



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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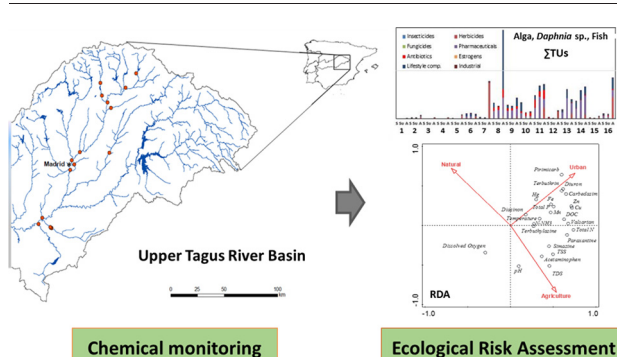
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HIGHLIGHTS

- Chemical status evaluated in 16 sites of the Tagus river basin over three seasons.
- Land use had a stronger influence than season on contamination patterns.
- Copper and zinc may exert acute toxicity to primary producers and invertebrates.
- Diuron, chlorpyrifos and imidacloprid identified as priority hazardous compounds.
- Only 30% of the sampled sites showed good chemical status as per the WFD.

GRAPHICAL ABSTRACT



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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,

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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/EU).

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., 2016; 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, 2019). Studies are still needed to better understand the temporal and spatial distribution of chemical contaminants in these ecosystems and to assess their risks for freshwater organisms.

The Tagus River is the longest river in the Iberian Peninsula (1092 km) and holds the third largest catchment (81,947 km²). 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. (Submitted) 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 (Fig. 1). All sampling sites were close to the Tagus River Basin Authority monitoring flow gauges, from which flow data series for 2016 were obtained.

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 × 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 (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

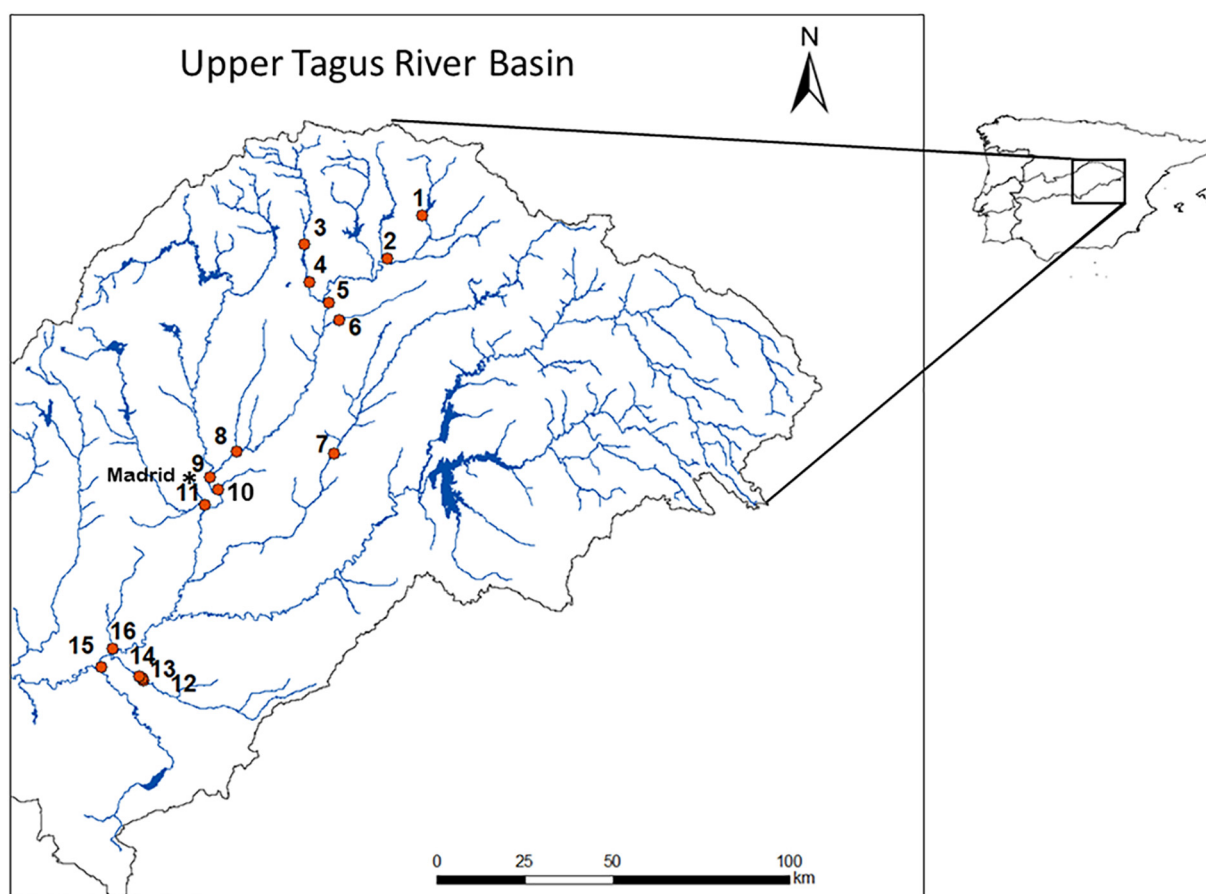


Fig. 1. Map of the study area and sampling sites in the upper Tagus river basin.

reported in Table 1. The 16 sub-basins have a diverse range of surface area (from 467 to >8000 km²). 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 m³/s) to very small creeks (annual average water flow <1 m³/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.

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

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.

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 ($\text{pH} < 2$) 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 72 h. 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 ($^\circ\text{C}$), 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^-), ammonia (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, AWWA, WEF, 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 $\mu\text{g/L}$ 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 Supplementary material): 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., Submitted). 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. (Submitted). The physico-chemical properties of the selected organic contaminants are reported in the Supplementary material (Table S1).

2.6. Spatiotemporal 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-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 Šmilauer, 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 (Quantitative Structure-Activity Relationships). Details on the selected endpoints and the QSARS used for the estimation of toxicity data are provided in the Supplementary material. 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 $\text{LOD}/2$. 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).

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 geo-chemical 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 Supplementary material.

Table 2

Variance partitioning analysis performed for each parameter group based on RDAs. LU U 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 \cap 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 U SE	Residual variance	LU SE	SE LU	LU \cap 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 , * $0.05 \geq p$ -value ≥ 0.01 , ^amarginally significant $0.1 \geq p$ -value >0.05 .

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 $100 \mu\text{g/L}$), with a maximum value of $>700 \mu\text{g/L}$ 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 $50 \mu\text{g/L}$, were measured in sites 1 to 5.

Nutrients did not show significant seasonal patterns although they may also reach surface waters through 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 Supplementary material.

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 Fig. S1 of the Supplementary material.

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. 1 to 5) and much higher concentrations in the most impacted ones (e.g. sites 8 to 11, and 16). This is also evident from Fig. 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 Supplementary material (Figs. 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., Submitted).

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 Ccancapa 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).

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-NH ₄ ⁺ N-NH ₃ (mg/L)	0.73 (<0.001–15.3)	0.19 (0.03–6.60)	0.10 (<0.001–7.53)
N-NH ₃ (mg/L)	0.05 (<0.001–0.21)	0.03 (<0.001–0.75)	0.008 (<0.001–0.05)
N-NO ₂ (mg/L)	0.0076 (<0.001–0.23)	0.03 (0.001–1.67)	0.02 (<0.001–0.57)
N-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-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

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; Fig. 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,

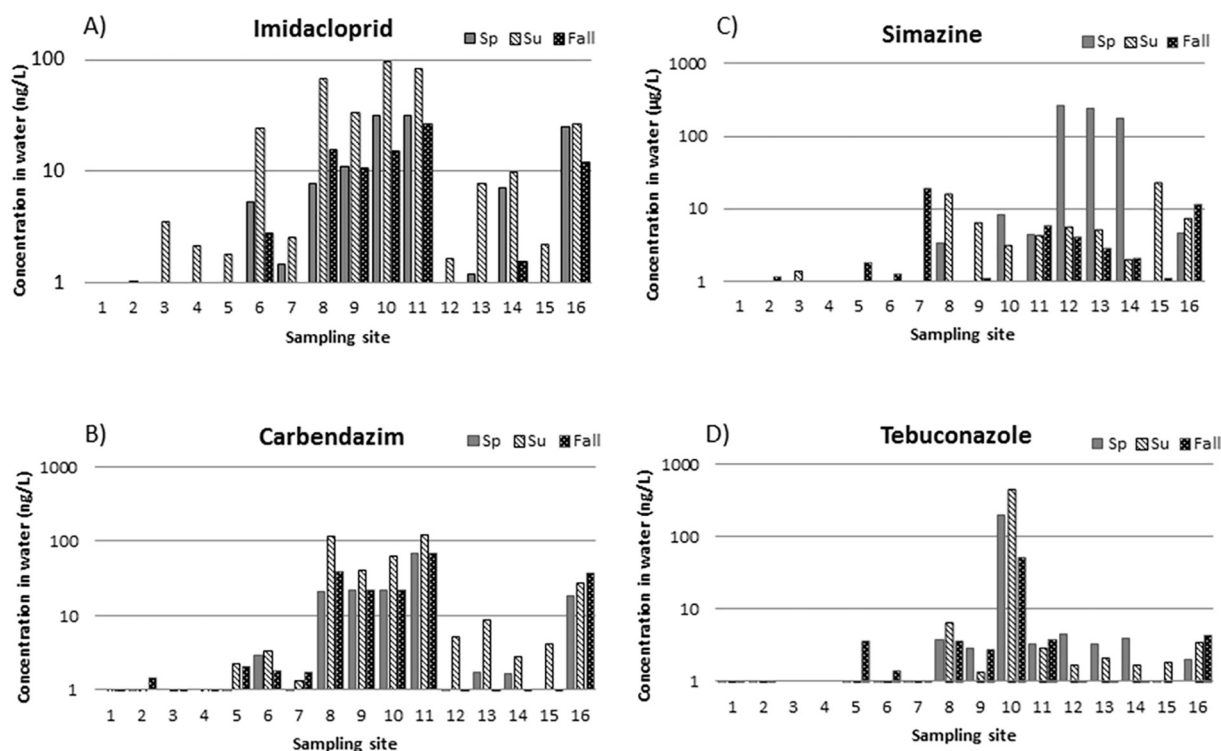


Fig. 2. Concentrations for pesticides with high occurrence percentages in river water grab samples. Only values over 1 ng/L are shown for the 16 sampling stations and for each sampling period (spring, summer, autumn).

sulfamethoxazole, trimethoprim) in autumn downstream of urban areas (Rico et al., Submitted). The concentrations of pharmaceuticals and antibiotics (Fig. 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 $\mu\text{g/L}$. 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., Submitted).

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 Fig. 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 (Fig. 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.

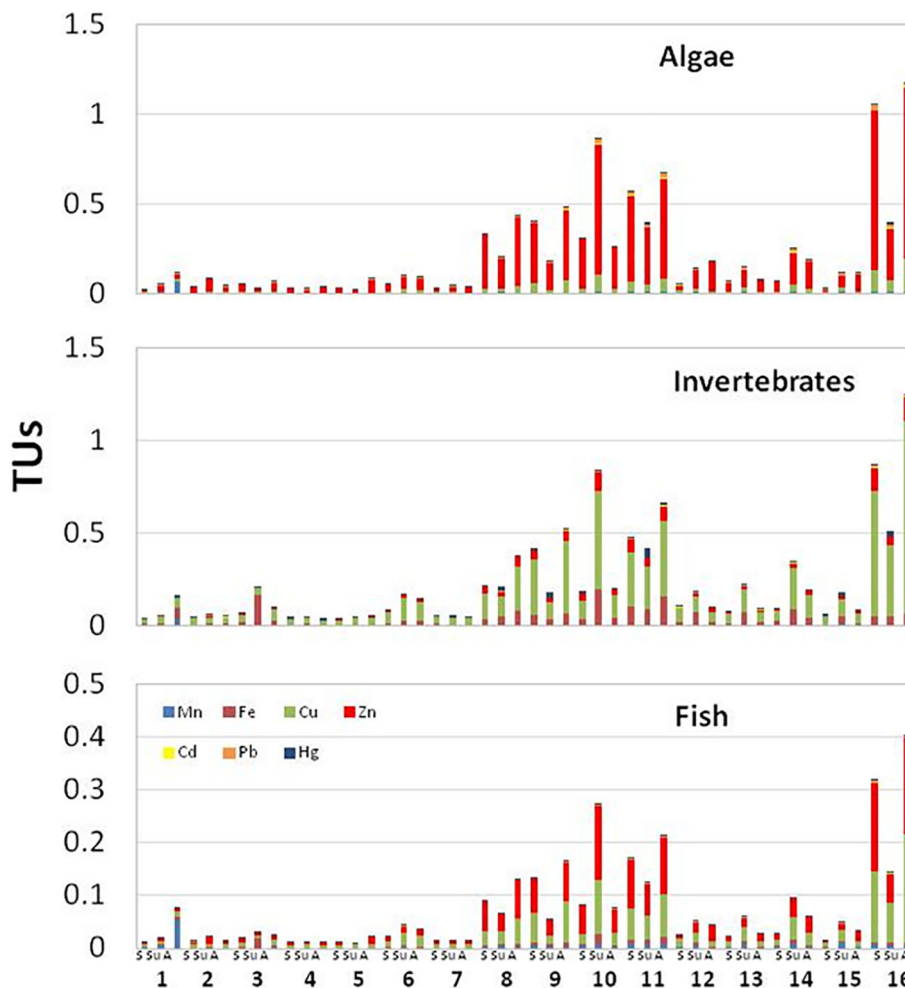


Fig. 3. TU values for the mixture (as the sum of TUs for individual metals) and for individual metals calculated for the different taxonomic groups. For algae, iron was excluded due to a lack of toxicity data.

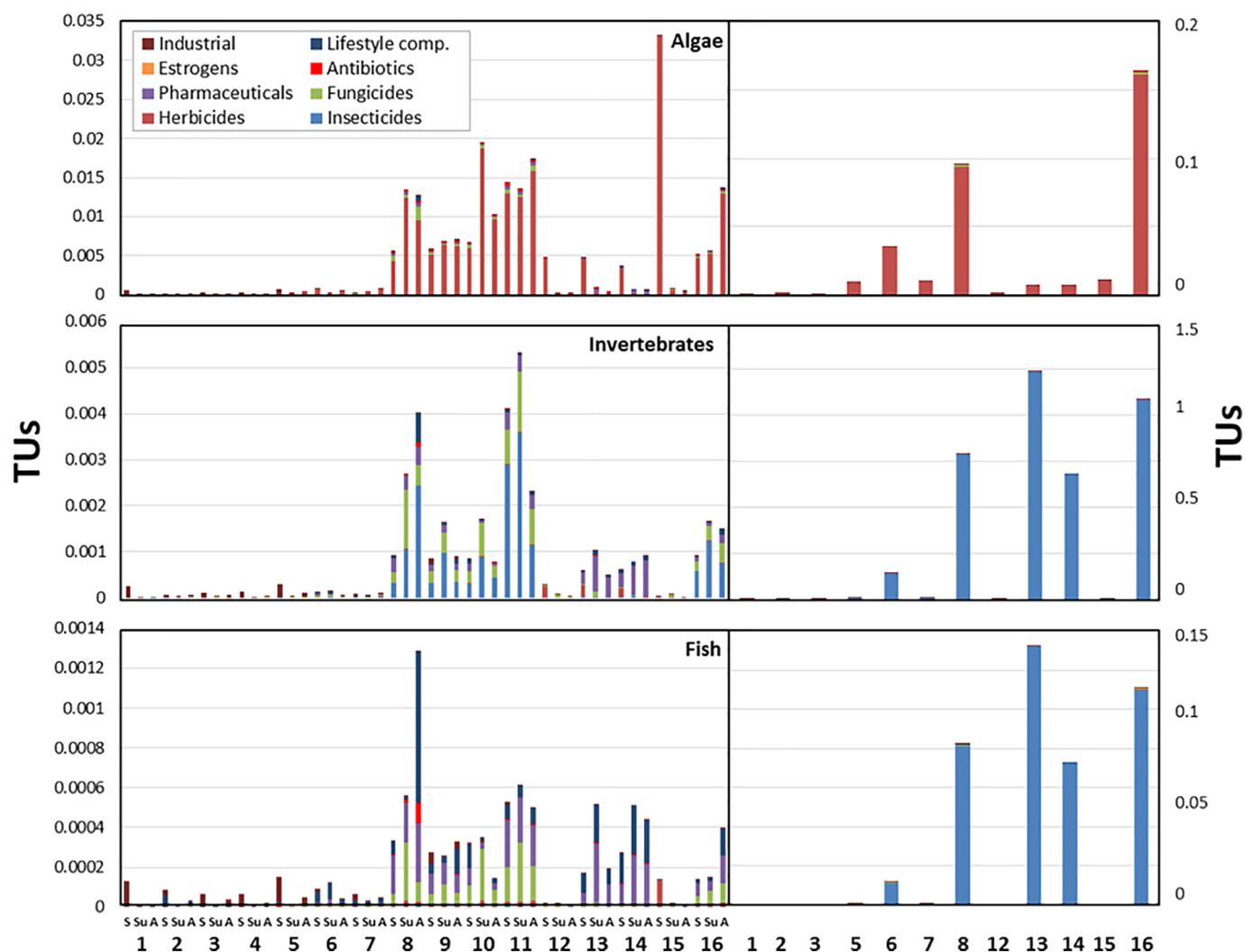


Fig. 4. Values of TUs for the total mixture and the different groups of organic contaminants (as the sum of TUs for sub-groups of pesticides and point-source contaminants) calculated for the different taxonomic groups in grab samples (left side of the figure and left scale) and in POCIS samples (right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

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 Fig. 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 (Fig. 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.

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 terbuthrin, 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 (Fig. 4), mainly due to the presence of the insecticides pirimicarb and diazinon, the fungicide carbendazim, and to a lesser extent the analgesic

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
Terbuthrin	13	Pirimicarb	24	Paraxantane	8
Simazine	11	Carbendazim	15		
Terbuthylazine	11	Acetaminophen	10		
		Diazinon	7		
		Valsartan	6		

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 (Fig. 4), due to the high paraxanthine 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., Submitted), 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.

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., Submitted). 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, Júcar and Guadalquivir basins. Chlorpyrifos has been ranked as top priority compound in several monitoring studies performed in the Júcar river basin (Cancapá 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 Fig. 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; Fig. 5, left).

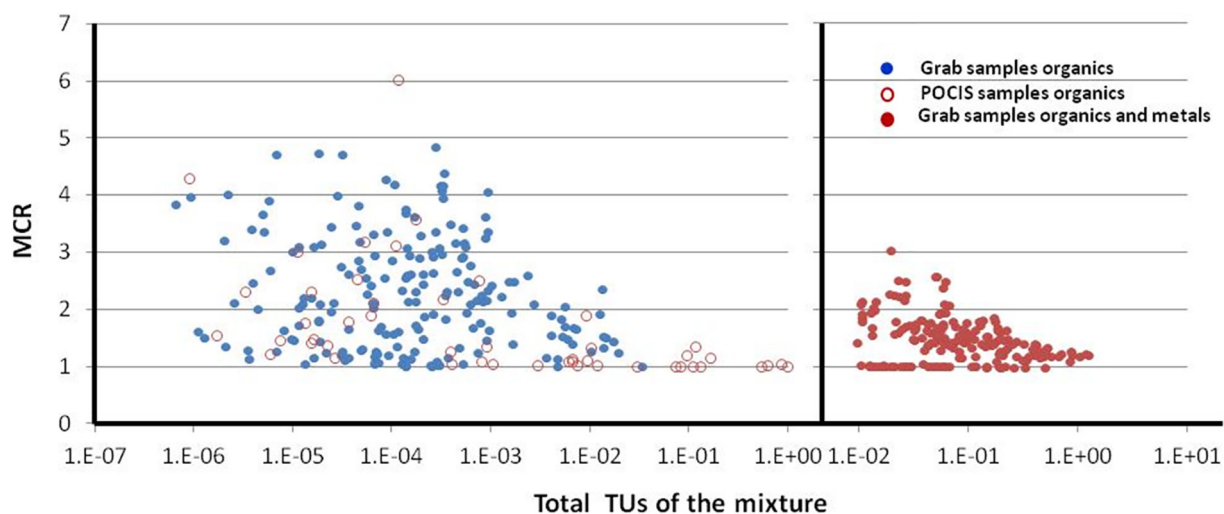


Fig. 5. Values of the Maximum Cumulative Ratio (MCR) as a function of the total potency of the mixture expressed as toxic units (TUs). Three types of mixtures are considered: mixture of organic contaminants in grab samples; mixture of organic contaminants in POCIS samples; mixture of organic contaminants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

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+Terbuthrin 2. Diuron+Terbuthrin+Terbuthylazine 3. Simazine 4. Terbuthylazine (n = 9)	1. Pirimicarb+Carbendazim (n = 1)	
Summer	1. Diuron+Terbuthrin 2. Diuron+Terbuthrin+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+Terbuthrin 2. Diuron+Terbuthrin+Terbuthylazine (n = 5)	1. Pirimicarb+Carbendazim+Valsartan 2. Diazinon+Pirimicarb+Carbendazim (n = 3)	1. Paraxantine+Nicotine+Valsartan+ Carbendazim+Naproxen (n = 1)

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 (Fig. 5, right).

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).

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 Fig. 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 through 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, ammonia, nitrate and phosphate have been set by the Spanish Ministry of Agriculture, Food and Environment (BOE, 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 Supplementary material. 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 (NH₃). Water quality criteria for un-dissociated ammonia have been

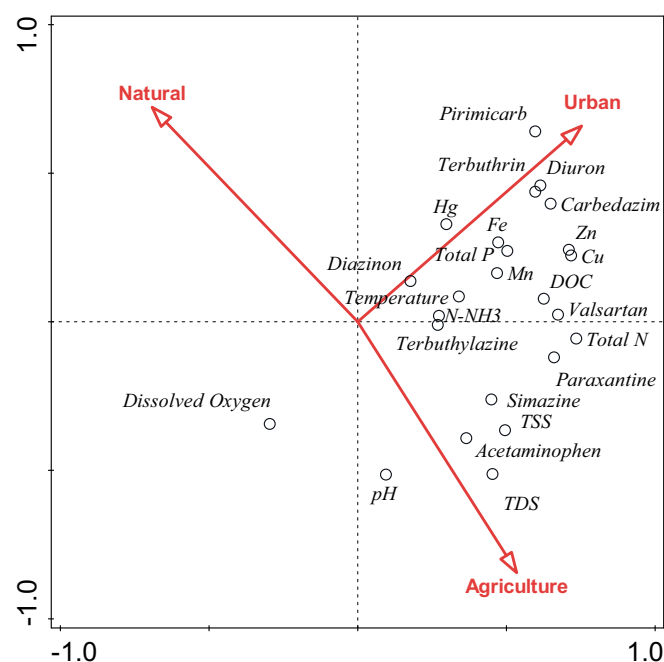


Fig. 6. RDA showing the relationship between land use, physico-chemical variables and the selected inorganic and organic contaminants. Land use explains 41% of the variance, of which 79% is represented in the x-axis and 21% in the y-axis (Monte Carlo p-value: 0.002).

set by various international agencies at 20 µg N-NH₃/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-NH₃/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 (chlorpyrifos, diuron, simazine, terbuthrin) 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 terbuthrin the AA-QS in 2 sites (8 and 16) (Fig. S4 of the Supplementary material). 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.

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 performed 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2019.02.286>.

References

- American Public Health Association (APHA), 2005. Standard Methods for the Examination of Water and Wastewater. 21th edition. 2005. APHA, AWWA, WEF.
- Arenas-Sánchez, A., Rico, A., Vighi, M., 2016. Effects of water scarcity and chemical pollution in aquatic ecosystems: state of the art. *Sci. Total Environ.* 572, 390–403.
- Arenas-Sánchez, A., López-Heras, I., Nozal, L., Vighi, M., Rico, A., 2019. Effects of increased temperature, drought, and an insecticide on freshwater zooplankton communities. *Environ. Toxicol. Chem.* 38, 396–411.
- Ashbolt, N.J., Amézquita, A., Backhaus, T., Borriello, P., Brandt, K.K., Collignon, P., Lawrence, J.R., 2013. Human health risk assessment (HHRA) for environmental development and transfer of antibiotic resistance. *Environ. Health Perspect.* 121, 993–1001.
- Backhaus, T., Altenburger, R., Boedeker, W., Faust, M., Scholze, M., Grimme, L.H., 2000. Predictability of the toxicity of a multiple mixture of dissimilarly acting chemicals to *Vibrio fischeri*. *Environ. Toxicol. Chem.* 19, 2348–2356.
- Barceló, D., Petrovic, M., 2007. Challenges and achievements of LC-MS in environmental analysis: 25 years on. *TrAC Trends Anal. Chem.* 26, 2–11.
- Bengtsson-Palme, J., Larsson, D.J., 2016. Concentrations of antibiotics predicted to select for resistant bacteria: proposed limits for environmental regulation. *Environ. Int.* 86, 140–149.
- Benito, G., Díez-Herrero, A., De Villalta, M.F., 2003. Magnitude and frequency of flooding in the Tagus basin (Central Spain) over the last millennium. *Clim. Chang.* 58, 171–192.
- BOE (Boletín Oficial del Estado), 2015. Real Decreto 817/2015, de 11 de septiembre, por el que se establecen los criterios de seguimiento y evaluación del estado de las aguas superficiales y las normas de calidad ambiental. *Boletín Oficial del Estado*, N 219, Sec. 1, Pgg. 80582–80677.
- Boedeker, W., Drescher, K., Altenburger, R., Faust, M., Grimme, L.H., 1993. Combined effects of toxicants: the need and soundness of assessment approaches in ecotoxicology. *Sci. Total Environ.* 134, 931–938.
- Brooks, B.W., 2014. Fish on Prozac (and Zoloft): ten years later. *Aquat. Toxicol.* 151, 61–67.
- Bundschuh, M., Weyers, A., Ebeling, M., Elsaesser, D., Schulz, R., 2016. Narrow pH range of surface water bodies receiving pesticide input in Europe. *Bull. Environ. Contam. Toxicol.* 96, 3–8.
- Ccancappa, A., Masiá, A., Andreu, V., Picó, Y., 2016. Spatio-temporal patterns of pesticide residues in the Turia and Júcar Rivers (Spain). *Sci. Total Environ.* 540, 200–210.
- Corine land cover. http://www.ifen.fr/clc/CORINE_Land_Cover_Condition_Utilisation.htm.
- Crane, M., Watts, C., Boucard, T., 2006. Chronic aquatic environmental risks from exposure to human pharmaceuticals. *Sci. Total Environ.* 367, 23–41.
- Crommentuijn, T., Polder, M.D., Van de Plassche, E.J., 1997. Maximum permissible concentrations and negligible concentrations for metals, taking background concentrations into account. RIVM Report No. 601501 001.
- De Schampelaere, K.A.C., Janssen, C.R., 2002. A biotic ligand model predicting acute copper toxicity to *Daphnia* sp. magna: the effects of calcium, magnesium, sodium, potassium and pH. *Environ. Sci. Technol.* 36, 48–84.

- Di Toro, D.M., Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P.R., Santore, R.C., 2001. A biotic ligand model of the acute toxicity of metals I. Technical basis. *Environ. Toxicol. Chem.* 20, 2383–2396.
- EC (European Commission), 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *Off. J. Eur. Communities* (327/1 22/12/2000).
- EC (European Commission), 2003. Common Implementation Strategy for the Water Framework Directive (2000/60/EC). Guidance Document No 7. Monitoring Under the Water Framework Directive. Office for Official Publications of the European Communities, Luxembourg.
- EC (European Commission), 2011. Technical Guidance for Deriving Environmental Quality Standards. Common Implementation Strategy for the Water Framework Directive (2000/60/EC). Guidance Document 27. European Communities <https://doi.org/10.2779/43816> ISBN: 978-92-79-16228-2.
- EC (European Commission), 2013. Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. *Off. J. Eur. Union* (24.8.2013, L226/1–17).
- EINECS, 1990. European Inventory of Existing Chemical Substances. EEC O.J., C164 A, June 15, 1990.
- ELINCS, 2017. European List of Notified Chemical Substances. Last updated 11 August 2017. <https://www.echa.europa.eu/information-on-chemicals/ec-inventory>.
- Fernández, C., González-Doncel, M., Pro, J., Carbonell, G., Tarazona, J.V., 2010. Occurrence of pharmaceutically active compounds in surface waters of the Henares-Jarama-Tajo river system (Madrid, Spain) and a potential risk characterization. *Sci. Total Environ.* 408, 543–551.
- Förstner, U., Wittmann, G.T., 2012. *Metal Pollution in the Aquatic Environment*. Springer Science and Business Media, Berlin, Germany (475 pp.).
- Ginebreda, A., Muñoz, I., de Alda, M.L., Brix, R., López-Doval, J., Barceló, D., 2010. Environmental risk assessment of pharmaceuticals in rivers: relationships between hazard indexes and aquatic macroinvertebrate diversity indexes in the Llobregat River (NE Spain). *Environ. Int.* 36, 153–162.
- Guo, J., Boxall, A., Selby, K., 2015. Do pharmaceuticals pose a threat to primary producers? *Crit. Rev. Environ. Sci. Technol.* 45, 2565–2610.
- Guo, J., Selby, K., Boxall, A.B.A., 2016. Comparing the sensitivity of chlorophytes, cyanobacteria and diatoms to major-use antibiotics. *Environ. Toxicol. Chem.* 35, 2587–2596.
- Henning-De Jong, I., Van Zelm, R., Huijbregts, M.A.J., DeZwart, D., van der Linden, T.M.A., Wintersen, A., Posthuma, L., van de Meent, D., 2008. Ranking of agricultural pesticides in the Rhine-Meuse-Scheldt basin based on toxic pressure in marine ecosystems. *Environ. Toxicol. Chem.* 27, 737–745.
- Hering, D., Carvalho, L., Argillier, C., Beklioglu, M., Borja, A., Cardoso, A.C., Hellsten, S., 2015. Managing aquatic ecosystems and water resources under multiple stress—an introduction to the MARS project. *Sci. Total Environ.* 503, 10–21.
- Kuzmanović, M., Ginebreda, A., Petrović, M., Barceló, D., 2015. Risk assessment based prioritization of 200 organic micropollutants in 4 Iberian rivers. *Sci. Total Environ.* 503, 289–299.
- López-Doval, J.C., De Castro-Català, N., Andrés-Doménech, I., Blasco, J., Ginebreda, A., Muñoz, I., 2012. Analysis of monitoring programmes and their suitability for ecotoxicological risk assessment in four Spanish basins. *Sci. Total Environ.* 440, 194–203.
- Malaj, E., Peter, C., Grote, M., Kühne, R., Mondy, C.P., Usseglio-Polatera, P., Schäfer, R.B., 2014. Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. *Proc. Natl. Acad. Sci.* 111, 9549–9554.
- Muir, D.C., Howard, P.H., 2007. Erratum: are there other persistent organic pollutants? A challenge for environmental chemists (*Environmental Science and Technology* (2006) 40(23), 7157–7166). *Environ. Sci. Technol.* 41, 3030–3030.
- Osorio, V., Marcé, R., Pérez, S., Ginebreda, A., Cortina, J.L., Barceló, D., 2012. Occurrence and modeling of pharmaceuticals on a sewage-impacted Mediterranean river and their dynamics under different hydrological conditions. *Sci. Total Environ.* 440, 3–13.
- Price, P.S., Han, X., 2011. Maximum cumulative ratio (MCR) as a tool for assessing the value of performing a cumulative risk assessment. *Int. J. Environ. Res. Public Health* 8, 2212–2225.
- Raby, M., Nowierski, M., Perlov, D., Zhao, X., Hao, C., Poirier, D.G., Sibley, P.K., 2018. Acute toxicity of 6 neonicotinoid insecticides to freshwater invertebrates. *Environ. Toxicol. Chem.* 37, 1430–1445.
- Rico, A., Van den Brink, P.J., Leitner, P., Graf, W., Focks, A., 2016. Relative influence of chemical and non-chemical stressors on invertebrate communities: a case study in the Danube River. *Sci. Total Environ.* 571, 1370–1382.
- Rico, A., Jacobs, R., Van den Brink, P.J., Tello, A., 2017. A probabilistic approach to assess antibiotic resistance development risks in environmental compartments and its application to an intensive aquaculture production scenario. *Environ. Pollut.* 231, 918–928.
- Rico, A., Arenas-Sánchez, A., Pasqualini, J., García-Astillerio, A., Cherta, L., Nozal, L., Vighi, M., 2018a. Effects of imidacloprid and a neonicotinoid mixture on aquatic invertebrate communities under Mediterranean conditions. *Aquat. Toxicol.* 204, 130–143.
- Rico, A., Zhao, W., Gillissen, F., Lüring, M., Van den Brink, P.J., 2018b. Effects of temperature, genetic variation and species competition on the sensitivity of algae populations to the antibiotic enrofloxacin. *Ecotoxicol. Environ. Saf.* 148, 228–236.
- Rico, A., Arenas-Sánchez, A., Alonso, C., López-Heras, I., Nozal, L., Rivas, D., Vighi, M. Identification of contaminants of concern in the upper Tagus river basin (central Spain). Part 1: screening, quantitative analysis and comparison of sampling methods. *Sci. Total Environ.* (Submitted).
- Schwarzenbach, R.P., Escher, B.I., Fenner, K., Hofstetter, T.B., Johnson, C.A., Von Gunten, U., Wehrli, B., 2006. The challenge of micropollutants/contaminants in aquatic systems. *Science* 313, 1072–1077.
- Silva, E., Daam, M.A., Cerejeira, M.J., 2015. Aquatic risk assessment of priority and other river basin specific pesticides in surface waters of Mediterranean river basins. *Chemosphere* 135, 394–402.
- Ter Braak, C.J.F., Šmilauer, P., 2012. *Canoco Reference Manual and User's Guide: Software for Ordination (Version 5.0)*. Microcomputer Power, Ithaca, NY, USA (496 pp.).
- Tsaboula, A., Papadakis, E.N., Vryzas, Z., Kotopoulou, A., Kintzikoglou, K., Papadopoulou-Mourkidou, E., 2016. Environmental and human risk hierarchy of pesticides: a prioritization method, based on monitoring, hazard assessment and environmental fate. *Environ. Int.* 91, 78–93.
- US EPA, 2013. *Aquatic Life Ambient Water Quality Criteria for Ammonia – Freshwater*. U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Washington, DC.
- Valcárcel, Y., Alonso, S.G., Rodríguez-Gil, J.L., Gil, A., Catalá, M., 2011. Detection of pharmaceutically active compounds in the rivers and tap water of the Madrid Region (Spain) and potential ecotoxicological risk. *Chemosphere* 84, 1336–1348.
- Valcárcel, Y., Alonso, S.G., Rodríguez-Gil, J.L., Castaño, A., Montero, J.C., Criado-Alvarez, J.J., Catalá, M., 2013. Seasonal variation of pharmaceutically active compounds in surface (Tagus River) and tap water (Central Spain). *Environ. Sci. Pollut. Res.* 20, 1396–1412.
- Verro, R., Finizio, A., Otto, S., Vighi, M., 2009. Predicting pesticide environmental risk in intensive agricultural areas. II: Screening level risk assessment of complex mixtures in surface waters. *Environ. Sci. Technol.* 43, 530–537.
- Von der Ohe, P.C., Dulio, V., Slobodnik, J., De Deckere, E., Kühne, R., Ebert, R.U., Brack, W., 2011. A new risk assessment approach for the prioritization of 500 classical and emerging organic microcontaminants as potential river basin specific pollutants/contaminants under the European Water Framework Directive. *Sci. Total Environ.* 409, 2064–2077.