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Analysis of monitoring programs and their suitability for ecotoxicological risk assessment in four Spanish basins

Julio C. López-Doval^{a*}, Núria De Castro-Català^{a**}, Ignacio Andrés-Doménech^b, Julian Blasco^c, Antoni Ginebreda^d, Isabel Muñoz^a.

^a Department of Ecology, University of Barcelona,. 643 Diagonal Ave., 08028 Barcelona, Spain

^b Department of Hydraulic Engineering and Environment, Polytechnic University of Valencia, Cno. de Vera s/n 46022, Valencia, Spain

^c Department of Ecology and Coastal Management Institute of Marine Sciences of Andalusia, Spanish Council for Scientific Research (CSIC), Campus Rio San Pedro. 11510 Puerto Real, Cadiz, Spain

^d Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research, Spanish Council for Scientific Research (IDAEA-CSIC), 18-26 Jordi Girona Str., 08034 Barcelona, Spain

Corresponding authors:

* e-mail: jclopezdoval@ub.edu; tel.: +34 00 93 402 15 07

** e-mail: ndecastro@ub.edu; tel.: +34 00 93 402 15 07

1
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3 Abstract
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6 Data from four Spanish basin management authorities were analysed. Chemical and
7 biological data from four Spanish basin management authorities were analysed,
8 focusing on three consecutive years.. Aims were to i) determine the chemicals most
9 likely responsible for the environmental toxicological risk in the four Spanish basins
10 and ii) investigate the relationships between toxicological risk and biological status in
11 these catchments. The toxicological risk of chemicals was evaluated using the Toxic
12 Units (TU) concept. With these data we considered if the potential risk properly reflects
13 the risk to the community or, alternatively, if new criteria should be developed to
14 improve risk assessment. Data study revealed inadequacies in processing and
15 monitoring that should be improved (e.g., site coincidence for chemical and biological
16 sampling). Analysis of the chemical data revealed high potential toxicological risk in
17 the majority of sampling points, to which metals were the main contributors to this risk.
18 However, clear relationships between biological quality and chemical risk were found
19 only in one river. Further investigation of metal toxicity may be necessary, and future
20 analyses are necessary to accurately estimate the risk to the environment.
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38 Keywords: toxic units, risk assessment, metals, pesticides, Mediterranean rivers
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1. Introduction

The European Water Framework Directive (WFD) aims to protect the aquatic ecosystems of the Member States through the achievement of ‘good status’ for surface water bodies by the year 2015. Assessment of ecological status is based on three quality elements: biological, physico-chemical and hydromorphological. Biological Quality Elements include phytoplankton, macrophytes and phytobenthos, benthic invertebrates, and fish. These elements are evaluated in combination with hydromorphological quality (hydrological, regime, river continuity and morphological conditions) and physico-chemical elements (temperature, oxygenation, salinity, acidity and nutrients). The chemical status is assessed on the basis of the Environmental Quality Standards (EQS) for 33 regulated compounds (priority substances) and eight priority hazardous substances identified from previous legislation and EQS for basin-specific pollutants (European Commission, 2008). Each Member State is responsible for developing appropriate monitoring and assessment methodologies to determine the status of each water body and the amount of those specific compounds that could endanger natural communities. Specific pollutants are considered among other parameters in designating the ecological status classification. Compliance with EQSs for specific pollutants is evaluated in the assessment of ecological status. If the EQSs for these substances are not met, the status of the water body cannot be classified as ‘good’, even if biological quality is high.

In assessing chemical stress, water agencies develop different programs (survey, operational and investigative) to monitor water quality. These data are not used directly for EQS derivation but can help identify critical data, critical sites or sensitive taxa to the implementation of effective control measures. For example, Crane et al. (2007) describe techniques for estimating a threshold for metal toxicity in the field based on chemical exposure and biological data from matched locations and sampling efforts. These programs provide large chemical and biological data sets of intrinsic value extending beyond data compilation to control the accomplishment of regulated EQS. However, risk management of chemicals is complex due to the numerous non-priority contaminants in the aquatic environment, most of which are not immediately targeted in

1 monitoring programs. Likewise, assessment of the effects of pollutants on ecosystems is
2 complicated by insufficient information about their effects, their different bioavailability
3 pathways, and their interactions with other stressors (e.g., nutrients, Holmstrup et al.
4 2010) and toxicants (mixtures) (Van Gestel et al. 2010, Muñoz et al. 2012). According
5 to the WFD, the primary causes of ecological impairment in rivers must be determined
6 and the necessary improvements to ameliorate the ecological status proposed. To this
7 end, monitoring data should be analysed, and the results should be used to inform basin
8 managers and policymakers of the risk of specific pollutants, the most adversely
9 affected water bodies, and the cause-effect relationships in biological communities
10 related to chemicals or other parameters. Moreover, due to time and budget constraints,
11 there is a need to develop efficient programs for risk assessment and monitoring in the
12 context of the WFD.
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23 Different approaches have been developed to identify compounds of environmental
24 concern and to establish priorities for monitoring. Most of these approaches are based
25 on the occurrence of such compounds in natural systems and on their ecological and
26 toxicological effects. The first list of priority substance in the WFD is an example of
27 prioritisation, and its revision (still underway) involved a monitoring (INERIS, French
28 National Institute for Industrial Environment and Risks) and modelling study (Daginnus
29 et al., 2010) that evaluated 339 and 2034 substances, respectively. Von der Ohe et al.
30 (2011) proposed the frequency of exceedance and the lowest PNEC as indicators for
31 prioritisation; these indicators have been adopted in four European basins. The toxic
32 unit (TU) concept (Sprague 1970, Von der Ohe et al., 2009, Altenburger and Greco
33 2009) is based on the concentration addition (CA) assumption (i.e., that all compounds
34 have the same mode of action) and quantifies the toxic stress associated with a mixture
35 of pollutants. It is generally accepted as a good first approach for quantifying the toxic
36 stress associated with a mixture of pollutants. The TU is defined as the ratio of a
37 chemical's concentration and its observed LC₅₀ (the lethal concentration for 50% of
38 individuals). Recently, the European Commission (2011) recommended the TU for
39 approximating the EQS for substances occurring in mixtures. Another way to express
40 the toxic pressure could be by means the multi-substance Potentially Affected Fraction
41 (msPAF; De Zwart and Posthuma, 2005), which is defined as the fraction of taxa in a
42 community that would potentially suffer from the exposure to the local mixture of
43 toxicants and is based on the Species Sensitivity Distribution methodology (SSD,
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1 Posthuma et al., 2002). The msPAFs express the acute toxic pressure of whole mixtures
2 of contaminants and estimate the fractions of taxa that would be potentially affected by
3 the local mixtures.
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6 The present study addresses two main objectives: i) to identify those chemicals most
7 likely responsible for the environmental toxicological risk in four Spanish
8 Mediterranean basins (as case studies) and ii) to investigate the relationships between
9 toxicological risk and biological status in these catchments. The toxicological risk for
10 the stream-dwelling macroinvertebrate community in the Ebro, Guadalquivir, Júcar and
11 Llobregat river basins has been evaluated through analysis of data compiled by different
12 water authorities and applying the TU concept. The analysis of TU results in each
13 monitoring site is intended to localise and evaluate the potential toxicological risks for
14 the communities. For the second objective, biological data compiled by water
15 authorities was compared with TU results obtained. These analyses permit to evaluate
16 whether a potential risk (measured as TU) accurately reflects the risk to the community
17 or, alternatively, whether new criteria should be developed to improve the risk
18 assessment.
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31 2. Materials and methods

32 2.1 Study sites

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34 The four rivers and their tributaries of the present study are classified as Mediterranean
35 rivers (Fig. 1; table 1). They are characterised by strong seasonality of rainfall and air
36 temperature, and predictable disturbances in riverine flow regimes, such as floods and
37 droughts, can be distinguished. Variations in water discharge are associated with large
38 inter-annual variability in river sediment flux (frequently several orders of magnitude).
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41 The Ebro River basin is the largest river basin in Spain (17.3% of the Iberian territory).
42 It is 928 km in length and has a drainage basin of 85,534 km² and serve to a population
43 of 3,019,176. It is largely regulated by dams and channels, which have altered its
44 hydrological and sedimentary regime. During the 20th century, the mean annual flow
45 has decreased by approximately 30% due to dam construction, increasing demands for
46 irrigation, evaporation from reservoirs and land use changes within the basin (i.e.,
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1 reforestation). The abstraction of ground and surface water for irrigation and industrial
2 activities concentrated close to the main cities has also deteriorated soil and water
3 quality.
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6 The Guadalquivir River is located in southwestern Spain and is 657 km in length.
7 Together with its tributaries, it is the main water source of the region, serving over 7
8 million inhabitants. As a result, the river receives materials of both natural and
9 anthropogenic origin that deteriorate water quality. The river is navigable as far as
10 Seville (approximately 90 km upstream), a major inland port. The river basin is also
11 affected by reservoirs and dams, and its regime is heavily modified. Over 700,000 ha of
12 the basin are devoted to agriculture (rice, olive and fruit trees), with the corresponding
13 environmental effects on the river. It discharges into the Atlantic Ocean, and its estuary
14 is heavily affected by tidal patterns.
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24 The Júcar river basin is located in eastern Spain. The basin covers 21,632 km² and
25 includes a main stream approximately 500 km long. Urban water use totals 118.64
26 hm³/year for 1,030,979 people, and the region irrigated by this basin spans 187,855 ha
27 and withdraws 1,394 hm³/year. It is a highly regulated basin with a total reservoir
28 capacity of 2,643 hm³. The main problems regarding water use are those typical of
29 semiarid zones with intensive water demands. In the lower part of the basin,
30 urbanisation, industry, and agriculture are intensive and negatively impact water quality.
31 The Júcar basin was designated as a European Pilot River Basin for the implementation
32 of the WFD.
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42 The Llobregat River is the second longest river in Catalonia (NE Spain), spanning over
43 170 km. The river is heavily managed in its lower course. Water at the mouth is
44 currently pumped upstream to augment the natural flow, recharge the delta wetlands and
45 control seawater incursion. This river is one major drinking water resources for
46 Barcelona and surroundings, with a population over 3,000,000 inhabitants. The
47 Llobregat basin receives extensive urban and industrial waste water discharge as well as
48 surface runoff from agricultural areas that are not diluted by natural flow.
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59 2.2 River basin database 60 61 62 63 64 65

1 Data were compiled from existing monitoring databases covering the four river basins
2 under study: Ebro, Guadalquivir, Júcar and Llobregat. Data were provided by the
3 following water agencies: Confederación Hidrográfica del Ebro (CHE), Confederación
4 Hidrográfica del Guadalquivir (CHG) and Agencia Andaluza del Agua, Confederación
5 Hidrográfica del Júcar (CHJ) and Agència Catalana de l'Aigua (ACA).
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10 Quality monitoring programs compile approximately 160000 data entries per year,
11 which are recorded at 1100 monitoring sites along the four basins. Physical, chemical
12 and biological data are collected. The most complete physical and chemical data records
13 date from the 1990s; however, use of biological quality indices began primarily in 2000
14 as a consequence of the WFD implementation. In this study, we focused on water
15 column data from 2008 to 2010. These years encompass the most complete data set,
16 including priority substances. The EQS normative for these compounds is applicable
17 from 2008. In addition, for biological indices, complete data are available only from the
18 last 3 years (2008-2010). The biological parameters included in these analyses are
19 related with measures of benthic macroinvertebrates, including the abundance of
20 different taxonomic groups and biological indices (IBMWP, Iberian Biomonitoring
21 Working Party). For the Guadalquivir basin, no chemical or biological data were
22 available from 2010 or later, so the analysis was conducted using data from 2007-2009.
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35 For metal analysis, water samples were filtered to obtain dissolved concentrations. For
36 analysis of organic pollutants, samples were unfiltered (Ministerio de Medio Ambiente,
37 2006) except for those from Llobregat. Biological samples were collected from different
38 habitats in the river using the Kick method for a set time period, providing semi-
39 qualitative measures of abundance at the taxonomic level of family.
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45 The sites studied here were those where priority substances and other hazardous
46 substances were periodically measured (Table 1). To study the relationship between
47 toxicological risk and biological quality, those sites where macroinvertebrates were also
48 sampled were selected. Unexpectedly, only 106 sites (28 in the Ebro, 50 in
49 Guadalquivir, 8 in Júcar and 25 in Llobregat) had both chemical and biological data.
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59 2.3 Assessment of ecotoxicological data 60 61 62 63 64 65

1 To evaluate the toxicological risk at each sampling point, the Toxic Unit (TU, Sprague
2 1970) concept was used. The TU quantifies the toxic stress associated with a mixture of
3 pollutants and is defined as the ratio of a chemical's concentration and its observed
4 LC₅₀ (the lethal concentration for 50% of individuals). In a mixture of chemicals, TU_{sum}
5 will be the sum of the concentrations (*C_i*) of *n* individual compounds in the mixture
6 expressed as a fraction of their respective LC₅₀, assuming an additive behaviour of all
7 components and representing the maximal expected effect of a mixture:
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$$17 \text{ ToxicUnits} = TU = \sum_{i=1}^n TU_i = \sum_{i=1}^n \frac{C_i}{LC50_i} \quad (1)$$

21 The TU allows the toxic risk of sites with different chemical exposure profiles to be
22 compared. To derive the respective TUs, the measured compound concentrations can be
23 scaled to the toxicity of each compound to standard test organisms (e.g., the invertebrate
24 *Daphnia magna*, the algae *Selenastrum capricornutum* and the fish *Pimephales*
25 *promelas*) representing all trophic levels (Liess and Von der Ohe, 2005). The resulting
26 three values indicate the risks to aquatic biota and can be used for management
27 prioritisation purposes. Given that focus is on macroinvertebrate communities, LC₅₀
28 values from previous acute toxicity tests of *Daphnia magna*, a representative aquatic
29 invertebrate, were used. This cladoceran is widely used as a model organism and is
30 among the taxa most sensitive to organic toxicants and metal compounds.
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41 In the few cases for which these values were unavailable, the EC₅₀ (effective
42 concentration for 50% of individuals; when more than one value was available, the
43 lowest concentration was used), was used. This information was gathered primarily
44 from the SPEAR calculator (Liess et al., 2008), the IPCS database (IPCS, 2008), the
45 Pesticide Properties Database (PPDB Management Team, 2009), the ECOTOX
46 database (USEPA, 2008) and Von der Ohe et al. (2011).
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54 For each year and sampling point, for calculation of the TU of each compound of the
55 mixture, the maximum annual concentration measured was used (representing a 'worst
56 case' scenario, in adherence to the precautionary principle). The total risk for each
57 sampling point, as sum of all TU values, was calculated. Compounds that have never
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been detected above the limit of quantification (LOQ) were excluded from the analyses of the TU estimate.

For each sampling point, the contribution (in percentage) of each pollutant to the total toxicity was calculated. This contribution was measured as TU, to identify the most toxic compounds in the mixture.

2.4 Relationship between chemical and biological status

For sites where both chemical and biological data were available, their correlation coefficient was calculated. The value for the IBMWP index was provided by the Water Agencies. This index is based on the principle that aquatic invertebrates have different tolerances to general organic pollution (primarily eutrophication); therefore, the presence or absence of different taxonomic groups is an indicator of the level of water pollution (Hellowell, 1986; Alba-Tercedor and Sanchez-Ortega, 1988). Each family receives a different score depending on their tolerance, and the final value is the sum of the scores.

An additional biological index, the SPEAR index (calculated following Beketov et al., 2009), was included to compare biological and chemical status. The main advantage of the SPEAR index is that it is based on the biological traits of stream invertebrates and not on taxonomic units or abundance parameters like many other conventional bioassessment indices. Therefore, the SPEAR index is relatively free of confounding factors, and its application is unconstrained by geographical and geomorphological influences on biological communities (Liess et al., 2008). Species are classified according to their vulnerability to pesticides and organic compounds. The ecological traits used to define these groups include sensitivity to toxicants, generation time, migration ability, and presence of aquatic stages during the time of maximum pesticide application. Species with long generation times and low migration abilities will be considered at risk due to their limited ability to avoid chemical exposure (Liess and von der Ohe, 2005)

Calculations were made using lists of families and abundances from the various Water Agencies and the program SPEAR Calculator (UFZ, Leipzig, Germany), which is freely

1 available at <http://www.systemecology.eu/SPEAR/Start.html>. The threshold index value
2 of an invertebrate community at risk corresponds to 30% of the species being at risk.
3 Values above this threshold are considered indicative of good ecological status because
4 a higher percentage of species are sensitive to pollution (i.e., are species at risk). Below
5 this threshold, the more sensitive species have disappeared and few or no remaining
6 species are at risk. To compare chemical stress with biological quality, the logarithm of
7 TUs was used to represent toxic stress resulting from the total mixture of toxicants (log
8 TU) or the organics (log TU_{org}) for each site and year.

$$\log TU = \log \sum_{i=1}^n TU_i \quad (2)$$

23 We assume that log TU indicates water contamination according to the range presented
24 below (after Beketov et al., 2009):

- 25 – Uncontaminated (log TU < -4)
- 26 – Slightly contaminated (-4 < log TU < -2)
- 27 – Highly contaminated (log TU > -2)

28 The lower end of the toxicity range was set at log TU= -4 corresponding to 1/10000 of
29 the acute LC₅₀. This concentration was assumed to be a protective concentration level,
30 without expected effects on communities (compared with PNEC).

31 Spearman's correlation coefficient (significant correlation for $p > 0.05$) among log TU
32 values and biological indexes IBMWP and SPEAR, were determined using SPSS
33 version 2.0.

34 As metals and organic compounds are expected to have different effects on
35 communities, we performed calculated log TU separately for metals (log TU_{metal}) and
36 organic compounds (log TU_{org}), as suggested by Höss et al. (2011).

3. Results

3.1 River basin database

In table 2 is depicted, in each basin, the number of sampling stations, number of parameters analyzed, number of stations where biological or chemical data are monitored, number of priority substances analyzed number of compounds detected and number of compounds above EQS. The analyses revealed a lack of consistency in the names of chemicals between the four basins studied; **in some cases, for the same chemical compound, no equal nomenclature exists thorough the basins.** In addition, the parameters analysed varied by sampling point and by basin. For the chemical parameters, spatial coverage and sampling frequency varied by parameter, year and basin. For example, in the Júcar basin, most sites containing chemicals did not extend throughout the entire basin but were primarily located in lower stretches. Almost all of the 33 priority substances were analysed periodically in all basins (ranging from 4 times a year to monthly depending on substance, site and basin). In the Ebro River, measurement of all priority substances occurred at only three sites (0027 Ebro-Tortosa, 0087 Jalón-Grisén, 0163 Ebro-Ascó). In this river, sampling of priority substances was divided into different monitoring networks with different sampling sites. In the Jucar River in 2010, of the 14 sampling stations for chemicals substances, only one (JUU619) measured pesticides but not other organic compounds or metals. In contrast, in four stations in this river (JUK625, JUK627, JUL508, JUL621), pesticides such as Alaclor, Aldrin, Diuron, Endosulfan or Simazine were not measured; the remaining 9 stations were sampled more exhaustively. The Guadalquivir basin between 2007 and 2009 also contained sampling stations with non-exhaustive sampling of compounds. Llobregat was the river basin with the most complete sampling of chemical data (organic and inorganic compounds), encompassing a large number of sites distributed along the catchment. **In table 3 is summarized the number of stations intended for monitoring metals, organic compounds or both.**

In contrast, biological parameters were monitored using a standardised procedure and were easy to compare and adopt in new calculations. Unfortunately, despite a large number of sites with available biological or chemical data (Table 2), only 106 sites (28 in the Ebro, 50 in Guadalquivir, 8 in Júcar and 25 in Llobregat) contained both. These

1 numbers slightly changed over time because some stations were removed and/or new
2 ones were added to the network. In most sites with biological data, only basic
3 environmental parameters were included in the databases (e.g., oxygen, conductivity,
4 nutrients).
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9 Relatively few sites exceeded the EQS values for priority substances (Table 2). In the
10 Ebro basin, the compounds most often responsible for exceeding the threshold value
11 were chlorpyrifos, nickel, mercury, and nonylphenol. In the Guadalquivir basin, the
12 compounds responsible for non-compliance were cadmium in 2008, simazine in 2009
13 and mercury in 2010. In the Júcar basin, chlorpyrifos exceeded the EQS in 2010, and in
14 the Llobregat basin, a value of trifluralin above the EQS was observed in 2009.
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23 Most of the chemicals analysed occurred (at times or consistently) at levels below the
24 limit of quantification (LOQ). The LOQ values for a given substance also varied with
25 year and sampling period.
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32 33 3.2 Assessment of toxicological data 34

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36 A total of 339 chemical substances were analysed in the four basins. Toxicity data for
37 macroinvertebrates (LC₅₀ for *Daphnia magna*) were available for 159 compounds
38 (almost 40% of the total). Few chemicals were consistently measured at levels above
39 their LOQ (Table 1). Finally, for the four basins, between 13 and 24 compounds were
40 selected for risk assessment and were evaluated as TU. This limitation did not
41 underestimate the toxicity risk because the computed compounds represented more than
42 the 95% of the toxicity (see supplementary material). As shown in supplementary
43 material, the highest toxicity risk is associated with the presence of metals (higher
44 values of log TU_{metal} than log TU_{org}) for almost all sites. At almost all sites, the log TU
45 metal value exceeds the threshold of high contamination (log TU > -2). The heavy metals
46 that contributed the most to the TU values were zinc and copper (Figures 2 and 3). In
47 contrast, with few exceptions, organic contaminants were associated with lower risk
48 (log TU_{org} values ranging between -2 and -4). The risk due to organics was appreciable
49 at some sites, especially at the Ebro (stations 0025, 0060, 0225, 0226, from 2008 to
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2010; and others sites in punctual years: Fig. 2 and supplementary material), Júcar (station JUJ619 in 2008 to 2010 and JUK616 in 2008) and Guadalquivir (10201 in 2008 and 2009 and 10203 and 10209 only in 2008) stations; at these sites, log TU_{org} values approached -1 and were higher than levels of metal toxicity (Fig. 3 and supplementary material). At these sampling points, pesticides or their derivatives were the most important compounds contributing to risk toxicity, with Chlorpyrifos being the most important. Desethylatrazine, Diuron and Isoproturon were also present. Sites with good chemical quality (values of log TU < -4) were rare. Guadalquivir was the basin with the greatest number of points of good chemical status (in 2008, sampling points 10202, 10303, 30506; in 2009, sampling points 10203, 20802, 41101), while the Ebro basin contained only one sampling point (0038) of good quality in 2010. At these sites, pesticides appeared to be the most important chemical substances, although their concentrations were low.

No pattern of pollution level change (in TU) was observed over time. In all basins, the average TU total value is within the high pollution range (supplementary material). In figures 2 and 3, we show the change in levels over time at the most polluted stations in 2008. Supplementary material list the compounds that accumulated more than the 95% of the total toxicological risk. The total risk (expressed as TU for the different sampling stations) did not change appreciably over time. Small changes in the contribution of the various pollutants to the total toxicity in each sampling point were observed, but only a few sampling points exhibited improvements over time (0574, 0577, 0627 in Ebro; JUJ617 in Jucar; 440, 700, 710, 840 in Llobregat; and 41601 in Guadalquivir). In contrast, other points decreased in quality over time.

Although the TUs indicated high toxic risk, especially for metals, the biological quality calculated with the SPEAR and IBMWP indexes varied considerably. The only basin exhibiting a relationship between biological and chemical quality was that of the Llobregat basin (Fig. 4). In this basin, biological quality (SPEAR index) and log TU for pesticides were correlated (R= -0.59, p= 0.002, n=26). The IBMWP index and TUs for all compounds (organics and metals) were also significantly correlated (R= -0.45, p<0.0001, n= 65, Fig. 4). Six sites (440, 800, 850, 860, 890 and 900) exhibited both low biological quality (SPEAR<20) and high organic toxicity (Log TU >-2) over the 3 year

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period; most had high metal toxicity as well. Site 340 exhibited low biological quality unrelated to toxicity.

For the Ebro basin, the correlation coefficient between SPEAR and $\log TU_{org}$ was small ($R = -0.07$, $n = 75$) and not statistically significant ($p = 0.66$). For values of $\log TU$ between 0 and -2, there was high dispersion of biological values (from near 0 to 80 % of species at risk, Fig. 4). Similar results were observed when comparing IBMWP and $\log TU$ for all compounds. Three sites were classified as ‘at risk’ with respect to organic toxicants (low % of species at risk and high organic toxicity): sites 87 and 225 in all years and site 60 in 2010. Site 565 also showed low biological quality correlated (only) with metal toxicity. Several sites with low-to-moderate biological quality presented no evidence of toxicity risk: 5, 38, 572 and 574.

In Júcar basin, site 272 exhibited both organic and metal pollution, which pose a risk for the invertebrate community as was observed with the lowest values of the biological indexes (Fig. 5). Another characteristic of this basin was the low percentage of species at risk (< 50% at all sites). However, associations between biological and toxicological data could not be made, due to the low number of sites with both biological and toxicological data.

Guadalquivir basin also exhibited low biological quality, but this was not consistently related to metal or organic pollution. Only two sites yielded values above 80% of species at risk. This basin had the highest number of sites with high TUs values for organic pollution, which, in several cases, presented a risk to the community (e.g., sites 10802, 10803, 10201, 10203, 10502, 40901, 51613) with percentages less than 15% of species at risk (Fig. 5).

4. Discussion

In this study, biological and chemical data from four Spanish basins was analysed. The toxicological risk at each sampling point and the relationships between toxicological risk and biological quality were assessed and evaluated. The sampling routines of the Water Agencies were sufficient to monitor the water quality under WFD specifications. Nevertheless, improvements can be made to increase the efficiency and quality of the

1 data mining process. The data sets were not homogeneous across basins. The use of
2 standardised chemical codes (e.g., CAS number) can facilitate comparisons between
3 basins and the application of scientific assessment tools. This is especially important
4 under the WFD, where intercalibration exercises between basins are required.
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8 It is also important to consider the implementation of sampling sites where chemical
9 and biological data could be taken together. In the present study, both types of sampling
10 were not available for many points, preventing the analysis of correlations between
11 biological and chemical quality; such analyses are important for identifying risk to
12 communities. It is desirable to have complete, high quality data sets for each sampling
13 site (hydromorphological parameters and chemical, biological and general water quality
14 measures), even at the cost of reducing the number of sampling sites.
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22 Assuming a significant relationship between predicted toxic pressure and observed
23 ecological status, even in a multi-stressor context, sufficient data are required from the
24 same sites to evaluate ecosystem changes in response to dynamic stressors and,
25 therefore, to assess the effects of measurement programs on improvements to ecological
26 status. Water Agencies, using a cost-effective approach, should revise their monitoring
27 programs (survey, operational and investigative) in light of these recommendations.
28 With respect to field data sets, the collection of long-term data is also recommended.
29 These data are urgently needed now and in the future.
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38 The large number of substances with below-LOQ values limits the assessment of their
39 chemical status. There is a risk that very toxic substances that are widely analysed but
40 rarely found in the environment could be considered of high priority but present a low
41 actual risk (von der Ohe 2011). Furthermore, some priority substances with EQS under
42 the LOQ could be underestimated. Therefore, for an accurate evaluation of risk, the
43 analytical procedures for some of these substances need improvement. In addition, in
44 the databases, different LOQ values for the same substances, depending on the year,
45 was found. This situation must be assumed since analytical methods are continuously
46 improving, but presently, the compounds with sufficient data for environmental quality
47 assessment are limited, both within and among basins. **Inconsistencies over time in
48 analytical methods and labs pose problems for intercalibration** exercises and
49 comparisons of toxicological risk.
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1 The use of TU with respect to the effects on *D. magna* enabled classification of
2 compounds by their potential toxicity to the macroinvertebrate community. With few
3 exceptions, the contribution of metals (especially zinc and copper) on total toxicity was
4 paramount in all basins. The TU values of these inorganic compounds (TU_{metal}) are high
5 and indicate a potential risk to the macroinvertebrate community. Where organic
6 pollutants contributed to the total toxicity, the pesticide Chlorpyrifos was the compound
7 presenting the highest risk in all basins. Other organic pollutants present were also
8 pesticides or their derivatives, such as Desethylatrazine, Diuron and Isoproturon.
9 Chlorpyrifos is one of the substances associated with non-compliance in both the Ebro
10 and Júcar basins. The assessment used in this work was conservative, calculating TU as
11 the ratio of maximum concentration to LC_{50} . Other works evaluate risk using other
12 criteria, such as hazard quotients (HQ). HQ is defined as the ratio between predicted
13 (PEC) or measured environmental concentrations (MEC) and their chronic toxicity and
14 is usually expressed as NOEC (non-observed effect concentrations) or PNEC (predicted
15 non-effect concentrations) values (European Commission, 2003; Ginebreda et al.,
16 2010). These approaches can be considered variations of the application of the
17 concentration addition (CA) hypothesis. In using maximum concentration values for the
18 chemical compounds, the worst case scenario is depicted. By omitting data below LOQ
19 in the analyses, the effects of those substances could be underestimated; however,
20 including them in the analyses would introduce uncertainty in the evaluation of
21 potential risk. To cover a wider range of effects on other groups of organisms, TUs
22 based on algae and fish toxicity should also be considered. The present study evaluates
23 the risk to the macroinvertebrate community, but the potential risks of the chemical
24 substances studied here to other groups, such as algae and fishes, are expected to be
25 different and significant, as observed by Köck et al. (2010) and Köck-Schulmeyer et al.
26 (2012) in the Ebro River. Priority pollutants, other compounds and other stressors need
27 to be considered simultaneously (Holmstrup et al. 2010). In recent years, the scientific
28 literature has highlighted the presence and effects of emerging substances in fluvial
29 systems (Farré et al., 2008, Caliman and Gavrilescu, 2009; Muñoz et al., 2009).
30 Agencies should include new emerging pollutants in their monitoring programmes to
31 improve the evaluation of chemical quality and to more reliably quantify risk in the
32 river. The potential risk of other unmonitored substances is apparent from studies of
33 pharmaceuticals in the Llobregat (Ginebreda et al., 2009), Guadalquivir (Martín et al.,
34 2011) and Ebro (Gros et al., 2011) basins.

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In contrast to the potential risk measured using TU values, especially for metals, a significant relationship between TU and community index (IBMWP and SPEAR) values was found only for the Llobregat basin. The SPEAR index is recognised as a good biotic descriptor in studying the effects of organics in rivers (von der Ohe et al. 2009, Schäfer et al. 2007). Results show that poor biological quality correlates with high chemical pollution at only few sites. Despite the poor chemical quality (as measured by TUs) generally observed across the basins, especially with respect to metals, the invertebrate community is not affected. This result likely reflects the lack of bioavailability of these inorganic compounds (Stockdale et al. 2010). Total recoverable metals (i.e., the sum of dissolved, colloidal, and solid metal that can be liberated via extraction with mineral acid) from a water sample are not good predictors of toxicity to aquatic organisms (Schmidt et al., 2010), although this is the method recommended by the Spanish authorities (Ministerio de Medio Ambiente, 2006). Roig et al. (2011) encouraged the use of passive samplers because this method permits efficient sampling of the bioavailable fraction of metals and, therefore, an accurate evaluation of the risk arising from metals. The bioavailability and toxicity of metals are influenced by water hardness, pH, and dissolved organic carbon; therefore, the effects and safe concentrations of metals will vary accordingly.

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Carafa et al. (2011) assessed the toxicological risk in Catalan rivers using data from the ACA and applying bioavailability models to calculate the available fractions of each toxicant depending on substance properties and local environmental conditions. They found a clear chemical risk in almost the sites but only a relatively small part of the variability in terms of effects on fluvial macroinvertebrates was explained by toxic pressure. It is possible that the LC₅₀ values of some metals obtained in laboratory tests are overestimated. Some recent studies have discussed the toxicological thresholds obtained in laboratory experiments for some heavy metals in comparison to field data on metal levels and macroinvertebrate communities. Crane et al. (2007) discussed the suitability of using EQS values obtained from laboratory observations in the risk assessment of macroinvertebrate communities in clean and polluted rivers. From the mixture of heavy metals, in the studied basins, zinc is the metal that presents (in terms of TU) the greatest apparent risk to macroinvertebrates. Iwakasi et al. (2012) proposed to increase the threshold of zinc in freshwater bodies after observing no obvious effects on macroinvertebrate communities at the levels recommended by the European Union

1 (17.8 $\mu\text{g L}^{-1}$). Other works have found, in experimental conditions, that the toxicity of
2 single metals can be modified when they are in mixtures (Gaete and Chavez, 2008).
3 Under conditions of chronic exposure, benthic invertebrates regulate, detoxify, and
4 eliminate metals (Schmidt et al., 2010), and these adaptive processes could be
5 responsible for the apparent high quality of invertebrate populations. Because metals are
6 naturally occurring, baseline concentrations of metals can contribute to these adaptive
7 mechanisms. These results reflect the need for improved chemical analysis and for the
8 inclusion of metal speciation to reliably evaluate risk, at least in sites with high
9 concentrations. In addition, sediments act as sources and sinks of pollutants, affecting
10 the organisms dwelling upon them (Ingersoll et al., 1995). Sediment analyses are
11 currently included in quality monitoring programs but are limited in frequency and
12 number, limiting the assessment of water and biological quality. Moreover, EQS for
13 sediments are not yet established by existing regulations.
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24 At sites with conflicting chemical and biological quality measures (high chemical risk
25 but no evidence of effects on communities), alternatives to the biotic indexes could be
26 explored. For example, the use of in situ and laboratory bio-assays (Damasio et al.,
27 2007) in addition to biological indices could be advantageous because they more closely
28 approximate natural conditions (especially with respect to the contamination scenario)
29 and permit more rapid detection of effects (Maltby et al. 2002). These tests would allow
30 researchers to determine whether the absence of an observed effect in the natural
31 community is because of an absence of risk or because the community is adapted to the
32 pollutants. These experiments are not yet common practice in Europe's river basins.
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45 5 Conclusions

46 Based on an analysis of data gathered by water agencies, the authors propose some
47 potential improvements to data collection in Spanish rivers that would allow integrated
48 and efficient assessments of river basins, in compliance with WFD. Chemical status,
49 toxic stress, morphological degradation and eutrophication should be considered
50 simultaneously with biological status at the same sampling sites or reaches for the
51 assessment of the global ecological status of those sites. Effective management
52 measures could then be implemented to improve ecological quality in a sustainable,
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1 cost-efficient way. Improvements in analytical approaches could reduce the large
2 number of substances with below-LOQ values and be applied consistently among
3 basins. Further investigation on metal-related toxicity may be necessary to reliably
4 estimate the risk to the environment. Agencies should include new emerging pollutants
5 in their monitoring programs to improve evaluations of chemical quality. The collection
6 of long-term data (survey monitoring) is essential for the assessment of global changes
7 in fluvial systems. Long-term data collection is recommended, at least for some selected
8 sites sufficiently representative of the basin, depending on basin length and spatial
9 heterogeneity.
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4 Figure 1. Locations of the studied basins in the Iberian Peninsula. 1 Llobregat, 2 Ebro, 3
5 Júcar and 4 Guadalquivir.
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12 Figure 2. Toxic Units (TU) level changes in sites over time. Depicted are changes in
13 stations with high toxicological risk from 2008 to 2010 in Llobregat and Ebro basins.
14 The compounds represented are those that contribute more than 95% to the total
15 toxicological risk. Data are represented in Toxic Units (TU).
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24 Figure 3. Toxic Units (TU) level changes in sites over time. Depicted are changes in the
25 stations with high toxicological risk from 2008 to 2010 in Júcar basin and from 2007 to
26 2009 in the Guadalquivir basin. The compounds represented are those that contribute
27 more than 95% to the total toxicological risk. Data are represented in Toxic Units (TU).
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35 Figure 4. Relationships between toxicological risk and biological quality in Llobregat
36 and Ebro. Toxicological risk is shown as $\log TU_{org}$ for organics and $\log TU$ for all
37 compounds; biological quality is shown as SPEAR or IBMWP index. R= Spearman
38 correlation coefficient; p= significance level.
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47 Figure 5. Relationships between toxicological risk and biological quality in Jucar
48 Guadalquivir. Toxicological risk is shown as $\log TU_{org}$ for organics and $\log TU$ for all
49 compounds; biological quality is shown as SPEAR or IBMWP index. R= Spearman
50 correlation coefficient; p= significance level.
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Table 1. Description of the different basins and impacts of dams.

basin	area (km ²)	number of tributaries	number of dams	total dam capacity (hm ³)
Ebro	85,534	31	200	7,507
Guadalquivir	56,978	15	114	8,247
Júcar	21,632	21	13	2,734
Llobregat	4,948	8	4	213

Table 2. Overview of the monitoring data available in the Water Agencies databases in 2010.

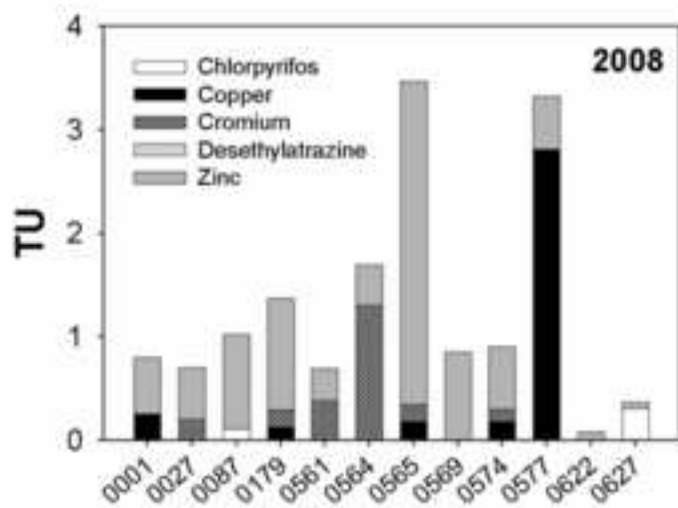
	Llobregat	Júcar	Ebro	Guadalquivir
Total number of monitoring sites	80	263	497	263
Total data entries per year	21200	40000	50000	50000
Total number of chemical monitoring sites	80	263	490	235
Total number of biological monitoring sites	59	156	342	164
Total number of sites where PS and other hazardous substances were periodically measured	45	49	37	108
Number of parameters measured (without biological parameters)	152	382	232	122
Number of biological parameters measured	4	9	10	4
Number of chemical compounds analyzed	115	332	96	117
Number of chemical compounds >LOQ	29	28	20	6
Number of PS analyzed	37	39	36	38
Number of PS above maximum EQS (only for 2008-2010)	1	8	9	1

Table 3. The number and group of chemical compounds analyzed is not the same in all the basins and sampling points, in the table is summarized this information. * In addition, cyanides are analyzed in these stations, ** all the organic compounds analyzed are pesticides

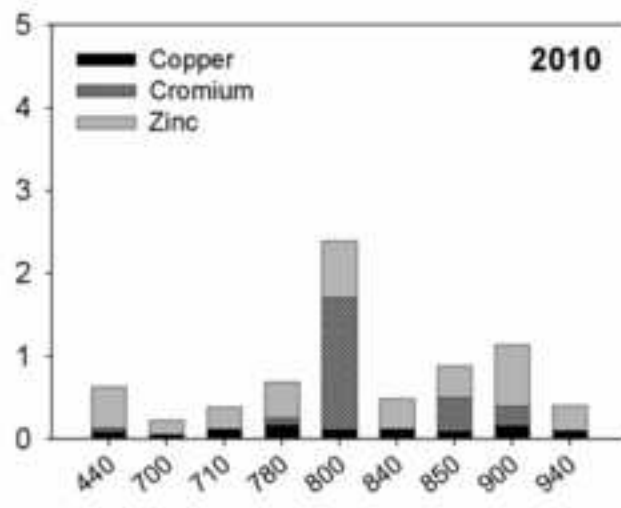
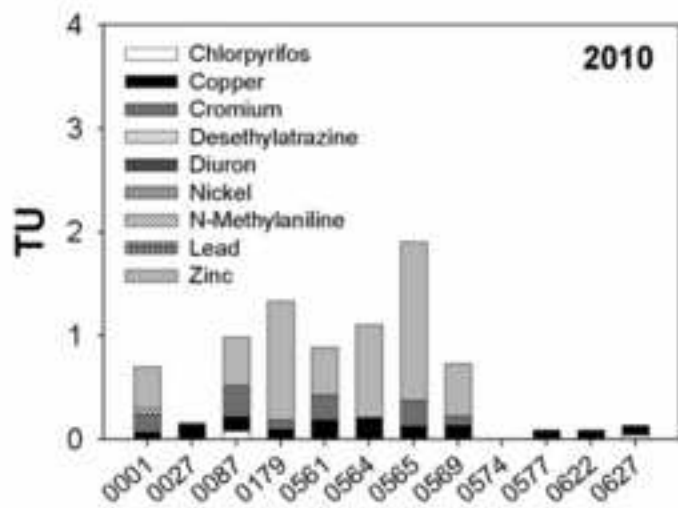
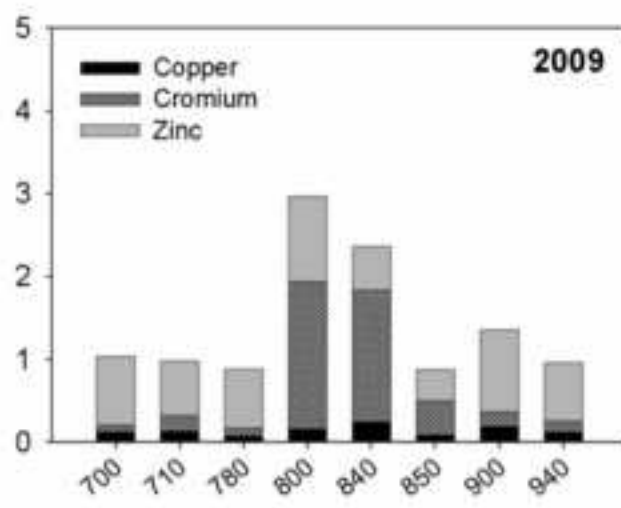
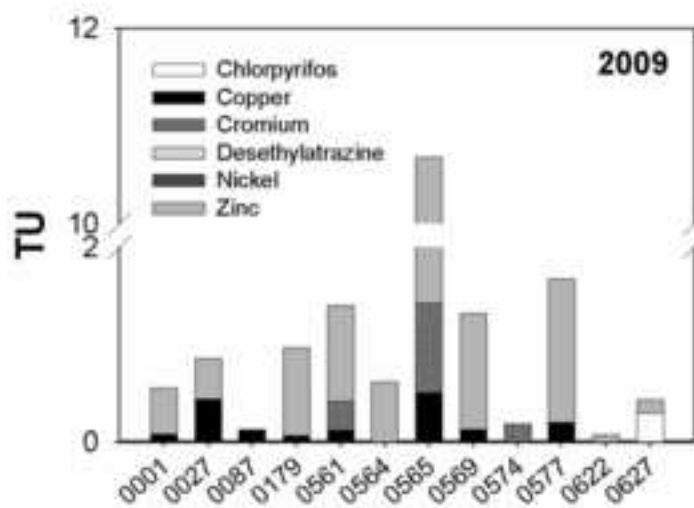
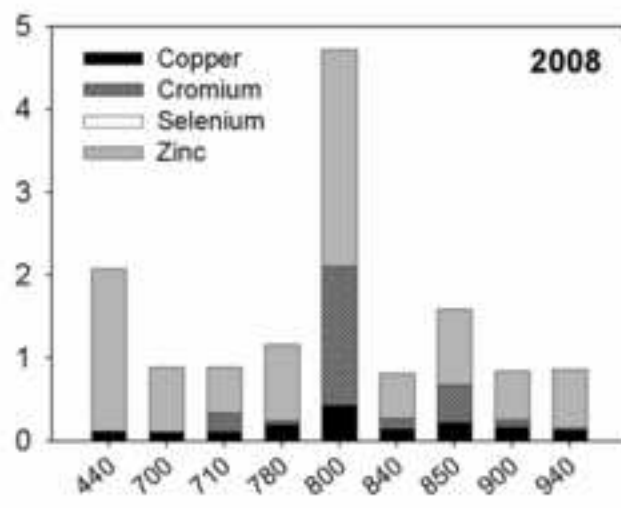
	Year	Number of stations	Maximum number of compounds analyzed	Minimum number of compounds analyzed	Stations where only metals are analyzed	Stations where only organic are analyzed	Stations where both are analyzed
Ebro	2008	33	84	30			33
	2009	34	85	28			34
	2010	35	83	25			35
Guadalquivir	2007	162	96	2	24	6	132
	2008	132	83	2	17*	27	88
	2009	127	81	2	14*	29	84
Júcar	2008	6	110	32		2**	4
	2009	12	105	37		1**	11
	2010	14	98	40		1**	13
Llobregat	2008	55	107	17	13		42
	2009	38	107	17	22		16
	2010	45	107	17	8		37



(A) Ebro basin



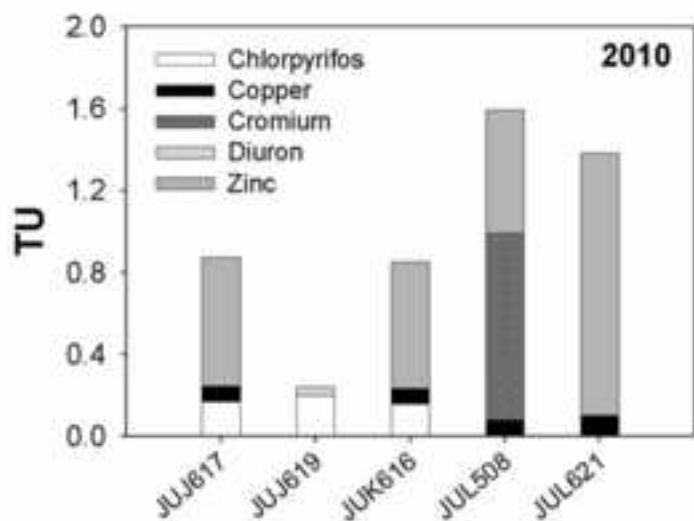
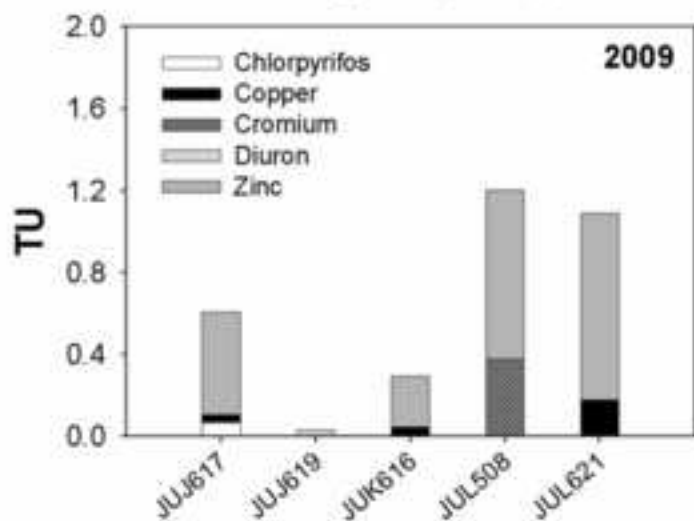
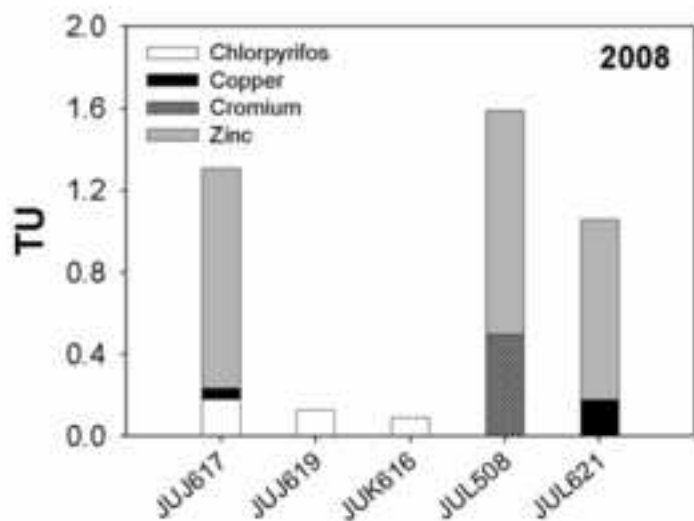
(B) Llobregat basin



CHE sampling points

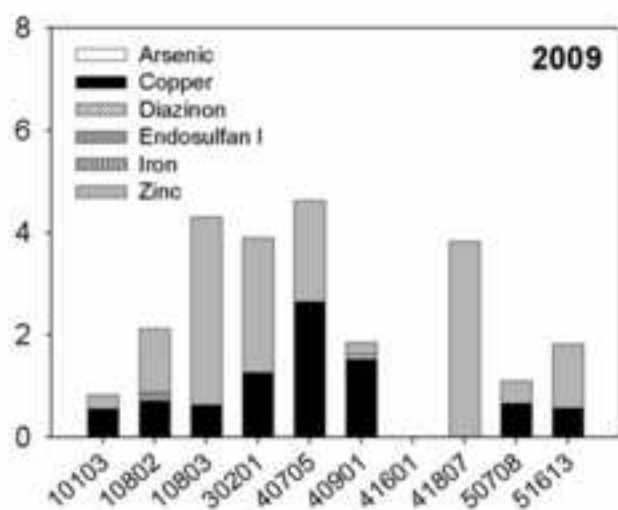
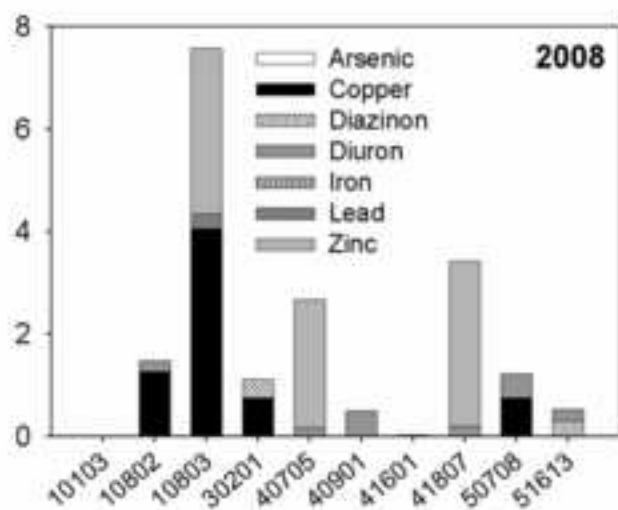
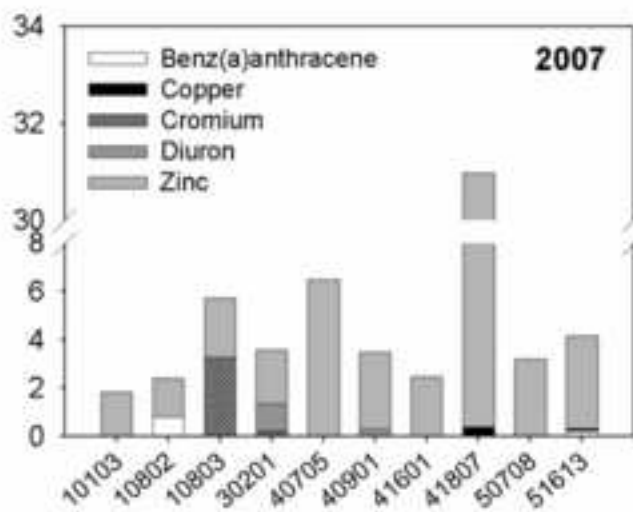
ACA sampling points

(A) Júcar basin



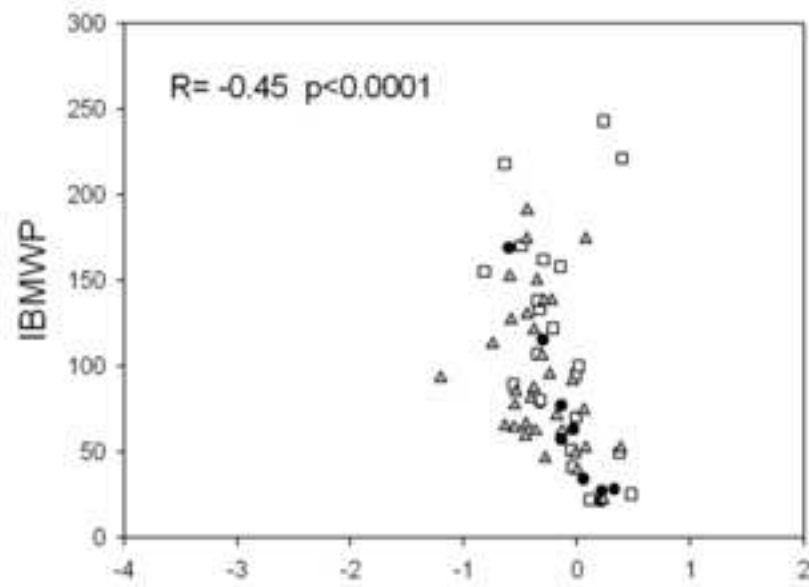
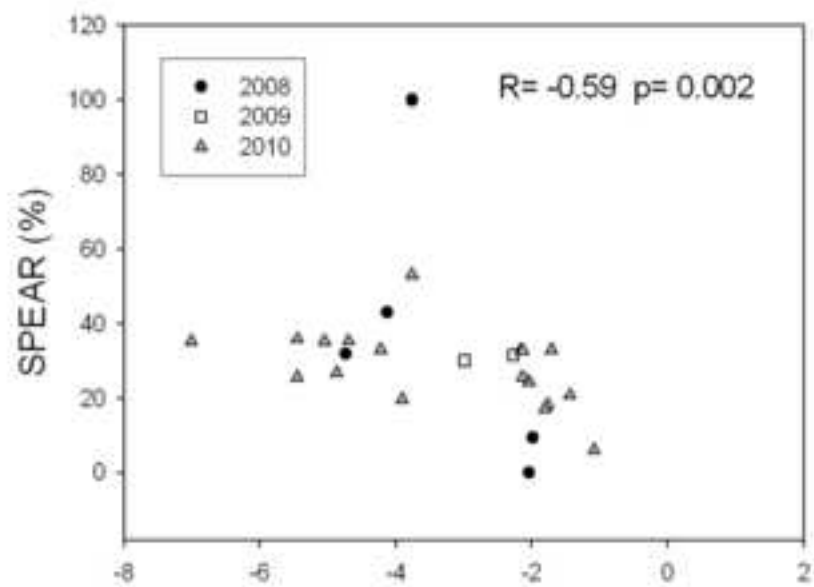
CHJ sampling points

(B) Guadalquivir basin

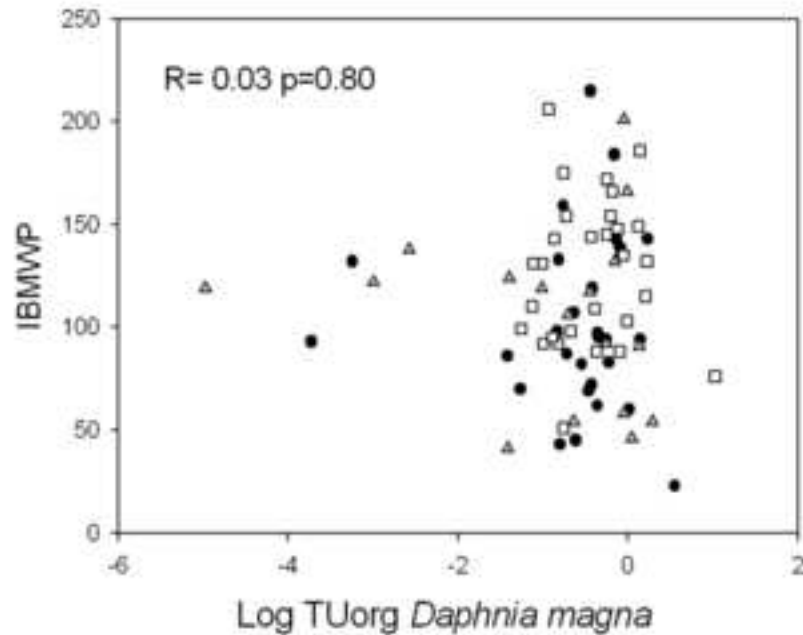
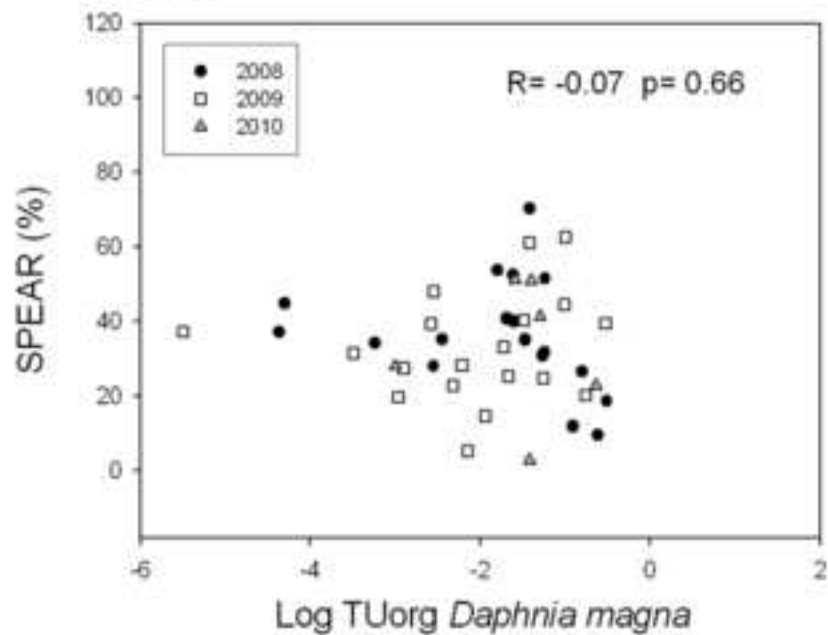


CHG sampling points

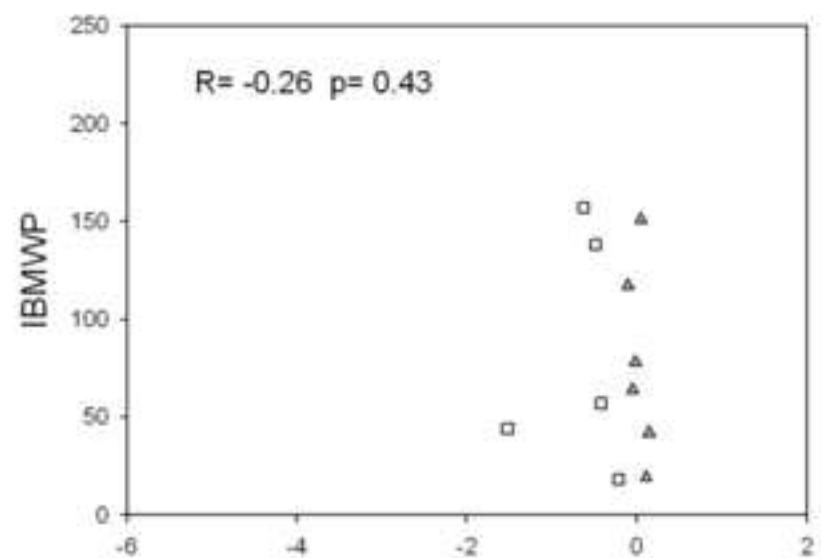
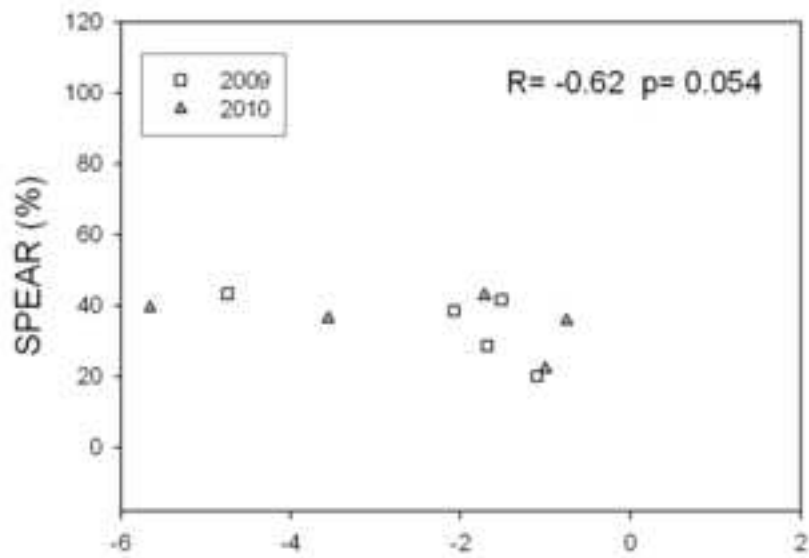
Llobregat



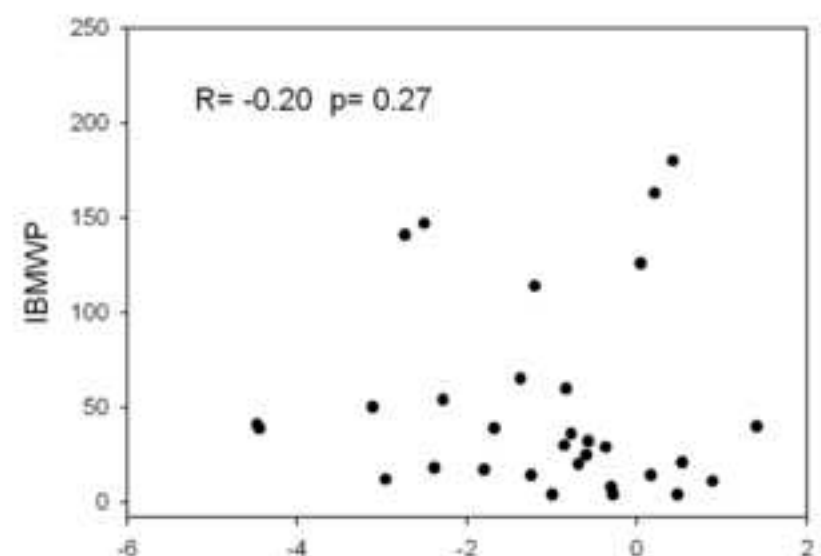
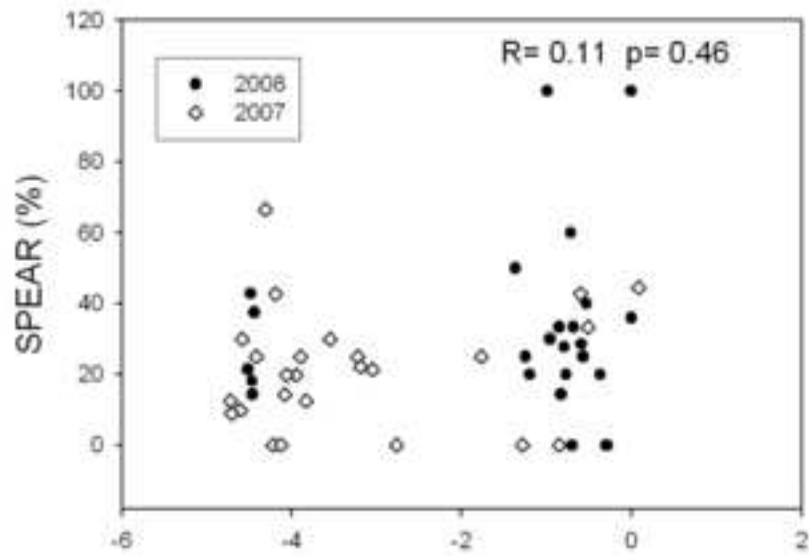
Ebro



Júcar



Guadalquivir



Log TUorg *Daphnia magna*

Log TUorg *Daphnia magna*