0	2.13E-09	5.26E-08	1.47E-08	7.77E-08	1.11E-07	3.12E-07	3.18E-07	5.18E-09	1.19E-08	7.12E-08	9.7E-10	2.5E-07
Malathion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Metolcarb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pirimicarb	0	0	0	0	0	0	0	1.35E-10	1.89E-10	1.34E-10	1.88E-09	0	0	0	0	2.81E-10
Spinosin-A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Insecticides	0	7.8E-09	9.65E-09	0	6.21E-09	5.26E-08	1.78E-08	1.03E-07	1.43E-07	3.45E-07	5.46E-07	5.67E-09	1.65E-08	7.5E-08	9.7E-10	3.08E-07
Chlorturon	5.45E-06	3.11E-06	0	0	5.31E-06	0	0	0.000388	0.000115	7.54E-06	4.29E-05	0	0	0	0	0.000518
Diuron	0	2.23E-05	0	0	1.99E-05	0	5.23E-05	0.002683	0.003971	0.004808	0.009473	0	0.000363	0	0	0.002765
Metribuzine	0	1.4E-06	0	0	4.22E-05	0.000383	0	2.18E-05	0	1.11E-05	1.28E-05	0	0	0	0	1.36E-05
Simazine	0	2.54E-06	1.56E-06	0	2.48E-06	0	3.69E-06	6.01E-05	4.05E-06	0.000147	7.65E-05	0.004579	0.004149	0.003085	9.24E-06	8.01E-05
Terbuthrin	0	9.51E-06	0	8.19E-06	1.08E-05	0.000331	2.69E-05	0.000961	0.000973	0.000954	0.003118	1.14E-05	2.05E-05	0.00024	1.46E-05	0.000735
Terbuthylazine	0	1.86E-05	0	0	5.42E-05	0	1.07E-05	0.000155	4.01E-05	3.96E-05	0.000196	1.71E-05	1.91E-05	2.48E-05	0.033172	0.000558
Total Herbicides	5.45E-06	5.75E-05	1.56E-06	8.19E-06	0.000135	0.000714	9.36E-05	0.004269	0.005103	0.005967	0.012919	0.004608	0.004552	0.00335	0.033196	0.00467
Carbedazim	0	1.33E-07	0	0	1.25E-07	2.26E-06	5.6E-07	1.57E-05	1.7E-05	1.65E-05	5.15E-05	3.48E-07	1.33E-06	1.25E-06	0	1.41E-05
Kresoxim methyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propiconazole	0	0	0	0	0	0	0	0.000666	0.000209	0.000291	0.000507	0	0	0	0	0.000196
Spiroxaminie	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tebuconazole	7.24E-08	5.68E-08	0	0	8.59E-08	8.92E-08	8.74E-08	1.34E-06	1.04E-06	7.15E-05	1.18E-06	1.58E-06	1.19E-06	1.42E-06	7.86E-08	7.2E-07
Total Fungicides	7.24E-08	1.9E-07	0	0	2.11E-07	2.35E-06	6.48E-07	0.000683	0.000227	0.000379	0.00056	1.93E-06	2.52E-06	2.67E-06	7.86E-08	0.000211
TOTAL PESTICIDES	5.52E-06	5.77E-05	1.57E-06	8.19E-06	0.000135	0.000717	9.43E-05	0.004952	0.00533	0.006346	0.013479	0.00461	0.004554	0.003353	0.033196	0.004881

Table S4 (cont.)

Summer	Toxic Units (C/EC50) Algae															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Carbofuran	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorpyrifos ethyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diazinon	0	0	0	0	0	1.98E-09	0	3.52E-08	6.33E-09	2.89E-08	1.07E-08	0	2.37E-09	3.41E-09	0	4.73E-09
Dimethoate	0	5.9E-09	0	0	8.95E-10	2.04E-09	3.46E-10	1.51E-08	1.38E-07	1.8E-08	4.8E-08	4.43E-10	1.76E-09	2.37E-09	0	4.78E-08
Imidacloprid	4.36E-09	1.04E-08	3.51E-08	2.15E-08	1.81E-08	2.4E-07	2.53E-08	6.73E-07	3.36E-07	9.7E-07	8.21E-07	1.66E-08	7.82E-08	9.89E-08	2.2E-08	2.67E-07
Malathion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Metolcarb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pirimicarb	0	0	0	0	0	0	0	4.81E-10	5.99E-10	4.17E-10	2.47E-09	0	0	0	0	8.33E-10
Spinosin-A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Insecticides	4.36E-09	1.63E-08	3.51E-08	2.15E-08	1.9E-08	2.44E-07	2.56E-08	7.24E-07	4.8E-07	1.02E-06	8.82E-07	1.71E-08	8.23E-08	1.05E-07	2.2E-08	3.2E-07
Chlorturon	0	0	0	0	9.35E-06	0	0	5.11E-05	2.02E-05	0	0.0003	0	5.69E-06	0	0	8.33E-05
Diuron	0	3.3E-05	0	0	0	0	0.000191	0.005445	0.004067	0.015574	0.008797	0	5.95E-05	0	0	0.003276
Metribuzine	0	0	0	0	0	8.31E-05	3.81E-06	1.79E-05	1.39E-05	2.01E-05	1.99E-05	0	5.08E-06	0	0	0
Simazine	6.65E-06	4.68E-06	2.41E-05	3.46E-06	5.95E-06	4.79E-06	6.71E-06	0.000276	0.000111	5.57E-05	7.64E-05	9.88E-05	9E-05	3.46E-05	0.000388	0.00013
Terbuthrin	9.22E-06	1.38E-05	0	0	8.91E-06	3.23E-05	8.92E-05	0.005676	0.002074	0.003035	0.003301	3.49E-05	6.25E-05	8.83E-05	0.000119	0.000817
Terbuthylazine	1.41E-05	1E-05	4.55E-06	0	0.000132	2.45E-06	1.09E-05	0.000975	1.99E-05	1.16E-05	1.65E-05	2E-05	1.91E-05	1.92E-05	0.000135	0.001005
Total Herbicides	2.99E-05	6.15E-05	2.86E-05	3.46E-06	0.000157	0.000123	0.000301	0.01244	0.006306	0.018696	0.01251	0.000154	0.000242	0.000142	0.000642	0.005311
Carbedazim	3.41E-07	3.07E-07	7.61E-07	1.52E-07	1.72E-06	2.55E-06	1.02E-06	8.86E-05	3.05E-05	4.71E-05	9.05E-05	4E-06	6.68E-06	2.11E-06	3.09E-06	2.08E-05
Kresoxim methyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propiconazole	0	0	0	0	0	0	0	0.000255	0.000125	0.000262	0.000226	0	0	1.82E-05	0	6.71E-05
Spiroxaminie	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tebuconazole	1.24E-07	9.43E-08	0	0	6.61E-08	3.37E-07	1.56E-07	2.36E-06	4.75E-07	0.00016	1.05E-06	5.95E-07	7.49E-07	5.95E-07	6.57E-07	1.22E-06
Total Fungicides	4.65E-07	4.02E-07	7.61E-07	1.52E-07	1.79E-06	2.89E-06	1.17E-06	0.000346	0.000156	0.000469	0.000317	4.59E-06	7.42E-06	2.09E-05	3.75E-06	8.92E-05
TOTAL PESTICIDES	3.04E-05	6.19E-05	2.94E-05	3.63E-06	0.000158	0.000126	0.000302	0.012786	0.006462	0.019166	0.012828	0.000158	0.000249	0.000163	0.000646	0.0054

Table S4 (cont.)

Autumn	Toxic Units (C/EC50) Algae															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Carbofuran	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorpyrifos ethyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diazinon	0	0	0	0	0	0	2.49E-09	2.11E-07	6.61E-09	4.36E-08	2.07E-08	0	0	0	0	1.25E-08
Dimethoate	0	0	0	0	0	0	0	1.35E-09	1.04E-08	9.45E-10	8.4E-08	0	0	1.6E-08	0	5.32E-08
Imidacloprid	0	3.98E-09	3.29E-09	5.51E-10	3.62E-09	2.83E-08	9.02E-09	1.57E-07	1.08E-07	1.52E-07	2.65E-07	3.37E-09	9.28E-09	1.58E-08	0	1.2E-07
Malathion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Metolcarb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pirimicarb	0	0	0	0	0	0	0	2.37E-10	1.94E-10	0	6.33E-10	0	0	0	0	4.28E-10
Spinosin-A	0	0	0	0	0	0	0	6.97E-08	0	0	0	0	0	0	0	0
Total Insecticides	0	3.98E-09	3.29E-09	5.51E-10	3.62E-09	2.83E-08	1.15E-08	4.39E-07	1.25E-07	1.96E-07	3.7E-07	3.37E-09	9.28E-09	3.19E-08	0	1.86E-07
Chlorturon	8.63E-06	0	2.42E-05	0	1.25E-05	2.15E-05	1.04E-05	0.000207	9.34E-05	4.99E-05	0.000322	0	0	0	0	0.000625
Diuron	0	0	0	0	0.000114	0.000199	0.000282	0.006582	0.004333	0.008068	0.012086	0	0	0	0	0.01027
Metribuzine	0	0	0	0	3.45E-06	1.32E-05	1.72E-06	4.43E-06	4.86E-06	6.96E-06	1.1E-05	0	0	0	0	9.41E-06
Simazine	4.44E-06	2.04E-05	9.99E-06	7.5E-06	3.25E-05	2.25E-05	0.000336	1.29E-05	1.93E-05	1E-05	0.000102	7.15E-05	4.98E-05	3.72E-05	1.95E-05	0.000206
Terbuthrin	0	1.53E-05	0	0	3.09E-05	0.000107	4E-05	0.002657	0.001764	0.00146	0.003205	1.67E-05	2.05E-05	4.18E-05	1.84E-05	0.001433
Terbuthylazine	1.1E-05	7.83E-06	3.08E-05	5.75E-06	7.44E-05	2.46E-05	8.79E-06	0.000105	3.27E-05	8.62E-06	0.000117	7E-06	7.56E-06	7.43E-06	0.000322	0.000422
Total Herbicides	2.41E-05	4.36E-05	6.5E-05	1.32E-05	0.000268	0.000387	0.000679	0.009568	0.006247	0.009604	0.015843	9.52E-05	7.78E-05	8.65E-05	0.00036	0.012964
Carbedazim	4.43E-07	1.11E-06	2.46E-07	6.82E-07	1.56E-06	1.36E-06	1.31E-06	2.95E-05	1.67E-05	1.7E-05	5.3E-05	4.28E-07	4.32E-07	4.21E-07	8.22E-08	2.86E-05
Kresoxim methyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propiconazole	0	0	0	0	0	1.78E-05	0	0.001089	0.000248	0.000319	0.000621	0	0	0	0	0.000203
Spiroxaminie	0	0	0	0	0	0	0	0.000626	0	0	0	0	0	0	0	0
Tebuconazole	2.21E-07	1.5E-07	0	0	1.3E-06	4.97E-07	2.48E-07	1.33E-06	1E-06	1.89E-05	1.36E-06	2.4E-07	3.21E-07	3.62E-07	0	1.59E-06
Total Fungicides	6.64E-07	1.26E-06	2.46E-07	6.82E-07	2.86E-06	1.97E-05	1.56E-06	0.001746	0.000266	0.000355	0.000675	6.68E-07	7.53E-07	7.83E-07	8.22E-08	0.000233
TOTAL PESTICIDES	2.48E-05	4.48E-05	6.53E-05	1.39E-05	0.000271	0.000407	0.000681	0.011315	0.006513	0.009959	0.016518	9.59E-05	7.86E-05	8.73E-05	0.00036	0.013198

Table S4 (cont.)

Spring	Toxic Units (C/EC50) Daphnia															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Carbofuran	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorpyrifos ethyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diazinon	0	0	0	0	0	0	0	0.000126	5.1E-05	0.000106	0.000145	0	3.24E-05	3.23E-05	0	0.000155
Dimethoate	0	1.41E-06	4.69E-06	0	2.04E-06	0	1.57E-06	6.33E-06	1.35E-05	1.09E-05	0.000106	2.45E-07	6.79E-07	3.21E-07	0	2.11E-05
Imidacloprid	0	2.93E-08	1.66E-09	0	1.25E-08	3.09E-07	8.64E-08	4.57E-07	6.52E-07	1.84E-06	1.87E-06	3.05E-08	7.01E-08	4.19E-07	5.71E-09	1.47E-06
Malathion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Metolcarb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pirimicarb	0	0	0	0	0	0	0	0.000189	0.000265	0.000187	0.002634	0	0	0	0	0.000393
Spinosin-A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Insecticides	0	1.43E-06	4.69E-06	0	2.06E-06	3.09E-07	1.66E-06	0.000322	0.00033	0.000306	0.002887	2.75E-07	3.32E-05	3.3E-05	5.71E-09	0.00057
Chlorturon	2.6E-09	1.49E-09	0	0	2.54E-09	0	0	1.85E-07	5.51E-08	3.6E-09	2.05E-08	0	0	0	0	2.48E-07
Diuron	0	8.69E-09	0	0	7.74E-09	0	2.03E-08	1.04E-06	1.54E-06	1.87E-06	3.68E-06	0	1.41E-07	0	0	1.08E-06
Metribuzine	0	1.25E-08	0	0	3.75E-07	3.4E-06	0	1.94E-07	0	9.82E-08	1.14E-07	0	0	0	0	1.21E-07
Simazine	0	1.45E-07	8.88E-08	0	1.41E-07	0	2.1E-07	3.43E-06	2.31E-07	8.38E-06	4.36E-06	0.000261	0.000237	0.000176	5.27E-07	4.56E-06
Terbuthrin	0	2.82E-08	0	2.43E-08	3.19E-08	9.82E-07	7.98E-08	2.85E-06	2.88E-06	2.83E-06	9.24E-06	3.39E-08	6.08E-08	7.12E-07	4.31E-08	2.18E-06
Terbuthylazine	0	1.42E-08	0	0	4.13E-08	0	8.17E-09	1.18E-07	3.06E-08	3.02E-08	1.5E-07	1.31E-08	1.46E-08	1.89E-08	2.53E-05	4.25E-07
Total Herbicides	2.6E-09	2.1E-07	8.88E-08	2.43E-08	5.99E-07	4.38E-06	3.19E-07	7.81E-06	4.74E-06	1.32E-05	1.76E-05	0.000261	0.000237	0.000177	2.58E-05	8.61E-06
Carbedazim	0	1.92E-06	0	0	1.81E-06	3.27E-05	8.1E-06	0.000227	0.000246	0.000238	0.000744	5.03E-06	1.92E-05	1.8E-05	0	0.000203
Kresoxim methyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propiconazole	0	0	0	0	0	0	0	1.53E-06	4.8E-07	6.69E-07	1.17E-06	0	0	0	0	4.5E-07
Spiroxaminie	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tebuconazole	1.84E-08	1.45E-08	0	0	2.19E-08	2.27E-08	2.22E-08	3.42E-07	2.63E-07	1.82E-05	3.01E-07	4.03E-07	3.03E-07	3.61E-07	2E-08	1.83E-07
Total Fungicides	1.84E-08	1.94E-06	0	0	1.83E-06	3.27E-05	8.12E-06	0.000228	0.000247	0.000257	0.000746	5.43E-06	1.95E-05	1.84E-05	2E-08	0.000204
TOTALPESTICIDES	2.1E-08	3.58E-06	4.78E-06	2.43E-08	4.49E-06	3.74E-05	1.01E-05	0.000558	0.000582	0.000577	0.00365	0.000267	0.000289	0.000228	2.59E-05	0.000783

Table S4 (cont.)

Summer	Toxic Units (C/EC50) Daphnia															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Carbofuran	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorpyrifos ethyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diazinon	0	0	0	0	0	1.98E-05	0	0.000352	6.33E-05	0.000289	0.000107	0	2.37E-05	3.41E-05	0	4.73E-05
Dimethoate	0	2.95E-06	0	0	4.47E-07	1.02E-06	1.73E-07	7.57E-06	6.89E-05	9.01E-06	2.4E-05	2.21E-07	8.82E-07	1.19E-06	0	2.39E-05
Imidacloprid	2.57E-08	6.15E-08	2.07E-07	1.26E-07	1.06E-07	1.41E-06	1.49E-07	3.96E-06	1.97E-06	5.71E-06	4.83E-06	9.78E-08	4.6E-07	5.82E-07	1.3E-07	1.57E-06
Malathion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Metolcarb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pirimicarb	0	0	0	0	0	0	0	0.000673	0.000838	0.000584	0.003455	0	0	0	0	0.001166
Spinosin-A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Insecticides	2.57E-08	3.01E-06	2.07E-07	1.26E-07	5.54E-07	2.22E-05	3.22E-07	0.001036	0.000972	0.000888	0.003591	3.19E-07	2.51E-05	3.58E-05	1.3E-07	0.001239
Chlorturon	0	0	0	0	4.47E-09	0	0	2.44E-08	9.65E-09	0	1.43E-07	0	2.72E-09	0	0	3.98E-08
Diuron	0	1.28E-08	0	0	0	0	7.41E-08	2.12E-06	1.58E-06	6.06E-06	3.42E-06	0	2.31E-08	0	0	1.27E-06
Metribuzine	0	0	0	0	0	7.39E-07	3.39E-08	1.59E-07	1.24E-07	1.78E-07	1.77E-07	0	4.51E-08	0	0	0
Simazine	3.79E-07	2.67E-07	1.37E-06	1.97E-07	3.39E-07	2.73E-07	3.82E-07	1.57E-05	6.33E-06	3.17E-06	4.35E-06	5.63E-06	5.13E-06	1.97E-06	2.21E-05	7.42E-06
Terbuthrin	2.73E-08	4.09E-08	0	0	2.64E-08	9.58E-08	2.64E-07	1.68E-05	6.14E-06	8.99E-06	9.78E-06	1.03E-07	1.85E-07	2.62E-07	3.53E-07	2.42E-06
Terbuthylazine	1.07E-08	7.66E-09	3.47E-09	0	1.01E-07	1.87E-09	8.27E-09	7.43E-07	1.51E-08	8.87E-09	1.26E-08	1.53E-08	1.45E-08	1.47E-08	1.03E-07	7.66E-07
Total Herbicides	4.17E-07	3.28E-07	1.38E-06	1.97E-07	4.71E-07	1.11E-06	7.63E-07	3.56E-05	1.42E-05	1.84E-05	1.79E-05	5.75E-06	5.4E-06	2.25E-06	2.26E-05	1.19E-05
Carbedazim	4.92E-06	4.44E-06	1.1E-05	2.2E-06	2.49E-05	3.68E-05	1.47E-05	0.001279	0.00044	0.00068	0.001308	5.78E-05	9.64E-05	3.05E-05	4.47E-05	0.000301
Kresoxim methyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propiconazole	0	0	0	0	0	0	0	5.86E-07	2.87E-07	6.02E-07	5.19E-07	0	0	4.17E-08	0	1.54E-07
Spiroxaminie	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tebuconazole	3.16E-08	2.4E-08	0	0	1.68E-08	8.57E-08	3.96E-08	6E-07	1.21E-07	4.06E-05	2.69E-07	1.52E-07	1.91E-07	1.52E-07	1.67E-07	3.1E-07
Total Fungicides	4.96E-06	4.46E-06	1.1E-05	2.2E-06	2.49E-05	3.69E-05	1.47E-05	0.00128	0.000441	0.000721	0.001308	5.79E-05	9.66E-05	3.07E-05	4.48E-05	0.000302
TOTAL PESTICIDES	5.4E-06	7.8E-06	1.26E-05	2.53E-06	2.59E-05	6.03E-05	1.58E-05	0.002352	0.001427	0.001627	0.004918	6.4E-05	0.000127	6.88E-05	6.75E-05	0.001552

Table S4 (cont.)

Autumn	Toxic Units (C/EC50) Daphnia															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Carbofuran	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorpyrifos ethyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diazinon	0	0	0	0	0	0	2.49E-05	0.002105	6.61E-05	0.000436	0.000207	0	0	0	0	0.000125
Dimethoate	0	0	0	0	0	0	0	6.75E-07	5.18E-06	4.72E-07	0.000042	0	0	8.01E-06	0	2.66E-05
Imidacloprid	0	2.34E-08	1.94E-08	3.24E-09	2.13E-08	1.67E-07	5.3E-08	9.25E-07	6.36E-07	8.93E-07	1.56E-06	1.98E-08	5.46E-08	9.31E-08	0	7.06E-07
Malathion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Metolcarb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pirimicarb	0	0	0	0	0	0	0	0.000331	0.000272	0	0.000886	0	0	0	0	0.0006
Spinosin-A	0	0	0	0	0	0	0	3.68E-07	0	0	0	0	0	0	0	0
Total Insecticides	0	2.34E-08	1.94E-08	3.24E-09	2.13E-08	1.67E-07	2.49E-05	0.002439	0.000344	0.000437	0.001137	1.98E-08	5.46E-08	8.11E-06	0	0.000752
Chlorturon	4.12E-09	0	1.16E-08	0	5.95E-09	1.03E-08	4.97E-09	9.87E-08	4.46E-08	2.38E-08	1.54E-07	0	0	0	0	2.99E-07
Diuron	0	0	0	0	4.44E-08	7.73E-08	1.1E-07	2.56E-06	1.68E-06	3.14E-06	4.7E-06	0	0	0	0	3.99E-06
Metribuzine	0	0	0	0	3.07E-08	1.17E-07	1.53E-08	3.94E-08	4.32E-08	6.19E-08	9.74E-08	0	0	0	0	8.37E-08
Simazine	2.53E-07	1.16E-06	5.7E-07	4.28E-07	1.85E-06	1.28E-06	1.92E-05	7.34E-07	1.1E-06	5.73E-07	5.84E-06	4.08E-06	2.84E-06	2.12E-06	1.11E-06	1.17E-05
Terbuthrin	0	4.54E-08	0	0	9.16E-08	3.16E-07	1.19E-07	7.87E-06	5.23E-06	4.33E-06	9.5E-06	4.94E-08	6.08E-08	1.24E-07	5.44E-08	4.24E-06
Terbuthylazine	8.41E-09	5.96E-09	2.35E-08	4.38E-09	5.67E-08	1.88E-08	6.7E-09	7.98E-08	2.49E-08	6.56E-09	8.9E-08	5.34E-09	5.76E-09	5.66E-09	2.45E-07	3.21E-07
Total Herbicides	2.65E-07	1.22E-06	6.05E-07	4.32E-07	2.08E-06	1.82E-06	1.94E-05	1.14E-05	8.13E-06	8.13E-06	2.04E-05	4.13E-06	2.9E-06	2.25E-06	1.41E-06	2.07E-05
Carbedazim	6.4E-06	1.6E-05	3.55E-06	9.86E-06	2.26E-05	1.96E-05	1.89E-05	0.000427	0.000242	0.000246	0.000766	6.18E-06	6.24E-06	6.08E-06	1.19E-06	0.000414
Kresoxim methyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propiconazole	0	0	0	0	0	4.09E-08	0	2.5E-06	5.7E-07	7.32E-07	1.43E-06	0	0	0	0	4.67E-07
Spiroxaminie	0	0	0	0	0	0	0	3.76E-06	0	0	0	0	0	0	0	0
Tebuconazole	5.62E-08	3.82E-08	0	0	3.31E-07	1.27E-07	6.3E-08	3.37E-07	2.55E-07	4.81E-06	3.45E-07	6.1E-08	8.17E-08	9.21E-08	0	4.05E-07
Total Fungicides	6.46E-06	1.6E-05	3.55E-06	9.86E-06	2.29E-05	1.98E-05	1.9E-05	0.000433	0.000243	0.000251	0.000768	6.25E-06	6.32E-06	6.17E-06	1.19E-06	0.000414
TOTAL PESTICIDES	6.73E-06	1.73E-05	4.17E-06	1.03E-05	2.5E-05	2.18E-05	6.33E-05	0.002883	0.000595	0.000697	0.001926	1.04E-05	9.28E-06	1.65E-05	2.6E-06	0.001187

Table S4 (cont.)

Spring	Toxic Units (C/EC50) Fish															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Carbofuran	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorpyrifos ethyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diazinon	0	0	0	0	0	0	0	3.15E-07	1.27E-07	2.65E-07	3.62E-07	0	8.1E-08	8.07E-08	0	3.87E-07
Dimethoate	0	4.68E-08	1.56E-07	0	6.81E-08	0	5.23E-08	2.11E-07	4.49E-07	3.64E-07	3.53E-06	8.16E-09	2.26E-08	1.07E-08	0	7.04E-07
Imidacloprid	0	4.99E-09	2.82E-10	0	2.13E-09	5.26E-08	1.47E-08	7.77E-08	1.11E-07	3.12E-07	3.18E-07	5.18E-09	1.19E-08	7.12E-08	9.7E-10	2.5E-07
Malathion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Metolcarb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pirimicarb	0	0	0	0	0	0	0	6.53E-10	9.13E-10	6.47E-10	9.08E-09	0	0	0	0	1.35E-09
Spinosin-A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Insecticides	0	5.18E-08	1.56E-07	0	7.03E-08	5.26E-08	6.7E-08	6.04E-07	6.88E-07	9.41E-07	4.22E-06	1.33E-08	1.16E-07	1.63E-07	9.7E-10	1.34E-06
Chlorturon	4.98E-09	2.85E-09	0	0	4.86E-09	0	0	3.55E-07	1.05E-07	6.9E-09	3.92E-08	0	0	0	0	4.74E-07
Diuron	0	2.79E-08	0	0	2.49E-08	0	6.53E-08	3.35E-06	4.96E-06	6.01E-06	1.18E-05	0	4.54E-07	0	0	3.46E-06
Metribuzine	0	7.38E-10	0	0	2.22E-08	2.01E-07	0	1.15E-08	0	5.82E-09	6.73E-09	0	0	0	0	7.17E-09
Simazine	0	1.45E-09	8.88E-10	0	1.41E-09	0	2.1E-09	3.43E-08	2.31E-09	8.38E-08	4.36E-08	2.61E-06	2.37E-06	1.76E-06	5.27E-09	4.56E-08
Terbuthrin	0	3.81E-08	0	3.28E-08	4.3E-08	1.33E-06	1.08E-07	3.85E-06	3.89E-06	3.81E-06	1.25E-05	4.58E-08	8.21E-08	9.61E-07	5.82E-08	2.94E-06
Terbuthylazine	0	7.44E-08	0	0	2.17E-07	0	4.29E-08	6.2E-07	1.61E-07	1.58E-07	7.85E-07	6.86E-08	7.65E-08	9.92E-08	0.000133	2.23E-06
Total Herbicides	4.98E-09	1.45E-07	8.88E-10	3.28E-08	3.13E-07	1.53E-06	2.18E-07	8.22E-06	9.12E-06	1.01E-05	2.52E-05	2.72E-06	2.98E-06	2.82E-06	0.000133	9.15E-06
Carbedazim	0	4.32E-07	0	0	4.07E-07	7.36E-06	1.82E-06	5.1E-05	5.54E-05	5.36E-05	0.000167	1.13E-06	4.33E-06	4.06E-06	0	4.57E-05
Kresoxim methyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propiconazole	0	0	0	0	0	0	0	2.51E-06	7.88E-07	1.1E-06	1.91E-06	0	0	0	0	7.39E-07
Spiroxaminie	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tebuconazole	4.61E-08	3.62E-08	0	0	5.46E-08	5.67E-08	5.56E-08	8.54E-07	6.59E-07	4.55E-05	7.53E-07	1.01E-06	7.59E-07	9.02E-07	5E-08	4.58E-07
Total Fungicides	4.61E-08	4.69E-07	0	0	4.62E-07	7.42E-06	1.88E-06	5.43E-05	5.68E-05	0.0001	0.00017	2.14E-06	5.09E-06	4.96E-06	5E-08	4.69E-05
TOTAL PESTICIDES	5.1E-08	6.66E-07	1.57E-07	3.28E-08	8.45E-07	9E-06	2.16E-06	6.32E-05	6.66E-05	0.000111	0.000199	4.88E-06	8.18E-06	7.94E-06	0.000133	5.74E-05

Table S4 (cont.)

Summer	Toxic Units (C/EC50) Fish															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Carbofuran	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorpyrifos ethyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diazinon	0	0	0	0	0	4.95E-08	0	8.79E-07	1.58E-07	7.23E-07	2.68E-07	0	5.93E-08	8.52E-08	0	1.18E-07
Dimethoate	0	9.84E-08	0	0	1.49E-08	3.39E-08	5.77E-09	2.52E-07	2.3E-06	3E-07	8E-07	7.38E-09	2.94E-08	3.96E-08	0	7.96E-07
Imidacloprid	4.36E-09	1.04E-08	3.51E-08	2.15E-08	1.81E-08	2.4E-07	2.53E-08	6.73E-07	3.36E-07	9.7E-07	8.21E-07	1.66E-08	7.82E-08	9.89E-08	2.2E-08	2.67E-07
Malathion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Metolcarb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pirimicarb	0	0	0	0	0	0	0	2.32E-09	2.89E-09	2.01E-09	1.19E-08	0	0	0	0	4.02E-09
Spinosin-A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Insecticides	4.36E-09	1.09E-07	3.51E-08	2.15E-08	3.3E-08	3.24E-07	3.11E-08	1.81E-06	2.79E-06	2E-06	1.9E-06	2.4E-08	1.67E-07	2.24E-07	2.2E-08	1.19E-06
Chlorturon	0	0	0	0	8.55E-09	0	0	4.67E-08	1.85E-08	0	2.74E-07	0	5.2E-09	0	0	7.62E-08
Diuron	0	4.12E-08	0	0	0	0	2.38E-07	6.81E-06	5.08E-06	1.95E-05	1.1E-05	0	7.44E-08	0	0	4.09E-06
Metribuzine	0	0	0	0	0	4.37E-08	2.01E-09	9.4E-09	7.33E-09	1.06E-08	1.05E-08	0	2.67E-09	0	0	0
Simazine	3.79E-09	2.67E-09	1.37E-08	1.97E-09	3.39E-09	2.73E-09	3.82E-09	1.57E-07	6.33E-08	3.17E-08	4.35E-08	5.63E-08	5.13E-08	1.97E-08	2.21E-07	7.42E-08
Terbuthrin	3.69E-08	5.52E-08	0	0	3.57E-08	1.29E-07	3.57E-07	2.27E-05	8.29E-06	1.21E-05	1.32E-05	1.4E-07	2.5E-07	3.53E-07	4.77E-07	3.27E-06
Terbuthylazine	5.63E-08	4.02E-08	1.82E-08	0	5.29E-07	9.82E-09	4.34E-08	3.9E-06	7.95E-08	4.66E-08	6.62E-08	8.01E-08	7.62E-08	7.7E-08	5.42E-07	4.02E-06
Total Herbicides	9.7E-08	1.39E-07	3.19E-08	1.97E-09	5.77E-07	1.86E-07	6.44E-07	3.36E-05	1.35E-05	3.17E-05	2.46E-05	2.76E-07	4.6E-07	4.5E-07	1.24E-06	1.15E-05
Carbedazim	1.11E-06	9.99E-07	2.47E-06	4.96E-07	5.6E-06	8.29E-06	3.3E-06	0.000288	9.91E-05	0.000153	0.000294	1.3E-05	2.17E-05	6.86E-06	1.01E-05	6.77E-05
Kresoxim methyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propiconazole	0	0	0	0	0	0	0	9.61E-07	4.72E-07	9.89E-07	8.52E-07	0	0	6.85E-08	0	2.53E-07
Spiroxaminie	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tebuconazole	7.9E-08	6E-08	0	0	4.2E-08	2.14E-07	9.9E-08	1.5E-06	3.02E-07	0.000102	6.71E-07	3.79E-07	4.76E-07	3.79E-07	4.18E-07	7.76E-07
Total Fungicides	1.19E-06	1.06E-06	2.47E-06	4.96E-07	5.64E-06	8.5E-06	3.4E-06	0.00029	9.98E-05	0.000256	0.000296	1.34E-05	2.22E-05	7.31E-06	1.05E-05	6.88E-05
TOTAL PESTICIDES	1.29E-06	1.31E-06	2.54E-06	5.19E-07	6.25E-06	9.01E-06	4.07E-06	0.000326	0.000116	0.000289	0.000322	1.37E-05	2.28E-05	7.98E-06	1.17E-05	8.15E-05

Table S4 (cont.)

Autumn	Toxic Units (C/EC50) Fish															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Carbofuran	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorpyrifos ethyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diazinon	0	0	0	0	0	0	6.22E-08	5.26E-06	1.65E-07	1.09E-06	5.19E-07	0	0	0	0	3.11E-07
Dimethoate	0	0	0	0	0	0	0	2.25E-08	1.73E-07	1.57E-08	1.4E-06	0	0	2.67E-07	0	8.87E-07
Imidacloprid	0	3.98E-09	3.29E-09	5.51E-10	3.62E-09	2.83E-08	9.02E-09	1.57E-07	1.08E-07	1.52E-07	2.65E-07	3.37E-09	9.28E-09	1.58E-08	0	1.2E-07
Malathion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Metolcarb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pirimicarb	0	0	0	0	0	0	0	1.14E-09	9.39E-10	0	3.06E-09	0	0	0	0	2.07E-09
Spinosin-A	0	0	0	0	0	0	0	8.37E-07	0	0	0	0	0	0	0	0
Total Insecticides	0	3.98E-09	3.29E-09	5.51E-10	3.62E-09	2.83E-08	7.12E-08	6.28E-06	4.47E-07	1.26E-06	2.19E-06	3.37E-09	9.28E-09	2.83E-07	0	1.32E-06
Chlorturon	7.89E-09	0	2.21E-08	0	1.14E-08	1.97E-08	9.52E-09	1.89E-07	8.54E-08	4.56E-08	2.95E-07	0	0	0	0	5.71E-07
Diuron	0	0	0	0	1.43E-07	2.49E-07	3.53E-07	8.23E-06	5.42E-06	1.01E-05	1.51E-05	0	0	0	0	1.28E-05
Metribuzine	0	0	0	0	1.82E-09	6.96E-09	9.06E-10	2.33E-09	2.56E-09	3.66E-09	5.77E-09	0	0	0	0	4.95E-09
Simazine	2.53E-09	1.16E-08	5.7E-09	4.28E-09	1.85E-08	1.28E-08	1.92E-07	7.34E-09	1.1E-08	5.73E-09	5.84E-08	4.08E-08	2.84E-08	2.12E-08	1.11E-08	1.17E-07
Terbuthrin	0	6.13E-08	0	0	1.24E-07	4.27E-07	1.6E-07	1.06E-05	7.06E-06	5.84E-06	1.28E-05	6.67E-08	8.2E-08	1.67E-07	7.34E-08	5.73E-06
Terbuthylazine	4.41E-08	3.13E-08	1.23E-07	2.3E-08	2.98E-07	9.86E-08	3.52E-08	4.19E-07	1.31E-07	3.45E-08	4.67E-07	2.8E-08	3.02E-08	2.97E-08	1.29E-06	1.69E-06
Total Herbicides	5.45E-08	1.04E-07	1.51E-07	2.73E-08	5.96E-07	8.13E-07	7.5E-07	1.95E-05	1.27E-05	1.6E-05	2.88E-05	1.35E-07	1.41E-07	2.18E-07	1.37E-06	2.09E-05
Carbedazim	1.44E-06	3.6E-06	7.99E-07	2.22E-06	5.08E-06	4.42E-06	4.26E-06	9.6E-05	5.44E-05	5.53E-05	0.000172	1.39E-06	1.4E-06	1.37E-06	2.67E-07	9.31E-05
Kresoxim methyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propiconazole	0	0	0	0	0	6.72E-08	0	4.11E-06	9.36E-07	1.2E-06	2.34E-06	0	0	0	0	7.66E-07
Spiroxaminie	0	0	0	0	0	0	0	2.21E-07	0	0	0	0	0	0	0	0
Tebuconazole	1.41E-07	9.54E-08	0	0	8.28E-07	3.17E-07	1.58E-07	8.44E-07	6.39E-07	1.2E-05	8.62E-07	1.52E-07	2.04E-07	2.3E-07	0	1.01E-06
Total Fungicides	1.58E-06	3.69E-06	7.99E-07	2.22E-06	5.91E-06	4.8E-06	4.42E-06	0.000101	5.6E-05	6.85E-05	0.000176	1.54E-06	1.61E-06	1.6E-06	2.67E-07	9.48E-05
TOTAL PESTICIDES	1.64E-06	3.8E-06	9.53E-07	2.25E-06	6.51E-06	5.65E-06	5.24E-06	0.000127	6.91E-05	8.58E-05	0.000207	1.68E-06	1.76E-06	2.1E-06	1.64E-06	0.000117

Table S4 (cont.)

POCIS-Summer	Toxic Units (C/EC50) Algae															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Carbofuran	0	0	0		0	0	0	0				0	0	0	0	0
Chlorpyrifos ethyl	0	5.52E-08	0		1.05E-07	1.63E-06	1.41E-07	1.16E-05				0	1.88E-05	1.03E-05	0	1.57E-05
Diazinon	2E-09	2.72E-09	0		3.4E-08	1.47E-07	2.6E-09	9.45E-07				3.2E-09	1.13E-08	1.18E-08	1.5E-08	9.27E-08
Dimethoate	0	2.08E-08	0		2.5E-08	1.32E-07	4.28E-09	6.95E-08				1.29E-09	1.08E-07	2.27E-07	4.54E-08	3.51E-06
Imidacloprid	8.48E-09	3.92E-08	3.5E-07		6.33E-08	1.74E-06	2.91E-07	1.27E-06				1.77E-08	5.53E-07	5.77E-07	1.12E-07	3.42E-06
Malathion	0	0	0		0	0	0	0				0	0	0	0	0
Metolcarb	0	0	0		0	0	0	0				0	0	0	0	0
Pirimicarb	0	0	0		0	1.93E-08	0	7.38E-09				0	0	0	0	2.64E-08
Spinosin-A	0	0	0		0	7.37E-08	0	2.19E-06				0	0	2.96E-08	0	2.72E-08
Total Insecticides	1.05E-08	1.18E-07	3.5E-07		2.27E-07	3.74E-06	4.39E-07	1.61E-05				2.21E-08	1.95E-05	1.12E-05	1.72E-07	2.28E-05
Chlorturon	2.94E-05	7.55E-06	0		0.000215	0.000281	0	0.002372				0	0	0	0	0.003063
Diuron	0	0.000676	0		0.002885	0.020055	0.00844	0.0793				0.0003	0.006074	0.005887	0	0.142124
Metribuzine	0	0	0		0.000382	0.010965	1.88E-05	0.001309				0	0	0	0.000379	0.000397
Simazine	4.57E-06	2.11E-05	2.22E-06		2.67E-05	0.000132	7.41E-05	0.000271				0.000184	0.000156	0.000104	0.001064	0.002794
Terbuthrin	3.51E-05	0.00014	0		0.000324	0.00266	0.000804	0.008246				0.00015	0.00021	0.00049	0.001122	0.009685
Terbuthylazine	3.19E-05	3.3E-05	1.59E-05		0.004821	0.000458	5.76E-05	0.002024				0.000117	2.39E-05	7.95E-05	0.007554	0.003762
Total Herbicides	0.000101	0.000878	1.81E-05		0.008653	0.034551	0.009395	0.093521				0.000752	0.006464	0.006561	0.010119	0.161827
Carbedazim	6.02E-07	2.39E-06	1.56E-06		9.19E-06	6.86E-05	6.74E-06	0.000112				1.05E-06	1.12E-05	1.18E-05	5.42E-06	0.00021
Kresoxim methyl	0	0	0		0	0	0	0				0	0	0	0	0
Propiconazole	0	2.56E-05	0		0.000528	0.000222	2.93E-05	0.001373				0	3.83E-05	3.95E-05	0	0.001223
Spiroxamine	0	0	0		0	0	0	0				0	0	0	0	0
Tebuconazole	9.69E-07	8.09E-07	7.57E-08		9.45E-06	2.75E-05	6.61E-07	1.9E-05				2.87E-06	4.88E-06	4.52E-06	5.34E-06	1.1E-05
Total Fungicides	1.57E-06	2.88E-05	1.64E-06		0.000547	0.000318	3.67E-05	0.001505				3.92E-06	5.44E-05	5.58E-05	1.08E-05	0.001444
TOTAL PESTICIDES	0.000103	0.000907	2.01E-05		0.0092	0.034873	0.009432	0.095042				0.000756	0.006538	0.006628	0.01013	0.163294

Table S4 (cont.)

POCIS-Summer	Toxic Units (C/EC50) Daphnia															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Carbofuran	0	0	0		0	0	0	0				0	0	0	0	0
Chlorpyrifos ethyl	0	0.0029	0		0.0055	0.085476	0.007377	0.609369				0	0.98945	0.54295	0	0.822921
Diazinon	0.00002	2.72E-05	0		0.00034	0.001473	2.6E-05	0.009451				3.2E-05	0.000113	0.000118	0.00015	0.000927
Dimethoate	0	1.04E-05	0		1.25E-05	6.61E-05	2.14E-06	3.47E-05				6.46E-07	5.41E-05	0.000113	2.27E-05	0.001754
Imidacloprid	4.99E-08	2.31E-07	2.06E-06		3.72E-07	1.02E-05	1.71E-06	7.44E-06				1.04E-07	3.25E-06	3.39E-06	6.56E-07	2.01E-05
Malathion	0	0	0		0	0	0	0				0	0	0	0	0
Metolcarb	0	0	0		0	0	0	0				0	0	0	0	0
Pirimicarb	0	0	0		0	0.026993	0	0.010338				0	0	0	0	0.036943
Spinosin-A	0	0	0		0	3.89E-07	0	1.16E-05				0	0	1.56E-07	0	1.43E-07
Total Insecticides	2E-05	0.002938	2.06E-06		0.005853	0.114019	0.007407	0.629211				3.27E-05	0.98962	0.543185	0.000173	0.862565
Chlorturon	1.4E-08	3.61E-09	0		1.03E-07	1.34E-07	0	1.13E-06				0	0	0	0	1.46E-06
Diuron	0	2.63E-07	0		1.12E-06	7.8E-06	3.28E-06	3.08E-05				1.17E-07	2.36E-06	2.29E-06	0	5.53E-05
Metribuzine	0	0	0		3.39E-06	9.75E-05	1.67E-07	1.16E-05				0	0	0	3.36E-06	3.53E-06
Simazine	2.6E-07	1.2E-06	1.26E-07		1.52E-06	7.55E-06	4.22E-06	1.54E-05				1.05E-05	8.87E-06	5.92E-06	6.06E-05	0.000159
Terbuthrin	1.04E-07	4.15E-07	0		9.6E-07	7.88E-06	2.38E-06	2.44E-05				4.46E-07	6.23E-07	1.45E-06	3.33E-06	2.87E-05
Terbuthylazine	2.43E-08	2.52E-08	1.21E-08		3.67E-06	3.49E-07	4.39E-08	1.54E-06				8.9E-08	1.82E-08	6.06E-08	5.76E-06	2.87E-06
Total Herbicides	4.03E-07	1.91E-06	1.38E-07		1.08E-05	0.000121	1.01E-05	8.5E-05				1.12E-05	1.19E-05	9.73E-06	7.31E-05	0.000251
Carbedazim	8.7E-06	3.45E-05	2.25E-05		0.000133	0.000991	9.74E-05	0.001623				1.52E-05	0.000162	0.00017	7.82E-05	0.003039
Kresoxim methyl	0	0	0		0	0	0	0				0	0	0	0	0
Propiconazole	0	5.89E-08	0		1.21E-06	5.1E-07	6.74E-08	3.16E-06				0	8.8E-08	9.09E-08	0	2.81E-06
Spiroxamine	0	0	0		0	0	0	0				0	0	0	0	0
Tebuconazole	2.47E-07	2.06E-07	1.93E-08		2.4E-06	7.01E-06	1.68E-07	4.84E-06				7.3E-07	1.24E-06	1.15E-06	1.36E-06	2.79E-06
Total Fungicides	8.95E-06	3.48E-05	2.26E-05		0.000136	0.000998	9.76E-05	0.001631				1.59E-05	0.000164	0.000171	7.96E-05	0.003044
TOTAL PESTICIDES	2.94E-05	0.002975	2.48E-05		0.006	0.115138	0.007514	0.630927				5.98E-05	0.989796	0.543366	0.000326	0.865861

Table S4 (cont.)

POCIS-Summer	Toxic Units (C/EC50) Fish															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Carbofuran	0	0	0		0	0	0	0				0	0	0	0	0
Chlorpyrifos ethyl	0	0.000387	0		0.000733	0.011397	0.000984	0.081249				0	0.131927	0.072393	0	0.109723
Diazinon	5E-08	6.81E-08	0		8.51E-07	3.68E-06	6.51E-08	2.36E-05				7.99E-08	2.82E-07	2.94E-07	3.75E-07	2.32E-06
Dimethoate	0	3.47E-07	0		4.17E-07	2.2E-06	7.13E-08	1.16E-06				2.15E-08	1.8E-06	3.78E-06	7.56E-07	5.85E-05
Imidacloprid	8.48E-09	3.92E-08	3.5E-07		6.33E-08	1.74E-06	2.91E-07	1.27E-06				1.77E-08	5.53E-07	5.77E-07	1.12E-07	3.42E-06
Malathion	0	0	0		0	0	0	0				0	0	0	0	0
Metolcarb	0	0	0		0	0	0	0				0	0	0	0	0
Pirimicarb	0	0	0		0	9.31E-08	0	3.56E-08				0	0	0	0	1.27E-07
Spinosin-A	0	0	0		0	8.84E-07	0	2.63E-05				0	0	3.55E-07	0	3.26E-07
Total Insecticides	5.85E-08	0.000387	3.5E-07		0.000735	0.011405	0.000984	0.081302				1.19E-07	0.131929	0.072398	1.24E-06	0.109787
Chlorturon	2.69E-08	6.9E-09	0		1.97E-07	2.57E-07	0	2.17E-06				0	0	0	0	2.8E-06
Diuron	0	8.45E-07	0		3.61E-06	2.51E-05	1.05E-05	9.91E-05				3.75E-07	7.59E-06	7.36E-06	0	0.000178
Metribuzine	0	0	0		2.01E-07	5.77E-06	9.89E-09	6.89E-07				0	0	0	1.99E-07	2.09E-07
Simazine	2.6E-09	1.2E-08	1.26E-09		1.52E-08	7.55E-08	4.22E-08	1.54E-07				1.05E-07	8.87E-08	5.92E-08	6.06E-07	1.59E-06
Terbuthrin	1.4E-07	5.6E-07	0		1.3E-06	1.06E-05	3.22E-06	3.3E-05				6.02E-07	8.41E-07	1.96E-06	4.49E-06	3.87E-05
Terbuthylazine	1.28E-07	1.32E-07	6.37E-08		1.93E-05	1.83E-06	2.31E-07	8.09E-06				4.67E-07	9.57E-08	3.18E-07	3.02E-05	1.5E-05
Total Herbicides	2.97E-07	1.56E-06	6.49E-08		2.46E-05	4.36E-05	1.41E-05	0.000143				1.55E-06	8.62E-06	9.7E-06	3.55E-05	0.000236
Carbedazim	1.96E-06	7.77E-06	5.07E-06		2.99E-05	0.000223	2.19E-05	0.000365				3.42E-06	3.65E-05	3.83E-05	1.76E-05	0.000684
Kresoxim methyl	0	0	0		0	0	0	0				0	0	0	0	0
Propiconazole	0	9.67E-08	0		1.99E-06	8.38E-07	1.11E-07	5.18E-06				0	1.44E-07	1.49E-07	0	4.61E-06
Spiroxamine	0	0	0		0	0	0	0				0	0	0	0	0
Tebuconazole	6.17E-07	5.15E-07	4.82E-08		6.01E-06	1.75E-05	4.21E-07	1.21E-05				1.82E-06	3.11E-06	2.87E-06	3.4E-06	6.98E-06
Total Fungicides	2.57E-06	8.38E-06	5.12E-06		3.79E-05	0.000241	2.24E-05	0.000382				5.25E-06	3.98E-05	4.13E-05	2.1E-05	0.000695
TOTAL PESTICIDES	2.93E-06	0.000397	5.53E-06		0.000797	0.01169	0.001021	0.081827				6.92E-06	0.131978	0.072449	5.78E-05	0.110719

Table S5. Toxic Units (TUs) for individual point sources chemicals and for mixtures.

Spring	Toxic Units (C/EC50) Algae															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Acetaminophen	1.08E-08	6.46E-08	5.27E-08	2.4E-08	1.02E-07	5.96E-06	8.61E-07	3.98E-07	5.56E-07	5.98E-06	2.04E-07	5.96E-08	3.5E-05	3.54E-05	6.16E-08	1.01E-07
Atenolol	3.1E-09	2.53E-08	5.3E-09	0	1.59E-08	6.13E-07	4.41E-08	3.05E-06	9.17E-07	1.61E-06	2.23E-06	1.68E-08	2.64E-07	5.24E-07	1.64E-08	8.68E-07
Carbamazepine	1.8E-09	1.34E-08	4.4E-09	6E-10	8.2E-09	4.12E-08	2.98E-08	1.78E-07	3.42E-06	1.28E-07	1.27E-06	7.28E-08	9.24E-08	1.03E-07	2.65E-08	3.14E-07
Citalopram	1.26E-05	4.95E-07	0	5.5E-08	4.16E-06	1.29E-06	1.38E-06	8.8E-06	4.13E-06	2.36E-06	6.35E-06	1.96E-06	2.92E-06	3.65E-06	1.2E-06	1.29E-05
Diclofenac	0	1.38E-08	0	0	0	1.68E-08	2.76E-08	5.48E-07	3.97E-07	5.54E-07	2.01E-06	9.14E-09	8.33E-08	1.45E-07	0	4.35E-07
Gemfibrocil	0	7.97E-07	0	0	1.71E-07	5.49E-07	1.53E-06	7.47E-05	3.42E-05	5.22E-05	0.000135	1.66E-07	2.2E-06	1.23E-05	1.69E-08	3.69E-05
Ibuprofen	2.46E-08	5.28E-08	2.91E-08	3.26E-08	6.37E-08	1.21E-06	2.25E-07	2.31E-06	9.07E-06	3.64E-06	1.27E-06	1.47E-08	3.26E-06	8.44E-06	1.61E-08	1.44E-07
Ketoprofen	0	0	0	0	0	3.06E-08	0	9.61E-07	1.32E-07	2.45E-07	1.78E-06	0	3.58E-08	3.75E-08	0	2.69E-08
Loratadine	0	0	0	0	0	0	0	9.2E-06	0	0	0	3.5E-06	0	0	0	0
Naproxen	0	2.13E-08	0	0	0	6.86E-07	3.99E-07	5.07E-06	2.76E-06	0	5.62E-06	0	0	4.76E-06	0	0
Omeprazole	0	0	0	0	0	0	0	1.79E-06	9.3E-09	1.77E-08	4.64E-08	7.5E-09	0	2.1E-09	0	0
Salbutamol	0	6E-10	1E-09	0	0	7E-10	1.4E-09	3.82E-08	2.82E-08	3.55E-08	8.91E-08	0	6.4E-08	3.9E-09	0	4.12E-08
Valsarten	3.66E-07	3.81E-06	0	0	1.66E-06	3.19E-06	9.35E-06	0.000181	8.71E-05	0.000104	0.000281	3.28E-06	4.99E-05	7.03E-05	0	8.15E-05
Venlafaxine	0	3.02E-07	0	0	4.2E-07	0	3.43E-07	5.12E-05	5.84E-06	3.11E-06	1.34E-05	8.83E-07	3.58E-07	1.66E-06	0	1.99E-06
Total Pharmac	1.3E-05	5.59E-06	9.25E-08	1.12E-07	6.6E-06	1.36E-05	1.42E-05	0.000339	0.000149	0.000174	0.000451	9.97E-06	9.42E-05	0.000137	1.33E-06	0.000135
Amoxicillin	0	0	0	0	0	0	0	3.62E-08	7.9E-09	0	0	0	0	0	0	0
Azithromycin	1.57E-07	1.21E-07	0	0	8.83E-07	1.76E-07	9.64E-08	2.77E-06	0	9.58E-08	1.06E-07	4.14E-07	5.69E-07	1.89E-07	7.67E-08	1.09E-07
Ciprofloxacin	3.13E-07	2.72E-07	0	0	7.06E-07	5.73E-07	8.75E-07	5.88E-05	1.32E-06	1.99E-06	2.46E-06	3.04E-06	5.94E-07	1.2E-06	6.82E-07	1.46E-06
Erythromycin	0	3.33E-06	0	0	3.33E-06	1.33E-06	3.17E-06	0.000082	8.63E-05	0.000047	0.000282	1.33E-05	1.5E-06	0.000008	0	1.55E-05
Lincomycin	5.71E-07	0	0	0	0	0	0	1.37E-05	5.13E-05	2.49E-05	5.96E-05	0.000003	3.57E-06	3.57E-06	0	2.11E-05
Metronidazole	0	0	0	0	0	0	0	1.27E-06	7.15E-07	6.58E-07	3.03E-06	0	6.78E-08	2.19E-06	0	1.21E-07
Sulfamethoxazole	5.8E-09	2.07E-08	0	0	7.6E-09	1.71E-07	4.59E-08	1.45E-06	8.23E-07	3.72E-07	1.89E-06	4.05E-08	5.7E-08	1.07E-07	2.9E-09	3.65E-07
Trimethoprim	1.6E-09	3.4E-09	9E-10	5.4E-09	3.8E-09	1.5E-09	2.6E-09	4.75E-07	2.09E-07	2.19E-07	8.71E-07	4E-09	2.39E-08	4.42E-08	5.7E-09	1.32E-07
Tylosin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Antibiotics	1.05E-06	3.75E-06	9E-10	5.4E-09	4.93E-06	2.25E-06	4.19E-06	0.000161	0.000141	7.52E-05	0.00035	1.98E-05	6.38E-06	1.53E-05	7.67E-07	3.88E-05
Estradiol, 17-beta-(E2)	0	0	0	0	0	0	0	9.6E-08	0	0	1.6E-07	0	0	1.64E-07	0	0
Estrone	1.54E-09	3.69E-09	1.32E-08	0	0	3.08E-09	4.62E-09	8.11E-08	8.02E-08	4.98E-08	8.6E-08	9.38E-09	2.05E-08	4.48E-08	3.08E-09	6.77E-09
Progesterone	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Testosterone	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Estrogens	1.54E-09	3.69E-09	1.32E-08	0	0	3.08E-09	4.62E-09	1.77E-07	8.02E-08	4.98E-08	2.46E-07	9.38E-09	2.05E-08	2.09E-07	3.08E-09	6.77E-09
Amphetamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caffeine	2.06E-07	4.81E-07	1.16E-07	6.38E-08	2.96E-07	2.93E-06	1.36E-06	4.24E-06	3.53E-06	9.37E-06	4.52E-06	1.14E-06	3.51E-06	7.06E-06	5.96E-08	1.17E-06
Nicotine	5.67E-07	2.21E-06	3.92E-07	1.33E-07	6.2E-07	1.07E-06	4.94E-07	6.65E-07	7.75E-07	1.03E-06	1.07E-06	1.66E-08	0.000003	3.23E-06	2.63E-08	7.44E-08
Paraxantine	6.67E-08	3.3E-06	2.81E-08	2.37E-08	1.75E-07	2.45E-05	8.93E-06	4.28E-05	2.62E-05	8.18E-05	4.04E-05	3.82E-06	2.07E-05	6.58E-05	1.48E-07	1.59E-05
Total LC	8.4E-07	5.99E-06	5.36E-07	2.21E-07	1.09E-06	2.85E-05	1.08E-05	4.77E-05	3.05E-05	9.22E-05	4.6E-05	4.98E-06	2.72E-05	7.61E-05	2.34E-07	1.71E-05
Tributyl-phosphate	0.000494	0.000111	0.000247	0.000284	0.000597	5.67E-05	0.000134	0.000014	0.000252	4.28E-05	0.000075	1.29E-05	1.41E-05	3.63E-06	6.61E-06	1.69E-05
Total Industrial	0.000494	0.000111	0.000247	0.000284	0.000597	5.67E-05	0.000134	0.000014	0.000252	4.28E-05	0.000075	1.29E-05	1.41E-05	3.63E-06	6.61E-06	1.69E-05
Total PSC	0.000509	0.000126	0.000248	0.000285	0.00061	0.000101	0.000164	0.000561	0.000572	0.000384	0.000922	4.77E-05	0.000142	0.000233	8.95E-06	0.000208

Table S5 (cont.)

Summer	Toxic Units (C/EC50) Algae															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Acetaminophen	0	0	3.12E-08	0	5.6E-08	2.25E-06	1.08E-07	3.06E-08	9.49E-08	6.27E-08	2.16E-07	2.08E-08	6.71E-05	5.66E-05	6.8E-09	3.13E-08
Atenolol	0	2.73E-08	0	0	2.13E-08	2.95E-07	5.88E-08	5.3E-06	1.02E-06	7.44E-07	4.39E-06	7.9E-09	8.57E-07	9.28E-07	0	5.45E-07
Carbamazepine	5.1E-09	1.35E-08	2.7E-09	8.84E-08	7.2E-09	2.53E-07	5.32E-08	4.98E-07	7.69E-07	1.2E-06	1.74E-06	9.27E-08	2.23E-07	2.33E-07	1.34E-07	4.51E-07
Citalopram	2.8E-07	2.5E-07	4E-08	1.35E-07	4.2E-07	2.2E-07	5.45E-07	9.1E-06	2.36E-06	2.16E-06	6.5E-06	5.15E-07	3.23E-06	9.5E-07	3.55E-07	1.16E-06
Diclofenac	0	1.4E-08	0	0	0	1.53E-07	1.91E-08	0	5.02E-07	1.62E-06	2.3E-06	1.14E-08	3.47E-07	4.12E-07	0	2.06E-07
Gemfibrocil	0	6E-07	0	0	1.71E-07	3.61E-06	3.66E-07	7.75E-05	7.58E-05	8.05E-06	0.000134	0	1.76E-05	2.1E-05	2.54E-08	1.99E-05
Ibuprofen	4.1E-09	6.6E-09	9.7E-09	9.3E-09	1.42E-08	2.41E-06	5.18E-08	2.04E-06	9.77E-06	6.45E-08	2.76E-05	1.37E-08	6.7E-06	1.13E-05	5.2E-09	4.41E-07
Ketoprofen	0	0	0	0	0	8.35E-08	0	2.77E-06	8.56E-08	3.14E-07	1.15E-06	0	3.99E-07	1.38E-07	0	0
Loratadine	0	0	0	0	0	0	0	3.74E-05	3.14E-06	0	0	0	0	0	0	0
Naproxen	0	0	0	0	0	2.37E-06	3.9E-07	6.05E-06	1.81E-06	0	4.91E-06	0	6.45E-06	1.06E-05	0	1.11E-06
Omeprazole	0	0	0	0	0	0	0	4.43E-07	3E-09	2.18E-08	3.36E-08	0	1.31E-08	3.9E-09	0	0
Salbutamol	0	5E-10	0	0	0	2E-09	1.5E-09	9.2E-08	5.28E-08	8.21E-08	1.02E-07	0	2.3E-09	6.2E-09	0	4.1E-08
Valsarten	2.89E-07	4.74E-06	5.09E-07	0	2.43E-06	1.93E-05	1.19E-05	0.000209	9.09E-05	3.58E-05	0.000182	6.06E-06	0.000417	0.000271	8.53E-07	6.93E-05
Venlafaxine	1.86E-07	3.63E-07	2.23E-07	2.3E-07	1.48E-07	4.47E-07	4.13E-07	2.08E-05	6.37E-06	5.05E-06	1.77E-05	1.52E-07	2.67E-06	2.82E-06	0	2.43E-06
Total Pharmac	7.64E-07	6.01E-06	8.16E-07	4.63E-07	3.26E-06	3.13E-05	1.39E-05	0.000371	0.000193	5.51E-05	0.000382	6.88E-06	0.000523	0.000376	1.38E-06	9.56E-05
Amoxicillin	0	0	0	0	0	0	0	2.63E-08	0	0	2.9E-09	0	0	0	0	4.3E-09
Azithromycin	3.89E-07	1.52E-07	0	2.12E-07	1.31E-08	7.58E-08	8.75E-08	2.68E-06	0	7.64E-08	8.47E-08	9.47E-08	3.08E-07	6.36E-08	2.53E-08	1.41E-07
Ciprofloxacin	8.1E-07	6.49E-07	0	4.27E-07	0	0	2.72E-07	6.81E-05	0	1.79E-06	3.25E-06	0	2.72E-05	1.19E-05	2.84E-07	1.28E-06
Erythromycin	0	1.67E-06	1.17E-06	0	8.33E-07	1.17E-06	1.83E-06	7.27E-05	0.000064	7.45E-05	0.000178	0.000003	3.17E-06	3.5E-06	1.33E-06	3.55E-05
Lincomycin	1.29E-06	0	0	0	0	0	0	3.16E-05	5.87E-05	3.39E-05	0.000101	0	0	0.000002	1.57E-06	2.44E-05
Metronidazole	0	0	0	0	0	0	0	6.18E-07	5.65E-07	2.39E-07	3.1E-06	0	7.2E-08	5.68E-08	0	8.4E-08
Sulfamethoxazole	5.8E-09	4.23E-08	0	0	7.4E-09	7.82E-07	4.84E-08	1.13E-06	3.47E-07	4.99E-06	1.24E-06	5.5E-08	2.94E-07	4.75E-07	1.22E-08	7.84E-07
Trimethoprim	0	0	0	7E-10	0	4.7E-09	1.8E-09	3.23E-06	2.51E-07	5.12E-07	1.16E-06	1E-09	1.51E-07	2.97E-07	0	1.02E-07
Tylosin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Antibiotics	2.49E-06	2.51E-06	1.17E-06	6.39E-07	8.54E-07	2.03E-06	2.24E-06	0.00018	0.000124	0.000116	0.000289	3.15E-06	3.12E-05	1.83E-05	3.23E-06	6.23E-05
Estradiol, 17-beta-(E2)	0	0	0	0	0	0	0	0	6.8E-08	0	1.16E-07	0	0	2.4E-07	0	0
Estrone	4.46E-09	0	0	0	0	3.85E-09	0	4.48E-08	5.88E-08	9.08E-09	1.14E-07	5.69E-09	4.94E-08	7.06E-08	0	7.38E-09
Progesterone	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Testosterone	0	0	0	0	0	0	0	0	0	0	0	0	5.19E-07	4.6E-07	0	0
Total Estrogens	4.46E-09	0	0	0	0	3.85E-09	0	4.48E-08	1.27E-07	9.08E-09	2.3E-07	5.69E-09	5.68E-07	7.71E-07	0	7.38E-09
Amphetamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caffeine	1.71E-07	9.95E-07	5.4E-07	5.95E-08	4.36E-07	4.61E-06	9.39E-07	4.65E-07	3.02E-06	4.24E-07	4.97E-06	1.94E-07	2.21E-05	2.41E-05	6.6E-07	7.1E-07
Nicotine	3.47E-08	6.9E-08	1.71E-07	3.61E-08	7.88E-08	2.12E-06	2.66E-07	4.46E-07	3.94E-07	3.49E-07	2.08E-07	1.46E-08	4.11E-06	5.99E-06	7.13E-08	9.24E-08
Paraxantine	5.87E-08	6.95E-06	2.93E-07	2.39E-08	2.55E-07	2.48E-05	5.94E-06	9.54E-06	2.08E-05	5.06E-06	4.92E-05	5.79E-07	6.68E-05	7.72E-05	2.19E-06	8.43E-06
Total LC	2.64E-07	8.01E-06	1E-06	1.2E-07	7.69E-07	3.16E-05	7.15E-06	1.05E-05	2.42E-05	5.83E-06	5.43E-05	7.88E-07	9.3E-05	0.000107	2.92E-06	9.23E-06
Tributyl-phosphate	2.93E-06	6.28E-06	4.53E-05	6.11E-05	4.52E-06	1.36E-05	2.41E-05	6.33E-06	2.74E-05	8.28E-06	1.22E-05	1.86E-06	2.11E-06	0	3.01E-06	5.12E-06
Total Industrial	2.93E-06	6.28E-06	4.53E-05	6.11E-05	4.52E-06	1.36E-05	2.41E-05	6.33E-06	2.74E-05	8.28E-06	1.22E-05	1.86E-06	2.11E-06	0	3.01E-06	5.12E-06
Total PSC	6.45E-06	2.28E-05	4.83E-05	6.23E-05	9.41E-06	7.86E-05	4.74E-05	0.000567	0.000368	0.000185	0.000738	1.27E-05	0.00065	0.000503	1.05E-05	0.000172

Table S5 (cont.)

Autumn	Toxic Units (C/EC50) Algae															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Acetaminophen	2.63E-08	2.71E-07	1.65E-08	7.4E-09	6.45E-08	1.05E-06	1.18E-06	7.5E-07	8.76E-07	1.87E-07	5.15E-07	4.85E-08	5.84E-05	9.83E-05	3.67E-08	2.58E-07
Atenolol	4.4E-08	3.25E-08	9.8E-09	0	1.41E-08	1.3E-07	2.73E-07	6.73E-06	1.37E-06	9.92E-07	3.97E-06	7.1E-09	2.92E-07	5.29E-07	4.5E-09	1.67E-06
Carbamazepine	4E-09	2.3E-08	2.3E-09	1.2E-09	1.24E-08	5.08E-08	7.99E-08	2.36E-07	5.78E-07	3.33E-07	1.25E-06	8.33E-08	1.19E-07	1.78E-07	1.57E-08	4.44E-07
Citalopram	2.21E-06	2.2E-06	0	3.3E-07	2.15E-07	5.2E-07	4.85E-07	6.05E-06	3.74E-06	2.01E-06	1E-05	2.53E-06	2.63E-06	3.97E-06	1.3E-06	1.15E-06
Diclofenac	0	4.2E-08	0	0	0	2.74E-08	5.28E-08	1.26E-06	5.65E-07	6.18E-07	2.37E-06	1.82E-08	6.74E-08	1.74E-07	0	1.22E-06
Gemfibrocil	0	3.56E-06	9.49E-08	0	1.64E-07	1.27E-06	1.42E-06	5.8E-05	3.92E-05	1.86E-05	9.34E-05	8.64E-08	6.78E-06	1.67E-05	1.86E-08	4.75E-05
Ibuprofen	5.2E-09	5.32E-08	9.3E-09	1.15E-08	1.49E-08	7.84E-07	5.31E-07	1.52E-05	8.28E-06	8.11E-08	1.14E-06	2.49E-08	5.53E-06	1.03E-05	8E-09	3.03E-06
Ketoprofen	0	4.32E-08	0	0	0	1.21E-07	3.18E-08	3.56E-06	1.52E-06	4.19E-07	1.71E-06	0	3.2E-07	6.45E-07	0	3.27E-07
Loratadine	0	0	0	0	0	0	0	9.01E-06	6.36E-06	0	0	0	0	0	0	0
Naproxen	0	0	0	0	0	0	9.76E-07	1.4E-05	3.08E-06	0	5.56E-06	0	2.01E-06	3.7E-06	0	8.09E-06
Omeprazole	5.5E-09	0	2.5E-09	2.2E-09	2.4E-09	8E-09	1.06E-08	3.92E-06	2.5E-08	0	5.39E-08	0	0	2.8E-09	0	1.37E-08
Salbutamol	0	1.2E-09	0	0	0	7E-10	2.9E-09	6.12E-08	3.11E-08	5.38E-08	7.99E-08	0	1E-09	3.2E-09	0	7.99E-08
Valsarten	4.04E-07	1.21E-05	4.26E-07	2.89E-07	2.67E-06	9.59E-06	1.43E-05	0.000301	9.14E-05	3.89E-05	0.000231	3.46E-06	7.33E-05	0.000178	0	0.000153
Venlafaxine	2.99E-07	1.16E-06	0	2.19E-07	2.74E-07	2.23E-07	5.25E-07	2.85E-05	3.22E-06	3.7E-06	1.41E-05	2.53E-07	4.53E-07	8.32E-07	6.79E-07	4.04E-06
Total Pharmac	3E-06	1.95E-05	5.62E-07	8.6E-07	3.43E-06	1.38E-05	1.99E-05	0.000449	0.00016	6.59E-05	0.000365	6.51E-06	0.00015	0.000313	2.06E-06	0.000221
Amoxicillin	0	0	0	0	0	0	0	4.35E-08	0	0	7.1E-09	0	0	1.51E-07	0	0
Azithromycin	6.44E-07	3.53E-08	4.36E-08	1.8E-07	1.23E-07	1.67E-07	5.25E-08	2.87E-05	1.31E-07	9.08E-08	4.62E-07	9.23E-07	2.72E-07	5.67E-06	1.49E-07	4E-08
Ciprofloxacin	1.03E-06	5.54E-07	0	0	0	3.4E-07	7.82E-07	0.000117	2.1E-06	3.34E-06	6.25E-06	6.33E-07	1.3E-05	3.6E-05	2.01E-06	1.84E-06
Erythromycin	0	4.83E-06	0	0	7.33E-06	1.33E-06	0.000002	2.03E-05	8.67E-05	0.000105	0.000296	4.5E-06	0.000027	2.42E-05	0.000004	3.02E-05
Lincomycin	7.14E-07	1.29E-06	0	0	7.14E-07	0	0	1.34E-05	0.000158	1.49E-05	7.31E-05	1.71E-06	0.000002	2.14E-06	0	5.41E-05
Metronidazole	0	0	0	0	0	0	6.5E-08	5.27E-07	1.4E-06	7.63E-07	3.27E-06	0	1.68E-08	4.23E-08	0	3.07E-07
Sulfamethoxazole	1.04E-08	4.59E-08	0	0	1.76E-08	3.8E-09	3.96E-08	5.96E-05	4.91E-07	1.04E-06	1.65E-06	2.21E-08	8.1E-08	1.34E-07	2.8E-09	8.98E-07
Trimethoprim	0	2.2E-09	0	0	4E-09	0	3E-09	1.29E-05	2.25E-07	2.58E-07	9.96E-07	7E-10	4.9E-08	8.49E-08	1.4E-09	1.88E-07
Tylosin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Antibiotics	2.4E-06	6.76E-06	4.36E-08	1.8E-07	8.19E-06	1.84E-06	2.94E-06	0.000253	0.000249	0.000125	0.000382	7.79E-06	4.25E-05	6.84E-05	6.16E-06	8.76E-05
Estradiol, 17-beta-(E2)	0	0	0	0	0	0	0	0	0	0	3.32E-07	0	0	2.56E-07	0	0
Estrone	2.62E-09	0	0	0	1.38E-09	7.85E-09	5.54E-09	4.92E-08	7.08E-08	1.58E-08	2.65E-07	1E-08	2.42E-08	6.08E-08	2.15E-09	1.74E-08
Progesterone	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Testosterone	0	0	0	0	0	0	0	0	0	0	0	0	0	2.88E-07	0	0
Total Estrogens	2.62E-09	0	0	0	1.38E-09	7.85E-09	5.54E-09	4.92E-08	7.08E-08	1.58E-08	5.97E-07	1E-08	2.42E-08	6.04E-07	2.15E-09	1.74E-08
Amphetamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caffeine	1.67E-07	4.31E-07	1.14E-07	8.58E-08	3.32E-07	1.77E-06	2.04E-06	5.87E-05	1.07E-05	1.34E-06	4.07E-06	2.36E-07	3.22E-06	8.17E-06	1.24E-07	6.24E-06
Nicotine	6.05E-08	2.83E-07	1.57E-07	6.21E-08	1.25E-07	4.24E-07	4.07E-07	4.97E-06	1.37E-06	3.47E-07	8.5E-07	1.48E-07	1.9E-06	5.54E-06	5.86E-08	4.85E-07
Paraxantine	6.22E-08	5.75E-06	3.35E-08	2.2E-08	1.67E-07	1.25E-05	1.21E-05	0.000576	7.88E-05	8.1E-06	5.59E-05	1.34E-06	2.72E-05	6.78E-05	5.69E-07	0.000113
Total LC	2.89E-07	6.47E-06	3.05E-07	1.7E-07	6.24E-07	1.47E-05	1.45E-05	0.00064	9.09E-05	9.79E-06	6.08E-05	1.72E-06	3.23E-05	8.15E-05	7.51E-07	0.000119
Tributyl-phosphate	0	1E-05	0.000138	7.72E-05	0.000169	2.38E-05	7.05E-06	5.52E-06	0.000179	2.28E-05	2.82E-05	3.59E-06	2.32E-06	3.5E-06	2.94E-06	8.34E-06
Total Industrial	0	1E-05	0.000138	7.72E-05	0.000169	2.38E-05	7.05E-06	5.52E-06	0.000179	2.28E-05	2.82E-05	3.59E-06	2.32E-06	3.5E-06	2.94E-06	8.34E-06
Total PSC	5.69E-06	4.27E-05	0.000139	7.84E-05	0.000181	5.4E-05	4.44E-05	0.001346	0.00068	0.000224	0.000837	1.96E-05	0.000227	0.000467	1.19E-05	0.000436

Table S5 (cont.)

Spring	Toxic Units (C/EC50) Daphnia															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Acetaminophen	6.75E-08	4.04E-07	3.29E-07	1.5E-07	6.4E-07	3.73E-05	5.38E-06	2.49E-06	3.48E-06	3.74E-05	1.28E-06	3.73E-07	0.000219	0.000221	3.85E-07	6.31E-07
Atenolol	3.1E-09	2.53E-08	5.3E-09	0	1.59E-08	6.13E-07	4.41E-08	3.05E-06	9.17E-07	1.61E-06	2.23E-06	1.68E-08	2.64E-07	5.24E-07	1.64E-08	8.68E-07
Carbamazepine	1.8E-09	1.34E-08	4.4E-09	6E-10	8.2E-09	4.12E-08	2.98E-08	1.78E-07	3.42E-06	1.28E-07	1.27E-06	7.28E-08	9.24E-08	1.03E-07	2.65E-08	3.14E-07
Citalopram	6.3E-06	2.48E-07	0	2.75E-08	2.08E-06	6.43E-07	6.88E-07	4.4E-06	2.06E-06	1.18E-06	3.18E-06	9.8E-07	1.46E-06	1.82E-06	5.98E-07	6.43E-06
Diclofenac	0	2.94E-08	0	0	0	3.6E-08	5.91E-08	1.17E-06	8.48E-07	1.18E-06	4.29E-06	1.95E-08	1.78E-07	3.09E-07	0	9.31E-07
Gemfibrocil	0	6.71E-07	0	0	1.44E-07	4.63E-07	1.29E-06	0.000063	2.89E-05	0.000044	0.000114	1.4E-07	1.86E-06	1.04E-05	1.43E-08	3.11E-05
Ibuprofen	2.46E-08	5.28E-08	2.91E-08	3.26E-08	6.37E-08	1.21E-06	2.25E-07	2.31E-06	9.07E-06	3.64E-06	1.27E-06	1.47E-08	3.26E-06	8.44E-06	1.61E-08	1.44E-07
Ketoprofen	0	0	0	0	0	3.06E-08	0	9.61E-07	1.32E-07	2.45E-07	1.78E-06	0	3.58E-08	3.75E-08	0	2.69E-08
Loratadine	0	0	0	0	0	0	0	6.44E-06	0	0	0	2.45E-06	0	0	0	0
Naproxen	0	2.6E-08	0	0	0	8.36E-07	4.86E-07	6.18E-06	3.37E-06	0	6.85E-06	0	0	5.8E-06	0	0
Omeprazole	0	0	0	0	0	0	0	1.79E-06	9.3E-09	1.77E-08	4.64E-08	7.5E-09	0	2.1E-09	0	0
Salbutamol	0	6E-10	1E-09	0	0	7E-10	1.4E-09	3.82E-08	2.82E-08	3.55E-08	8.91E-08	0	6.4E-08	3.9E-09	0	4.12E-08
Valsarten	2.93E-07	3.04E-06	0	0	1.33E-06	2.55E-06	7.48E-06	0.000145	6.97E-05	8.32E-05	0.000225	2.63E-06	3.99E-05	5.62E-05	0	6.52E-05
Venlafaxine	0	3.62E-07	0	0	5.04E-07	0	4.12E-07	6.14E-05	7.01E-06	3.74E-06	1.61E-05	1.06E-06	4.3E-07	1.99E-06	0	2.39E-06
Total Pharmac	6.69E-06	4.88E-06	3.69E-07	2.11E-07	4.78E-06	4.37E-05	1.61E-05	0.000298	0.000129	0.000176	0.000377	7.76E-06	0.000266	0.000307	1.06E-06	0.000108
Amoxicillin	0	0	0	0	0	0	0	3.62E-08	7.9E-09	0	0	0	0	0	0	0
Azithromycin	1.11E-07	8.53E-08	0	0	6.24E-07	1.24E-07	6.8E-08	1.95E-06	0	6.76E-08	7.47E-08	2.92E-07	4.02E-07	1.34E-07	5.41E-08	7.69E-08
Ciprofloxacin	2.1E-08	1.82E-08	0	0	4.73E-08	3.84E-08	5.86E-08	3.94E-06	8.82E-08	1.33E-07	1.65E-07	2.04E-07	3.98E-08	8.03E-08	4.57E-08	9.78E-08
Erythromycin	0	2E-09	0	0	2E-09	8E-10	1.9E-09	4.92E-08	5.18E-08	2.82E-08	1.69E-07	8E-09	9E-10	4.8E-09	0	9.3E-09
Lincomycin	5.56E-09	0	0	0	0	0	0	1.33E-07	4.99E-07	2.42E-07	5.79E-07	2.92E-08	3.47E-08	3.47E-08	0	2.06E-07
Metronidazole	0	0	0	0	0	0	0	5.07E-07	2.86E-07	2.63E-07	1.21E-06	0	2.71E-08	8.77E-07	0	4.85E-08
Sulfamethoxazole	5.8E-09	2.07E-08	0	0	7.6E-09	1.71E-07	4.59E-08	1.45E-06	8.23E-07	3.72E-07	1.89E-06	4.05E-08	5.7E-08	1.07E-07	2.9E-09	3.65E-07
Trimethoprim	1.6E-09	3.4E-09	9E-10	5.4E-09	3.8E-09	1.5E-09	2.6E-09	4.75E-07	2.09E-07	2.19E-07	8.71E-07	4E-09	2.39E-08	4.42E-08	5.7E-09	1.32E-07
Tylosin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Antibiotics	1.45E-07	1.3E-07	9E-10	5.4E-09	6.84E-07	3.36E-07	1.77E-07	8.54E-06	1.96E-06	1.32E-06	4.96E-06	5.78E-07	5.85E-07	1.28E-06	1.08E-07	9.35E-07
Estradiol, 17-beta-(E2)	0	0	0	0	0	0	0	8.28E-08	0	0	1.38E-07	0	0	1.41E-07	0	0
Estrone	1.41E-09	3.38E-09	1.21E-08	0	0	2.82E-09	4.23E-09	7.42E-08	7.34E-08	4.56E-08	7.87E-08	8.59E-09	1.87E-08	4.1E-08	2.82E-09	6.2E-09
Progesterone	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Testosterone	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Estrogens	1.41E-09	3.38E-09	1.21E-08	0	0	2.82E-09	4.23E-09	1.57E-07	7.34E-08	4.56E-08	2.17E-07	8.59E-09	1.87E-08	1.82E-07	2.82E-09	6.2E-09
Amphetamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caffeine	2.06E-07	4.81E-07	1.16E-07	6.38E-08	2.96E-07	2.93E-06	1.36E-06	4.24E-06	3.53E-06	9.37E-06	4.52E-06	1.14E-06	3.51E-06	7.06E-06	5.96E-08	1.17E-06
Nicotine	5.67E-07	2.21E-06	3.92E-07	1.33E-07	6.2E-07	1.07E-06	4.94E-07	6.65E-07	7.75E-07	1.03E-06	1.07E-06	1.66E-08	0.000003	3.23E-06	2.63E-08	7.44E-08
Paraxantine	6.67E-08	3.3E-06	2.81E-08	2.37E-08	1.75E-07	2.45E-05	8.93E-06	4.28E-05	2.62E-05	8.18E-05	4.04E-05	3.82E-06	2.07E-05	6.58E-05	1.48E-07	1.59E-05
Total LC	8.4E-07	5.99E-06	5.36E-07	2.21E-07	1.09E-06	2.85E-05	1.08E-05	4.77E-05	3.05E-05	9.22E-05	4.6E-05	4.98E-06	2.72E-05	7.61E-05	2.34E-07	1.71E-05
Tributyl-phosphate	0.00024	5.38E-05	0.00012	0.000138	0.000291	2.76E-05	6.54E-05	6.81E-06	0.000122	2.08E-05	3.65E-05	6.27E-06	6.86E-06	1.76E-06	3.22E-06	8.22E-06
Total Industrial	0.00024	5.38E-05	0.00012	0.000138	0.000291	2.76E-05	6.54E-05	6.81E-06	0.000122	2.08E-05	3.65E-05	6.27E-06	6.86E-06	1.76E-06	3.22E-06	8.22E-06
Total PSC	0.000248	6.48E-05	0.000121	0.000139	0.000297	0.0001	9.25E-05	0.000361	0.000284	0.000291	0.000465	1.96E-05	0.000301	0.000386	4.62E-06	0.000134

Table S5 (cont.)

Summer	Toxic Units (C/EC50) Daphnia															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Acetaminophen	0	0	1.95E-07	0	3.5E-07	1.41E-05	6.74E-07	1.91E-07	5.93E-07	3.92E-07	1.35E-06	1.3E-07	0.000419	0.000354	4.25E-08	1.96E-07
Atenolol	0	2.73E-08	0	0	2.13E-08	2.95E-07	5.88E-08	5.3E-06	1.02E-06	7.44E-07	4.39E-06	7.9E-09	8.57E-07	9.28E-07	0	5.45E-07
Carbamazepine	5.1E-09	1.35E-08	2.7E-09	8.84E-08	7.2E-09	2.53E-07	5.32E-08	4.98E-07	7.69E-07	1.2E-06	1.74E-06	9.27E-08	2.23E-07	2.33E-07	1.34E-07	4.51E-07
Citalopram	1.4E-07	1.25E-07	2E-08	6.75E-08	2.1E-07	1.1E-07	2.73E-07	4.55E-06	1.18E-06	1.08E-06	3.25E-06	2.58E-07	1.61E-06	4.75E-07	1.78E-07	5.78E-07
Diclofenac	0	3E-08	0	0	0	3.26E-07	4.08E-08	0	1.07E-06	3.46E-06	4.92E-06	2.44E-08	7.43E-07	8.82E-07	0	4.41E-07
Gemfibrocil	0	5.06E-07	0	0	1.44E-07	3.04E-06	3.09E-07	6.53E-05	6.39E-05	6.79E-06	0.000113	0	1.49E-05	1.77E-05	2.14E-08	1.68E-05
Ibuprofen	4.1E-09	6.6E-09	9.7E-09	9.3E-09	1.42E-08	2.41E-06	5.18E-08	2.04E-06	9.77E-06	6.45E-08	2.76E-05	1.37E-08	6.7E-06	1.13E-05	5.2E-09	4.41E-07
Ketoprofen	0	0	0	0	0	8.35E-08	0	2.77E-06	8.56E-08	3.14E-07	1.15E-06	0	3.99E-07	1.38E-07	0	0
Loratadine	0	0	0	0	0	0	0	2.62E-05	2.2E-06	0	0	0	0	0	0	0
Naproxen	0	0	0	0	0	2.89E-06	4.76E-07	7.38E-06	2.21E-06	0	5.99E-06	0	7.87E-06	1.3E-05	0	1.35E-06
Omeprazole	0	0	0	0	0	0	0	4.43E-07	3E-09	2.18E-08	3.36E-08	0	1.31E-08	3.9E-09	0	0
Salbutamol	0	5E-10	0	0	0	2E-09	1.5E-09	9.2E-08	5.28E-08	8.21E-08	1.02E-07	0	2.3E-09	6.2E-09	0	4.1E-08
Valsarten	2.31E-07	3.79E-06	4.07E-07	0	1.94E-06	1.54E-05	9.52E-06	0.000167	7.27E-05	2.86E-05	0.000146	4.85E-06	0.000334	0.000217	6.82E-07	5.54E-05
Venlafaxine	2.23E-07	4.36E-07	2.68E-07	2.76E-07	1.77E-07	5.36E-07	4.95E-07	0.000025	7.64E-06	6.06E-06	2.12E-05	1.82E-07	3.2E-06	3.38E-06	0	2.91E-06
Total Pharmac	6.03E-07	4.93E-06	9.02E-07	4.41E-07	2.86E-06	3.94E-05	1.2E-05	0.000307	0.000163	4.88E-05	0.00033	5.56E-06	0.00079	0.000619	1.06E-06	7.92E-05
Amoxicillin	0	0	0	0	0	0	0	2.63E-08	0	0	2.9E-09	0	0	0	0	4.3E-09
Azithromycin	2.75E-07	1.07E-07	0	1.49E-07	9.22E-09	5.35E-08	6.18E-08	1.89E-06	0	5.39E-08	5.98E-08	6.69E-08	2.18E-07	4.49E-08	1.78E-08	9.92E-08
Ciprofloxacin	5.43E-08	4.35E-08	0	2.86E-08	0	0	1.82E-08	4.56E-06	0	1.2E-07	2.18E-07	0	1.82E-06	7.99E-07	1.9E-08	8.59E-08
Erythromycin	0	1E-09	7E-10	0	5E-10	7E-10	1.1E-09	4.36E-08	3.84E-08	4.47E-08	1.07E-07	1.8E-09	1.9E-09	2.1E-09	8E-10	2.13E-08
Lincomycin	1.25E-08	0	0	0	0	0	0	3.07E-07	5.71E-07	3.29E-07	9.86E-07	0	0	1.94E-08	1.53E-08	2.38E-07
Metronidazole	0	0	0	0	0	0	0	2.47E-07	2.26E-07	9.56E-08	1.24E-06	0	2.88E-08	2.27E-08	0	3.36E-08
Sulfamethoxazole	5.8E-09	4.23E-08	0	0	7.4E-09	7.82E-07	4.84E-08	1.13E-06	3.47E-07	4.99E-06	1.24E-06	5.5E-08	2.94E-07	4.75E-07	1.22E-08	7.84E-07
Trimethoprim	0	0	0	7E-10	0	4.7E-09	1.8E-09	3.23E-06	2.51E-07	5.12E-07	1.16E-06	1E-09	1.51E-07	2.97E-07	0	1.02E-07
Tylosin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Antibiotics	3.47E-07	1.94E-07	7E-10	1.79E-07	1.71E-08	8.41E-07	1.31E-07	1.14E-05	1.43E-06	6.15E-06	5.01E-06	1.25E-07	2.52E-06	1.66E-06	6.51E-08	1.37E-06
Estradiol, 17-beta-(E2)	0	0	0	0	0	0	0	0	5.86E-08	0	1E-07	0	0	2.07E-07	0	0
Estrone	4.08E-09	0	0	0	0	3.52E-09	0	4.1E-08	5.38E-08	8.31E-09	1.04E-07	5.21E-09	4.52E-08	6.46E-08	0	6.76E-09
Progesterone	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Testosterone	0	0	0	0	0	0	0	0	0	0	0	0	4.15E-07	3.68E-07	0	0
Total Estrogens	4.08E-09	0	0	0	0	3.52E-09	0	4.1E-08	1.12E-07	8.31E-09	2.04E-07	5.21E-09	4.6E-07	6.4E-07	0	6.76E-09
Amphetamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caffeine	1.71E-07	9.95E-07	5.4E-07	5.95E-08	4.36E-07	4.61E-06	9.39E-07	4.65E-07	3.02E-06	4.24E-07	4.97E-06	1.94E-07	2.21E-05	2.41E-05	6.6E-07	7.1E-07
Nicotine	3.47E-08	6.9E-08	1.71E-07	3.61E-08	7.88E-08	2.12E-06	2.66E-07	4.46E-07	3.94E-07	3.49E-07	2.08E-07	1.46E-08	4.11E-06	5.99E-06	7.13E-08	9.24E-08
Paraxantine	5.87E-08	6.95E-06	2.93E-07	2.39E-08	2.55E-07	2.48E-05	5.94E-06	9.54E-06	2.08E-05	5.06E-06	4.92E-05	5.79E-07	6.68E-05	7.72E-05	2.19E-06	8.43E-06
Total LC	2.64E-07	8.01E-06	1E-06	1.2E-07	7.69E-07	3.16E-05	7.15E-06	1.05E-05	2.42E-05	5.83E-06	5.43E-05	7.88E-07	9.3E-05	0.000107	2.92E-06	9.23E-06
Tributyl-phosphate	1.42E-06	3.05E-06	2.21E-05	2.97E-05	2.2E-06	6.62E-06	1.17E-05	3.08E-06	1.34E-05	4.03E-06	5.92E-06	9.05E-07	1.03E-06	0	1.46E-06	2.49E-06
Total Industrial	1.42E-06	3.05E-06	2.21E-05	2.97E-05	2.2E-06	6.62E-06	1.17E-05	3.08E-06	1.34E-05	4.03E-06	5.92E-06	9.05E-07	1.03E-06	0	1.46E-06	2.49E-06
Total PSC	2.64E-06	1.62E-05	2.4E-05	3.05E-05	5.85E-06	7.84E-05	3.1E-05	0.000332	0.000202	6.48E-05	0.000395	7.38E-06	0.000887	0.000728	5.51E-06	9.23E-05

Table S5 (cont.)

Autumn	Toxic Units (C/EC50) Daphnia															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Acetaminophen	1.64E-07	1.7E-06	1.03E-07	4.63E-08	4.03E-07	6.55E-06	7.4E-06	4.69E-06	5.47E-06	1.17E-06	3.22E-06	3.03E-07	0.000365	0.000614	2.29E-07	1.61E-06
Atenolol	4.4E-08	3.25E-08	9.8E-09	0	1.41E-08	1.3E-07	2.73E-07	6.73E-06	1.37E-06	9.92E-07	3.97E-06	7.1E-09	2.92E-07	5.29E-07	4.5E-09	1.67E-06
Carbamazepine	4E-09	2.3E-08	2.3E-09	1.2E-09	1.24E-08	5.08E-08	7.99E-08	2.36E-07	5.78E-07	3.33E-07	1.25E-06	8.33E-08	1.19E-07	1.78E-07	1.57E-08	4.44E-07
Citalopram	1.11E-06	1.1E-06	0	1.65E-07	1.08E-07	2.6E-07	2.43E-07	3.03E-06	1.87E-06	1.01E-06	5.01E-06	1.27E-06	1.32E-06	1.98E-06	6.5E-07	5.75E-07
Diclofenac	0	8.98E-08	0	0	0	5.85E-08	1.13E-07	2.7E-06	1.21E-06	1.32E-06	5.06E-06	3.89E-08	1.44E-07	3.72E-07	0	2.61E-06
Gemfibrocil	0	3E-06	8E-08	0	1.39E-07	1.07E-06	1.2E-06	4.89E-05	0.000033	1.57E-05	7.87E-05	7.29E-08	5.72E-06	1.41E-05	1.57E-08	0.00004
Ibuprofen	5.2E-09	5.32E-08	9.3E-09	1.15E-08	1.49E-08	7.84E-07	5.31E-07	1.52E-05	8.28E-06	8.11E-08	1.14E-06	2.49E-08	5.53E-06	1.03E-05	8E-09	3.03E-06
Ketoprofen	0	4.32E-08	0	0	0	1.21E-07	3.18E-08	3.56E-06	1.52E-06	4.19E-07	1.71E-06	0	3.2E-07	6.45E-07	0	3.27E-07
Loratadine	0	0	0	0	0	0	0	6.31E-06	4.45E-06	0	0	0	0	0	0	0
Naproxen	0	0	0	0	0	0	1.19E-06	1.71E-05	3.76E-06	0	6.78E-06	0	2.45E-06	4.51E-06	0	9.87E-06
Omeprazole	5.5E-09	0	2.5E-09	2.2E-09	2.4E-09	8E-09	1.06E-08	3.92E-06	2.5E-08	0	5.39E-08	0	0	2.8E-09	0	1.37E-08
Salbutamol	0	1.2E-09	0	0	0	7E-10	2.9E-09	6.12E-08	3.11E-08	5.38E-08	7.99E-08	0	1E-09	3.2E-09	0	7.99E-08
Valsarten	3.23E-07	9.67E-06	3.41E-07	2.31E-07	2.13E-06	7.68E-06	1.15E-05	0.000241	7.32E-05	3.11E-05	0.000185	2.77E-06	5.86E-05	0.000142	0	0.000122
Venlafaxine	3.59E-07	1.39E-06	0	2.63E-07	3.29E-07	2.67E-07	6.3E-07	3.42E-05	3.86E-06	4.44E-06	1.69E-05	3.04E-07	5.44E-07	9.98E-07	8.15E-07	4.85E-06
Total Pharmac	2.01E-06	1.71E-05	5.48E-07	7.2E-07	3.16E-06	1.7E-05	2.32E-05	0.000388	0.000139	5.66E-05	0.000309	4.87E-06	0.00044	0.00079	1.74E-06	0.000187
Amoxicillin	0	0	0	0	0	0	0	4.35E-08	0	0	7.1E-09	0	0	1.51E-07	0	0
Azithromycin	4.55E-07	2.49E-08	3.08E-08	1.27E-07	8.71E-08	1.18E-07	3.71E-08	2.02E-05	9.27E-08	6.41E-08	3.26E-07	6.52E-07	1.92E-07	0.000004	1.05E-07	2.82E-08
Ciprofloxacin	6.92E-08	3.71E-08	0	0	0	2.28E-08	5.24E-08	7.86E-06	1.41E-07	2.24E-07	4.19E-07	4.24E-08	8.74E-07	2.41E-06	1.34E-07	1.24E-07
Erythromycin	0	2.9E-09	0	0	4.4E-09	8E-10	1.2E-09	1.22E-08	5.2E-08	6.3E-08	1.78E-07	2.7E-09	1.62E-08	1.45E-08	2.4E-09	1.81E-08
Lincomycin	6.94E-09	1.25E-08	0	0	6.94E-09	0	0	1.31E-07	1.54E-06	1.44E-07	7.11E-07	1.67E-08	1.94E-08	2.08E-08	0	5.26E-07
Metronidazole	0	0	0	0	0	0	2.6E-08	2.11E-07	5.61E-07	3.05E-07	1.31E-06	0	6.7E-09	1.69E-08	0	1.23E-07
Sulfamethoxazole	1.04E-08	4.59E-08	0	0	1.76E-08	3.8E-09	3.96E-08	5.96E-05	4.91E-07	1.04E-06	1.65E-06	2.21E-08	8.1E-08	1.34E-07	2.8E-09	8.98E-07
Trimethoprim	0	2.2E-09	0	0	4E-09	0	3E-09	1.29E-05	2.25E-07	2.58E-07	9.96E-07	7E-10	4.9E-08	8.49E-08	1.4E-09	1.88E-07
Tylosin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Antibiotics	5.41E-07	1.26E-07	3.08E-08	1.27E-07	1.2E-07	1.45E-07	1.59E-07	0.000101	3.1E-06	2.1E-06	5.6E-06	7.36E-07	1.24E-06	6.83E-06	2.46E-07	1.91E-06
Estradiol, 17-beta-(E2)	0	0	0	0	0	0	0	0	0	0	2.86E-07	0	0	2.21E-07	0	0
Estrone	2.39E-09	0	0	0	1.27E-09	7.18E-09	5.07E-09	4.51E-08	6.48E-08	1.45E-08	2.43E-07	9.15E-09	2.21E-08	5.56E-08	1.97E-09	1.59E-08
Progesterone	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Testosterone	0	0	0	0	0	0	0	0	0	0	0	0	0	2.3E-07	0	0
Total Estrogens	2.39E-09	0	0	0	1.27E-09	7.18E-09	5.07E-09	4.51E-08	6.48E-08	1.45E-08	5.29E-07	9.15E-09	2.21E-08	5.06E-07	1.97E-09	1.59E-08
Amphetamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caffeine	1.67E-07	4.31E-07	1.14E-07	8.58E-08	3.32E-07	1.77E-06	2.04E-06	5.87E-05	1.07E-05	1.34E-06	4.07E-06	2.36E-07	3.22E-06	8.17E-06	1.24E-07	6.24E-06
Nicotine	6.05E-08	2.83E-07	1.57E-07	6.21E-08	1.25E-07	4.24E-07	4.07E-07	4.97E-06	1.37E-06	3.47E-07	8.5E-07	1.48E-07	1.9E-06	5.54E-06	5.86E-08	4.85E-07
Paraxantine	6.22E-08	5.75E-06	3.35E-08	2.2E-08	1.67E-07	1.25E-05	1.21E-05	0.000576	7.88E-05	8.1E-06	5.59E-05	1.34E-06	2.72E-05	6.78E-05	5.69E-07	0.000113
Total LC	2.89E-07	6.47E-06	3.05E-07	1.7E-07	6.24E-07	1.47E-05	1.45E-05	0.00064	9.09E-05	9.79E-06	6.08E-05	1.72E-06	3.23E-05	8.15E-05	7.51E-07	0.000119
Tributyl-phosphate	0	4.87E-06	6.73E-05	3.76E-05	8.22E-05	1.16E-05	3.43E-06	2.69E-06	8.73E-05	1.11E-05	1.37E-05	1.75E-06	1.13E-06	1.7E-06	1.43E-06	4.06E-06
Total Industrial	0	4.87E-06	6.73E-05	3.76E-05	8.22E-05	1.16E-05	3.43E-06	2.69E-06	8.73E-05	1.11E-05	1.37E-05	1.75E-06	1.13E-06	1.7E-06	1.43E-06	4.06E-06
Total PSC	2.84E-06	2.86E-05	6.82E-05	3.86E-05	8.61E-05	4.33E-05	4.12E-05	0.001131	0.00032	7.96E-05	0.00039	9.08E-06	0.000475	0.000881	4.17E-06	0.000312

Table S5 (cont.)

Spring	Toxic Units (C/EC50) Fish															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Acetaminophen	1.08E-08	6.46E-08	5.27E-08	2.4E-08	1.02E-07	5.96E-06	8.61E-07	3.98E-07	5.56E-07	5.98E-06	2.04E-07	5.96E-08	3.5E-05	3.54E-05	6.16E-08	1.01E-07
Atenolol	3.1E-09	2.53E-08	5.3E-09	0	1.59E-08	6.13E-07	4.41E-08	3.05E-06	9.17E-07	1.61E-06	2.23E-06	1.68E-08	2.64E-07	5.24E-07	1.64E-08	8.68E-07
Carbamazepine	9E-09	6.7E-08	2.2E-08	3E-09	4.1E-08	2.06E-07	1.49E-07	8.9E-07	1.71E-05	6.4E-07	6.35E-06	3.64E-07	4.62E-07	5.15E-07	1.33E-07	1.57E-06
Citalopram	3.6E-06	1.41E-07	0	1.57E-08	1.19E-06	3.67E-07	3.93E-07	2.51E-06	1.18E-06	6.73E-07	1.81E-06	5.6E-07	8.34E-07	1.04E-06	3.41E-07	3.67E-06
Diclofenac	0	3.61E-08	0	0	0	4.41E-08	7.24E-08	1.44E-06	1.04E-06	1.45E-06	5.25E-06	2.39E-08	2.18E-07	3.79E-07	0	1.14E-06
Gemfibrocil	0	3.62E-07	0	0	7.77E-08	2.49E-07	6.93E-07	3.39E-05	1.55E-05	2.37E-05	6.14E-05	7.54E-08	0.000001	5.57E-06	7.69E-09	1.68E-05
Ibuprofen	2.46E-08	5.28E-08	2.91E-08	3.26E-08	6.37E-08	1.21E-06	2.25E-07	2.31E-06	9.07E-06	3.64E-06	1.27E-06	1.47E-08	3.26E-06	8.44E-06	1.61E-08	1.44E-07
Ketoprofen	0	0	0	0	0	3.06E-08	0	9.61E-07	1.32E-07	2.45E-07	1.78E-06	0	3.58E-08	3.75E-08	0	2.69E-08
Loratadine	0	0	0	0	0	0	0	3.22E-06	0	0	0	1.23E-06	0	0	0	0
Naproxen	0	1.12E-07	0	0	0	3.61E-06	2.1E-06	2.67E-05	1.45E-05	0	2.96E-05	0	0	2.51E-05	0	0
Omeprazole	0	0	0	0	0	0	0	5.77E-06	3E-08	5.71E-08	1.5E-07	2.42E-08	0	6.77E-09	0	0
Salbutamol	0	6E-10	1E-09	0	0	7E-10	1.4E-09	3.82E-08	2.82E-08	3.55E-08	8.91E-08	0	6.4E-08	3.9E-09	0	4.12E-08
Valsarten	1.54E-07	1.6E-06	0	0	7.01E-07	1.34E-06	3.94E-06	7.61E-05	3.67E-05	4.38E-05	0.000118	1.38E-06	0.000021	2.96E-05	0	3.43E-05
Venlafaxine	0	2.26E-07	0	0	3.15E-07	0	2.58E-07	3.84E-05	4.38E-06	2.34E-06	1.01E-05	6.63E-07	2.69E-07	1.24E-06	0	1.49E-06
Total Pharmac	3.8E-06	2.69E-06	1.1E-07	7.53E-08	2.5E-06	1.36E-05	8.73E-06	0.000196	0.000101	8.41E-05	0.000239	4.41E-06	6.24E-05	0.000108	5.76E-07	6.01E-05
Amoxicillin	0	0	0	0	0	0	0	3.62E-08	7.9E-09	0	0	0	0	0	0	0
Azithromycin	1.2E-07	9.26E-08	0	0	6.77E-07	1.35E-07	7.38E-08	2.12E-06	0	7.34E-08	8.11E-08	3.17E-07	4.36E-07	1.45E-07	5.87E-08	8.34E-08
Ciprofloxacin	2.1E-08	1.82E-08	0	0	4.73E-08	3.84E-08	5.86E-08	3.94E-06	8.82E-08	1.33E-07	1.65E-07	2.04E-07	3.98E-08	8.03E-08	4.57E-08	9.78E-08
Erythromycin	0	2E-09	0	0	2E-09	8E-10	1.9E-09	4.92E-08	5.18E-08	2.82E-08	1.69E-07	8E-09	9E-10	4.8E-09	0	9.3E-09
Lincomycin	4E-10	0	0	0	0	0	0	9.6E-09	3.59E-08	1.74E-08	4.17E-08	2.1E-09	2.5E-09	2.5E-09	0	1.48E-08
Metronidazole	0	0	0	0	0	0	0	5.07E-07	2.86E-07	2.63E-07	1.21E-06	0	2.71E-08	8.77E-07	0	4.85E-08
Sulfamethoxazole	5.8E-09	2.07E-08	0	0	7.6E-09	1.71E-07	4.59E-08	1.45E-06	8.23E-07	3.72E-07	1.89E-06	4.05E-08	5.7E-08	1.07E-07	2.9E-09	3.65E-07
Trimethoprim	1.6E-09	3.4E-09	9E-10	5.4E-09	3.8E-09	1.5E-09	2.6E-09	4.75E-07	2.09E-07	2.19E-07	8.71E-07	4E-09	2.39E-08	4.42E-08	5.7E-09	1.32E-07
Tylosin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Antibiotics	1.49E-07	1.37E-07	9E-10	5.4E-09	7.37E-07	3.46E-07	1.83E-07	8.59E-06	1.5E-06	1.11E-06	4.43E-06	5.76E-07	5.87E-07	1.26E-06	1.13E-07	7.51E-07
Estradiol, 17-beta-(E2)	0	0	0	0	0	0	0	6.86E-08	0	0	1.14E-07	0	0	1.17E-07	0	0
Estrone	1E-09	2.4E-09	8.6E-09	0	0	2E-09	3E-09	5.27E-08	5.21E-08	3.24E-08	5.59E-08	6.1E-09	1.33E-08	2.91E-08	2E-09	4.4E-09
Progesterone	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Testosterone	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Estrogens	1E-09	2.4E-09	8.6E-09	0	0	2E-09	3E-09	1.21E-07	5.21E-08	3.24E-08	1.7E-07	6.1E-09	1.33E-08	1.46E-07	2E-09	4.4E-09
Amphetamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caffeine	2.06E-07	4.81E-07	1.16E-07	6.38E-08	2.96E-07	2.93E-06	1.36E-06	4.24E-06	3.53E-06	9.37E-06	4.52E-06	1.14E-06	3.51E-06	7.06E-06	5.96E-08	1.17E-06
Nicotine	1.42E-05	5.53E-05	9.8E-06	3.33E-06	1.55E-05	2.68E-05	1.24E-05	1.66E-05	1.94E-05	2.58E-05	2.68E-05	4.15E-07	0.000075	8.08E-05	6.58E-07	1.86E-06
Paraxantine	6.67E-08	3.3E-06	2.81E-08	2.37E-08	1.75E-07	2.45E-05	8.93E-06	4.28E-05	2.62E-05	8.18E-05	4.04E-05	3.82E-06	2.07E-05	6.58E-05	1.48E-07	1.59E-05
Total LC	1.44E-05	5.9E-05	9.94E-06	3.41E-06	1.6E-05	5.42E-05	2.26E-05	6.37E-05	4.91E-05	0.000117	7.17E-05	5.38E-06	9.92E-05	0.000154	8.65E-07	1.89E-05
Tributyl-phosphate	0.000111	2.49E-05	5.56E-05	6.4E-05	0.000134	1.28E-05	3.03E-05	3.15E-06	5.66E-05	9.64E-06	1.69E-05	2.9E-06	3.18E-06	8.16E-07	1.49E-06	3.8E-06
Total Industrial	0.000111	2.49E-05	5.56E-05	6.4E-05	0.000134	1.28E-05	3.03E-05	3.15E-06	5.66E-05	9.64E-06	1.69E-05	2.9E-06	3.18E-06	8.16E-07	1.49E-06	3.8E-06
Total PSC	0.00013	8.67E-05	6.57E-05	6.75E-05	0.000154	8.09E-05	6.18E-05	0.000271	0.000208	0.000212	0.000332	1.33E-05	0.000165	0.000264	3.04E-06	8.36E-05

Table S5 (cont.)

Summer	Toxic Units (C/EC50) Fish															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Acetaminophen	0	0	3.12E-08	0	5.6E-08	2.25E-06	1.08E-07	3.06E-08	9.49E-08	6.27E-08	2.16E-07	2.08E-08	6.71E-05	5.66E-05	6.8E-09	3.13E-08
Atenolol	0	2.73E-08	0	0	2.13E-08	2.95E-07	5.88E-08	5.3E-06	1.02E-06	7.44E-07	4.39E-06	7.9E-09	8.57E-07	9.28E-07	0	5.45E-07
Carbamazepine	2.55E-08	6.75E-08	1.35E-08	4.42E-07	3.6E-08	1.27E-06	2.66E-07	2.49E-06	3.85E-06	5.98E-06	8.71E-06	4.64E-07	1.12E-06	1.17E-06	6.7E-07	2.26E-06
Citalopram	8E-08	7.14E-08	1.14E-08	3.86E-08	1.2E-07	6.29E-08	1.56E-07	2.6E-06	6.73E-07	6.16E-07	1.86E-06	1.47E-07	9.21E-07	2.71E-07	1.01E-07	3.3E-07
Diclofenac	0	3.68E-08	0	0	0	4E-07	5E-08	0	1.31E-06	4.24E-06	6.03E-06	2.99E-08	9.1E-07	1.08E-06	0	5.41E-07
Gemfibrocil	0	2.72E-07	0	0	7.77E-08	1.64E-06	1.66E-07	3.52E-05	3.44E-05	3.65E-06	6.07E-05	0	0.000008	9.54E-06	1.15E-08	9.05E-06
Ibuprofen	4.1E-09	6.6E-09	9.7E-09	9.3E-09	1.42E-08	2.41E-06	5.18E-08	2.04E-06	9.77E-06	6.45E-08	2.76E-05	1.37E-08	6.7E-06	1.13E-05	5.2E-09	4.41E-07
Ketoprofen	0	0	0	0	0	8.35E-08	0	2.77E-06	8.56E-08	3.14E-07	1.15E-06	0	3.99E-07	1.38E-07	0	0
Loratadine	0	0	0	0	0	0	0	1.31E-05	1.1E-06	0	0	0	0	0	0	0
Naproxen	0	0	0	0	0	1.25E-05	2.05E-06	3.18E-05	9.53E-06	0	2.58E-05	0	3.39E-05	0.000056	0	5.84E-06
Omeprazole	0	0	0	0	0	0	0	1.43E-06	9.68E-09	7.03E-08	1.08E-07	0	4.23E-08	1.26E-08	0	0
Salbutamol	0	5E-10	0	0	0	2E-09	1.5E-09	9.2E-08	5.28E-08	8.21E-08	1.02E-07	0	2.3E-09	6.2E-09	0	4.1E-08
Valsarten	1.22E-07	1.99E-06	2.14E-07	0	1.02E-06	8.11E-06	5.01E-06	8.78E-05	3.83E-05	1.51E-05	7.66E-05	2.55E-06	0.000176	0.000114	3.59E-07	2.92E-05
Venlafaxine	1.39E-07	2.73E-07	1.68E-07	1.73E-07	1.11E-07	3.35E-07	3.09E-07	1.56E-05	4.78E-06	3.79E-06	1.32E-05	1.14E-07	0.000002	2.11E-06	0	1.82E-06
Total Pharmac	3.71E-07	2.75E-06	4.48E-07	6.62E-07	1.46E-06	2.93E-05	8.23E-06	0.0002	0.000105	3.47E-05	0.000227	3.35E-06	0.000298	0.000253	1.15E-06	5.01E-05
Amoxicillin	0	0	0	0	0	0	0	2.63E-08	0	0	2.9E-09	0	0	0	0	4.3E-09
Azithromycin	2.98E-07	1.16E-07	0	1.62E-07	1E-08	5.81E-08	6.7E-08	2.05E-06	0	5.85E-08	6.49E-08	7.26E-08	2.36E-07	4.87E-08	1.94E-08	1.08E-07
Ciprofloxacin	5.43E-08	4.35E-08	0	2.86E-08	0	0	1.82E-08	4.56E-06	0	1.2E-07	2.18E-07	0	1.82E-06	7.99E-07	1.9E-08	8.59E-08
Erythromycin	0	1E-09	7E-10	0	5E-10	7E-10	1.1E-09	4.36E-08	3.84E-08	4.47E-08	1.07E-07	1.8E-09	1.9E-09	2.1E-09	8E-10	2.13E-08
Lincomycin	9E-10	0	0	0	0	0	0	2.21E-08	4.11E-08	2.37E-08	7.1E-08	0	0	1.4E-09	1.1E-09	1.71E-08
Metronidazole	0	0	0	0	0	0	0	2.47E-07	2.26E-07	9.56E-08	1.24E-06	0	2.88E-08	2.27E-08	0	3.36E-08
Sulfamethoxazole	5.8E-09	4.23E-08	0	0	7.4E-09	7.82E-07	4.84E-08	1.13E-06	3.47E-07	4.99E-06	1.24E-06	5.5E-08	2.94E-07	4.75E-07	1.22E-08	7.84E-07
Trimethoprim	0	0	0	7E-10	0	4.7E-09	1.8E-09	3.23E-06	2.51E-07	5.12E-07	1.16E-06	1E-09	1.51E-07	2.97E-07	0	1.02E-07
Tylosin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Antibiotics	3.59E-07	2.03E-07	7E-10	1.91E-07	1.79E-08	8.45E-07	1.37E-07	1.13E-05	9.04E-07	5.84E-06	4.1E-06	1.3E-07	2.54E-06	1.65E-06	5.25E-08	1.16E-06
Estradiol, 17-beta-(E2)	0	0	0	0	0	0	0	0	4.86E-08	0	8.29E-08	0	0	1.71E-07	0	0
Estrone	2.9E-09	0	0	0	0	2.5E-09	0	2.91E-08	3.82E-08	5.9E-09	7.4E-08	3.7E-09	3.21E-08	4.59E-08	0	4.8E-09
Progesterone	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Testosterone	0	0	0	0	0	0	0	0	0	0	0	0	2.31E-07	2.04E-07	0	0
Total Estrogens	2.9E-09	0	0	0	0	2.5E-09	0	2.91E-08	8.68E-08	5.9E-09	1.57E-07	3.7E-09	2.63E-07	4.22E-07	0	4.8E-09
Amphetamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caffeine	1.71E-07	9.95E-07	5.4E-07	5.95E-08	4.36E-07	4.61E-06	9.39E-07	4.65E-07	3.02E-06	4.24E-07	4.97E-06	1.94E-07	2.21E-05	2.41E-05	6.6E-07	7.1E-07
Nicotine	8.68E-07	1.73E-06	4.28E-06	9.03E-07	1.97E-06	5.31E-05	6.65E-06	1.12E-05	9.85E-06	8.73E-06	5.2E-06	3.65E-07	0.000103	0.00015	1.78E-06	2.31E-06
Paraxantine	5.87E-08	6.95E-06	2.93E-07	2.39E-08	2.55E-07	2.48E-05	5.94E-06	9.54E-06	2.08E-05	5.06E-06	4.92E-05	5.79E-07	6.68E-05	7.72E-05	2.19E-06	8.43E-06
Total LC	1.1E-06	9.67E-06	5.11E-06	9.86E-07	2.66E-06	8.25E-05	1.35E-05	2.12E-05	3.36E-05	1.42E-05	5.93E-05	1.14E-06	0.000192	0.000251	4.63E-06	1.15E-05
Tributyl-phosphate	6.59E-07	1.41E-06	1.02E-05	1.38E-05	1.02E-06	3.06E-06	5.43E-06	1.43E-06	6.18E-06	1.86E-06	2.74E-06	4.19E-07	4.75E-07	0	6.76E-07	1.15E-06
Total Industrial	6.59E-07	1.41E-06	1.02E-05	1.38E-05	1.02E-06	3.06E-06	5.43E-06	1.43E-06	6.18E-06	1.86E-06	2.74E-06	4.19E-07	4.75E-07	0	6.76E-07	1.15E-06
Total PSC	2.49E-06	1.4E-05	1.58E-05	1.56E-05	5.15E-06	0.000116	2.73E-05	0.000234	0.000146	5.66E-05	0.000293	5.04E-06	0.000493	0.000506	6.52E-06	6.38E-05

Table S5 (cont.)

Autumn	Toxic Units (C/EC50) Fish															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Acetaminophen	2.63E-08	2.71E-07	1.65E-08	7.4E-09	6.45E-08	1.05E-06	1.18E-06	7.5E-07	8.76E-07	1.87E-07	5.15E-07	4.85E-08	5.84E-05	9.83E-05	3.67E-08	2.58E-07
Atenolol	4.4E-08	3.25E-08	9.8E-09	0	1.41E-08	1.3E-07	2.73E-07	6.73E-06	1.37E-06	9.92E-07	3.97E-06	7.1E-09	2.92E-07	5.29E-07	4.5E-09	1.67E-06
Carbamazepine	2E-08	1.15E-07	1.15E-08	6E-09	6.2E-08	2.54E-07	4E-07	1.18E-06	2.89E-06	1.66E-06	6.25E-06	4.17E-07	5.93E-07	8.92E-07	7.85E-08	2.22E-06
Citalopram	6.31E-07	6.29E-07	0	9.43E-08	6.14E-08	1.49E-07	1.39E-07	1.73E-06	1.07E-06	5.74E-07	2.86E-06	7.23E-07	7.51E-07	1.13E-06	3.71E-07	3.29E-07
Diclofenac	0	1.1E-07	0	0	0	7.17E-08	1.38E-07	3.31E-06	1.48E-06	1.62E-06	6.2E-06	4.76E-08	1.77E-07	4.56E-07	0	3.2E-06
Gemfibrocil	0	1.62E-06	4.31E-08	0	7.46E-08	5.78E-07	6.46E-07	2.63E-05	1.78E-05	8.46E-06	4.24E-05	3.92E-08	3.08E-06	7.6E-06	8.46E-09	2.15E-05
Ibuprofen	5.2E-09	5.32E-08	9.3E-09	1.15E-08	1.49E-08	7.84E-07	5.31E-07	1.52E-05	8.28E-06	8.11E-08	1.14E-06	2.49E-08	5.53E-06	1.03E-05	8E-09	3.03E-06
Ketoprofen	0	4.32E-08	0	0	0	1.21E-07	3.18E-08	3.56E-06	1.52E-06	4.19E-07	1.71E-06	0	3.2E-07	6.45E-07	0	3.27E-07
Loratadine	0	0	0	0	0	0	0	3.16E-06	2.23E-06	0	0	0	0	0	0	0
Naproxen	0	0	0	0	0	0	5.13E-06	7.39E-05	1.62E-05	0	2.93E-05	0	1.06E-05	1.95E-05	0	4.26E-05
Omeprazole	1.77E-08	0	8.06E-09	7.1E-09	7.74E-09	2.58E-08	3.42E-08	1.26E-05	8.06E-08	0	1.74E-07	0	0	9.03E-09	0	4.42E-08
Salbutamol	0	1.2E-09	0	0	0	7E-10	2.9E-09	6.12E-08	3.11E-08	5.38E-08	7.99E-08	0	1E-09	3.2E-09	0	7.99E-08
Valsarten	1.7E-07	5.09E-06	1.79E-07	1.22E-07	1.12E-06	4.04E-06	6.03E-06	0.000127	3.85E-05	1.64E-05	9.74E-05	1.46E-06	3.08E-05	7.49E-05	0	6.43E-05
Venlafaxine	2.24E-07	8.69E-07	0	1.64E-07	2.06E-07	1.67E-07	3.94E-07	2.14E-05	2.42E-06	2.77E-06	1.06E-05	1.9E-07	3.4E-07	6.24E-07	5.09E-07	3.03E-06
Total Pharmac	1.14E-06	8.83E-06	2.78E-07	4.12E-07	1.63E-06	7.37E-06	1.49E-05	0.000297	9.47E-05	3.32E-05	0.000202	2.95E-06	0.000111	0.000215	1.02E-06	0.000143
Amoxicillin	0	0	0	0	0	0	0	4.35E-08	0	0	7.1E-09	0	0	1.51E-07	0	0
Azithromycin	4.93E-07	2.7E-08	3.34E-08	1.38E-07	9.45E-08	1.28E-07	4.02E-08	2.2E-05	1.01E-07	6.96E-08	3.54E-07	7.07E-07	2.08E-07	4.34E-06	1.14E-07	3.06E-08
Ciprofloxacin	6.92E-08	3.71E-08	0	0	0	2.28E-08	5.24E-08	7.86E-06	1.41E-07	2.24E-07	4.19E-07	4.24E-08	8.74E-07	2.41E-06	1.34E-07	1.24E-07
Erythromycin	0	2.9E-09	0	0	4.4E-09	8E-10	1.2E-09	1.22E-08	5.2E-08	6.3E-08	1.78E-07	2.7E-09	1.62E-08	1.45E-08	2.4E-09	1.81E-08
Lincomycin	5E-10	9E-10	0	0	5E-10	0	0	9.4E-09	1.11E-07	1.04E-08	5.12E-08	1.2E-09	1.4E-09	1.5E-09	0	3.79E-08
Metronidazole	0	0	0	0	0	0	2.6E-08	2.11E-07	5.61E-07	3.05E-07	1.31E-06	0	6.7E-09	1.69E-08	0	1.23E-07
Sulfamethoxazole	1.04E-08	4.59E-08	0	0	1.76E-08	3.8E-09	3.96E-08	5.96E-05	4.91E-07	1.04E-06	1.65E-06	2.21E-08	8.1E-08	1.34E-07	2.8E-09	8.98E-07
Trimethoprim	0	2.2E-09	0	0	4E-09	0	3E-09	1.29E-05	2.25E-07	2.58E-07	9.96E-07	7E-10	4.9E-08	8.49E-08	1.4E-09	1.88E-07
Tylosin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Antibiotics	5.73E-07	1.16E-07	3.34E-08	1.38E-07	1.21E-07	1.55E-07	1.62E-07	0.000103	1.68E-06	1.97E-06	4.97E-06	7.76E-07	1.24E-06	7.15E-06	2.55E-07	1.42E-06
Estradiol, 17-beta-(E2)	0	0	0	0	0	0	0	0	0	0	2.37E-07	0	0	1.83E-07	0	0
Estrone	1.7E-09	0	0	0	9E-10	5.1E-09	3.6E-09	3.2E-08	4.6E-08	1.03E-08	1.73E-07	6.5E-09	1.57E-08	3.95E-08	1.4E-09	1.13E-08
Progesterone	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Testosterone	0	0	0	0	0	0	0	0	0	0	0	0	0	1.28E-07	0	0
Total Estrogens	1.7E-09	0	0	0	9E-10	5.1E-09	3.6E-09	3.2E-08	4.6E-08	1.03E-08	4.1E-07	6.5E-09	1.57E-08	3.5E-07	1.4E-09	1.13E-08
Amphetamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caffeine	1.67E-07	4.31E-07	1.14E-07	8.58E-08	3.32E-07	1.77E-06	2.04E-06	5.87E-05	1.07E-05	1.34E-06	4.07E-06	2.36E-07	3.22E-06	8.17E-06	1.24E-07	6.24E-06
Nicotine	1.51E-06	7.07E-06	3.93E-06	1.55E-06	3.12E-06	1.06E-05	1.02E-05	0.000124	3.43E-05	8.68E-06	2.13E-05	3.71E-06	4.75E-05	0.000139	1.47E-06	1.21E-05
Paraxantine	6.22E-08	5.75E-06	3.35E-08	2.2E-08	1.67E-07	1.25E-05	1.21E-05	0.000576	7.88E-05	8.1E-06	5.59E-05	1.34E-06	2.72E-05	6.78E-05	5.69E-07	0.000113
Total LC	1.74E-06	1.33E-05	4.08E-06	1.66E-06	3.62E-06	2.48E-05	2.43E-05	0.000759	0.000124	1.81E-05	8.12E-05	5.29E-06	7.79E-05	0.000214	2.16E-06	0.000131
Tributyl-phosphate	0	2.25E-06	3.11E-05	1.74E-05	0.000038	5.35E-06	1.59E-06	1.24E-06	4.04E-05	5.13E-06	6.35E-06	8.08E-07	5.21E-07	7.88E-07	6.63E-07	1.88E-06
Total Industrial	0	2.25E-06	3.11E-05	1.74E-05	0.000038	5.35E-06	1.59E-06	1.24E-06	4.04E-05	5.13E-06	6.35E-06	8.08E-07	5.21E-07	7.88E-07	6.63E-07	1.88E-06
Total PSC	3.46E-06	2.44E-05	3.55E-05	1.96E-05	4.34E-05	3.77E-05	4.1E-05	0.001159	0.000261	5.84E-05	0.000295	9.83E-06	0.000191	0.000438	4.09E-06	0.000277

Table S5 (cont.)

POCIS-Summer	Toxic Units (C/EC50) Algae															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Acetaminophen	4.83E-08	0	0		0	1.08E-06	3.66E-08	1.22E-07				8.49E-10	1.57E-06	1.08E-06	0	0
Atenolol	2.08E-09	3.31E-08	0		1.92E-08	1.14E-06	6.89E-08	1.31E-06				2.36E-08	3.4E-07	1.65E-07	5.11E-09	7.58E-07
Carbamazepine	1.23E-09	1.04E-08	8.64E-10		3.42E-09	3.71E-07	2.3E-08	2.09E-07				1.55E-08	7.42E-08	7.18E-08	3.94E-08	7.07E-07
Citalopram	0	1.13E-07	1.42E-08		9.79E-08	9.85E-07	4.02E-07	5.1E-06				7.83E-09	3E-06	1.84E-07	0	1.65E-06
Diclofenac (*)	0	8.82E-09	0		3.99E-09	1.46E-07	9.55E-09	2.63E-07				0	2.08E-07	1.18E-07	0	6.26E-07
Gemfibrocil	0	1.36E-06	0		4.51E-07	2.41E-05	9.28E-07	5.24E-05				0	2.78E-05	1.84E-05	7.59E-07	7.41E-05
Ibuprofen	0	4.17E-08	1.07E-09		4.93E-09	7.12E-06	1.32E-07	1.68E-06				0	2.95E-06	3.2E-06	0	1.85E-06
Ketoprofen	0	0	0		0	5.46E-07	0	1.02E-06				0	2.3E-07	1.31E-07	0	6.76E-08
Loratadine	0	0	0		0	6.81E-06	0	8.35E-05				0	0	0	0	5.3E-07
Naproxen	0	0	0		0	4.34E-06	3.95E-07	1.79E-06				0	7.91E-07	0	0	0
Omeprazole	0	0	0		0	3.14E-07	0	2.01E-06				0	0	5.39E-09	0	1.52E-09
Salbutamol	0	1.17E-10	0		0	2.17E-09	0	4.47E-09				0	5.39E-10	5.96E-10	0	1.19E-08
Valsarten	6.75E-08	2.13E-06	2.15E-08		1.32E-06	1.96E-05	2.76E-06	4.81E-05				3.71E-07	3.96E-05	2.94E-05	1.09E-07	6.21E-05
Venlafaxine	0	6.29E-08	0		7.08E-08	8.35E-07	1.35E-07	3.29E-06				0	0	1.84E-08	0	6.57E-07
Total Pharmaceuticals	1.19E-07	3.76E-06	3.76E-08		1.97E-06	6.74E-05	4.89E-06	0.000201				4.19E-07	7.66E-05	5.28E-05	9.13E-07	0.000143
Amoxicillin	0	0	0		0	0	0	6.11E-09				0	0	0	0	0
Azithromycin	4.4E-09	9.44E-09	0		1.2E-08	0	1.94E-08	0.000133				1.74E-08	1.5E-08	1.43E-08	1.72E-08	0
Ciprofloxacin	0	0	0		0	4.19E-06	1.05E-07	8.46E-06				0	0	1.46E-07	0	0
Erythromycin	9.24E-08	0	0		0	2.8E-07	3.37E-07	5.04E-05				0	0	0	0	6.44E-05
Lincomycin	3.76E-07	2.26E-07	0		0	2.67E-06	1.38E-07	7.99E-06				2.68E-07	4.44E-07	1.14E-05	1.37E-05	0
Metronidazole	0	0	0		0	0	0	2.21E-08				1.36E-08	0	0	0	8.87E-09
Sulfamethoxazole	2.15E-09	3.61E-08	0		5.37E-09	1.08E-06	1.61E-08	3.99E-06				5.09E-09	1.6E-07	3.41E-10	4.11E-10	2.58E-08
Trimethoprim	0	5.03E-10	6.22E-11		1.63E-10	1E-07	1.05E-09	7.81E-07				0	8.17E-08	6.73E-08	2.76E-10	1.34E-07
Tylosin	0	0	0		0	2.9E-09	0	0				0	0	0	0	0
Total Antibiotics	4.75E-07	2.72E-07	6.22E-11		1.75E-08	8.3E-06	6.17E-07	0.000205				3.04E-07	7.01E-07	1.17E-05	1.37E-05	6.45E-05
Estradiol, 17-beta-(E2)	0	0	0		0	8.65E-08	0	1.18E-07				0	1.73E-07	2.12E-07	0	0
Estrone	1.59E-08	1.36E-08	2.12E-09		2.77E-08	7.81E-08	5.92E-09	1.39E-07				1.2E-08	1.35E-07	8.65E-08	1.74E-08	1.38E-07
Progesterone	0	0	0		0	0	0	0				0	6.95E-08	0	0	0
Testosterone	0	0	0		0	2.75E-08	1.55E-08	0				0	6.07E-08	3.29E-08	1.29E-08	0
Total Estrogens	1.59E-08	1.36E-08	2.12E-09		2.77E-08	1.92E-07	2.15E-08	2.57E-07				1.2E-08	4.38E-07	3.31E-07	3.03E-08	1.38E-07
Amphetamine	0	0	0		0	0	0	0				0	0	0	0	0
Caffeine	6.19E-07	2.64E-07	1.8E-07		1.69E-07	5.71E-06	7.82E-07	1.12E-06				1.3E-07	2.02E-06	1.85E-06	2.22E-07	9.18E-07
Nicotine	2.6E-07	2.15E-07	2.06E-08		1.85E-07	2.11E-06	2.4E-07	4.98E-07				1.48E-07	6.93E-07	7.51E-07	2.06E-07	2.42E-07
Paraxantine	4.8E-06	1.4E-06	1.1E-06		4.72E-07	8.07E-06	7.42E-06	2.33E-06				0	6.66E-06	6.96E-06	0	3.28E-06
Total Lifestyle comp.	5.68E-06	1.88E-06	1.3E-06		8.26E-07	1.59E-05	8.44E-06	3.95E-06				2.77E-07	9.37E-06	9.56E-06	4.28E-07	4.44E-06
Tributyl-phosphate	8.36E-07	8.58E-07	5.51E-07		7.24E-07	1.03E-06	3.44E-07	1.47E-06				6.18E-07	4.81E-07	1.95E-07	7.84E-07	8.58E-06
Total Inustrial	8.36E-07	8.58E-07	5.51E-07		7.24E-07	1.03E-06	3.44E-07	1.47E-06				6.18E-07	4.81E-07	1.95E-07	7.84E-07	8.58E-06
Total PSC	7.12E-06	6.79E-06	1.89E-06		3.56E-06	2896.825	1.43E-05	0.000411				1.63E-06	8.76E-05	7.45E-05	1.59E-05	0.000221

Table S5 (cont.)

POCIS-Summer	Toxic Units (C/EC50) Daphnia															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Acetaminophen	3.02E-07	0	0		0	6.77E-06	2.29E-07	7.6E-07				5.3E-09	9.83E-06	6.72E-06	0	0
Atenolol	2.08E-09	3.31E-08	0		1.92E-08	1.14E-06	6.89E-08	1.31E-06				2.36E-08	3.4E-07	1.65E-07	5.11E-09	7.58E-07
Carbamazepine	1.23E-09	1.04E-08	8.64E-10		3.42E-09	3.71E-07	2.3E-08	2.09E-07				1.55E-08	7.42E-08	7.18E-08	3.94E-08	7.07E-07
Citalopram	0	5.66E-08	7.1E-09		4.9E-08	4.92E-07	2.01E-07	2.55E-06				3.92E-09	1.5E-06	9.2E-08	0	8.23E-07
Diclofenac (*)	0	1.89E-08	0		8.53E-09	3.12E-07	2.04E-08	5.63E-07				0	4.45E-07	2.52E-07	0	1.34E-06
Gemfibrocil	0	1.14E-06	0		3.8E-07	2.03E-05	7.82E-07	4.42E-05				0	2.35E-05	1.55E-05	6.4E-07	6.25E-05
Ibuprofen	0	4.17E-08	1.07E-09		4.93E-09	7.12E-06	1.32E-07	1.68E-06				0	2.95E-06	3.2E-06	0	1.85E-06
Ketoprofen	0	0	0		0	5.46E-07	0	1.02E-06				0	2.3E-07	1.31E-07	0	6.76E-08
Loratadine	0	0	0		0	4.76E-06	0	5.85E-05				0	0	0	0	3.71E-07
Naproxen	0	0	0		0	5.3E-06	4.82E-07	2.18E-06				0	9.64E-07	0	0	0
Omeprazole	0	0	0		0	3.14E-07	0	2.01E-06				0	0	5.39E-09	0	1.52E-09
Salbutamol	0	1.17E-10	0		0	2.17E-09	0	4.47E-09				0	5.39E-10	5.96E-10	0	1.19E-08
Valsarten	5.4E-08	1.71E-06	1.72E-08		1.05E-06	1.57E-05	2.21E-06	3.85E-05				2.97E-07	3.17E-05	2.35E-05	8.73E-08	4.97E-05
Venlafaxine	0	7.54E-08	0		8.5E-08	1E-06	1.62E-07	3.95E-06				0	0	2.21E-08	0	7.88E-07
Total Pharmaceuticals	3.59E-07	3.09E-06	2.62E-08		1.6E-06	6.41E-05	4.31E-06	0.000157				3.45E-07	7.15E-05	4.97E-05	7.72E-07	0.000119
Amoxicillin	0	0	0		0	0	0	6.11E-09				0	0	0	0	0
Azithromycin	3.1E-09	6.67E-09	0		8.47E-09	0	1.37E-08	9.38E-05				1.22E-08	1.06E-08	1.01E-08	1.21E-08	0
Ciprofloxacin	0	0	0		0	2.81E-07	7.01E-09	5.67E-07				0	0	9.81E-09	0	0
Erythromycin	5.55E-11	0	0		0	1.68E-10	2.02E-10	3.03E-08				0	0	0	0	3.86E-08
Lincomycin	3.65E-09	2.2E-09	0		0	2.59E-08	1.34E-09	7.77E-08				2.6E-09	4.32E-09	1.11E-07	1.33E-07	0
Metronidazole	0	0	0		0	0	0	8.82E-09				5.43E-09	0	0	0	3.55E-09
Sulfamethoxazole	2.15E-09	3.61E-08	0		5.37E-09	1.08E-06	1.61E-08	3.99E-06				5.09E-09	1.6E-07	3.41E-10	4.11E-10	2.58E-08
Trimethoprim	0	5.03E-10	6.22E-11		1.63E-10	1E-07	1.05E-09	7.81E-07				0	8.17E-08	6.73E-08	2.76E-10	1.34E-07
Tylosin	0	0	0		0	2.9E-09	0	0				0	0	0	0	0
Total Antibiotics	8.96E-09	4.54E-08	6.22E-11		1.4E-08	1.49E-06	3.94E-08	9.93E-05				2.54E-08	2.57E-07	1.99E-07	1.46E-07	2.02E-07
Estradiol, 17-beta-(E2)	0	0	0		0	7.46E-08	0	1.02E-07				0	1.49E-07	1.82E-07	0	0
Estrone	1.45E-08	1.25E-08	1.94E-09		2.53E-08	7.15E-08	5.42E-09	1.27E-07				1.1E-08	1.24E-07	7.92E-08	1.6E-08	1.26E-07
Progesterone	0	0	0		0	0	0	0				0	4.63E-08	0	0	0
Testosterone	0	0	0		0	2.2E-08	1.24E-08	0				0	4.86E-08	2.63E-08	1.03E-08	0
Total Estrogens	1.45E-08	1.25E-08	1.94E-09		2.53E-08	1.68E-07	1.79E-08	2.29E-07				1.1E-08	3.68E-07	2.88E-07	2.63E-08	1.26E-07
Amphetamine	0	0	0		0	0	0	0				0	0	0	0	0
Caffeine	6.19E-07	2.64E-07	1.8E-07		1.69E-07	5.71E-06	7.82E-07	1.12E-06				1.3E-07	2.02E-06	1.85E-06	2.22E-07	9.18E-07
Nicotine	2.6E-07	2.15E-07	2.06E-08		1.85E-07	2.11E-06	2.4E-07	4.98E-07				1.48E-07	6.93E-07	7.51E-07	2.06E-07	2.42E-07
Paraxantine	4.8E-06	1.4E-06	1.1E-06		4.72E-07	8.07E-06	7.42E-06	2.33E-06				0	6.66E-06	6.96E-06	0	3.28E-06
Total Lifestyle comp.	5.68E-06	1.88E-06	1.3E-06		8.26E-07	1.59E-05	8.44E-06	3.95E-06				2.77E-07	9.37E-06	9.56E-06	4.28E-07	4.44E-06
Tributyl-phosphate	4.07E-07	4.17E-07	2.68E-07		3.52E-07	4.99E-07	1.67E-07	7.16E-07				3.01E-07	2.34E-07	9.5E-08	3.82E-07	4.17E-06
Total Inustrial	4.07E-07	4.17E-07	2.68E-07		3.52E-07	4.99E-07	1.67E-07	7.16E-07				3.01E-07	2.34E-07	9.5E-08	3.82E-07	4.17E-06
Total PSC	6.47E-06	5.45E-06	1.59E-06		2.82E-06	8.22E-05	1.3E-05	0.000262				9.6E-07	8.17E-05	5.98E-05	1.75E-06	0.000128

Table S5 (cont.)

POCIS-Summer	Toxic Units (C/EC50) Fish															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Acetaminophen	4.83E-08	0	0		0	1.08E-06	3.66E-08	1.22E-07				8.49E-10	1.57E-06	1.08E-06	0	0
Atenolol	2.08E-09	3.31E-08	0		1.92E-08	1.14E-06	6.89E-08	1.31E-06				2.36E-08	3.4E-07	1.65E-07	5.11E-09	7.58E-07
Carbamazepine	6.17E-09	5.19E-08	4.32E-09		1.71E-08	1.86E-06	1.15E-07	1.05E-06				7.76E-08	3.71E-07	3.59E-07	1.97E-07	3.54E-06
Citalopram	0	3.24E-08	4.06E-09		2.8E-08	2.81E-07	1.15E-07	1.46E-06				2.24E-09	8.58E-07	5.26E-08	0	4.71E-07
Diclofenac (*)	0	2.31E-08	0		1.05E-08	3.82E-07	2.5E-08	6.89E-07				0	5.45E-07	3.09E-07	0	1.64E-06
Gemfibrocil	0	6.16E-07	0		2.05E-07	1.09E-05	4.21E-07	2.38E-05				0	1.26E-05	8.37E-06	3.44E-07	3.36E-05
Ibuprofen	0	4.17E-08	1.07E-09		4.93E-09	7.12E-06	1.32E-07	1.68E-06				0	2.95E-06	3.2E-06	0	1.85E-06
Ketoprofen	0	0	0		0	5.46E-07	0	1.02E-06				0	2.3E-07	1.31E-07	0	6.76E-08
Loratadine	0	0	0		0	2.38E-06	0	2.92E-05				0	0	0	0	1.85E-07
Naproxen	0	0	0		0	2.29E-05	2.08E-06	9.41E-06				0	4.16E-06	0	0	0
Omeprazole	0	0	0		0	1.01E-06	0	6.49E-06				0	0	1.74E-08	0	4.92E-09
Salbutamol	0	1.17E-10	0		0	2.17E-09	0	4.47E-09				0	5.39E-10	5.96E-10	0	1.19E-08
Valsarten	2.84E-08	8.99E-07	9.05E-09		5.54E-07	8.27E-06	1.16E-06	2.03E-05				1.56E-07	1.67E-05	1.24E-05	4.6E-08	2.61E-05
Venlafaxine	0	4.72E-08	0		5.31E-08	6.27E-07	1.01E-07	2.47E-06				0	0	1.38E-08	0	4.92E-07
Total Pharmaceuticals	8.5E-08	1.74E-06	1.85E-08		8.92E-07	5.85E-05	4.26E-06	9.9E-05				2.61E-07	4.03E-05	2.61E-05	5.93E-07	6.88E-05
Amoxicillin	0	0	0		0	0	0	6.11E-09				0	0	0	0	0
Azithromycin	3.37E-09	7.23E-09	0		9.19E-09	0	1.49E-08	0.000102				1.33E-08	1.15E-08	1.1E-08	1.32E-08	0
Ciprofloxacin	0	0	0		0	2.81E-07	7.01E-09	5.67E-07				0	0	9.81E-09	0	0
Erythromycin	5.55E-11	0	0		0	1.68E-10	2.02E-10	3.03E-08				0	0	0	0	3.86E-08
Lincomycin	2.63E-10	1.58E-10	0		0	1.87E-09	9.66E-11	5.59E-09				1.87E-10	3.11E-10	8E-09	9.6E-09	0
Metronidazole	0	0	0		0	0	0	8.82E-09				5.43E-09	0	0	0	3.55E-09
Sulfamethoxazole	2.15E-09	3.61E-08	0		5.37E-09	1.08E-06	1.61E-08	3.99E-06				5.09E-09	1.6E-07	3.41E-10	4.11E-10	2.58E-08
Trimethoprim	0	5.03E-10	6.22E-11		1.63E-10	1E-07	1.05E-09	7.81E-07				0	8.17E-08	6.73E-08	2.76E-10	1.34E-07
Tylosin	0	0	0		0	2.9E-09	0	0				0	0	0	0	0
Total Antibiotics	5.83E-09	4.4E-08	6.22E-11		1.47E-08	1.47E-06	3.94E-08	0.000107				2.4E-08	2.54E-07	9.64E-08	2.34E-08	2.02E-07
Estradiol, 17-beta-(E2)	0	0	0		0	6.18E-08	0	8.43E-08				0	1.23E-07	1.51E-07	0	0
Estrone	1.03E-08	8.87E-09	1.38E-09		1.8E-08	5.07E-08	3.85E-09	9.03E-08				7.8E-09	8.8E-08	5.63E-08	1.13E-08	8.95E-08
Progesterone	0	0	0		0	0	0	0				0	2.32E-08	0	0	0
Testosterone	0	0	0		0	1.22E-08	6.91E-09	0				0	2.7E-08	1.46E-08	5.73E-09	0
Total Estrogens	1.03E-08	8.87E-09	1.38E-09		1.8E-08	1.25E-07	1.08E-08	1.75E-07				7.8E-09	2.61E-07	2.22E-07	1.71E-08	8.95E-08
Amphetamine	0	0	0		0	0	0	0				0	0	0	0	0
Caffeine	6.19E-07	2.64E-07	1.8E-07		1.69E-07	5.71E-06	7.82E-07	1.12E-06				1.3E-07	2.02E-06	1.85E-06	2.22E-07	9.18E-07
Nicotine	6.5E-06	5.38E-06	5.16E-07		4.61E-06	5.27E-05	6.01E-06	1.24E-05				3.7E-06	1.73E-05	1.88E-05	5.15E-06	6.04E-06
Paraxantine	4.8E-06	1.4E-06	1.1E-06		4.72E-07	8.07E-06	7.42E-06	2.33E-06				0	6.66E-06	6.96E-06	0	3.28E-06
Total Lifestyle comp.	1.19E-05	7.05E-06	1.79E-06		5.26E-06	6.64E-05	1.42E-05	1.59E-05				3.83E-06	2.6E-05	2.76E-05	5.37E-06	1.02E-05
Tributyl-phosphate	1.88E-07	1.93E-07	1.24E-07		1.63E-07	2.31E-07	7.73E-08	3.31E-07				1.39E-07	1.08E-07	4.39E-08	1.76E-07	1.93E-06
Total Inustrial	1.88E-07	1.93E-07	1.24E-07		1.63E-07	2.31E-07	7.73E-08	3.31E-07				1.39E-07	1.08E-07	4.39E-08	1.76E-07	1.93E-06
Total PSC	1.22E-05	9.04E-06	1.94E-06		6.34E-06	0.000127	1.86E-05	0.000223				4.26E-06	6.69E-05	5.4E-05	6.18E-06	8.12E-05

Table S6. Comparison of measured values of some parameters with the limits proposed by the Spanish Ministry of Agriculture, Food and Environment corresponding to the different river tipologies.

		Spanish R-T05 Limits		**Measured values**				**Spanish R-T11 Limits**		**Measured values**		**Spanish R-T12 Limits**		**Measured values**				
		Class 1	Class 2	**Spring**				Class 1	Class 2	**Spring**		Class 1	Class 2	**Spring**				
		Very good/good	Good/moderate	**15**	**16**			Very good/good	Good/moderate	**3**	**4**	Very good/good	Good/moderate	**1**	**2**	**6**	**7**	**10**
pH		6.5-8.7	6-9	7.9	8.1	pH		6.5-8.7	6-9	7.3	8.2	6.5-8.7	6-9	8.2	8.4	8.4	8.4	8.4
O2	mg/L		5	9.7	7.3	O2	mg/L		5	10.6	11.0		5	10.1	10.3	10.0	9.9	9.8
O2	%	70-100	60-120	88	73	O2	%	70-100	60-120	88	95	70-100	60-120	89	95	90	89	93
NH4	mg/L	0.2	0.6	<0.001	1.83	NH4	mg/L	0.2	0.6	<0.001	<0.001	0.2	0.6	<0.001	<0.001	0.10	0.002	0.69
NO3	mg/L	20	25	17.24	3.82	NO3	mg/L	20	25	0.12	0.32	20	25	1.42	3.79	4.31	3.55	3.03
PO4	mg/L	0.2	0.4	0.01	0.84	PO4	mg/L	0.2	0.4	<0.003	<0.003	0.2	0.4	<0.003	0.01	0.01	0.01	0.38
				Summer										**Summer**				
pH		6.5-8.7	6-9	7.6	9.0	pH		6.5-8.7	6-9	7.3	8.5	6.5-8.7	6-9	8.3	8.5	8.7	9.6	8.0
O2	mg/L		5	7.9	7.6	O2	mg/L		5	8.6	10.2		5	7.9	8.3	8.2	9.7	8.2
O2	%	70-100	60-120	82	88	O2	%	70-100	60-120	89	98	70-100	60-120	80	85	85	104	90
NH4	mg/L	0.2	0.6	0.17	2.90	NH4	mg/L	0.2	0.6	0.04	0.06	0.2	0.6	0.14	0.32	0.34	0.18	0.11
NO3	mg/L	20	25	8.80	7.66	NO3	mg/L	20	25	0.55	0.50	20	25	0.47	2.65	5.85	4.51	5.33
PO4	mg/L	0.2	0.4	0.01	0.80	PO4	mg/L	0.2	0.4	<0.003	<0.003	0.2	0.4	<0.003	0.01	0.01	0.01	0.30
				Autumn										**Autumn**				
pH		6.5-8.7	6-9	7.0	7.8	pH		6.5-8.7	6-9	6.2	7.2	6.5-8.7	6-9	8.0	7.5	8.0	8.1	8.1
O2	mg/L		5	7.7	8.3	O2	mg/L		5	9.0	8.4		5	8.1	7.9	9.2	9.1	8.9
O2	%	70-100	60-120	69	75	O2	%	70-100	60-120	75	75	70-100	60-120	70	69	78	79	78
NH4	mg/L	0.2	0.6	0.110	4.460	NH4	mg/L	0.2	0.6	<0.001	<0.001	0.2	0.6	0.003	<0.001	0.128	0.025	0.069
NO3	mg/L	20	25	2.240	10.135	NO3	mg/L	20	25	0.466	0.367	20	25	0.597	3.291	5.097	4.136	5.446
PO4	mg/L	0.2	0.4	<0.003	0.800	PO4	mg/L	0.2	0.4	<0.003	<0.003	0.2	0.4	<0.003	0.005	0.006	0.014	0.300

Class 2

Worst than Class 2

Table S6 (cont.)

Spanish R-T13 Limits		**Measured values**			**Spanish R-T15 Limits**		**Measured values**		**Spanish R-T16 Limits**		**Measured values**	
Class 1	Class 2	**Spring**			Class 1	Class 2	**Spring**		Class 1	Class 2	**Spring**	
Very good/good	Good/moderate	**12**	**13**	**14**	Very good/good	Good/moderate	**9**	**11**	Very good/good	Good/moderate	**5**	**8**
6.5-8.7	6-9	8.6	8.5	8.5	6.5-8.7	6-9	8.0	7.7	6.5-8.7	6-9	8.5	7.9
	5	10.4	10.6	9.9		5	6.8	5.1		5	11.2	8.9
70-100	60-120	99	100	95	70-100	60-120	63	50	70-100	60-120	102	84
0.2	0.6	0.01	0.79	1.08	0.2	0.6	6.87	<0.001	0.2	0.6	<0.001	<0.001
20	25	5.21	5.45	5.13	20	25	1.45	2.13	20	25	3.09	2.77
0.2	0.4	0.02	0.01	0.01	0.2	0.4	0.15	0.16	0.2	0.4	<0.003	0.22
		Summer					**Summer**				**Summer**	
6.5-8.7	6-9	8.3	8.6	8.8	6.5-8.7	6-9	8.5	7.1	6.5-8.7	6-9	8.8	7.7
	5	8.0	8.5	2.2		5	5.1	4.6		5	10.6	7.2
70-100	60-120	88	95	25	70-100	60-120	57	52	70-100	60-120	110	84
0.2	0.6	0.18	1.60	3.60	0.2	0.6	7.00	8.50	0.2	0.6	0.05	5.60
20	25	5.85	5.71	5.15	20	25	1.86	5.13	20	25	2.71	7.95
0.2	0.4	0.01	0.17	0.32	0.2	0.4	0.50	0.39	0.2	0.4	<0.003	0.06
		Autumn					**Autumn**				**Autumn**	
6.5-8.7	6-9	7.9	7.7	7.8	6.5-8.7	6-9	6.7	6.8	6.5-8.7	6-9	7.8	7.1
	5	10.2	10.0	7.9		5	6.7	5.5		5	9.6	6.9
70-100	60-120	86	85	68	70-100	60-120	59	53	70-100	60-120	83	65
0.2	0.6	0.017	0.816	1.900	0.2	0.6	6.150	9.710	0.2	0.6	0.005	7.640
20	25	8.359	8.562	7.893	20	25	2.289	5.417	20	25	3.262	5.213
0.2	0.4	0.010	0.170	0.320	0.2	0.4	0.500	0.390	0.2	0.4	<0.003	0.060

Class 2

Worst than Class 2

Hydrological patterns

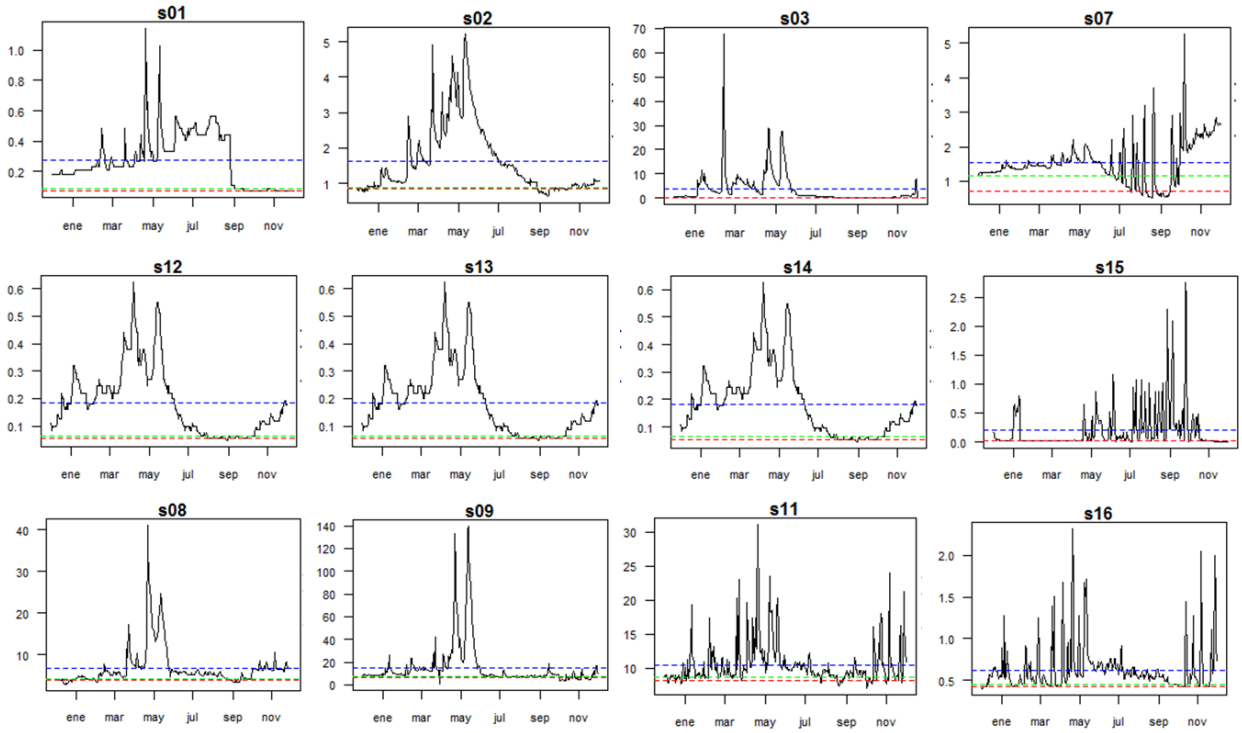


Figure S1. Daily flow measured in flow gauges corresponding to each sampling site. Criteria for water stressed sites (HighDrought or HD): >55days(15%) with flow <20%quantile of all flow data per site, being <1m3/s. Blue dashed line: mean flow; green line: flow value representing the 20% quantile of the whole set of data; red line: flow value representing the 10% quantile of the whole set of data.

Chironomidae data elaboration to compensate the mismatch at the level of identification for some samples

We determined the total % of each tribe or subfamily identified within the Chironomidae group (i.e. Orthocladiinae, Tanypodinae, Diamesinae, Tanitarsini, Chironomini), with respect to the total number of Chironomidae (considering all sites per season). Afterward, these percentages per group were applied to the measured Chironomidae abundance per site-season, adding up the counted value and the calculated one in each group. To determine whether this proportional calculation was having a differential effect on site distribution based on taxonomic composition with respect to the whole dataset, a Principal Component Analysis (PCA) was performed on raw and recalculated data. A correlation analysis with the 1st PCA axis scores of the two datasets showed a linear correlation with R^2=±0.999, confirming that this data elaboration would not influence the final results. There recalculated data were used as they give more consistent information on Chironomidae subgroups differences between seasons and solves the problem of Chironomidae overweight in the dataset.

PCA on substrate data

Table S1. Substrate matrix with percentages (%) of each substrate type based on qualitative observations.

Code	*Stone*	*Gravel*	*Sand*	*Silt*	*Macrophy*	*Mud*	*Debris*	*Algae*
s03Sp	60.0	40.0	0.0	0.0	0.0	0.0	0.0	0.0
s02Sp	15.0	10.0	26.7	10.0	38.3	0.0	0.0	0.0
s01Sp	26.7	16.7	5.0	5.0	46.7	0.0	0.0	0.0
s07Sp	0.0	31.7	20.0	23.3	13.3	10.0	0.0	1.7
s11Sp	0.0	36.7	63.3	0.0	0.0	0.0	0.0	0.0
s09Sp	33.3	0.0	10.0	0.0	20.0	36.7	0.0	0.0
s08Sp	0.0	0.0	46.7	46.7	6.7	0.0	0.0	0.0
s15Sp	0.0	0.0	43.3	10.0	3.3	26.7	16.7	0.0
s12Sp	0.0	0.0	60.0	0.0	21.7	6.7	0.0	11.7
s13Sp	0.0	13.3	10.0	0.0	0.0	76.7	0.0	0.0
s14Sp	6.7	0.0	10.0	10.0	46.7	26.7	0.0	0.0
s16Sp	0.0	0.0	93.3	0.0	0.0	6.7	0.0	0.0
s03Su	60.0	40.0	0.0	0.0	0.0	0.0	0.0	0.0
s02Su	6.7	0.0	20.0	20.0	43.3	0.0	0.0	10.0
s01Su	26.7	16.7	5.0	5.0	46.7	0.0	0.0	0.0
s07Su	0.0	31.7	20.0	23.3	13.3	10.0	0.0	1.7
s11Su	0.0	36.7	63.3	0.0	0.0	0.0	0.0	0.0
s09Su	33.3	0.0	10.0	0.0	20.0	36.7	0.0	0.0
s08Su	0.0	0.0	46.7	46.7	6.7	0.0	0.0	0.0
s15Su	0.0	0.0	43.3	10.0	3.3	26.7	16.7	0.0
s12Su	0.0	0.0	60.0	0.0	21.7	6.7	0.0	11.7
s13Su	0.0	13.3	10.0	0.0	0.0	76.7	0.0	0.0
s14Su	6.7	0.0	5.0	5.0	10.0	73.3	0.0	0.0
s16Su	0.0	0.0	93.3	0.0	0.0	6.7	0.0	0.0
s03Au	60.0	40.0	0.0	0.0	0.0	0.0	0.0	0.0
s02Au	6.7	0.0	20.0	20.0	43.3	0.0	0.0	10.0
s01Au	26.7	16.7	5.0	5.0	46.7	0.0	0.0	0.0
s07Au	0.0	31.7	20.0	23.3	13.3	10.0	0.0	1.7
s11Au	0.0	36.7	63.3	0.0	0.0	0.0	0.0	0.0
s09Au	33.3	0.0	10.0	0.0	20.0	36.7	0.0	0.0
s08Au	0.0	0.0	46.7	46.7	6.7	0.0	0.0	0.0
s15Au	0.0	0.0	43.3	10.0	3.3	26.7	16.7	0.0
s12Au	0.0	0.0	60.0	0.0	21.7	6.7	0.0	11.7
s13Au	0.0	13.3	10.0	0.0	0.0	76.7	0.0	0.0
s14Au	6.7	0.0	5.0	5.0	10.0	73.3	0.0	0.0
s16Au	0.0	0.0	93.3	0.0	0.0	6.7	0.0	0.0

Substrate data were analyzed by means of a PCA on the percentages of each substrate types in each site. PCA results on substrate data showed that this parameter was not a major driver despite some more (expected) muddy bottoms in impacted sites (Figure S2). Differences between groups tested by means of an ANOVA test showed significant differences between groups of sites on the 2nd axis (28% of the variance explained), with polluted groupings being only marginally different (p-value=0.05). Differences along the 1st axis (44% of the variance explained) were not significant.

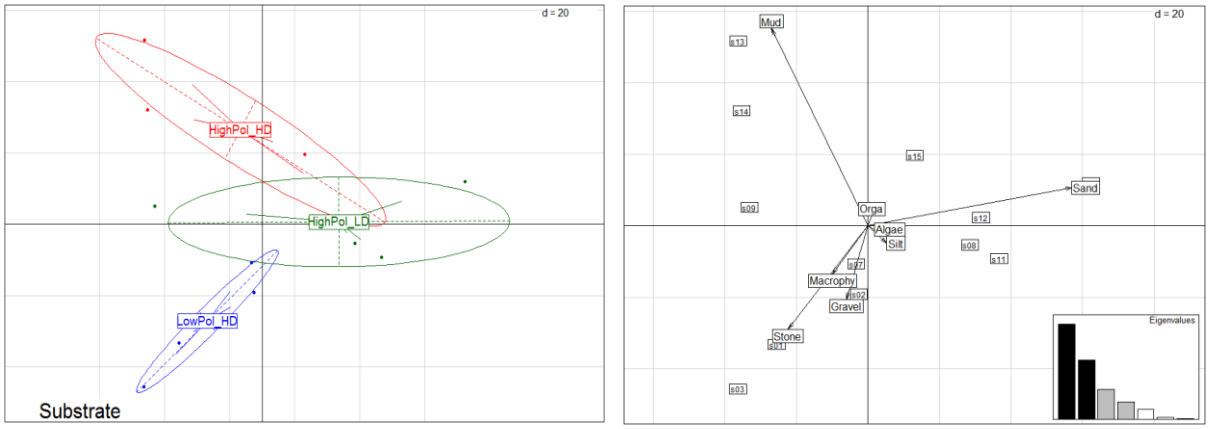


Figure S2. PCA on substrate types per site.

Environmental data

Table S2. Mean annual values of environmental variables, metals and organic microcontaminants for each group of sites, and ANOVA p-values testing seasonal differences and between groups differences.

	LowPol_HD	p-value (seasonal change)	*HighPol_HD*	p-value (seasonal change)	*HighPol_LD*	p-value (seasonal change)	p-value (between groups differences)
Flow ($m^3 s^{-1}$)	1.24±0.83	-	0.15±0.13	-	8.76±7.18	-	<0.001
Temperature (°C)	12.0±4.3	0.001	13.9±5.1	<0.001	16.7±5.91	<0.001	0.055
pH	7.98 ±0.84	-	8.10±0.53	0.053	7.70±0.68	-	0.321
Conductivity (µS cm^{-1})	1871±1718	-	5040±287	-	1383±869	-	<0.001
TSS (mg L^{-1})	24.2±31.3	-	113±114	<0.001	57.2±63.1	-	<0.001
O2sat (%)	84.3±10.2	0.043	81.7±20.6	-	66.9±13.5	-	0.022
DOC (mg L^{-1})	2.94±1.42	-	6.03±1.33	-	6.76±1.35	-	<0.001
N_NH3 (mg L^{-1})	0.01±0.02	0.043	0.08±0.18	-	0.19±0.26	0.069	<0.001
N_NO2 (mg L^{-1})	0.006±0.006	0.001	0.05±0.07	0.067	0.42±0.46	0.069	<0.001
N_NO3 (mg L^{-1})	1.65±1.3	-	5.53±2.87	-	3.61±2.16	-	<0.001
Total N (mg L^{-1})	1.7±1.32	-	6.24±2.80	-	10.2±3.65	-	<0.001
P_PO4 (mg L^{-1})	0.005±0.004	-	0.06±0.07	-	0.34±0.28	-	<0.001
TUMetals	0.08±0.05	-	0.15±0.08	0.019	0.51±0.30	-	<0.001
TUPestic	1E-05±2E-05	-	1E-04±1E-04	0.009	2E-03±1E-03	-	<0.001
TUPSC	6E-05±7E-05	-	3E-04±4E-04	-	4E-04±3E-04	-	0.006
TUTotal	7E-05±7E-05	-	4E-04±4E-04	-	2E-03±1E-03	-	<0.001

Table S3. Mean values in each site groupings for all abiotic variables, metals and organic microcontaminants in spring, summer and autumn, and ANOVA p-values testing for differences between groups.

	Spring				*Summer*				*Autumn*			
	LowPol_HD	*HighPol_HD*	*HighPol_LD*	p-value	*LowPol_HD*	*HighPol_HD*	*HighPol_LD*	p-value	*LowPol_HD*	*HighPol_HD*	*HighPol_LD*	p-value
Flow ($m^3 s^{-1}$)	1.44±0.83	0.270±0.161	11.8±10.3	0.020	0.86±0.48	0.07±0.03	5.57±3.70	0.015	1.41±1.15	0.12±0.08	8.85±6.51	0.027
Temperature (˚C)	9.5±1.7	13.1±1.0	14.7±1.4	0.007	17.5±0.9	20.1±0.5	23.0±0.8	0.016	9.1±1.1	8.8±1.5	12.5±1.9	0.027
pH	8.08±0.53	8.38±0.32	7.93±0.17	-	8.43±0.94	8.33±0.53	8.08±0.84	-	7.45±0.87	7.60±0.41	7.10±0.5	-
Conductivity (µS cm^{-1})	1581±1475	5266±63.7	1308±814	0.011	2104±2048	4873±432	1579±1118	0.064	1928±2066	4980±15	1262±882	0.034
TSS (mg L^{-1})	2.23±0.81	39.2±16.6	51.5±77.6	0.010	50.0±34.1	258.7±70.8	81.5±80.3	-	20.5±29.6	41.7±13.8	38.6±29.2	-
O2sat (%)	90.3±3.2	95.5±5.5	67.5±14.5	0.010	89.5±10.3	72.5±32.1	70.3±18.4	-	73.3±4.6	77.0±9.8	63.0±9.4	-
DOC (mg L^{-1})	3.13±1.72	6.70±0.93	6.78±1.37	0.011	2.45±1	6.25±1.55	7.20±1.57	0.007	3.23±1.72	5.13±1.22	6.29±1.34	0.063
N_NH3 (mg L^{-1})	3E-05±3E-05	0.03±0.03	0.15±0.08	0.009	0.03±0.04	0.20±0.29	0.39±0.37	-	0.0001±0.0001	0.01±0.01	0.02±0.02	0.027
N_NO2 (mg L^{-1})	0.0002±0	0.011±0.018	0.098±0.101	0.004	0.010±0.004	0.106±0.109	0.875±0.533	<0.001	0.007±0.009	0.041±0.037	0.272±0.206	0.006
N_NO3 (mg L^{-1})	1.72±1.36	6.4±4.7	1.97±0.78	0.056	1.59±1.5	4.95±1.27	4.38±2.19	0.061	1.65±1.45	5.24±2.35	4.47±2.52	-
Total N (mg L^{-1})	1.72±1.36	6.79±4.43	10.7±6.13	0.029	1.73±1.55	6.13±1.11	9.91±2.16	0.002	1.66±1.46	5.82±2.73	10.2±2.49	0.011
P_PO4 (mg L^{-1})	0.005±0.004	0.03±0.03	0.27±0.26	0.005	0.005±0.004	0.10±0.12	0.34±0.24	0.014	0.01±0.01	0.06±0.05	0.41±0.38	0.015
TUMetals	0.06±0.01	0.09±0.02	0.49±0.27	0.001	0.09±0.08	0.23±0.08	0.33±0.16	0.023	0.09±0.05	0.12±0.05	0.71±0.38	0.003
TUPestic	5E-06±4E-06	2E-04±1E-04	1E-03±2E-03	0.007	1E-05±1E-05	8E-05±3E-05	3E-03±2E-03	<0.001	2E-05±3E-05	10E-07±6E-06	2E-03±1E-03	<0.001
TUPSC	1E-04±1E-04	2E-04±2E-04	3E-04±1E-04	-	2E-05±1E-05	4E-04±5E-04	3E-04±1E-04	-	4E-05±3E-05	3E-04±4E-04	5E-04±4E-04	-
TUTotal	1E-04±1E-04	4E-04±3E-04	2E-03±2E-03	0.03	3E-05±2E-05	5E-04±5E-04	3E-03±2E-03	0.002	6E-05±4E-05	3E-04±4E-04	2E-03±1E-03	0.03

PCA and testing of the differences between groups of sites per season

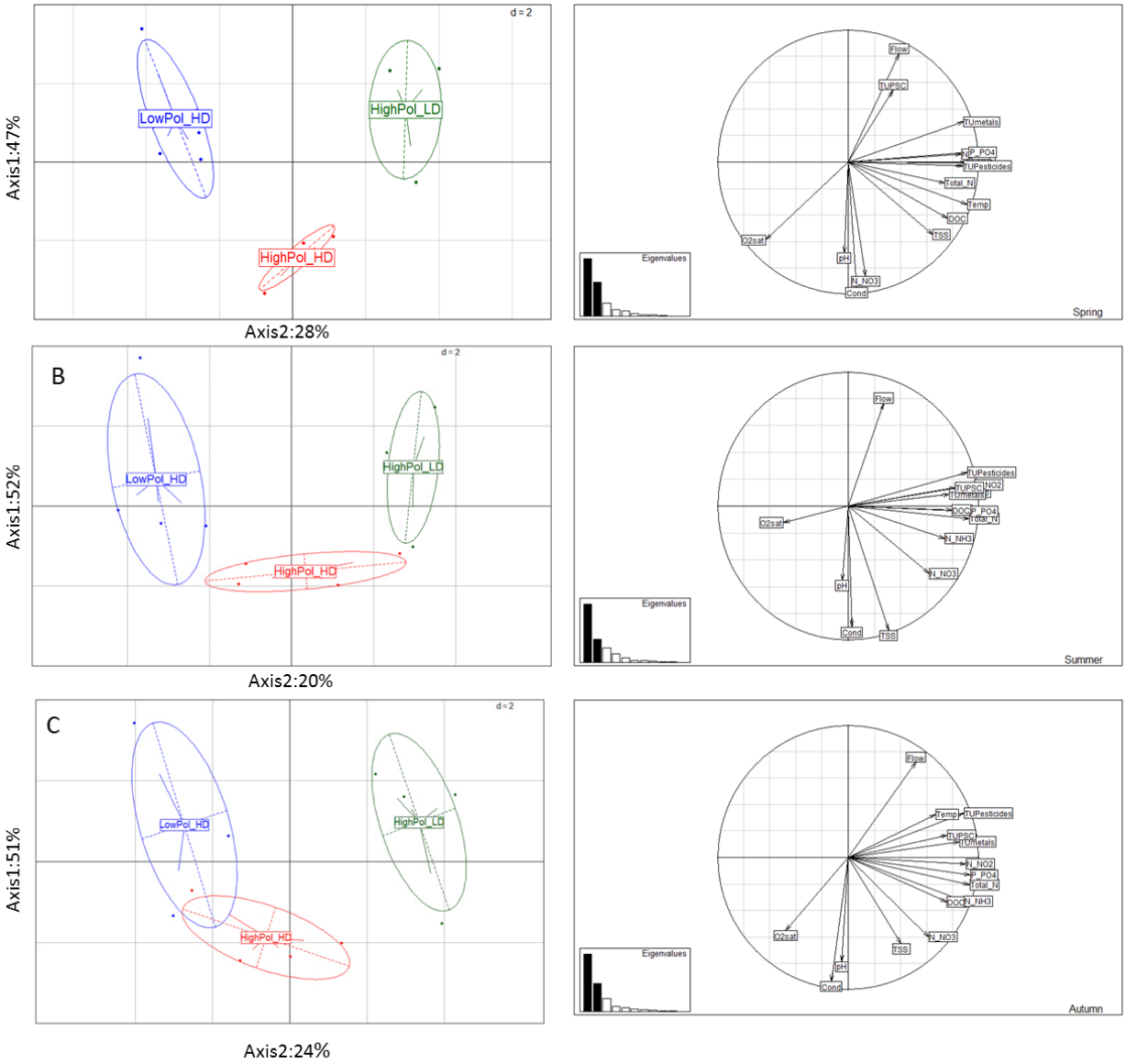


Figure S3. PCA of environmental data performed on sampling period. A: Spring; R^2=0.64, simulated p-value=0.010. B: Summer; R^2=0.57, simulated p-value =0.010. C: Autumn; 0.57, simulated p-value = 0.010.

Statistical tests performed on biological indexes

Table S4. P-values for ANOVA and t-test performed to test differences on biological indexes between groups of sites. Significant differences confirmed when P<0.05. A: LowPol_HD; B: HighPol_HD; C: HighPol_LD

		Spring				Summer				Autumn		
	ANOVA	t-test			ANOVA	t-test			ANOVA	t-test		
		A-B	A-C	B-C		A-B	A-C	B-C		A-B	A-C	B-C
T.Abund.												
Richness					0.086	0.007	0.015	-	0.048	0.022	0.051	
Diversity												
F.Rich.					0.019	0.043	0.073	-	0.069	0.045	0.043	
F.Div.					0.086	-	-	0.033	0.054	0.033		0.037
IBWMP	0.01	0.011	0.008	-	0.003	0.002	0.002	-	0.005	0.004	0.004	-

Biological indexes not showing significant results but with observable trends

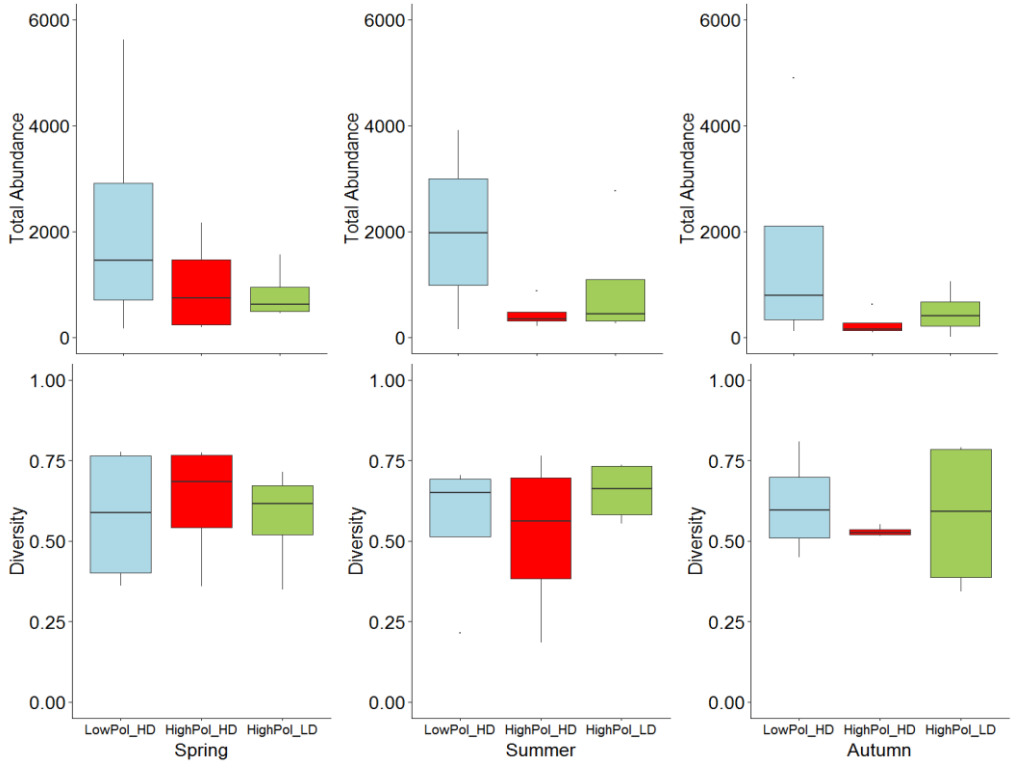


Figure S4. Total abundance and taxonomic diversity (Simpson) indexes in LowPol_HD, HighPol_HD and HighPol_LD groups of sites in spring, summer and autumn.

Table S5. Macroinvertebrate abundance data

Code	Press	Aeshnidae	Ancylidae	Athericidae	Baetidae	Brachycentridae	Caenidae	Capniidae	Chironomini	Diamesinae	Elmidae	Enchytraeidae	Ephemerellidae	Ephemeridae	Erpobdellidae
s03Sp	LowImp_HD	0	0	0	2	0	0	0	67	9	1	0	0	13	0
s02Sp	LowImp_HD	0	9	0	181	0	0	0	0	0	1	0	0	0	0
s01Sp	LowImp_HD	0	19	0	69	0	0	0	2	0	14	1	0	0	0
s07Sp	LowImp_HD	3	68	0	83	0	0	8	21	0	553	86	0	0	0
s11Sp	HighImp_LD	0	0	0	4	0	0	0	658	0	3	576	0	0	1
s09Sp	HighImp_LD	0	0	0	0	0	0	0	219	0	0	1	0	0	77
s08Sp	HighImp_LD	0	4	0	22	0	0	0	26	0	0	309	0	0	0
s16Sp	HighImp_LD	0	0	0	0	0	0	0	28	0	0	360	0	0	0
s12Sp	HighImp_HD	0	0	0	30	0	14	0	337	0	0	231	0	0	0
s13Sp	HighImp_HD	0	0	0	0	0	0	0	103	0	0	18	0	0	0
s14Sp	HighImp_HD	0	0	0	0	0	0	0	1708	0	1	262	0	0	0
s15Sp	HighImp_HD	0	6	0	1	0	23	0	46	0	0	14	0	0	0
s03Su	LowImp_HD	0	1	0	4	0	0	0	2	0	0	3	0	0	0
s02Su	LowImp_HD	1	55	0	46	4	1	0	0	0	94	1	12	0	5
s01Su	LowImp_HD	0	729	0	146	0	0	0	0	0	18	0	0	0	0
s07Su	LowImp_HD	11	116	0	47	0	0	0	0	0	93	5	17	0	0
s11Su	HighImp_LD	0	0	0	0	0	0	0	21	0	0	0	0	0	8
s09Su	HighImp_LD	0	0	0	39	0	0	0	204	0	0	0	0	0	88
s08Su	HighImp_LD	0	97	0	0	0	0	0	7	0	0	71	0	0	0
s16Su	HighImp_LD	0	0	0	2	0	0	0	120	0	0	217	0	0	0
s12Su	HighImp_HD	0	0	0	36	0	321	0	130	0	1	83	11	0	0
s13Su	HighImp_HD	0	0	0	0	0	0	0	300	0	0	0	0	0	0
s14Su	HighImp_HD	0	0	0	0	0	0	0	253	0	0	2	0	0	0
s15Su	HighImp_HD	0	0	0	0	0	11	0	0	0	0	0	0	0	0
s03Au	LowImp_HD	0	0	5	0	0	0	6	4	0	0	0	0	0	0
s02Au	LowImp_HD	0	8	0	4	0	0	0	1	0	16	0	0	0	0
s01Au	LowImp_HD	0	1	0	2	0	0	0	0	0	9	0	0	0	0
s07Au	LowImp_HD	4	8	0	51	0	0	0	0	0	792	0	0	0	0
s11Au	HighImp_LD	0	0	0	0	0	0	0	18	0	0	65	0	0	1
s09Au	HighImp_LD	0	0	0	0	0	0	0	4	0	0	158	0	0	32
s08Au	HighImp_LD	0	23	0	0	0	0	0	16	0	1	30	0	0	0
s16Au	HighImp_LD	0	0	0	2	0	0	0	0	0	0	4	0	0	0
s12Au	HighImp_HD	0	0	0	9	0	4	0	51	0	0	412	0	0	0
s13Au	HighImp_HD	0	0	0	0	0	0	0	9	0	0	11	0	0	0
s14Au	HighImp_HD	0	0	0	0	0	0	0	102	0	0	3	0	0	0
s15Au	HighImp_HD	0	0	0	0	0	15	0	7	0	0	0	0	0	0

Table S5 (cont.)

Code	Press	Gammaridae	Glossiphoniidae	Heptageniidae	Hydrachnellae	Hydrobiidae	Hydropsychidae	Hydroptilidae	Leptoceridae	Leptophlebiidae	Leuctridae	Limnephilidae	Lumbriculidae
s03Sp	LowImp_HD	0	0	8	0	0	0	0	0	0	8	0	3
s02Sp	LowImp_HD	246	0	14	0	254	0	0	0	0	0	0	2
s01Sp	LowImp_HD	247	0	0	0	1517	4	0	0	0	0	53	9
s07Sp	LowImp_HD	197	0	14	0	4449	11	50	0	0	0	23	4
s11Sp	HighImp_LD	0	8	0	0	0	0	0	0	0	0	0	2
s09Sp	HighImp_LD	0	0	0	0	0	0	0	0	0	0	0	0
s08Sp	HighImp_LD	0	1	0	0	0	1	0	0	0	0	0	81
s16Sp	HighImp_LD	0	0	0	0	12	0	0	0	0	0	0	0
s12Sp	HighImp_HD	20	0	0	0	0	1	0	0	0	0	0	6
s13Sp	HighImp_HD	0	0	0	0	0	0	0	0	0	0	0	1
s14Sp	HighImp_HD	0	0	0	0	0	0	0	0	0	0	0	0
s15Sp	HighImp_HD	6	0	0	0	104	0	0	0	0	0	0	1
s03Su	LowImp_HD	0	0	33	0	0	0	0	0	0	70	0	1
s02Su	LowImp_HD	1174	0	37	2	596	20	2	0	0	0	0	1
s01Su	LowImp_HD	232	0	0	2	48	1	0	0	0	0	0	6
s07Su	LowImp_HD	17	0	0	0	3465	5	5	0	3	0	0	36
s11Su	HighImp_LD	0	175	0	0	0	0	0	0	0	0	0	120
s09Su	HighImp_LD	0	105	0	0	0	1	0	0	0	0	0	1803
s08Su	HighImp_LD	0	4	0	0	2	0	0	0	0	0	0	10
s16Su	HighImp_LD	0	0	0	0	7	0	0	0	0	0	0	120
s12Su	HighImp_HD	40	0	0	0	2	0	0	0	0	0	0	223
s13Su	HighImp_HD	0	0	0	0	0	0	0	0	0	0	0	1
s14Su	HighImp_HD	0	0	0	0	0	0	0	0	0	0	0	58
s15Su	HighImp_HD	9	1	0	0	92	1	0	0	0	0	0	0
s03Au	LowImp_HD	0	0	3	0	0	0	0	0	44	0	0	13
s02Au	LowImp_HD	373	0	17	0	173	16	0	0	0	0	0	1
s01Au	LowImp_HD	256	0	0	0	114	0	0	0	0	0	0	2
s07Au	LowImp_HD	313	0	0	0	3529	33	10	0	0	0	0	0
s11Au	HighImp_LD	0	23	0	0	0	0	0	0	0	0	0	99
s09Au	HighImp_LD	0	59	0	0	0	0	0	0	0	0	0	0
s08Au	HighImp_LD	0	2	0	0	0	0	0	0	0	0	0	435
s16Au	HighImp_LD	0	0	0	0	1	0	0	0	0	0	0	0
s12Au	HighImp_HD	70	0	0	0	0	4	0	0	0	0	0	0
s13Au	HighImp_HD	0	0	0	0	0	4	0	0	0	0	0	0
s14Au	HighImp_HD	0	0	0	0	0	0	0	0	0	0	0	0
s15Au	HighImp_HD	1	0	0	0	94	0	0	0	0	0	0	0

Table S5 (cont.)

Code	Press	Lymnaeidae	Nemouridae	Orthocladiinae	Osmylidae	Perlodidae	Physidae	Planorbidae	Polycentropodidae	Potamanthidae	Psychodidae	Psychomyiidae	Rhyacophilidae
s03Sp	LowImp_HD	0	0	12	0	0	0	0	0	5	0	0	3
s02Sp	LowImp_HD	0	0	0	0	0	0	7	0	0	0	0	0
s01Sp	LowImp_HD	0	0	0	0	0	0	0	0	0	0	0	0
s07Sp	LowImp_HD	0	0	1	0	1	4	9	0	0	0	0	0
s11Sp	HighImp_LD	0	0	5	0	0	1	1	0	0	40	0	0
s09Sp	HighImp_LD	0	0	0	0	0	160	1	0	0	5	0	0
s08Sp	HighImp_LD	1	0	2	0	0	1	1	0	0	0	0	0
s16Sp	HighImp_LD	6	0	0	0	0	18	8	0	0	0	0	0
s12Sp	HighImp_HD	0	0	3	0	0	0	0	0	0	0	0	0
s13Sp	HighImp_HD	0	0	1	0	0	0	1	0	0	2	0	0
s14Sp	HighImp_HD	0	0	2	0	0	0	2	0	0	71	0	0
s15Sp	HighImp_HD	21	0	0	0	0	9	4	0	0	0	0	0
s03Su	LowImp_HD	0	0	0	0	0	0	0	1	39	0	0	0
s02Su	LowImp_HD	0	0	0	0	0	0	5	0	0	0	0	3
s01Su	LowImp_HD	0	0	0	0	0	0	0	1	0	0	0	0
s07Su	LowImp_HD	5	0	0	0	1	12	20	0	0	0	0	0
s11Su	HighImp_LD	0	0	0	0	0	9	0	0	0	0	0	0
s09Su	HighImp_LD	0	0	0	0	0	169	0	0	0	0	0	0
s08Su	HighImp_LD	0	0	0	0	0	1	0	0	0	0	0	0
s16Su	HighImp_LD	8	0	0	0	0	13	9	0	0	0	0	0
s12Su	HighImp_HD	0	0	0	0	0	0	0	0	0	2	0	0
s13Su	HighImp_HD	0	0	0	0	0	0	0	0	0	6	0	0
s14Su	HighImp_HD	1	0	0	0	0	28	1	0	0	0	0	0
s15Su	HighImp_HD	78	0	0	0	0	4	12	0	0	0	0	0
s03Au	LowImp_HD	0	8	9	0	0	0	1	3	0	0	2	0
s02Au	LowImp_HD	0	0	0	0	0	0	8	0	0	0	2	0
s01Au	LowImp_HD	0	0	0	0	0	0	0	0	0	0	0	0
s07Au	LowImp_HD	0	0	0	0	1	1	2	0	0	0	1	0
s11Au	HighImp_LD	0	0	1	0	0	1	0	0	0	26	0	0
s09Au	HighImp_LD	0	0	0	0	0	802	1	0	0	0	0	0
s08Au	HighImp_LD	0	0	2	0	0	4	2	0	0	0	0	0
s16Au	HighImp_LD	0	0	0	0	0	1	1	0	0	0	0	0
s12Au	HighImp_HD	0	0	6	0	0	0	2	0	0	0	0	0
s13Au	HighImp_HD	0	0	1	0	0	0	0	0	0	56	0	0
s14Au	HighImp_HD	0	0	1	0	0	1	1	0	0	41	0	0
s15Au	HighImp_HD	10	0	1	0	0	2	1	0	0	0	0	0

Table S5 (cont.)

Code	Press	Sericostomatidae	Sialidae	Simuliidae	Sphaeriidae	Stratiomyiidae	Tanypodinae	Tanytarsini	Tipulidae	Tubificidae
s03Sp	LowImp_HD	0	0	3	0	0	0	26	0	0
s02Sp	LowImp_HD	0	0	172	0	0	0	0	0	0
s01Sp	LowImp_HD	1	0	3	66	0	4	3	0	4
s07Sp	LowImp_HD	0	0	0	26	0	0	8	0	1
s11Sp	HighImp_LD	0	0	0	0	0	2	259	0	0
s09Sp	HighImp_LD	0	0	0	0	1	0	284	0	0
s08Sp	HighImp_LD	0	0	0	0	0	0	7	1	39
s16Sp	HighImp_LD	0	0	0	0	0	0	17	0	0
s12Sp	HighImp_HD	0	0	393	0	0	1	174	16	0
s13Sp	HighImp_HD	0	0	0	0	0	0	55	6	0
s14Sp	HighImp_HD	0	0	1	0	0	0	118	0	0
s15Sp	HighImp_HD	0	0	0	0	0	0	18	0	0
s03Su	LowImp_HD	0	0	0	0	0	0	0	0	1
s02Su	LowImp_HD	0	0	636	2	0	0	0	0	0
s01Su	LowImp_HD	0	1	2	72	0	0	1	0	0
s07Su	LowImp_HD	0	0	15	40	0	0	0	0	3
s11Su	HighImp_LD	0	0	0	0	0	0	1	0	0
s09Su	HighImp_LD	0	0	240	0	0	5	112	0	0
s08Su	HighImp_LD	0	0	0	1	0	0	9	0	53
s16Su	HighImp_LD	0	0	0	0	0	1	10	0	34
s12Su	HighImp_HD	0	0	0	0	0	2	22	0	0
s13Su	HighImp_HD	0	0	0	0	0	3	23	0	0
s14Su	HighImp_HD	0	0	0	0	0	0	9	0	0
s15Su	HighImp_HD	0	0	0	6	0	0	0	0	0
s03Au	LowImp_HD	0	0	0	0	0	0	15	1	2
s02Au	LowImp_HD	3	0	539	4	0	0	0	0	0
s01Au	LowImp_HD	0	1	0	26	0	0	0	0	0
s07Au	LowImp_HD	6	0	129	18	0	0	0	2	0
s11Au	HighImp_LD	0	0	15	0	0	0	28	0	5
s09Au	HighImp_LD	0	0	0	0	0	0	4	0	0
s08Au	HighImp_LD	0	0	1	2	0	0	20	0	1
s16Au	HighImp_LD	0	0	1	0	0	0	0	0	0
s12Au	HighImp_HD	0	0	21	0	0	0	40	0	0
s13Au	HighImp_HD	0	0	0	0	1	0	5	0	0
s14Au	HighImp_HD	0	0	0	0	4	0	5	0	0
s15Au	HighImp_HD	0	0	1	1	0	0	4	0	1

Supporting data for the assessment of trait categories distribution between classes per season

Table S6. Percentages of contribution of each trait category to the total variance explained on Axis 1 and 2 of co-inertia analysis. Yellow highlight means position on the positive side of the axis and grey highlight means position on the negative side of the axis.

		Axis1			Axis2		
Trait	***Trait category***	Spring	Summer	Autumn	Spring	Summer	Autumn
Size	<0.5cm	0.0	0.6	3.2	2.3	1.4	0.1
	0.5-1cm	0.3	0.1	0.7	0.0	1.9	1.1
	1-2cm	2.4	1.0	0.4	0.0	0.6	0.1
	2-4cm	0.3	0.7	0.0	0.2	5.4	1.3[a]
	>4cm	4.5	6.2	2.4[a]	5.4	0.3	1.3[a]
Life cycle duration	Short_LC	0.3	0.9	0.2	0.0	1.4	1.9
	Long_LC	0.5	1.7	0.4	0.1	2.8	3.8
Number of cycles p/y	Semivoltine	5.8	2.4	4.6	0.2	0.4	0.0
	Univoltine	0.5	1.2	0.4	2.0	0.8	0.1
	Plurivoltine	4.8	4.8	3.5	4.0	2.2	0.1
Reproduction	ovoviviparity	1.3	0.9	0.1	3.0	2.0	0.5
	free_eggs	0.3	1.2	0.1	5.4	0.3	5.0
	fixed_eggs	31.4	11.2	1.9	6.9	9.9	0.2
	fixed_clutches	3.5	0.8	0.1	5.2	1.6	0.3
	free_clutches	1.1	6.4	5.7	0.1	0.2	0.2
	veg_terr_clutches	0.0	1.6	2.8	0.1	0.4	0.7
	asexual	2.3	1.4	3.3[a]	4.0	0.5	3.4[a]
Dispersal	aquatic_passive	1.6	2.9	2.2	0.0	0.1	1.7
	aquatic_active	0.0	0.1	0.3	0.5	0.6	0.2
	aerial_passive	0.1	0.0	0.0	0.0	0.1	0.2
	aerial_active	3.0	5.9	2.8	1.0	0.4	4.3[c]
Resistance forms	resist_eggs	3.7	3.5	3.7	0.2	0.0	0.0
	cocoons	4.5[a]	6.6[a]	0.9	10.9	1.1	2.0[a]
	diapause	2.2	0.0	1.8[b]	0.7	0.1	5.3[b]
	none	1.2	0.0	0.1	0.4	0.0	0.9
Respiration	tegument	1.0	3.2	0.7	0.3	0.5	6.8[a]
	gills	4.6	5.6	9.1	0.2	0.0	0.2
	aerial	3.7	0.0	12.3[b]	0.3	6.2	29.9[b]
Substrate relation	surface_swimmer	1.3	0.7	10.1[b]	3.2	0.2	8.0[b]
	swimmer	0.4	0.2	0.4	0.2	0.1	0.0
	crawler	0.3	1.7	1.7	4.5	0.0	0.3
	burrower	0.0	3.7	0.2	7.2	1.6	1.6[a]
	interstitial	0.2	2.3	2.2[a]	3.4	1.8	2.4[a]
	temp_attached	0.3	0.5	0.5	0.0	6.0	0.1

[a]:Higher affinity for HighPol_LD; [b]; higher affinity for HighPol_HD; [c]: Higher frequency in LowPol_HD.

Table S6 (cont.)

		Axis1			Axis2		
Trait	***Trait category***	Spring	Summer	Autumn	Spring	Summer	Autumn
Food	DetritusL1mm	0.2	2.0	2.0	4.8	2.6	0.4
	DeadplantM1mm	0.5	0.6	0.1	0.0	1.6	2.7[c]
	Microphytes	0.0	0.2	0.1	0.0	0.0	0.1
	Macrophytes	0.4	1.8	1.9	0.2	0.2	0.3
	Dead_animM1mm	0.2	0.4	1.3	2.8	0.1	0.3
	Microinvertebrates	0.3	1.0	0.4	0.0	4.3	0.6
	Macroinvertebrates	1.2	1.2[a]	0.1	2.9	6.0	3.9[a]
Feeding type	deposit_feeder	2.3	6.0	11.8	10.2	12.9	0.4
	shredder	2.3	2.7	1.6	0.0	4.9	2.8[c]
	scraper	0.0	0.2	0.5	2.5	0.4	0.1
	filter_feeder	0.3	0.9	0.9	0.6	5.3	0.2
	piercer	4.7[a]	2.7[a]	0.1	2.0	6.0	3.7[a]
	predator	0.3	0.2	0.2	1.7	4.9	0.8

[a]:Higher affinity for HighPol_LD; [b]; higher affinity for HighPol_HD; [c]: Higher frequency in LowPol_HD.

Appendix E: SI Chapter 6

Section A: Lufenuron extraction method from water and sediment samples.

Lufenuron was extracted from water samples by solid-phase extraction (SPE). Before SPE, 10 mL of methanol (20% v/v) were added to 40 mL of water samples. The water samples were previously spiked with 100 µL of the internal standard (IS) novaluron (Sigma Aldrich, CAS 116714-46-6) in the range of 15 - 600 µg/L. The amount of IS added to samples varied according to the experimental requirements. Afterwards, the samples were transferred into a polypropylene tube and centrifuged (4000 rpm during 5 min). The SPE was performed using Clearnet IC-C18 cartridges (360 mg, 1 mL; Vaima 2000 Componentes, Madrid, Spain) pre-conditioned with 5 mL of acetonitrile, 5 mL of chloroform, 5 mL of acetonitrile and 5 mL of Milli-Q. The centrifuged water samples were loaded into the cartridges at low speed. After loading, the cartridges were dried-up under vacuum during 30 min to remove excess water and eluted with 5 mL of acetonitrile and 5 mL of chloroform. The extracts were evaporated to dryness, reconstituted with 1 mL of methanol:water 80:20 (v/v), filtered using a 0.22 µm PVDF syringe filter (Kinesis, Cambridgeshire, UK) and stored in amber glass vials.

Sediment samples were lyophilised and followed a two-step extraction procedure. First, 6 mL of acetone and 60 µL of IS (80-8330 µg/L) were added to 7 g of sediment, and the mixture was shaken for 2 h (230 mot/min). After that, the samples were centrifuged for 10 min at 2000 rpm. The supernatant was carefully transferred into a glass tube and 6 mL of acetone were added to the solid fraction in order to carry out a second extraction. Finally, both supernatants were mixed and evaporated to dryness. Samples were reconstituted in 4 mL of methanol and 1 mL of Milli-Q water, vortexed, filtered using a 0.20 µm PVDF syringe filter (Kinesis, Cambridgeshire, UK) and stored in amber glass vials.

Optimum parameters for the LC-MS/MS system and the Multiple Reaction Mode (MRM) transitions.

Table S1. Instrumental parameters for LC-MS/MS system.

Triple Quadrupole (MS/MS) parameters	
Ionization mode	Positive
Sheath gas temperature	350 °C
Sheath gas flow	11 L/min
Drying gas temperature	250 °C
Drying gas flow	13 L/min
Nebulizer press	25 psi
Capillary voltage	4000 V
Nozzle voltage	500 V
Δ EMV	400 V
Chromatographic parameters	
Mobile phases	A: 0.1% formic acid in water
Elution mode	Isocratic: 20% (v/v) A / 80% (v/v) B
Flow rate	0.4 mL/min
Column temperature	40 °C
Injection volume	20 µL

Table S2. Collision energies, precursors and product ions selected for the analysis of lufenuron and novaluron in Multiple Reaction Mode (MRM).

Compound	**Formula**	**Precursor**	**Product ion**	**Collision**	**MRM transition**
Lufenuron	$C_{17}H_8Cl_2F_8N_2O_3$	510.7	157.8	25	Quantifier (Q)
		510.7	140.8	50	qualifier (q)
Novaluron	$C_{17}H_9ClF_8N_2O_4$	492.8	157.8	20	Quantifier (Q)
		492.8	140.8	50	qualifier (q)

Measured lufenuron concentration in water and sediment

Table S3. Measured lufenuron concentrations in water (mean values; n=3) at different sampling dates, under different environmental scenarios (T20, T28, T28_Drought). n.d. not detected.

		T20		
Time (day)	C1(µg/L)	St.dev.	C2(µg/L)	St.dev.
-1	n.d.	n.d.	n.d.	n.d.
0.1	0.12[1]	-	0.95[1]	-
3	0.04[1]	-	0.46[1]	-
10.1	1.85	0.13	8.75	0.53
11	1.14	0.20	6.34	0.12
13	0.64	0.07	3.32	0.39
17	0.34	0.03	1.71	0.37
		T28		
Time (day)	C1 (µg/L)	St.dev.	C2 (µg/L)	St.dev.
-1	n.d.	n.d.	n.d.	n.d.
0.1	0.11[1]	-	1.03[1]	-
3	0.03[1]	-	0.30[1]	-
10.1	2.17	0.23	8.38	1.08
11	1.05	0.14	5.18	0.84
13	0.48	0.20	2.66	0.81
17	0.26	0.14	1.26	0.50
		T28_Drought		
Time (day)	C1 (µg/L)	St.dev.	C2 (µg/L)	St.dev.
-1	n.d.	n.d.	n.d.	n.d.
0.1	0.12	0.02	1.12	0.09
3	0.027	0.005	0.35	0.11
10.1	2.49	0.87	9.40	0.81
11	0.91	0.23	4.12	1.27
13	0.30	0.06	1.61	0.91
17	0.09	0.03	0.57	0.40

[1] Result based on one measurement (n=1) due to analytical problems.

Table S4. Measured lufenuron concentrations in sediment (mean values; n=3) at different sampling dates, under different environmental scenarios (T20, T28, T28_Drought). n.d. not detected.

		T20		
Time (day)	C1 (µg/kg)	St.dev.	C2 (µg/kg)	St.dev.
-1	n.d.	n.d.	n.d.	n.d.
4	0.42	0.16	2.29	0.20
14	8.29	3.73	38.5	8.6
21	8.73	0.41	36.7	4.4
46	4.47	1.14	20.6	3.5
60	3.16	2.04	25.2	5.3
73	3.14	0.33	11.3	0.8
		T28		
Time (day)	C1 (µg/kg)	St.dev.	C2(µg/kg)	St.dev.
-1	n.d.	n.d.	n.d.	n.d.
4	0.29	0.05	3.40	0.50
14	5.29	2.63	36.5	9.2
21	3.78	0.09	31.6	7.4
46	2.22	1.39	18.0	4.7
60	0.92	0.42	9.2	2.0
73	0.04*	0.04	10.4	2.0
		T28_Drought		
Time (day)	C1 (µg/kg)	St.dev	C2(µg/kg)	St.dev
-1	n.d.	n.d.	n.d.	n.d.
4	0.28	0.10	4.89	4.23
14	5.47	3.42	25.3	5.3
21	3.25	1.54	17.7	4.2
46	3.78	2.17	7.20	2.57
60	1.01	1.32	6.31	2.70
73	0.75	0.57	4.02	2.51

*Outlier, excluded from analysis.

Table S5. Calculated lufenuron concentration per g of OC contained in the sediment samples (mean values; n=3) at different sampling dates, under different environmental scenarios (T20, T28, T28_Drought). n.d. not detected.

		T20		
Time (day)	C1 (µg/g OC)	St.dev.	C2 (µg/g OC)	St.dev.
-1	n.d.	n.d.	n.d.	n.d.
4	24.1	9.4	131.4	11.6
14	476	214	2213	495
21	502	23	2107	255
46	257	66	1184	200
60	182	117	1447	305
73	181	19	648.2	42.8
		T28		
Time (day)	C1 (µg/g OC)	St.dev.	C2 (µg/g OC)	St.dev.
-1	n.d.	n.d.	n.d.	n.d.
4	16.5	3.1	197.3	31.3
14	304	151	2098	527
21	217	5	1814	427
46	128	80	1034	268
60	52.7	24.1	527.1	113.6
73	2.41	2.53	595.9	114.5
		T28_Drought		
Time (day)	C1 (µg/g OC)	St.dev.	C2 (µg/g OC)	St.dev.
-1	n.d.	n.d.	n.d.	n.d.
4	15.8	5.9	281	243
14	314	197	1452	305
21	187	89	1017	239
46	217	125	414	148
60	58.1	76.0	363	155
73	43.2	32.9	231	145

*Outlier, excluded from analysis.

Influence of single and combined effects of lufenuron and the evaluated environmental factors on water physico-chemical variables.

Table S6. Results of the two-way ANOVA test (p-values) considering the influence of lufenuron and temperature on DO, EC and pH. p-values below 0.05 are represented in bold. N/A: Not applicable, due to the absence of a stressor at that sampling time. n.s.: not significant.

	D-7	D-3	D0	D4	D7	D10	D14	D21	D28	D46	D53	D60	D73
Lufenuron													
DO	N/A	N/A	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
EC	N/A	N/A	n.s.	n.s.	n.s.	n.s.	n.s.	**0.018**	**0.003**	n.s.	n.s.	**0.018**	n.s.
pH	N/A	N/A	n.s.	n.s.	**0.013**	**<0.001**	**0.035**	**<0.001**	**<0.001**	**0.005**	n.s.	n.s.	n.s.
Temperature													
DO	**<0.001**	**<0.001**	**0.001**	n.s.	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**<0.001**	**0.001**
EC	n.s.	n.s.	n.s.	n.s.	**0.049**	n.s.	**0.002**	**<0.001**	**0.024**	n.s.	n.s.	n.s.	n.s.
pH	n.s.	**<0.001**	n.s.	**0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**<0.001**	**<0.001**	**<0.001**
*Lufenuron*Temperature*													
DO	N/A	N/A	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
EC	N/A	N/A	**0.034**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
pH	N/A	N/A	n.s.	**0.009**	**0.017**	**<0.001**	n.s.	**0.019**	**<0.001**	n.s.	n.s.	n.s.	**0.009**

Table S7. Results of the two-way ANOVA test (p-values) considering the influence of lufenuron and drought on DO, EC and pH. p-values below 0.05 are represented in bold. N/A: Not applicable, due to the absence of a stressor at that sampling time. n.s.: not significant.

	D-7	D-3	D0	D4	D7	D10	D14	D21	D28	D46	D53	D60	D73
Lufenuron													
DO	N/A	N/A	n.s	n.s	n.s	n.s	n.s	n.s	**0.045**	n.s	n.s	n.s	n.s
EC	N/A	N/A	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	**0.027**	n.s
pH	N/A	N/A	n.s	n.s	**0.033**	**0.011**	n.s	**0.003**	**<0.001**	n.s	n.s	n.s	n.s
Drought													
DO	n.s	n.s	n.s	n.s	n.s	**0.034**	**0.005**	n.s	n.s	**0.001**	n.s	n.s	**0.021**
EC	n.s	n.s	n.s	**0.024**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	n.s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
pH	n.s	n.s	n.s	n.s	n.s	n.s	**0.001**	**<0.001**	**0.005**	**0.046**	n.s	**0.001**	**0.007**
*Lufenuron*Drought*													
DO	N/A	N/A	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	**0.016**
EC	N/A	N/A	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
pH	N/A	N/A	n.s	n.s	n.s	**0.037**	n.s	n.s	n.s	n.s	n.s	n.s	n.s

Table S8. Results of the two-way ANOVA test (p-values) considering the influence of lufenuron and temperature on nutrient concentrations and DOC. p-values below 0.05 are represented in bold. N/A: Not applicable, due to the absence of a stressor at that sampling time. n.s.: not significant. n.e.: not statistically evaluated due to non-detectable levels in the two groups compared.

	D-3	D14	D28	D46	D60
Lufenuron					
Ammonia	N/A	n.s.	n.s.	n.e.	**0.043**
Nitrite	N/A	n.s.	n.s.	n.s.	n.s.
Nitrate	N/A	n.s.	n.s.	n.s.	**0.02**
Fosfate	N/A	n.s.	n.s.	**<0.001**	n.s.
Total N	N/A	n.s.	n.s.	n.s.	**0.018**
Total P	N/A	n.s.	n.s.	n.s.	n.s.
N/P	N/A	n.s.	n.s.	n.s.	n.s.
DOC	N/A	n.s.	**0.009**	n.s.	n.s.
Temperature					
Ammonia	n.s.	n.s.	n.s.	n.e.	n.s.
Nitrite	**<0.001**	n.s.	n.s.	n.s.	n.s.
Nitrate	**0.014**	**0.01**	**0.007**	**0.011**	**0.025**
Fosfate	**0.001**	n.s.	n.s.	n.s.	n.s.
Total N	**0.006**	**0.005**	**0.007**	**0.011**	**0.025**
Total P	n.s.	**0.002**	**0.005**	n.s.	n.s.
N/P	**0.006**	**0.006**	n.s.	n.s.	n.s.
DOC	**0.044**	**<0.001**	n.s.	n.s.	n.s.
*Lufernuron*Temperature*					
Ammonia	N/A	n.s.	n.s.	n.e.	n.s.
Nitrite	N/A	n.s.	n.s.	n.s.	n.s.
Nitrate	N/A	n.s.	n.s.	n.s.	n.s.
Fosfate	N/A	n.s.	n.s.	n.s.	n.s.
Total N	N/A	n.s.	n.s.	n.s.	n.s.
Total P	N/A	n.s.	n.s.	n.s.	n.s.
N/P	N/A	n.s.	n.s.	n.s.	n.s.
DOC	N/A	n.s.	n.s.	n.s.	n.s.

Table S9. Results of the two-way ANOVA test (p-values) considering the influence of lufenuron and drought on nutrients concentrations and DOC. p-values below 0.05 are represented in bold. N/A: Not applicable, due to the absence of a stressor at that sampling time. n.s.: not significant.

	D-3	D14	D28	D46	D60
Lufenuron					
Ammonia	N/A	n.s.	n.s.	n.s.	n.s.
Nitrite	N/A	n.s.	n.s.	n.s.	n.s.
Nitrate	N/A	n.s.	n.s.	n.s.	n.s.
Fosfate	N/A	n.s.	n.s.	**0.04**	n.s.
Total N	N/A	n.s.	n.s.	n.s.	n.s.
Total P	N/A	n.s.	**0.03**	n.s.	n.s.
N/P	N/A	n.s.	n.s.	n.s.	n.s.
DOC	N/A	n.s.	n.s.	n.s.	n.s.
Drought					
Ammonia	n.s.	n.s.	n.s.	n.s.	n.s.
Nitrite	n.s.	n.s.	n.s.	n.s.	n.s.
Nitrate	n.s.	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Fosfate	n.s.	n.s.	n.s.	n.s.	n.s.
Total N	n.s.	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Total P	**0.005**	n.s.	**0.001**	n.s.	n.s.
N/P	**0.037**	n.s.	n.s.	n.s.	n.s.
DOC	n.s.	**<0.001**	**<0.001**	**<0.001**	**0.002**
*Lufenuron*Drought*					
Ammonia	N/A	n.s.	n.s.	n.s.	**0.034**
Nitrite	N/A	n.s.	n.s.	n.s.	n.s.
Nitrate	N/A	n.s.	n.s.	n.s.	n.s.
Fosfate	N/A	n.s.	n.s.	n.s.	n.s.
Total N	N/A	n.s.	n.s.	n.s.	n.s.
Total P	N/A	n.s.	n.s.	n.s.	n.s.
N/P	N/A	n.s.	n.s.	n.s.	n.s.
DOC	N/A	n.s.	n.s.	n.s.	n.s.

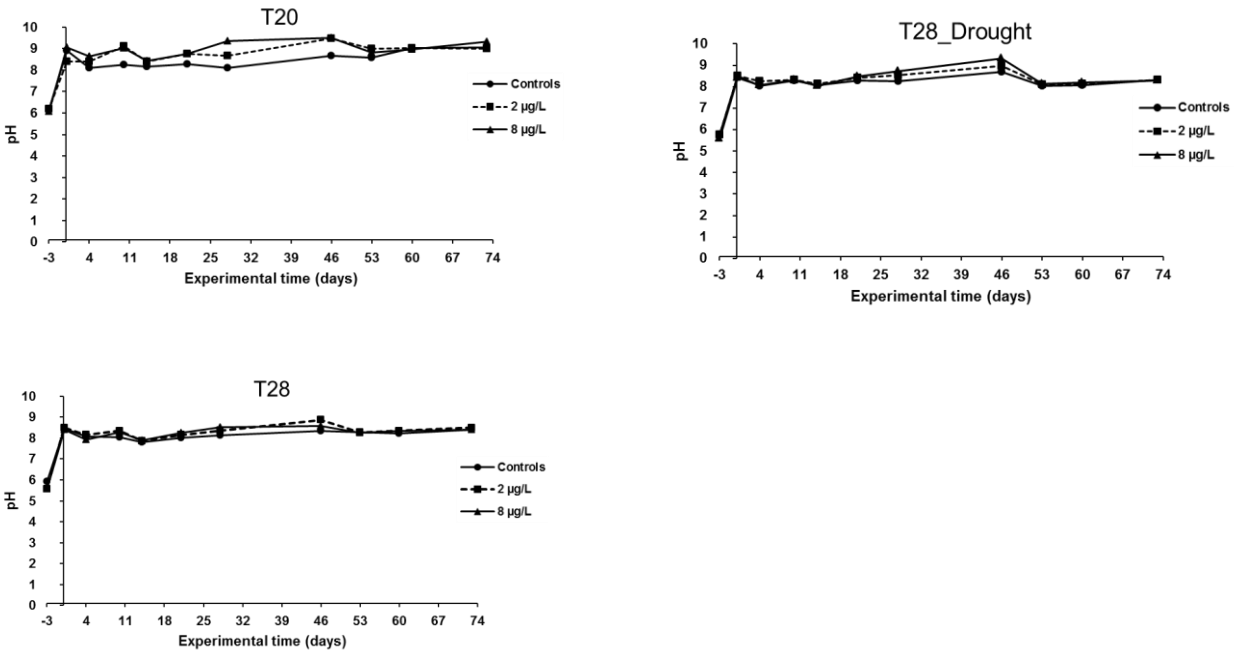


Figure S1. Measured pH (mean values; n=3) in microcosm water at different exposure levels under different environmental scenarios (i.e., T20, T28, T28_Drought).

Differences in community composition due to temperature and drought.

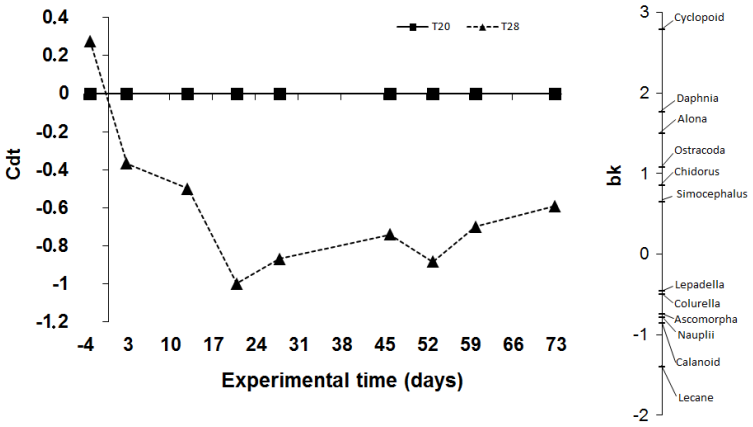


Figure S2. PRC indicating the differences in zooplankton species composition between the controls evaluated at T20 and T28. Of all variance, 37% could be attributed to sampling date; this is displayed on the horizontal axis. 20% of all variance could be attributed to different temperature. Of this variance, 44% is displayed on the vertical axis. Taxa weights between 0.4 and -0.4 are not shown. The Monte Carlo permutation test indicated that temperature had a marginally significant influence on the community composition (Monte Carlo test, p-vaue=0.09). Individual RDA with Monte Carlo permutation tests performed per sampling date revealed marginally significant results (0.05<p-value<0.1) on the majority of the sampling dates.

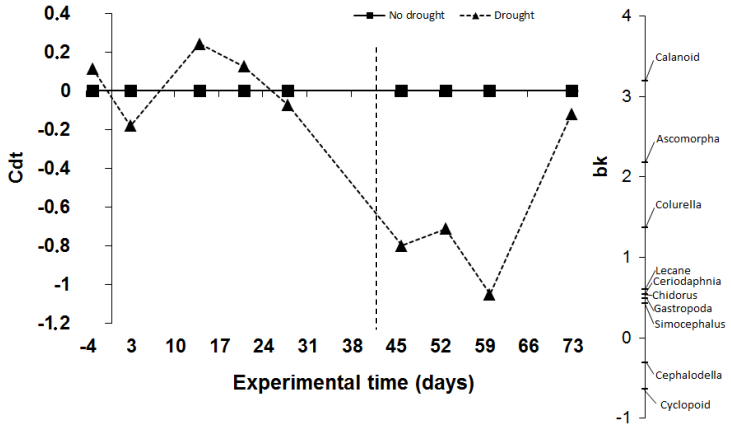


Figure S3. PRC indicating the differences in zooplankton species composition between the controls that did not undergo drought and ones that were affected by drought. Of all variance, 43% could be attributed to sampling date; this is displayed on the horizontal axis. 14% of all variance could be attributed to drought. Of this variance, 33% is displayed on the vertical axis. Taxa weights between 0.4 and -0.4 are not shown. Despite some taxa responding differently, the Monte Carlo permutation test indicated that the drought treatment had no significant influence on the community composition (Monte Carlo test, p-value=0.41). Individual RDA with Monte Carlo permutation test performed per sampling date, revealed marginally significant results (0.05<p-value<0.1) on Day 46 and 60, just after complete desiccation and refilling occurred.

Influence of single and combined effects of lufenuron and the evaluated environmental factors on zooplankton taxa.

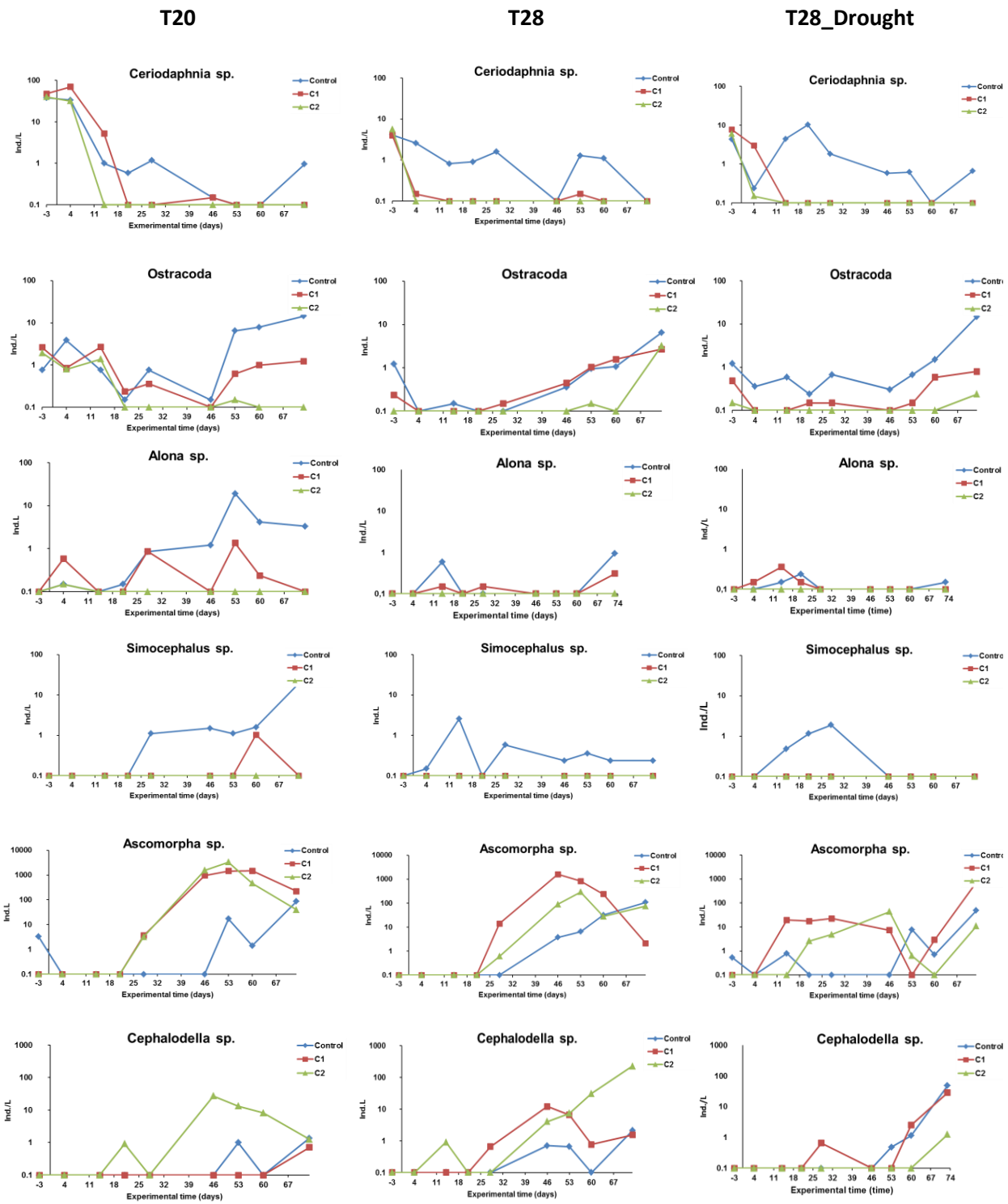


Figure S4. Density dynamics of other taxa showing different lufenuron-response patterns among environmental scenarios (individuals/L).

Table S10. Results of the two-way ANOVA analysis (p-values) performed with lufenuron and temperature as factors and selected zooplankton taxa (those with b_k values between 0.4 and -0.4). p-values below 0.05 are represented in bold. N/A: Not applicable, due to the absence of a stressor at that sampling time. n.s.: not significant. n.e.: not evaluated due to the absence of individuals.

	D-3	D4	D14	D21	D28	D46	D53	D60	D73
Lufenuron									
Ceriodaphnia	N/A	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Daphnia	N/A	n.s.	**<0.001**	**0.007**	**0.001**	**0.009**	**<0.001**	n.s.	n.s.
Chydorus	N/A	n.s.	**0.008**	**0.023**	**0.029**	**0.017**	**<0.001**	**<0.001**	**0.003**
Cyclopoid	N/A	n.s.	**0.001**	**0.02**	n.s.	n.s.	n.s.	n.s.	n.s.
Calanoid	N/A	**0.04**	**0.001**	**<0.001**	**0.003**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Ostracoda	N/A	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**0.008**	**0.024**
Alona	N/A	n.s.	n.s.	n.s.	n.s.	n.s.	**0.006**	**0.038**	n.s.
Simocephalus	N/A	n.s.	**0.003**	n.e.	**0.049**	**0.002**	**0.009**	n.s.	**<0.001**
Nauplii	N/A	**0.001**	**<0.001**	**<0.001**	**<0.001**	n.s.	n.s.	n.s.	n.s.
Ascomorpha	N/A	n.e.	n.e.	n.e.	n.s.	**<0.001**	**<0.001**	**0.025**	n.s.
Lecane	N/A	n.e.	**0.024**	n.s.	n.s.	**0.001**	n.s.	n.s.	**0.005**
Cephalodella	N/A	n.e.	n.s.	n.s.	n.s.	n.s.	n.s.	**0.001**	n.s.
Temperature									
Ceriodaphnia	**<0.001**	**<0.001**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Daphnia	**<0.001**	**<0.001**	**<0.001**	n.s.	**0.006**	**<0.001**	**<0.001**	n.s.	n.s.
Chydorus	n.s.	n.s.	**0.033**	n.s.	n.s.	n.s.	n.s.	n.s.	**0.044**
Cyclopoid	**<0.001**	**0.005**	**<0.001**	**<0.001**	**0.002**	n.s.	**0.035**	n.s.	n.s.
Calanoid	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**0.021**
Ostracoda	**0.003**	**0.005**	**<0.001**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Alona	n.e.	**0.041**	n.s.	n.s.	n.s.	n.s.	**0.001**	**0.017**	n.s.
Simocephalus	n.e.	n.s.	**0.008**	n.e.	n.s.	n.s.	n.s.	n.s.	**<0.001**
Nauplii	**0.044**	**0.019**	**0.019**	**0.044**	**0.001**	n.s.	n.s.	n.s.	n.s.
Ascomorpha	n.s.	n.e.	n.e.	n.e.	n.s.	n.s.	n.s.	n.s.	n.s.
Lecane	n.s.	n.e.	**<0.001**	**<0.001**	n.s.	n.s.	n.s.	n.s.	**0.005**
Cephalodella	n.e.	n.e.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Lufenuron*Temperature									
Ceriodaphnia	N/A	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Daphnia	N/A	n.s.	**0.011**[a]	n.s.	**0.027**[a]	**0.009**[*]	**<0.001**[*]	n.s.	n.s.
Chydorus	N/A	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Cyclopoid	N/A	**0.04**[a]	**0.037**[a]	**0.046**[a]	**0.005**[a]	n.s.	n.s.	n.s.	**0.045**[b]
Calanoid	N/A	n.s.	n.s.	n.s.	n.s.	n.s.	**0.028**[b]	n.s.	**0.01**[b]
Ostracoda	N/A	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Alona	N/A	n.s.	n.s.	n.s.	n.s.	n.s.	**0.006**[*]	**0.038**[*]	n.s.
Simocephalus	N/A	n.s.	**0.003**[b]	n.e.	n.s.	n.s.	n.s.	n.s.	**<0.001**[a]
Nauplii	N/A	**0.005**[a]	n.s.	n.s.	n.s.	**0.019**[b]	n.s.	n.s.	n.s.
Ascomorpha	N/A	n.e.	n.e.	n.e.	n.s.	n.s.	n.s.	n.s.	n.s.
Lecane	N/A	n.e.	**0.024**[a]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Cephalodella	N/A	n.e.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[a]Synergistic interaction. [*] Density declines in controls due to environmental factors (T) do not allow evaluating toxic effects.
[b]Antagonistic interaction.
n.s.:additive.

Table S11. Results of the two-way ANOVA analysis (p-values) performed with lufenuron and drought as factors and selected zooplankton taxa (those with b_k values between 0.4 and -0.4). p-values below 0.05 are represented in bold. N/A: Not applicable, due to the absence of a stressor at that sampling time. n.s.: not significant. n.e.: not evaluated due to the absence of individuals.

	D-3	D4	D14	D21	D28	D46	D53	D60	D73
Lufenuron									
Ceriodaphnia	N/A	n.s.	**0.048**	**0.011**	**0.036**	n.s.	n.s.	n.s.	n.s.
Daphnia	N/A	n.s.	**<0.001**	n.s.	**0.013**	n.e.	n.e.	n.e.	n.e.
Chydorus	N/A	n.s.	**0.006**	**0.034**	n.s.	n.s.	**0.005**	**0.009**	**0.007**
Cyclopoid	N/A	**0.001**	**<0.001**	**0.022**	n.s.	n.s.	n.s.	n.s.	n.s.
Calanoid	N/A	**0.03**	**<0.001**	**0.004**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	n.s.
Ostracoda	N/A	**0.047**	**0.041**	n.s.	n.s.	n.s.	n.s.	**0.049**	**0.042**
Alona	N/A	n.s.	n.s.	n.s.	n.s.	n.e.	n.e.	n.e.	n.s.
Simocephalus	N/A	n.s.	**0.008**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Nauplii	N/A	**<0.001**	**<0.001**	**<0.001**	**0.003**	n.s.	n.s.	n.s.	n.s.
Ascomorpha	N/A	n.e.	**0.01**	n.s.	n.s.	**0.001**	n.s.	n.s.	n.s.
Lecane	N/A	n.s.	n.s.	n.s.	n.s.	**0.012**	**0.005**	**0.021**	**0.045**
Cephalodella	N/A	n.e.	n.s.	n.e.	n.s.	n.s.	n.s.	n.s.	n.s.
Drought									
Ceriodaphnia	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Daphnia	n.s.	n.s.	**0.005**	n.s.	n.s.	n.e.	n.e.	n.e.	n.e.
Chydorus	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**0.025**	n.s.	n.s.
Cyclopoid	n.s.	n.s.	**0.005**	**0.011**	n.s.	n.s.	n.s.	n.s.	n.s.
Calanoid	n.s.	n.s.	n.s.	n.s.	n.s.	**<0.001**	**<0.001**	**<0.001**	n.s.
Ostracoda	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Alona	n.e.	n.s.	n.s.	n.s.	n.s.	n.e.	n.e.	n.e.	n.s.
Simocephalus	n.e.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Nauplii	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Ascomorpha	n.s.	n.e.	**0.002**	n.s.	n.s.	**0.005**	**<0.001**	**0.002**	n.s.
Lecane	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Cephalodella	n.e.	n.e.	n.s.	n.e.	n.s.	**0.013**	**0.031**	n.s.	n.s.
Lufenuron*Drought									
Ceriodaphnia	N/A	**0.045**[b]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Daphnia	N/A	n.s.	**0.001**[a]	n.s.	n.s.	n.e.	n.e.	n.e.	n.e.
Chydorus	N/A	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Cyclopoid	N/A	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Calanoid	N/A	n.s.	n.s.	n.s.	n.s.	**<0.001**[*]	**<0.001**[*]	**<0.001**[*]	n.s.
Ostracoda	N/A	**0.047**[b]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Alona	N/A	n.s.	n.s.	n.s.	n.s.	n.e.	n.e.	n.e.	n.s.
Simocephalus	N/A	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Nauplii	N/A	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Ascomorpha	N/A	n.e.	**0.01**[a]	n.s.	n.s.	n.s.	**0.002**[a]	n.s.	n.s.
Lecane	N/A	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Cephalodella	N/A	n.e.	n.s.	n.e.	n.s.	n.s.	n.s.	**0.05**[a]	**0.017**[a]

[a]Synergistic interaction. [*] Density declines in controls due to environmental factors (D) do not allow evaluating toxic effects.
[b]Antagonistic interaction.
n.s.:additive.

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Va por todos

Alba