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# Pesticides in the Ebro River basin: Occurrence and risk assessment☆

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## ABSTRACT

In this study, 50 pesticides were analyzed in the Ebro River basin in 2010 and 2011 to assess their impact in water, sediment and biota. A special emphasis was placed on the potential effects of both, individual pesticides and their mixtures, in three trophic levels (algae, daphnia and fish) using Risk Quotients (RQs) and Toxic Units (TUs) for water and sediments. Chlorpyrifos, diazinon and carbendazim were the most frequent in water (95, 95 and 70% of the samples, respectively). Imazalil (409.73 ng/L) and diuron (150 ng/L) were at the highest concentrations. Sediment and biota were less contaminated. Chlorpyrifos, diazinon and diclofenthion were the most frequent in sediments (82, 45 and 21% of the samples, respectively). The only pesticide detected in biota was chlorpyrifos (up to 840.2 ng $g^{-1}$). Ecotoxicological risk assessment through RQs showed that organophosphorus and azol presented high risk for algae; organophosphorus, benzimidazoles, carbamates, juvenile hormone mimic and other pesticides for daphnia, and organophosphorus, azol and juvenile hormone mimics for fish. The sum $TU_{site}$ for water and sediments showed values < 1 for the three bioassays. In both matrices, daphnia and fish were more sensitive to the mixture of pesticide residues present.

## 1. Introduction

Pesticides are a widespread group of chemical substances used to improve agricultural production. However, these substances could be persistent in water, accumulative in sediment or bioaccumulative in biota, depending on their solubility and Log $K_{OW}$. They are hazardous for living organisms, human health or environment, even at low concentrations (Campo et al., 2013; Claver et al., 2006; Damásio et al., 2011; Giordano et al., 2009; Masiá et al., 2015a). Furthermore, physical, chemical and biological processes degrade pesticides into one or more transformation products that could be more toxic or persistent than the parent one. There is a need of data on the real occurrence of pesticide residues in environmental matrices (De Gerónimo et al., 2014; Köck-Schulmeyer et al., 2014; Palma et al., 2014a; Bruzzoniti et al., 2014; Martínez-Domínguez et al., 2015; Masiá et al., 2014, 2015b; Wei et al., 2015).

The potential ecotoxicological risks associated with pesticide residue contamination are addressed through toxic units (TUs) and/or risk quotients (RQs) (EC, 2003; Ginebreda et al., 2014; Kökc et al., 2010). Their application in most studies is restricted to water samples (Ginebreda et al., 2014; Kuzmanović et al., 2016). However, pesticide residues can also be adsorbed into sediments (Masiá et al., 2015b). WFD (EC, 2000) and environmental quality standards (EQS) (EC, 2008; EU, 2013) unquestionably support to include sediments in the risk assessment. A variety of methods were proposed but only scarcely applied to evaluate the potential toxicity of sediments (e.g., toxic equivalent factor approach, TUs summation, hazard index) (Schwarzenbach and Westall, 1981; Booij et al., 2015; de Castro-Catalá et al., 2016).

Another problem caused by pesticides contamination is the simultaneous occurrence of several of them and the need to establish the real impact of these mixtures on biota (Cedergreen,

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2014; Roig et al., 2015), which can be predicted by independent action (IA) or concentration addition (CA). The former assumes that the components have different mechanisms of action —ignoring synergies/antagonisms and effect summation and therefore underestimating the effect— and the latter that have a similar one —overestimating the effects. (Cedergreen, 2014; Ginebreda et al., 2014; Kuzmanović et al., 2016). CA is often the recommended first step on a tiered process because presents the worst case scenario (even that synergies are not considered) (de Castro-Catalá et al., 2016).

Mediterranean area is one of the most affected by climatic fluctuations that alter hydrological conditions and originate the great wavering on concentrations of the cocktail of pesticide residues present in water (Batalla et al., 2004). Ebro River is the second largest river of the Iberian Peninsula and the first one that flows into the Mediterranean area of Spain. Previous studies performed in the Ebro River linking occurrence of pollutants, concentrations and toxicity, but most of them have focused on a single chemical family or select one environmental matrix (water, soils, sediments or biota) (Claver et al., 2006; Damásio et al., 2010; Köck-Schulmeyer et al., 2013; Köck et al., 2010; Navarro et al., 2010; Silva et al., 2011).

The objective of this study was to establish pesticide's occurrence, spatial distribution and transport and to evaluate the ecotoxicological risk in three trophic levels (Algae, daphnia and fish), using RQs for each pesticide and sumTUs for each sampling site. The partial objectives of this study were to (i) monitor the concentration of 50 pesticides and transformation products in the surface waters, sediments and biota of the Ebro River basin in two consecutive campaigns (2010–2011) (ii) compare the concentration of the pesticides found in the present study with those detected since 2001 and with the EQS values of the pesticides included in the Directive 2013/39/EU (EU, 2013), and (iii) perform an environmental risk assessment not only for water concentrations but also sediments based on the RQs and TUs methods.

## 2. Experimental design

### 2.1. Physical setting and sampling

The Ebro River is located at the northeast of Spain and drains an area of approximately 85,000 km$^2$. It has 928 km in length and receives waters from several tributaries, which altogether represent 12,000 km of waterway network, ending into Mediterranean Sea and forms a delta of more than 300 km$^2$ (Lacorte et al., 2006; Navarro et al., 2010; Roig et al., 2015). The basin is characterized by a Mediterranean valley, which forms a triangular morphological unit, surrounded by mountains. Mean annual precipitation and temperature vary with altitude, ranging respectively from 1800 mm to 8 °C in the Pyrenees to 320 mm and 18 °C in the Ebro valley. Traditionally, the Ebro River basin is agricultural land, but lately industry has been a growing sector. In 2008, one third of the total surface of the basin was agricultural and it is still the most irrigated area in Spain (906.000 ha) (Herrero-Hernández et al., 2013), the most important crops are herbaceous plants (all over the basin), grapes for wine production (La Rioja), fruit trees (Lleida) and rice (Ebro Delta) (Silva et al., 2011). The Spanish statistics estimated that ca. 14,000 T of pesticides were used in 2010 and ca. 13,500 T in 2011. The monitoring in this study comprised two sampling campaigns, 2010 and 2011, including 24 sampling stations for water and sediments covering the whole River Basin (see Fig. S-1 and S-2) and finally five for biota sampling in 2010. These sites are representative of the whole basin (geo – references are in Table S-2).

Samples were taken in October in both years. Grab water samples (2 L) were collected in clean amber glass bottles, from the middle of the river width. Each bottle was thoroughly rinsed with MilliQ water at the laboratory and with the river water at the sampling point before collection. Sediment samples (approx. 250 g) were taken in the same point as the water ones using a Van Veen grab sampler (0.5 L capacity). They were transferred and wrapped into an aluminum foil (previously washed with methanol and dried in oven at 100 °C) that was put inside an aluminum box. Fish samples were only collected in 2010 at five selected sites of the River course: EBR2, EBR3, EBR4, EBR5 and OCA using electro-fishing because the complexity of the basin, the difficulties to perform electrofishing and the small sample sizes obtained.

All samples were transported in hermetic boxes refrigerated with ice upon arrival at the laboratory. There, the water samples were kept at 4°°C and pre-treated and processed in a period not exceeding 5 days. Before the analysis, water samples were vacuum filtered through 1 μm glass fiber filters followed by 0.45 μm nylon membrane filters (VWR, Barcelona, Spain). Sediment and fish samples were frozen, lyophilized (Hetosicc CD4, Birkerod, Denmark), pulverized, thoroughly mixed, passed through a 2 mm Ø sieve and kept at −20 °C until the analysis that was performed within 3 months.

### 2.2. Extraction procedures and instrumental analysis: water, sediment and fish samples

For this study, 42 pesticides including some of their transformation products were determined in the 2010 campaign. Carbendazim, thiabendazole, terbumeton, terbumeton deethyl, terbuthylazine, terbuthylazine deethyl, terbuthylazine-2-hydroxy and tebuconazole were added in the next year. These pesticides belong to different chemical families, with a variety of uses as well as different physicochemical characteristics and toxicity (see Table S-1).

The water extraction was carried out according to Masiá et al. (2013b). Very briefly, water samples (200 mL) were extracted using an Oasis HLB solid-phase extraction (SPE) cartridge (200 mg sorbent/6 mL cartridge, Waters, Milford, MA, USA). The cartridge was dried under vacuum for 10 min and the analytes eluted with 10 mL of dichloromethane–methanol (50:50, v/v). The extract was evaporated to dryness and reconstituted with 1 mL of methanol. The fish and sediment samples were extracted using the QuEChERS method as described by Masiá et al. (2015b). Lyophilized sediment (1 g) or fish (2 g) were extracted with 8 ml of $H_2O$ MilliQ, 15 ml of acetonitrile, 6 g of $MgSO_4$ and 1.5 g of NaCl. Then, 2 mL of the resulting supernatant were cleaned-up by dispersive SPE with 0.3 g of $MgSO_4$, 0.1 g of PSA, 0.1 g of $C_{18}$ and 0.015 g of GCB. All samples were analyzed in triplicate. The results presented are the average of the three values.

The chromatographic instrument was an HP1200 series LC – automatic injector, degasser, quaternary pump and column oven – combined with an Agilent 6410 triple quadrupole (QQQ) mass spectrometer, equipped with an electrospray ionization interface (Agilent Technologies, Waldbronn, Germany). Data were processed using a MassHunter Workstation Software for qualitative and quantitative analysis (A GL Sciences, Tokyo, Japan). The detailed conditions are in the Supplementary material Tables S-3 and S-4).

### 2.3. Quality assurance and quality control

The analytical methods validation was detailed in the SM Table S-5. The method's limits of detection (MLDs) and quantification (MLQs) ranged from 0.01 to 2 ng L$^{-1}$ for water, from 0.03 to 1.67 ng g$^{-1}$ for sediment and from 0.08 to 3.75 ng g$^{-1}$ for biota. Recovery tests were carried out in quintuplicate in order to evaluate the precision of the method. In water samples, recoveries varied from 48% to 70% and precision was below 20% for all pesticides. In sediment and biota samples, recoveries were higher than 40% and precision ≤22%.

Pesticide concentrations were assessed though a comprehensive quality control scheme that included: laboratory and field blanks, matrix spikes and triplicate samples. Blank contamination is the most common problem observed in the determination of pesticides at trace levels. Thus, precautions were taken to prevent contamination from personnel, organic solvents, equipment and glassware. Blank assays were performed employing MilliQ water samples, to check for laboratory background levels of the studied compounds.

### 2.4. *Risk assessment*

The Toxic Units (TUs) and Risk Quotient (RQ) were calculated according to the European guidelines for each pesticide (EC, 2003) in at least three representative taxons (algae, *Daphnia magna*, and fish) of three trophic levels in the ecosystem. Acute 48 h EC50 for *D. magna,* 72 h EC50 for algae and 96 h LC50 for fish, as well as Chronic 96 h NOEC data for algae and 21 days NOEC for fish and *D. magna* of each chemical was collected from the website http://sitem.herts.ac.uk/aeru/ppdb/en/atoz.htm. In this database the EC50 for *D. magna* is refereed to immobilization, for algae (unknown species) to growth inhibition and for fish (*Oncorhynchus mykiss* mostly) to survival. Values of any compound not available in this site were calculated using the ECOSAR™ v. 1.11 (ECOlogical Structure Activity Relationship), in which the lowest toxicity prediction for each taxon was chosen to set in the worst-case scenario.

The toxic unit ($TU_i$) (Sprague, 1971) is used for the ecotoxicological risk assessment of measured concentrations of compounds ($C_i$). The TU of each compound was based on acute toxicity values. The following equation was applied for water and sediment samples.

$$TU_i\ (algae,\ daphnia,\ fish) = \frac{C_i}{EC50_i}$$

where TUi is the toxic unit of a compound i; $C_i$ measured concentration (ng $L^{-1}$) in the water samples; $EC50_i$ (ng $L^{-1}$) is the effective concentration of 50% of individuals when exposed to the substance concerned.

Site specific toxic stress (TUsite) was calculated by summing all the individual TUi of each detected compound at all of the 24 studied sites.

$$Sum\ TU_{site} = \sum_{i=1}^{n} TU_i$$

Sediment-associated pesticide concentrations were converted to pore-water concentrations according to the equilibrium-partitioning approach to comply with the sediment benchmark toxicity tests that are based on dissolved phase pesticides in pore water. Pore water concentrations from sediments were calculated according to Di Toro et al. (1991) as:

$$C_{pw} = \frac{C_s}{K_d}$$

where $K_d$ is the partitioning coefficient, $C_S$ is the sediment concentration and $C_{PW}$ the pore water concentration of the pesticide. $K_d$ was calculated as:

$$K_d = K_{oc} \times f_{oc}$$

where $K_{OC}$ is the dimensionless organic carbon–water partitioning coefficient for the pesticide and $f_{OC}$ is the fraction of total organic carbon measured in the sediment samples. The $K_{OC}$ was calculated as:

$$\log K_{OC} = a \times \log K_{OW} + b$$

where $K_{OW}$ is the octanol–water partitioning coefficient. The constants a and b were set to 0.72 and 0.49, respectively (Schwarzenbach and Westall, 1981). $TUs > 1$ indicates environmental concern.

RQ was calculated using the following equation:

$$RQ = EC/PNEC$$

where, EC is the mean or maximum concentration of pesticides detected in the water samples and PNEC is the predicted no-effect concentration. PNEC can be calculated for acute or chronic toxicity, dividing the lowest short-term EC50 or long-term NOEC respectively by an assessment factor (AF), in this case 1000. The AF is an arbitrary factor to consider the inherent uncertainty in the obtained laboratory toxicity data. If $RQ > 1$, harmful effects could be expected due to the presence of the pollutant in water. On the contrary, if $RQ < 0.1$, the environmental risk is low. The intermediate situation in which the RQ is between 0.1 and 1 involves medium risk.

## 3. Results and discussion

Pollutants were more frequent in water than in sediment and biota (more apolar matrices). The low frequency can be explained because of the 50 target pesticides, only 21 had values Log $K_{ow} > 3$ (high), 6 between 2.5 and 3 (moderate) and 17 had values < 2.5 (low). Tables 1–3 show the minimum, maximum, mean and frequency of detection of the studied pesticides in the water, sediment and biota samples, respectively.

### 3.1. *Residues of pesticides in water samples*

The frequency was higher in 2010 than 2011. Organophosphorus, juvenile hormone mimics, azols, triazines, ureas and other pesticides were detected in both campaigns (See Table 1). In 2010, pyriproxyphen, chlorpyrifos, diazinon, buprofezin and hexythiazox were the most frequents (>90% of the samples) followed by imazalil and prochloraz (70% of the samples). In 2011, carbendazim was the most frequent (70% of the samples), whereas, diazinon, terbuthylazine and terbutryn frequency was >45% of the samples. Chlorpyrifos (95% of the samples in 2010) was already reported as the most commonly detected pesticide in the Ebro River (Claver et al., 2006; Navarro et al., 2010) even though is not usually persistent in water systems. Diazinon had a high frequency in 2010 (95% of the samples) but a medium-low one in 2011 (45%). This compound is stable in water, moderately soluble and slightly volatile (Table S-1). In 2011, carbendazim (not analyzed in 2010) was present in 70% of the sampling points. This fungicide has a low water solubility, can be persistent in water under certain conditions and is moderately persistent in soil. Herbicides terbuthylazine and terbutryn not analyzed in 2010 were detected in 50% of the samples in 2011. On the legal or illegal use of pesticides, of 50 target compounds analyzed, 14 —withdrawn by the European Union— were detected in both campaigns including carbendazim, metolachlor, azinphos methyl, chlorfenvinphos, diazinon, fenitrothion, fenthion, omethoate, parathion-methyl, atrazine, propazine, simazine, terbumeton and terbutryn (See Table S-1).

The pollution profile in both campaigns was marked by azoles, organophosphorus and triazines (detailed concentration at each

**Table 1**
Minimum, maximum and mean concentrations and frequency of detection of the studied pesticides in water samples.

| Pollutants | 2010 | | | | 2011 | | | |
|---|---|---|---|---|---|---|---|---|
| | Concentration (ng $L^{-1}$) | | | | Concentration (ng $L^{-1}$) | | | |
| | Min | Max | Mean | Freq (%)[a] | Min | Max | Mean | Freq (%)[a] |
| **Azol** | | | | | | | | |
| Imazalil | 4.91 | 409.76 | 61.01 | 17 (70) | 1.28 | 121.70 | 7.50 | 8 (33) |
| Prochloraz | 2.24 | 34.47 | 15.59 | 17 (70) | 2.14 | 2.14 | 0.09 | 1 (4) |
| **Benzimidazole** | | | | | | | | |
| Carbendazim | n.a | n.a | n.a | n.a | 0.04 | 11.63 | 2.78 | 17 (70) |
| Thiabendazole | n.a | n.a | n.a | n.a | 0.43 | 48.77 | 3.58 | 5 (20) |
| **Carbamates** | | | | | | | | |
| 3-Hydroxycarbofuran | 8.47 | 8.47 | 0.35 | 1 (4) | 0.20 | 0.20 | 0.01 | 1 (4) |
| Methiocarb | n.d | n.d | n.d | n.d | 1.24 | 2.52 | 0.30 | 4 (16) |
| **Chloroacetanilide** | | | | | | | | |
| Metoalachlor | n.d | n.d | n.d | n.d | 1.10 | 4.86 | 0.55 | 7 (29) |
| **Juvenile Hormone Mimics** | | | | | | | | |
| Pyriproxyphen | 0.89 | 37.74 | 24.38 | 23 (95) | 4.76 | 4.76 | 0.20 | 1 (4) |
| **Neonicotinoid** | | | | | | | | |
| Imidacloprid | 1.84 | 2.77 | 1.06 | 11 (45) | 1.64 | 14.96 | 1.66 | 9 (37) |
| **Organophosphorus** | | | | | | | | |
| Azinphos Methyl | n.d | n.d | n.d | n.d | 2.31 | 2.31 | 0.10 | 1 (4) |
| Chlorfenvinphos | 2.54 | 41.24 | 17.97 | 18 (75) | 1.57 | 1.57 | 0.07 | 1 (4) |
| Chlorpyrifos | 2.64 | 16.40 | 5.97 | 23 (95) | 1.01 | 2.86 | 0.32 | 5 (20) |
| Diazinon | 0.12 | 13.58 | 5.65 | 23 (95) | 0.53 | 20.39 | 1.35 | 11 (45) |
| Diclofenthion | 13.62 | 22.73 | 12.86 | 18 (75) | n.d | n.d | n.d | n.d |
| Dimethoate | 2.33 | 3.19 | 0.47 | 4 (16) | 61.56 | 61.56 | 2.57 | 1 (4) |
| Fenitrothion | 2.64 | 2.64 | 0.11 | 1 (4) | 36.49 | 36.49 | 1.52 | 1 (4) |
| Fenoxon | 2.64 | 2.64 | 0.11 | 1 (4) | n.d | n.d | n.d | n.d |
| Fenoxon Sulfone | 2.64 | 2.64 | 0.11 | 1 (4) | n.d | n.d | n.d | n.d |
| Fenoxon Sulfoxide | 2.64 | 20.84 | 4.43 | 9 (37) | n.d | n.d | n.d | n.d |
| Fenthion | 2.64 | 2.64 | 0.11 | 1 (4) | 0.33 | 0.33 | 0.01 | 1 (4) |
| Fenthion Sulfone | 2.64 | 2.64 | 0.11 | 1 (4) | n.d | n.d | n.d | n.d |
| Fenthion Sulfoxide | 2.64 | 2.64 | 0.11 | 1 (4) | n.d | n.d | n.d | n.d |
| Malathion | n.d | n.d | n.d | n.d | 7.93 | 7.93 | 0.33 | 1 (4) |
| Omethoate | n.d | n.d | n.d | n.d | 3.47 | 3.47 | 0.14 | 1 (4) |
| Parathion-Ethyl | 14.01 | 14.45 | 1.19 | 2 (8) | n.d | n.d | n.d | n.d |
| Parathion-Methyl | n.d | n.d | n.d | n.d | 2.00 | 2.00 | 0.08 | 1 (4) |
| Tolclophos-Methyl | 8.30 | 16.07 | 3.50 | 7 (29) | 0.50 | 0.50 | 0.02 | 1 (4) |
| **Other Pesticides** | | | | | | | | |
| Buprofezin | 2.32 | 8.25 | 5.82 | 22 (91) | n.d | n.d | n.d | n.d |
| Hexythiazox | 1.90 | 10.57 | 7.41 | 22 (91) | 1.21 | 1.21 | 0.05 | 1 (4) |
| **Triazines** | | | | | | | | |
| Atrazine | 8.13 | 12.22 | 1.99 | 5 (20) | n.d | n.d | n.d | n.d |
| Deisopropylatrazine | 4.35 | 13.15 | 1.30 | 4 (16) | 6.96 | 19.16 | 2.72 | 6 (25) |
| Deethylatrazine | 6.57 | 58.82 | 7.67 | 7 (29) | 4.99 | 4.99 | 0.21 | 1 (4) |
| Propazine | 3.26 | 3.26 | 0.14 | 1 (4) | n.d | n.d | n.d | n.d |
| Simazine | 30.71 | 47.95 | 3.28 | 2 (8) | n.d | n.d | n.d | n.d |
| Terbumeton | n.a | n.a | n.a | n.a | 5.22 | 5.22 | 0.22 | 1 (4) |
| Terbumeton-Deethyl | n.a | n.a | n.a | n.a | 0.42 | 9.72 | 0.89 | 8 (33) |
| Terbuthylazine | n.a | n.a | n.a | n.a | 0.11 | 10.10 | 2.21 | 12 (50) |
| Terbuthylazine Deethyl | n.a | n.a | n.a | n.a | 0.77 | 2.41 | 0.29 | 4 (16) |
| Terbuthylazine-2 Hydroxy | n.a | n.a | n.a | n.a | 0.23 | 11.59 | 1.41 | 6 (25) |
| Terbutryn | 14.85 | 14.85 | 0.65 | 1 (4) | 0.92 | 30.54 | 7.66 | 12 (50) |
| **Triazole** | | | | | | | | |
| Tebuconazole | n.a | n.a | n.a | n.a | 1.66 | 15.38 | 2.36 | 8 (33) |
| **Ureas** | | | | | | | | |
| Diuron | 2.64 | 150.96 | 6.40 | 2 (8) | 7.52 | 24.47 | 1.95 | 3 (12) |
| Isoproturon | 2.58 | 25.48 | 1.60 | 4 (16) | 2.41 | 2.41 | 0.10 | 1 (4) |

**n.d** = Not detected.
**n.a** = Not analyzed.
[a] Number of findings (percentage of positive samples).

point is shown in Fig S3A), Samples of 2010 were more contaminated than those of 2011. The annual pesticide loads from the Ebro River to the Mediterranean Sea were estimated to be 4359 kg in 2010 and 1606 kg in 2011. These estimations correspond to the October–November period, which is characterized by lower pesticide discharge compared to spring time. These annual pesticide loads released to the sea could affect the Ebro Delta, biota and marine ecosystems. There are several estimations in different Mediterranean rivers of the pesticide loads that arrives yearly to the Sea: Jucar River 539 kg and Turía River 156 kg (Ccanccapa et al., 2016; Mai et al., 2013; Soubaneh et al., 2015). Mediterranean Sea receives already 2301 kg of pesticides yearly just from these three rivers. Tables 4 and 5 outline concentration of pesticides in water samples of the Ebro River and of other Mediterranean Rivers from 2001 to present. Regarding pollutants found in the Ebro River organophosphorus, carbamates, triazine, azol and ureas were always the most detected compounds. The concentrations were within the range from 3 to 12,597 ng $L^{-1}$. The main pesticides found were atrazine, molinate, propanil, diazinon, diuron, malathion, terbuthylazine, imidacloprid, tebuconalezole and dimethoate in

**Table 2**
Minimum, maximum and mean concentrations and frequency of detection of the studied pesticides in sediment samples.

| Pollutants | 2010 | | | | 2011 | | | |
|---|---|---|---|---|---|---|---|---|
| | Concentration (ng $g^{-1}$ dw) | | | | Concentration (ng $g^{-1}$ dw) | | | |
| | Min | Max | Mean | Freq (%)[a] | Min | Max | Mean | Freq (%)[a] |
| **Azol** | | | | | | | | |
| Imazalil | 7.35 | 7.35 | 0.33 | 1 (4) | 4.20 | 4.20 | 0.18 | 1 (4) |
| Prochloraz | 4.60 | 4.60 | 0.21 | 1 (4) | n.d | n.d | n.d | n.d |
| Chlorpyrifos | 0.18 | 9.59 | 1.06 | 10 (45) | 0.88 | 36.17 | 7.66 | 19 (82) |
| Diazinon | 0.28 | 8.85 | 0.63 | 10 (45) | 0.62 | 3.30 | 0.20 | 3 (13) |
| Diclofenthion | n.d | n.d | n.d | n.d | 1.44 | 28.82 | 1.73 | 5 (21) |
| Ethion | n.d | n.d | n.d | n.d | 5.10 | 5.10 | 0.22 | 1 (4) |
| Malathion | 1.84 | 1.84 | 0.08 | 1 (4) | n.d | n.d | n.d | n.d |
| **Other Pesticides** | | | | | | | | |
| Hexythiazox | n.d | n.d | n.d | n.d | 0.50 | 0.50 | 0.02 | 1 (4) |
| **Triazines** | | | | | | | | |
| Terbutryn | 3.97 | 21.61 | 1.16 | 2 (9) | 0.10 | 0.10 | 0.00 | 1 (4) |

[a] Number of findings (percentage of positive samples).**n.d** = Not detected.
**n.a** = Not analyzed.

agreement with this study. Although the profile of contamination is variable, since 2005 the pesticide residue concentration increased from 4680 ng $L^{-1}$ to 12,597 ng $L^{-1}$ in 2011.

The spatial distribution (See Fig. 1A) of pesticides along the Ebro River and its tributaries could be related to the land use (Belenguer et al., 2014; Ccanccapa et al., 2016; Vryzas et al., 2009). Pesticide concentrations were moderate to low in most of the river course. The most polluted sites are Zadorra (ZAD) in the head and Segre (SEG) as well as the Ebro Delta in the mouth. Station ZAD —located in Alava (Basque Country)— is part of the Natura 2000 Network but surrounding by cereals, sugar beets and potatoes crops and influenced by the Crispijana wastewater treatment plant. In this point, diuron exceed 100 ng $L^{-1}$, limit established for individual concentrations in drinking water according to EU legislation (EC, 1998). The sampling point of the Segre River (SEG) had the highest concentrations of all tributaries. In 2010, this point exceed 500 ng $L^{-1}$, limit established for group pesticides in drinking water, and imazalil exceed 100 ng $L^{-1}$, individual limit established (EC, 1998) and in 2011 the total concentration was 233.33 ng $L^{-1}$. These high concentrations are only punctual. Fruit trees, corn, wheat and barley crops are characteristics of this area. The high concentrations of fungicide imazalil in both campaigns could be related to the post-harvest treatments of apples and pears. The Ebro Delta receives a high load of pesticides because of the intensive agricultural activities that are carried out upstream and in the Delta itself (rice cultivation) (Kuster et al., 2008). The spatial distribution showed clearly the increasing concentration gradient for both campaigns in the points sampled EBR-7, EBR-8 and EBR-9 (see Fig. 1 -A). In 2010, the concentrations go up from 2.32 ng $L^{-1}$ to 109.24 ng $L^{-1}$ and in 2011 from 1.11 ng $L^{-1}$ to 30.54 ng $L^{-1}$ (Kuster et al., 2008; Ochoa et al., 2012).

**Table 3**
Minimum, maximum and mean concentrations and frequency of detection of the studied pesticides in biota samples.

| Pollutants | 2010 | | | |
|---|---|---|---|---|
| | Concentration ng $g^{-1}$ dw | | | |
| | Min | Max | Mean | Freq % |
| **ORGANOPHOSPHORUS** | | | | |
| *CHLORPYRIFOS* | n.d | n.d | n.d | n.d |
| Barbus (*Barbus guiraonis*) | n.d | n.d | n.d | n.d |
| Barbus (*Barbus guiraonis*): Adult | n.d | n.d | n.d | n.d |
| Barbus (*Barbus guiraonis*): Young | n.d | n.d | n.d | n.d |
| Carp (*Cyprinus carpus*) | 840.25 | 840.25 | 420.13 | 1 (20) |
| Carp (*Cyprinus carpus*): Adult | n.d | n.d | n.d | n.d |
| European catfish (*Silurus glanis*): Adult | 168.62 | 168.62 | 84.31 | 1 (20) |

**n.d** = Not detected.

The co-occurrence of different pesticides in the water samples are shown in Fig. S-4A. In 2010, 38% of the samples contained less than 5 pesticides and 22% of the samples contained more than 16 pesticides. This means that even though concentrations were low, and there was one point (SEG) that exceed the European threshold for drinking water, the number of pesticides in each sample was high. In 2011, 42% of the samples present less than 5 pesticides but 22% of the samples present among 6 to 16 pesticides. In 2011 the co-occurrence was lower than in 2010.

The differences between both sampling campaigns could be related to the river flow (see Table S-6). Considering all the flow measurements in the last ten years in each point where there are data available and normalizing them to 100, the water flow in the first campaign ranged from 0.03 $m^3 s^{-1}$ (MAT) to 213.40 $m^3 s^{-1}$ (EBR-7), these values represent percentiles 18% and 50% that could be considered medium–high. On the contrary, in 2011 the flows ranged from 0.01 $m^3 s^{-1}$ (MAT) to 155.43 $m^3 s^{-1}$ (EBR-7), percentiles 5% and 20%, respectively. These values are below of 50% percentile and could be considered low. Apparently, the higher flow, the greater frequency and co-occurrence of pesticides, and consequently in 2010 the frequency and co-occurrence was higher than 2011 (Table 1 and Fig. S-4A). Regarding the low flow, there are reports that point out that lower flows are related with higher concentrations (Masiá et al., 2015a). However, this work shows low concentrations also at low flows. The concentration could vary taking into account the physico-chemical properties of pesticides but also other environmental conditions as precipitation or temperatures (see Table S-1) (Ccanccapa et al., 2016).

### 3.2. *Residues of pesticides in sediment samples*

Pesticides detected in sediment samples in both campaigns are outlined in Table 2. Out of the 42 pesticides analyzed in 2010 and 50 pesticides in 2011, 6 and 7 respectively, were detected at the concentrations over the MLODs. In 2010, 14% of the analytes —imazalil, prochloraz, chlorpyrifos, diazinon, malathion and terbutryn— were found. The concentrations detected ranged from 1.84 to 21.61 ng $g^{-1}$ of dry weight (d.w). In 2011, pesticides detected were imazalil, chlorpyrifos, diazinon, diclofenthion, ethion, hexythiazox and terbutryn, and their concentrations ranged from 0.10 to 36.17 ng $g^{-1}$ of d.w.

Regarding the frequency, diazinon and chlorpyrifos were the most prevalent compounds in 2010, which appeared in 45% of the samples. In 2011, chlorpyrifos (82%) and diclofenthion (21%) were the most frequently detected compounds. These pesticides had high octanol/water partition coefficient (log $k_{ow}$) (see Table S-1), consequently, are hydrophobic, low water soluble and tend to accumulate in sediment. However, other factors influence pesticides accumulation such as the application moment and the time elapses before the next major storm event. Chlorpyrifos was detected at high frequency in both campaigns and there are other reports that also remark their presence in the Mediterranean area (Ccanccapa et al., 2016; Masiá et al., 2015a, 2013a).

The spatial distribution of pesticides in sediment is shown in Fig. 1B and the contribution of each family of pesticides is detailed in Fig. S-4B. In 2010, the most ubiquitous pesticides were organophosphorus (38.99 ng $L^{-1}$), triazine (25.57 ng $L^{-1}$) and azol (11.94 ng $L^{-1}$). However, in 2011 only organophosphorus (225.62 ng $L^{-1}$) were found in all points sampled. Regarding the highest concentrations, in 2010 were for terbutryn (21.61 ng $L^{-1}$) and chlorpyrifos (9.59 ng $L^{-1}$) in points sampled ZAD and EBR-9

**Table 4**
Historical data of the pesticides concentrations in the Ebro Basin.

| Year | Location | Family | Pesticide | Concentration ($ng\ L^{-1}$) | | Ref. |
|---|---|---|---|---|---|---|
| | | | | Max | Mean | |
| 2001–2004 | Ebro River | Urea | Diuron | – | 105 | (Claver et al., 2006) |
| | | Carbamates | Molinate | – | 751 | |
| | | Triazine | Atrazine | – | 451 | |
| | | Chloroacetanilide | Metolachlor | – | 200 | |
| | | Organophosphates | Chlorpyrifos | – | 312 | |
| 2004–2006 | Ebro River | Triazine | Atrazine | 825 | 62 | (Navarro et al., 2010) |
| | | Organophosphates | Dimethoate | 259 | 115 | |
| | | Chloroacetanilide | Alachlor | 272 | 32 | |
| | | Carbamate | Molinate | 344 | 107 | |
| | | Anilide | Propanil | 156 | 34 | |
| 2005 | Ebro Delta | Triazine | Triazines | 935 | 697 | (Damásio et al., 2010) |
| | | Anilide | Propanil | 4680 | 1757 | |
| | | Carbamate | Molinate | 485 | 318 | |
| 2007–2009 | Ebro Basin | Chloroacetanilide | Alachlor | 3 | 3 | (Köck-Schulmeyer et al., 2013) |
| | | Anilide | Propanil | 36 | 9 | |
| | | Organophosphates | Diazinon | 684 | 133 | |
| | | Urea | Diuron | 452 | 93 | |
| | | Triazine | Terbuthylazine | 71 | 21 | |
| 2008 | Ebro Delta | Organophosphates | Malathion | 5825 | 1072 | (Köck et al., 2010) |
| | | Urea | Diuron | 408 | 72 | |
| | | Carbamates | Molinate | 3590 | 526 | |
| | | Triazine | Terbuthylazine | 1550 | 250 | |
| 2011 | Ebro River | Triazine | Terbuthylazine | 12,597 | – | (Herrero-Hernández et al., 2013) |
| | | Urea | Diuron | 8551 | – | |
| | | Neonicotinoid | Imidacloprid | 656 | – | |
| | | Chloroacetanilide | Acetochlor | 314 | – | |
| | | Triazole | Tebuconazole | 3236 | – | |
| | | Organophosphates | Dimethoate | 7549 | – | |

**Table 5**
Historical data of pesticides concentration in the Mediterranean area.

| Year | Location | Family | Pesticides | Concentration ($ng\ L^{-1}$) | | Ref. |
|---|---|---|---|---|---|---|
| | | | | Max | Mean | |
| 2010 | Jucar River | Triazine | Atrazine-desethyl | 11 | – | (Belenguer et al., 2014) |
| | | Organophosphorus | Chlorfenvinphos | 93 | – | |
| | | Azol | Imazalil | 172 | – | |
| | | Other Pesticides | Hexythiazox | 21 | – | |
| | | Juvenile Hormone Mimics | Pyriproxyfen | 100 | – | |
| 2010–2011 | Guadalquivir River | Azole | Carbendazim | 11 | 1 | (Masiá et al., 2013a) |
| | | Juvenile hormone mimics | Imidacloprid | 19 | 2 | |
| | | Organophosphorus | Diazinon | 457 | 19 | |
| 2010–2011 | Llobregat River | Triazine | Terbuthylazine-2-hydroxy | 50 | 13 | (Masiá et al., 2015a) |
| | | Organophosphorus | Malathion | 320 | 58 | |
| | | Benzimidazole | Carbendazim | 697 | 273 | |
| | | Carbamates | Carbofuran | 7 | 3 | |
| | | Azol | Prochloraz | 10 | 10 | |
| | | Other Pesticides | Hexythiazox | 24 | 13 | |
| | | Neonicotinoid | Imidacloprid | 67 | 25 | |
| | | Urea | Diuron | 160 | 109 | |
| | | Chloroacetanilide | Metolachlor | 13 | 11 | |
| | | Juvenile Hormone Mimics | Pyriproxyphen | 2 | 2 | |
| 2012–2013 | Turia River | Anilide | Propanil | 46 | 2 | (Ccanccapa et al., 2016) |
| | | Azol | Imazalil | 750 | 43 | |
| | | Benzimidazole | Carbendazim | 382 | 23 | |
| | | Carbamates | Carbofuran | 6845 | 283 | |
| | | Chloroacetanilide | Metolachlor | 58 | 12 | |
| | | Juvenile Hormone Mimics | Pyriproxyfen | 3 | 0 | |
| | | Neonicotinoid | Imidacloprid | 207 | 23 | |
| | | Organophosphorus | Ethion | 350 | 13 | |
| | | Other Pesticides | Buprofezin | 25 | 12 | |
| | | Thiocarbamates | Molinate | 14 | 1 | |
| | | Triazine | Terbutylazine Deethyl | 59 | 10 | |
| | | Triazole | Tebuconazole | 21 | 3 | |
| | | Urea | Isoproturon | 13 | 2 | |

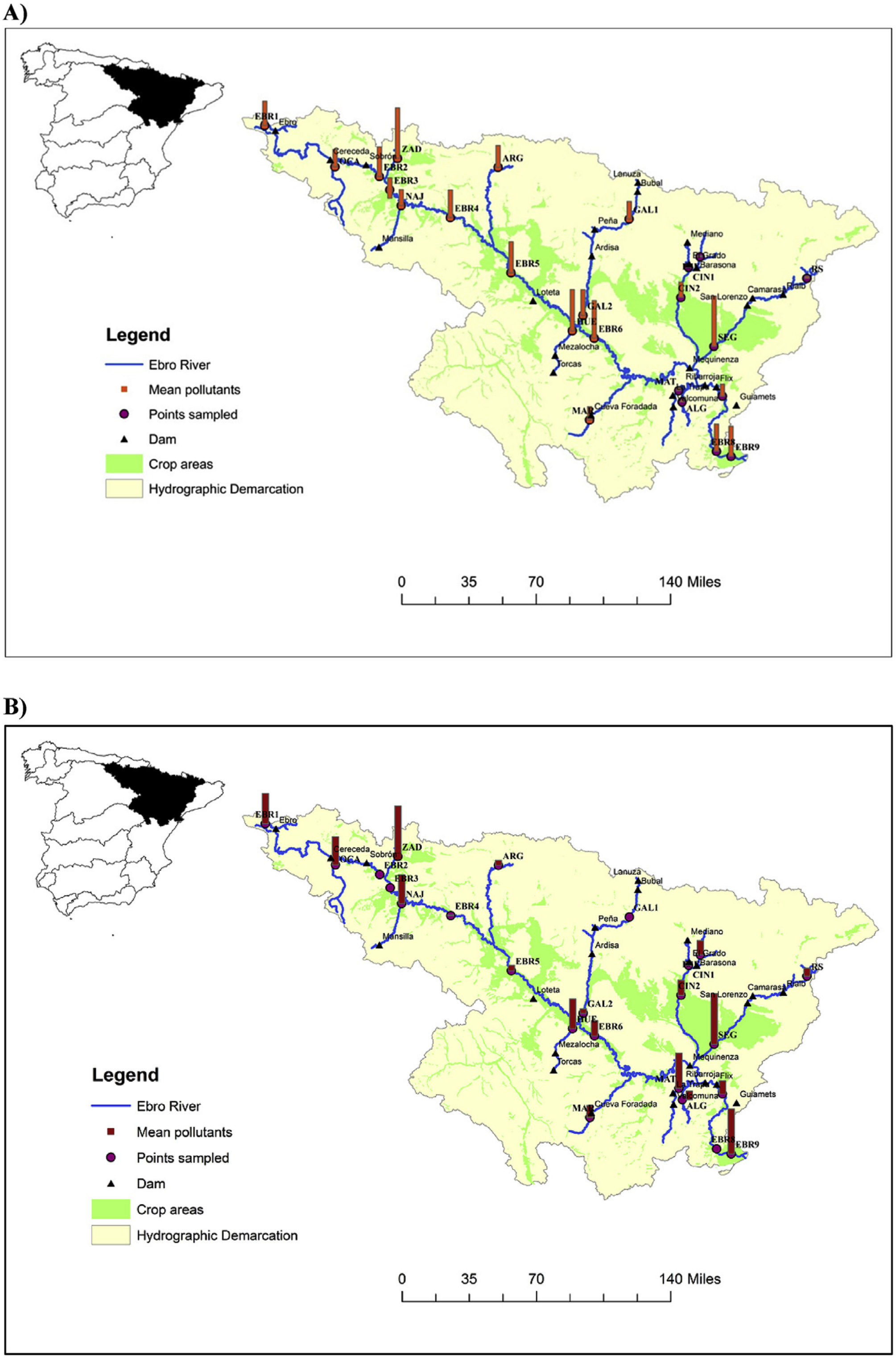

**Fig. 1.** Spatial distribution of pesticides in Ebro basin. A) 2010–2011 water samples and B) 2010–2011 sediment samples.

respectively. In 2011, chlorpyrifos (36.17 ng L$^{-1}$ in EBR-1) and diclofenthion (28.82 ng L$^{-1}$ in OCA) had the highest concentrations.

The co-occurrence of pesticides in sediments can be seen in the Fig. S-4B. In both campaigns, 86% of the sediment samples did not present pesticides. In 2010, 9% and 2011, 12% had at least 5 pesticides. Only 5% and 2% samples, consecutively, presented up to 10 pesticides.

### 3.3. Residues of pesticides in biota samples

Fish samples were taken at five points (EBR-2, EBR-3, EBR-4, EBR-5 and OCA) in one campaign (2010). The collected fish species include, barbus (*Barbus guiraonis*), carp (*Cyprinus carpus*) and european catfish (*Silurus glanis*) (see Table 3). Chlorpyrifos ($K_{ow} = 4$) was the only pesticide detected in two fish species (Carp and European catfish). The concentrations were high, carp presented 840.25 ng g$^{-1}$ dw and European catfish 168.62 ng g $^{-1}$ dw. These data indicated possible bioaccumulation of these pesticides in fish. There are studies carried out in Mediterranean Rivers that pointed out chlorpyrifos bioaccumulation's in different fish species (Belenguer et al., 2014; Masiá et al., 2015a). Chlorpyrifos is considered as highly toxic to aquatic organisms.

## 4. Toxic units and risk quotient for water and sediments

The Sum TU$_{site}$ could help to estimate the toxic effects of the mixture of pollutants per monitoring area by summing single compound TU for each sampling point as well as to study toxicity due to the contaminant present in sediments. However, the obtained Sum TU$_{site}$ for water (Table 6) and sediment (Table 7) were <1 in all sites, evidencing that there is no acute risk associated with pollution either in water or sediments. Among the studied sites EBR-6 (0.26), ARG (0.24), ZAD (0.21), SEG (0.12), HUE (0.21), EBR-5 (0.21) and EBR-2 (0.23) showed the highest Sum TU$_{site}$ values always for daphnia and water (See Fig. 2A). These sites reflected a dispersed pollution along the basin and a corresponding loss of ecological quality. The values do not reach the unit but are indicative of the sensitivity of *D. magn*a to the mixture of pesticide residues in comparison with the other trophic levels. In 2011 the values were very low. These results clearly pointed out that there are not acute effects due to the mixtures of contaminants. However, complex chronic effects and interactions can not be discharged.

Subsequently, to evaluate the impact of the pesticides on the Ebro River basin ecosystems, the risk quotient (RQ) method was used employing, whenever is possible, the NOEC values obtained from chronic toxicity tests for producing the corresponding PNECs. Table 8 (Detailed Table S-7) shows the results obtained for the pesticides exhibiting low to high risk at either average or extreme condition, as calculated from their corresponding mean and maximum concentrations (Masiá et al., 2015a; Palma et al., 2014b; Sánchez-Bayo et al., 2002). Hexythiazox and prochloraz were present in some samples at levels that involved a risk, mean and maximum concentrations (RQ values > 1) for algae. Carbendazim, chlorfenvinphos, chlorpyrifos, diazinon, dichlofenthion, fenitrothion, hexythiazox, imazalil, malathion, methiocarb, and pyriproxyfen showed also as a hazard for daphnia at mean and maximum concentrations. Finally, Chlorpyrifos, dichlofenthion, imazalil, and pyriproxyfen presented RQ > 1 for fish at both, mean and maximum concentrations. Chronic toxicity test showed the high risk caused by pesticides in three trophic levels (algae, daphnia and fish); this could cause changes in fish and invertebrate communities and the decrease of the most sensitive species or increase of the more resistant ones, with a consequent loss of biodiversity. On the other hand, out of the 6 pesticides found with values above RQ > 1 for algae, all those are herbicides and fungicides. These compounds affect photosynthesis in microalgae and its reduction in aquatic ecosystems (Booij et al., 2015). For daphnia, 16 pollutants (RQ > 1) —mostly insecticides and fungicides— could produce seriously effect in this trophic level. Finally, for fish, 8 pesticides exceed RQ > 1. Mixtures of organophosphate, azoles and carbamates pesticides were commonly found in water samples. These pesticides inhibit the activity of acetylcholinesterase and have potential to interfere with behaviors that may be essential for the survival of species. There are reports of the Carps exposed to mixtures containing some of the organophosphorus, azoles and carbamates showed concentration additive or synergistic neurotoxicity (Cedergreen, 2014; Wang et al., 2015). This implies that single-chemical assessments systematically underestimate actual risks to aquatic species in watersheds where insecticides mixtures occur. RQ and TU are important indexes to estimate the risk in different trophic levels and for the protection of aquatic ecosystems.

**Table 6**
Toxic units for the different sites and trophic levels for water samples.

| | Algae | | Daphnia | | Fish | |
|---|---|---|---|---|---|---|
| | 2010 | 2011 | 2010 | 2011 | 2010 | 2011 |
| MAR | E− | E− | 0.190 | E− | 0.028 | E− |
| ALG | E− | E− | 0.073 | 0.001 | 0.004 | E− |
| ARG | 0.002 | 0.006 | 0.240 | 0.001 | 0.020 | E− |
| CIN1 | E | E− | 0.053 | E− | 0.003 | E− |
| CIN2 | 0.001 | E− | 0.182 | E− | 0.017 | E− |
| EBR1 | 0.001 | E− | 0.139 | E− | 0.019 | E− |
| EBR2 | 0.001 | E− | 0.232 | 0.051 | 0.017 | 0.003 |
| EBR3 | 0.001 | E− | 0.172 | E− | 0.017 | E− |
| EBR4 | 0.001 | E− | 0.221 | E− | 0.019 | E− |
| EBR5 | 0.001 | 0.003 | 0.210 | 0.011 | 0.019 | 0.001 |
| EBR6 | 0.001 | E− | 0.263 | 0.001 | 0.020 | E− |
| EBR7 | E− | 0.004 | E− | 0.013 | E− | 0.001 |
| EBR8 | 0.001 | 0.004 | 0.204 | E− | 0.017 | E− |
| EBR9 | 0.001 | 0.004 | 0.167 | E− | 0.023 | E− |
| ESE | E− | E− | 0.041 | E− | 0.003 | E− |
| GAL1 | 0.001 | E− | 0.126 | 0.001 | 0.015 | E− |
| GAL2 | 0.002 | E− | 0.153 | 0.001 | 0.015 | E− |
| HUE | 0.001 | 0.004 | 0.215 | 0.036 | 0.021 | 0.001 |
| MAT | 0.001 | E− | 0.029 | 0.000 | 0.002 | E− |
| NAJ | E− | 0.001 | 0.149 | 0.001 | 0.019 | E− |
| OCA | E− | E− | 0.102 | E− | 0.024 | E− |
| RS | E− | E− | 0.077 | E− | 0.003 | E− |
| SEG | 0.001 | 0.002 | 0.122 | 0.016 | 0.019 | 0.001 |
| ZAD | 0.064 | 0.012 | 0.217 | 0.004 | 0.019 | E− |

$^{E-}$ More than four decimals.

**Table 7**
Toxic units for the different sites and trophic levels for sediment samples.

| | Algae | | Daphnia | | Fish | |
|---|---|---|---|---|---|---|
| | 2010 | 2011 | 2010 | 2011 | 2010 | 2011 |
| MAR | E− | E− | 0.003 | E− | E− | E− |
| ALG | E− | E− | 0.004 | E− | E− | E− |
| ARG | 0.001 | 0.001 | 0.008 | E− | E− | E− |
| CIN1 | E− | n.d | 0.001 | n.d | E− | n.d |
| CIN2 | E− | E− | 0.006 | E− | E− | E− |
| EBR1 | E− | E− | 0.003 | E− | E− | E− |
| EBR2 | E− | E− | 0.012 | 0.002 | E− | E− |
| EBR3 | E− | E− | 0.004 | E− | E− | E− |
| EBR4 | E− | E− | 0.022 | n.d | 0.001 | n.d |
| EBR5 | n.a | E− | n.a | E− | n.d | E− |
| EBR6 | E− | E− | 0.005 | E− | E− | E− |
| EBR7 | E− | E− | E− | E− | E− | E− |
| EBR8 | n.a | n.a | n.a | n.a | n.a | n.a |
| EBR9 | E− | 0.001 | 0.004 | E− | E− | E− |
| ESE | E− | E− | 0.006 | n.d | E− | n.d |
| GAL1 | E− | E− | 0.003 | E− | E− | E− |
| GAL2 | 0.002 | E− | 0.009 | E− | E− | E− |
| HUE | E− | 0.001 | 0.004 | 0.002 | E− | E− |
| MAT | 0.001 | E− | 0.003 | E− | E− | E− |
| NAJ | E− | E− | 0.002 | E− | E− | E− |
| OCA | E− | E− | 0.002 | E− | E− | E− |
| RS | E− | n.d | 0.006 | n.d | E− | n.d |
| SEG | 0.001 | E− | 0.008 | E− | E− | E− |
| ZAD | 0.003 | 0.001 | 0.002 | E− | E− | E− |

$^{E-}$ More than four decimals.
n.d: Not detected.
n.a: Not analyzed.

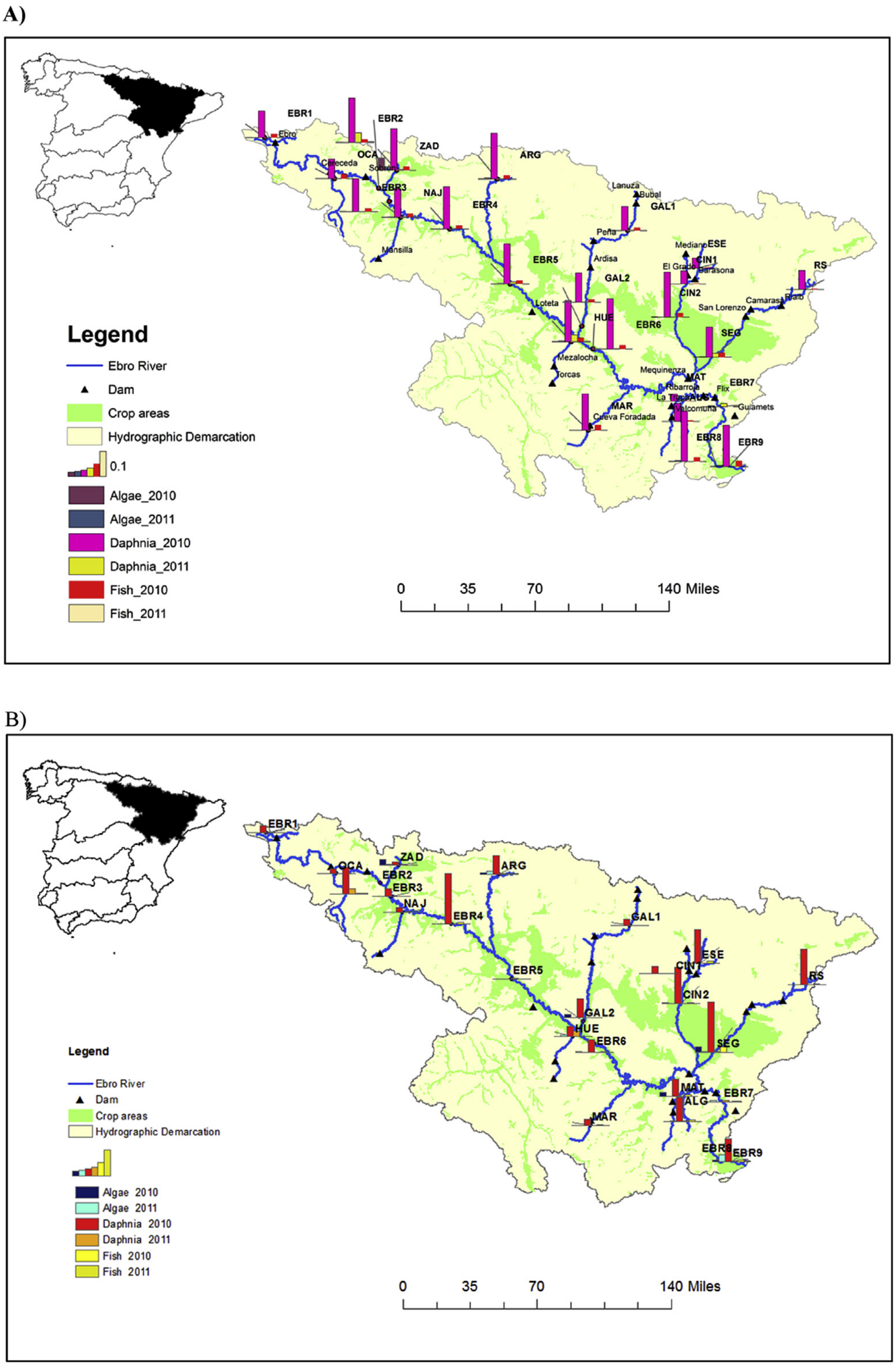

**Fig. 2.** Sum $TU_{site}$ in sampling site for algae, daphnia and fish 2010–2011 A) Water samples and B) Sediment samples.

## 5. Conclusions

The survey carried out in 2010 and 2011 in the Ebro River and its tributaries regarding determination, distribution and ecotoxicological effects of 50 pesticides showed a dispersed pattern of concentration and risk on the different trophic levels (algae, daphnia and fish) along the basin. Water samples were the most frequently contaminated in both campaigns and in lesser extent sediment and biota samples. The most ubiquitous pesticides were azoles, organophosphorus and triazines in both years. The annual loads of pesticides for the Ebro basin were estimated in 4359 kg in 2010 and 1606 kg in 2011. This estimation was made in October and

**Table 8**
RQ for algae, daphnia and fish.

| Pollutants | PNEC Ng L-1 | 2010 | | 2011 | |
|---|---|---|---|---|---|
| | | RQ-Mean | RQ-Max | RQ-Mean | RQ-Max |
| Chronic 96/72 h NOEC in Algae | | | | | |
| Alachlor | | | | | |
| Atrazine | 100 | <0.1 | 0.1 | | |
| Chlorpyrifos | 43 | 0.1 | 0.4 | <0.1 | <0.1 |
| Dichlofenthion | 204 | 0.1 | 0.1 | | |
| Diuron | 93 | 0.1 | 1.6 | <0.1 | 0.3 |
| Fenitrothion | 100 | <0.1 | <0.1 | <0.1 | 0.4 |
| Hexythiazox | 7 | 1.1 | 1.5 | <0.1 | 0.2 |
| Imazalil | 92 | 0.7 | 4.5 | 0.1 | 1.3 |
| Isoproturon | 52 | <0.1 | 0.5 | <0.1 | <0.1 |
| Metolachlor | 1 | | | 0.9 | 8.2 |
| Prochloraz | 10 | 1.6 | 3.4 | <0.1 | 0.2 |
| Propazine | 40 | <0.1 | 0.1 | | |
| Pyriproxyfen | 213 | 0.1 | 0.2 | <0.1 | <0.1 |
| Tebuconazole | 100 | | | <0.1 | 0.2 |
| Terbutryn | 28 | <0.1 | 0.5 | 0.3 | 1.1 |
| Chronic 96/72 h NOEC in Aquatic invertebrates (*Daphnia magna*) | | | | | |
| Azinphos Methyl | 0.4 | | | 0.2 | 5.8 |
| Buprofezin | 80 | 0.1 | 0.1 | | |
| Carbendazim | 1.5 | | | 1.9 | 7.8 |
| Chlorfenvinphos | 0.1 | 179.7 | 412.4 | 0.7 | 15.7 |
| Chlorpyrifos | 4.6 | 1.3 | 3.6 | 0.1 | 0.6 |
| Diazinon | 0.56 | 10.1 | 24.2 | 2.4 | 36.4 |
| Dimethoate | 40 | <0.1 | 0.1 | 0.1 | 1.5 |
| Diuron | 96 | 0.1 | 1.6 | <0.1 | 0.3 |
| Fenitrothion | 0.09 | 1.3 | 30.3 | 17.5 | 419.4 |
| Hexythiazox | 6.1 | 1.2 | 1.7 | <0.1 | 0.2 |
| Imazalil | 15 | 4.1 | 27.3 | 0.5 | 8.1 |
| Isoproturon | 120 | <0.1 | 0.2 | <0.1 | <0.1 |
| Malathion | 0.06 | | | 5.5 | 132.1 |
| Methiocarb | 0.1 | | | 3 | 25.2 |
| Prochloraz | 18 | 0.9 | 1.9 | <0.1 | 0.1 |
| Pyriproxyfen | 0.02 | 1625.6 | 2515.7 | 13.2 | 317 |
| Tebuconazole | 10 | | | 0.2 | 1.5 |
| Terbutryn | 205 | <0.1 | 0.1 | <0.1 | 0.1 |
| Thiabendazole | 42 | | | 0.1 | 1.2 |
| Chronic 21 days NOEC in Fish | | | | | |
| Azinphos Methyl | 0.17 | | | 0.6 | 13.6 |
| Buprofezin | 52 | 0.1 | 0.2 | | |
| Carbendazim | 3.2 | | | 0.9 | 3.6 |
| Chlorfenvinphos | 30 | 0.6 | 1.4 | <0.1 | 0.1 |
| Chlorpyrifos | 0.14 | 42.6 | 117.2 | 2.3 | 20.4 |
| Dichlofenthion | 4 | 3.2 | 5.7 | | |
| Dimethoate | 400 | <0.1 | <0.1 | <0.1 | 0.2 |
| Diuron | 410 | <0.1 | 0.4 | <0.1 | 0.1 |
| Fenitrothion | 88 | <0.1 | <0.1 | <0.1 | 0.4 |
| Fenoxon Sulfone | 23 | <0.1 | 0.1 | | |
| Hexythiazox | 40 | 0.2 | 0.3 | <0.1 | <0.1 |
| Imazalil | 43 | 1.4 | 9.5 | 0.2 | 2.8 |
| Malathion | 91 | | | <0.1 | 0.1 |
| Methiocarb | 50 | | | <0.1 | 0.1 |
| Prochloraz | 49 | 0.3 | 0.7 | <0.1 | <0.1 |
| Pyriproxyfen | 4.3 | 5.7 | 8.8 | <0.1 | 1.1 |
| Simazine | 700 | <0.1 | 0.1 | | |
| Tebuconazole | 12 | | | 0.2 | 1.3 |
| Terbutryn | 104 | <0.1 | 0.1 | 0.1 | 0.3 |
| Thiabendazole | 12 | | | 0.3 | 4.1 |

November; a period characterized by lower pesticide discharge, and in 24 points sampled, demonstrating a high impact in the delta and marine ecosystems. The ecotoxicological assessment point out that exist a chronic toxicity (RQ index) caused by pesticides (organophosphorus, azol, carbamates and juvenile hormone mimics) in three trophic levels (algae, daphnia and fish), specially in *Daphnia magna*. The Toxic unit for water and sediments, calculated to assess the effects of the cocktail of pesticide residues and know the specific sites impacted, showed the daphnia as the most sensitive in 2010 along the basin. According to the TUs, there are not acute effects due to pesticide concentrations either in water or sediments. However, several pesticides showed a RQ > 1 indicating that pesticide risk to the aquatic communities needs further study. A long-term chronic study on assessment of these mixtures is highly required.

## Acknowledgment

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2015.12.059.

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