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Multi-residues analysis of pre-emergence herbicides in fluvial sediments: application to the mid-Garonne River

Damien A. Devault, Georges Merlina, Puy Lim,* Jean-Luc Probst and Eric Pinelli

Contamination of man and ecosystems by pesticides has become a major environmental concern. Whereas many studies exist on contamination from agriculture, the effects of urban sources are usually omitted. Fluvial sediment is a complex matrix of pollutants but little is known of its recent herbicide content. This study proposes a method for a fast and reliable analysis of herbicides by employing the accelerated solvent extractor (ASE). The aim of the study is to show the impact of a major town (Toulouse) on the herbicide content in the river. In this study, three herbicide families (*i.e.* *s*-triazine, substituted ureas and anilides) were analysed in fluvial sediment fractions at 11 sampling sites along the mid-Garonne River and its tributaries. River water contamination by herbicides is minor, except for at three sites located in urban areas. Among the herbicidal families studied, urban and suburban areas are distinguished from rural areas and were found to be the most contaminated sites during the study period, a winter low-water event. The herbicide content of the coarse sediment fractions is about one third of that found in the fine fractions and usually ignored. The distribution of pesticide concentrations across the whole range of particle sizes was investigated to clarify the role of plant remains on the significant accumulation in the coarse fractions.

Introduction

Contamination of man and ecosystems by pesticides has become a major environmental concern. The impact of these products on human health and ecosystem integrity is well known.[1] Ecosystem contamination by agricultural practices and eradication procedures has been the subject of many studies, and more recently some have started to outline the impact of urban activities: Kimbrough and Litke[2] in Colorado (USA), CORPEN[3] in France, Gerecke *et al.*[4] in Switzerland, and Blanchoud *et al.*[5] in the surroundings of Paris (France). Braman *et al.*[6] showed that herbicides account for 85% of the pesticides applied in urban environments. In France, 10% of the pesticides consumed are used for non-agricultural uses, especially domestic (8%) and collective (2%) uses.[7] With two percent of the surface area of the Midi-Pyrenees region being urbanised, we can estimate that the urban ground receives about as much pesticide per unit of area as agricultural land. ANTEA[8] estimated that 26 g of pesticide were used per year per inhabitant in the Garonne basin. Among non-agrarian consumers, the greatest users of herbicides are as follows, greatest first: private individuals, municipal services (who use half as much as private individuals) and professional gardeners (who use half as much as municipal services) as reported by OPECST[7] and Blanchoud *et al.*[9] Unlike private individuals and *a fortiori* farmers, professionals use herbicides on impermeable surfaces (asphalt, concrete, gravel, sand) where streaming dominates (40 to 100% runoff).[5,10] Most of the herbicides used belong to *s*-triazine, substituted urea and anilide families. One of their advantages is that some of them can be used for winter treatment in agricultural or urban practices. However, some molecules such as atrazine have been forbidden since 2003 in France, or are under limitations as substituted ureas, *i.e.* linuron and isoproturon in the European Community (EC).

The fluvial sediment is well known to be an integrative matrix for pollutants.[11,12] Since there is little variation in the agricultural practice, an accumulation of these pesticides is conceivable in river bed sediment by direct agricultural and urban dissolved effluents as well as by deposition of suspended particulate matter (SPM)[13–15] contaminated by agricultural and urban use. However, accumulation of these molecules is seldom studied in sediment and biota because no reliable and easily implemented method exists.

Excluding specific sanitary reports, the pesticide concentration of the Garonne River has been the subject of only three studies restricted to the confluence of the Garonne and its tributaries, the Lot River[16] and the Dropt River[17] or to specific pesticides, *i.e.* organotin.[18] The upstream area of the Malause reservoir has never been covered. The aim of this study is to determine the herbicide levels in the water and sediment of the Garonne River for the first time and to assess the respective contributions of agricultural and urban areas to different herbicide concentrations in the sediments. For this purpose, a broad spectrum multi-residue analysis method based on ASE extraction coupled with mini column solid purification has been developed.

Ecole Nationale Supérieure Agronomique de Toulouse, Institut National Polytechnique, Laboratoire Agronomie Environnement Ecotoxicologie (AEE), Avenue de l'Agrobiopole, BP 32607, Auzeville tolosane, 31326 Castanet-Tolosan. E-mail: plim@ensat.fr; Tel: +33-5-62-19-39-04

1. Characteristics of the Garonne River basin

The Garonne River has an uncommonly "wild" character for a river in a developed country. Its hydrological regime is still natural, with an unstable river bed, easily creating oxbows and side-arms. This river is the least fitted, large French river.[19] Most of the smaller towns lie outside of its flood plain *i.e.* uphill or on the tributaries, especially between Toulouse and the Malause reservoir where the Garonne River overflows. The mean annual river discharge in Toulouse and Tonneins (last measurement site before the tidal part) is 191 $m^3\ s^{-1}$ and 613 $m^3\ s^{-1}$, respectively. The lowest daily temperature is 5.4 °C (January) and the average yearly temperature is 13.3 °C.[20] The studied area concerns the middle part of the river marked off by two dams with Mancies upstream and Malause downstream, making a 120 km long section centred on Toulouse, allowing the impact of the city area to be distinguished.

The country through which the mid-Garonne River runs is a coherent morpho-pedological, physiographical and agricultural area. Mid-Garonne is characterised by a low altitude range (100–500 m) with a smooth relief made of Quaternary flat and alluvial terraces and steep Tertiary clay-rock slopes.[21] Excluding the industrial effluent from the suburbs of Toulouse, the expected herbicide concentration of the Garonne water and sediment in this area could be attributed to the agricultural activities and particularly to the use of pre-emergence herbicides applied to crops: *s*-triazines, substituted ureas and anilides. The mid-Garonne area is favourable to farming of cereals (wheat, corn) and oilseed (sunflower), which are the main crops. Their respective agricultural surface areas are similar, at around 200 000 hectares each (data from the French services of statistics for agriculture and forestry).[22,23] In this basin, rapeseed is distributed over a total area of 20 000 hectares. A similar area for orchards is concentrated around the confluence of the Garonne and the Tarn Rivers, near the Malause dam (Fig. 1). Horticulture and market gardening is located north of Toulouse (14 000 hectares). Thus, different samples of river water and sediments were taken along the mid-course of this river and at the mouth of the main tributaries. The sampling sites were chosen for the nature of their agricultural or urban catchments, since these products can originate from both sources.[5,9]

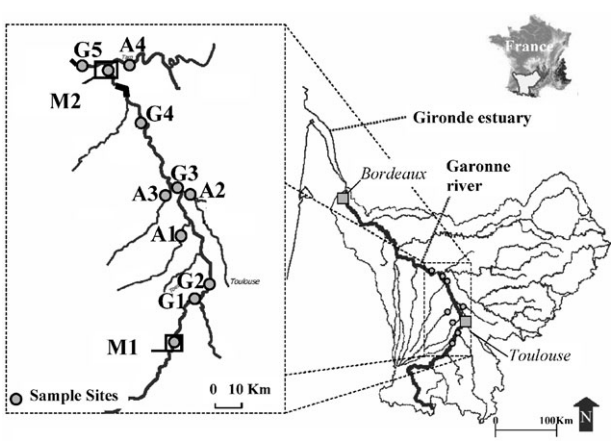

Fig. 1 Location of the eleven sampling sites. M1: Mancies reservoir, M2: Malause reservoir, G1: Portet-sur-Garonne, G2: Empalot, G3: Grenade, G4: Bourret, G5: Lamagistère, A1: Aussonnelle River, A2: Hers River, A3: Save River, A4: Tarn River.

Table 1 List of sampling sites, corresponding river, type of flow and human environment, upstream to downstream

Code	Sample site	River	Type	Environment
M1	Mancies	Garonne	Reservoir	Rural
G1	Portet-sur-Garonne	Garonne	Main river	Rural
G2	Empalot	Garonne	Main river	Urban
A1	Seilh	Aussonnelle	Tributary	Suburban
A2	St Jory	Hers	Tributary	Suburban
G3	Grenade	Garonne	Main river	Suburban
A3	Grenade	Save	Tributary	Rural
G4	Bourret	Garonne	Main river	Rural
A4	Ste Livrade	Tarn	Tributary	Rural
M2	Malause	Garonne	Reservoir	Rural
G5	Lamagistère	Garonne	Main river	Rural

2. Material

The bottom riverside sediments were collected on the same day at 5 sites on the Garonne River (2 urban/suburban, 3 agricultural) and 4 sites on the tributaries (2 suburban, 2 agricultural) (Table 1). Surface water samples were also collected at the same time. Sediments were sampled (3 pooled replicates for river sites, 6 pooled samples for reservoir M1 and 12 pooled samples for reservoir M2) with a 0.045 m^2 Eckmann bucket and water surface samples with stainless steel and glass equipment.

2.1 Sampling area

Sampling of sediment and the corresponding surface water was performed in the bed of the Garonne River (Fig. 1). The physicochemical evaluation, pedological evolution and human activity effects of this part of the Garonne basin have been described in other papers.[24–27] The sampling sites are referenced by the codes in Table 1, corresponding to the sites shown on the map (Fig. 1).

2.2 Sampling period

All sediment and water samples were collected the same day (16th March 2005), 54 days after the last rainfall. Sediment samples were collected in coves during the winter low-water event corresponding to the beginning of treatments and to maximum sedimentation under cold water conditions. Pre-emergence herbicides indicate recent pollution, urban as well as agricultural, during a typical period of spreading. This was the most marked low-water level event and the sediment to be collected was never out of water. The Garonne has a flow mode characterized by severe low-water levels[19]. The end of the winter season was selected for sampling, taking into consideration agricultural uses according to investigations of the regional service for plant protection (SRPV). This period corresponds to the beginning of spring, especially in 2005, when spring was particularly early and dry. The last relevant rain event induced a 568.91 $m^3\ s^{-1}$ flow (21/01/2005).

2.3 Sample treatment

The different sediment fractions were dried at room temperature and separated according to the Commission of Oslo and Paris (OSPAR) methods.[28–32] The sediment components were separated using an agate mortar and sifted with 2 mm and 63 μm mesh sieves (stainless steel sieves from Retsch GmbH and Co. KG, Haan, Germany) to distinguish silts and clays from sands and plant remains.

For water samples, dichloromethane (1 : 40; v/v) was added to the unfiltered water sample at the sampling site and stored in the dark until extraction the next day. Unfiltered water was analyzed according to normative directives and to a better appreciation of the exposure of the different biota to the contamination.

3. Method of analysis: multi-residue approach

3.1 Sample analysis

3.1.1 Chemicals and equipment. All solvents were of analytical grade for pesticide analysis ("Pestipur" by SDS Solvant Documents Synthèse, Peypin, France). Analytical grade anhydrous sodium sulfate was also from SDS. Pesticide standards (Mix 44) from Dr Ehrenstorfer GmbH (Augsburg, Germany) were purchased from C.I.L., Sainte-Foy-la-Grande, France. Reference soil (Eurosoil7) was from Sigma–Aldrich (St Louis, Missouri, USA). Florisil cartridges used for purification were from Waters Corporation (Milford, Massachusetts, USA).

Extraction was performed with a Dionex accelerated solvent extractor (ASE 200) (Dionex, Salt Lake City, Utah, USA). Diatomaceous earth (Hydromatrix®) was from Varian, Palo Alto, California, USA.

3.1.2 Sample extraction and purification

Sediment samples. From the initially described method for organo-chlorine insecticides in soils and whole sediments,[33] recovered by Concha-Graña *et al.*[34] on the Ehrenstorfer mix 20, recommended by Dionex[35] and approved by the U.S. Environmental Protection Agency, the method was adapted to the extraction of those pre-emergence herbicides. Sediment samples collected were analysed on arrival at the laboratory. For each sediment fraction, 10 g was extracted on an ASE 200. Briefly, three cycles of five minutes (static phase) were carried out for extraction. The extraction solvent was a mix of hexane and acetone (1 : 1, v/v) under two distinct temperatures, the first being at 50 °C and the second at 100 °C, according to pesticide sensitivity to high temperatures. The cell was flushed to 60% of its volume over a period of 60 s. Sediment extracts were not directly analysed because of their high chlorophyll content. Chlorophyll was removed on a Florisil cartridge according to a method derived from Müller *et al.*[36] Each extract was evaporated until dry and the residue dissolved in 2.5 mL of hexane. Compounds of interest were eluted in three separate fractions using 20 mL of *n*-hexane : diethylether (94 : 6, v/v), followed by 10 mL of *n*-hexane : acetone (9 : 1, v/v) and finally by 20 mL hexane : acetone (1 : 1, v/v). The elution speed was 0.5 mL min^{-1}. On the basis of spiking the reference material (Eurosoil 7) used as a surrogate standard at a concentration of 0.15 μg g^{-1}, the recovery yield was established for each compound and the efficiency was checked and confirmed by applying the same protocol to an organochlorine mix (mix 20, Dr Ehrenstorfer GmbH).

Water samples. Unfiltered water samples were liquid–liquid extracted using the shake-flask method with dichloromethane as a solvent for phase exchange. The total solvent : water ratio was 1 : 6, v/v. After extraction, dichloromethane was dried on anhydrous sodium sulfate (50 g) and evaporated under vacuum. The dried residue was dissolved in 2 mL of hexane.

3.1.3 Chromatographic conditions. The extracts were analysed using an HP 5890 Series II gas chromatograph coupled to an MSD HP 5971 mass detector. Chromatographic conditions in the splitless mode (injector temperature: 280 °C) were set up at an initial temperature of 45 °C. The first step had a temperature increase rate of 35 °C min^{-1} up to 180 °C, then a second step at 8 °C min^{-1} up to 280 °C and, finally, a 10 min plateau at 280 °C. The detection conditions were: temperature, 300 °C; E.M.V., 2600 V; the followed ions in SIM mode are presented in Table 2. In these conditions, diuron could not be detected since a cold on-column injection was not used.

3.1.4 Evaluation of the detection limit and the repeatability of the method. The detection limit established was 0.001 μg g^{-1} (Table 2) except for metobromuron, methabenthiazuron and metazachlor which showed higher detection values (0.01–0.005 μg g^{-1}) due to the cross contamination of some samples by phthalates, particularly of water samples. The relative standard deviations for four derivatives were compared to

Table 2 Studied herbicides and metabolites by family and their recovery percentage from a standard reference soil material (Eurosoil7) and the specific ions used in GC-MS. Detection limit was established for the HP5890 equipped with 5971MSD equipment

	Herbicides (Mix 44)	Recovery (%)	Specific ions	Detection limit/μg g^{-1}
s-Triazines	Desethylatrazine (DEA)	82.4%	146	0.005–0.001
	Hexazinone	102.0%	209-175-135	0.005
	Simazine	91.3%	172-187-145	0.001
	Atrazine	101.3%	164	0.005–0.001
	Terbuthylazine	101.2%	172-201	0.001–0.0001
	Sebuthylazine	96.7%	61	0.001
	Cyanazine	102.2%	162	0.005–0.001
Substituted ureas	Metobromuron	99.1%	172-214	0.01–0.005
	Isoproturon	95.9%	68-173-201	0.001
	Linuron	104.6%	61	0.001
	Methabenzthiazuron	97.7%	187	0.01–0.005
Anilides	Metazachlor	88.2%	132-160-209	0.01–0.005
	Metolachlor	102.2%	61	0.001

Table 3 Relative standard deviation observed on cyanazine, simazine and metolachlor herbicides and DEA metabolite from triplicates of sediment samples for two sites and triple injection of Mix44 standard

	Cyanazine	DEA	Simazine	Metolachlor
Site A	4.51%	7.88%	12.20%	11.86%
Site B	4.90%	7.30%	13.11%	12.51%
Standard	5.11%	9.01%	13.76%	13.50%

that of three injections of the standards mix and varied with an acceptable repeatability $<14\%$ as shown in Table 3.

3.1.5 Recovery on a reference material. For the studied herbicides, the recovery percentage after sample preparation, extraction and purification obtained for each pesticide varied from 82.4% to 104.6% (Table 2), leading to a mean recovery of $95.4 \pm 6.5\%$. The efficiency of this method is confirmed by the test on organo-chlorine derivatives which gave a mean recovery yield of 98.5% in accordance to methods used in other studies.[34]

3.2 Assessment of the used method

As the extraction method was applied to organo-chlorine pesticides with results in agreement to the previous studies of Richter *et al.*[33] and Concha-Graña *et al.*,[34] this method covers a broad spectrum of pesticides having varied physicochemical properties with fairly good recoveries. The extracted compounds can vary from quasi-insoluble residues to more soluble ones (from endosulfan: 0.32 mg L^{-1} to hexazinone: 33 000 mg L^{-1}), for lipophily or vapour pressure the same ranges are involved: DDT K_{ow}: 6.19 to hexazinone's one: 1.05, 0.2 μPa for cyanazine to 53 000 μPa for heptachlor. This can be attributed to the extraction solvent mixture; hexane collecting the most lipophilic residues like OCPs while a less hydro phobic solvent like acetone collects the least lipophilic residues like substituted ureas. Although being air-dried, the matrix contains a small quantity of bound water measured by a concomitant 105 °C drying (3% of dry weight). This low level of water does not represent a handicap in the process since drying on sodium sulfate could be eliminated but prohibits the use of a sole hydrophobic solvent such as hexane.

The duration of the sample treatment can be adapted to the study's objectives. If an overview of the contamination is needed, the first two steps *i.e.* drying at room temperature and a 2 mm sifting are convenient, but if an approach of the fate of pesticides in sediments is aimed at, the next step of a 63 μm sifting must be taken in account, even though it is time consuming. Since wet sifting is prohibited due to the solubility of the studied compounds, the air drying step (2–3 days) is sufficiently short to avoid the consideration of the decomposition of those weak half-life molecules explained by the possibility of an adsorption–protection process. When lipophilic molecules such as OCPs and PCBs are involved, the study of the fine fraction of the sediment is sufficient[11] for a global evaluation of sediment contamination, but in the case of those herbicidal compounds, further study is needed to evaluate the contribution of the coarse fraction in the total contamination of the sediment.

4. Application to the mid-Garonne River

4.1 Results

4.1.1 Herbicide content

4.1.1.1 Sediment. Herbicide concentrations of sediment are shown in Table 4. The sediment from the reservoirs presented the highest herbicide contamination with the most varied types of herbicide molecules and the highest concentrations. Except in dams, the fine fraction was more contaminated than the coarse fraction. Five molecules made a significant contribution to herbicide contamination: simazine (especially in the Garonne and tributaries), cyanazine, isoproturon (especially in reservoirs), linuron and metolachlor.

The concentrations of the herbicides in whole, unsieved sediment for the 3 main families are presented in Fig. 2. Malause (M2), Empalot (G2) and Mancies (M1) were the three most contaminated sites, whereas Portet (G1), Bourret (G4), and Save River (A3) sediments exhibited the lowest contents.

Except in dams, on examination of the herbicide distribution in the 2 subfractions (Table 4), the fine fraction was more contaminated than the coarse one. Five molecules made a significant contribution to herbicide contamination *i.e.* simazine (especially in the Garonne and tributaries), cyanazine, isoproturon (especially in the M2 reservoir), linuron and metolachlor. Total concentration range (between 2.63 μg g^{-1} for M1 and 2.41 μg g^{-1} for M2) and the highest number of detected molecules (11) were observed in the reservoir. Fine fractions of tributary sediment had a concentration range between 2.42 μg g^{-1} and 2.02 μg g^{-1}, except for in the Save River (A3). Sediment sampled in the main channel showed lower concentrations, even under the detection limit. The most contaminated site was G2 with 3.15 μg g^{-1}, followed by M2 and M1. Except in the reservoir sediments, only five herbicides and the metabolite desethylatrazine (DEA) were significantly detected in this Garonne area: isoproturon and metolachlor reached their maximum level at G2; the cyanazine level at this point was only exceeded by that at M2. Simazine and linuron were also at a maximum among Garonne samples at G2. At G1, the upstream sample site on the Garonne River, herbicides were under the detection limit. Maximum DEA levels were observed on the Garonne downstream from Toulouse (G3). For all sites *s*-triazines were the most abundant derivatives (54%), then substituted ureas (29%) and finally anilides (17%). On the basis of frequency of occurrence (number of detectable concentrations of a molecule over the total number of analyses), anilides dominated (41%) followed by substituted ureas (39%) and triazines (36%).

For the coarse fraction (Table 4), sediment collected behind dams showed the maximum concentration ranges (1.9 μg g^{-1} for M1 and 5.18 μg g^{-1} for M2) and the largest number of detected molecules (12). Coarse fractions of tributary sediments held ranges between 0.75 μg g^{-1} (A1) and 0.28 μg g^{-1} (A3). Coarse sediments sampled in the Garonne River bed showed the lowest ranges (0.01 μg g^{-1} for G1 to 0.72 μg g^{-1} for G2). The most contaminated sites were reservoirs: Malause (M2) with 5.18 μg g^{-1} followed by Mancies (M1) (1.90 μg g^{-1}) and the urban weir G2 (3.15 μg g^{-1}), which induced high sedimentation levels in this area. The least contaminated

Table 4 Herbicide concentration of fine (FF) and coarse (CF) fractions in the riverside sediment (μg g^{-1}) of the different stations (*cf.* Table 1)[a]

	Stations																					
	M1		G1		G2		A1		A2		G3		A3		G4		A4		M2		G5	
Herbicides	FF	CF	FF	CF	FF	CF	FF	CF	FF	CF	FF	CF	FF	CF	FF	CF	FF	CF	FF	CF	FF	CF
DEA	0.45	0.01	—	—	0.12	—	0.23	—	0.12	0.02	0.62	—	0.05	0.02	—	—	0.27	0.03	0.29	0.1	—	—
Simazine	0.17	0.22	—	—	0.66	0.05	0.93	—	0.93	0.03	0.53	—	0.01	—	—	—	0.77	—	0.19	T	—	0.08
Atrazine	0.38	0.07	—	—	—	—	—	—	—	—		—	—	—	—	0.06	—	0.01	0.08	—	—	—
Terbuthylazine	0.13	—	—	—	—	—	—	—	0.16	—		—	—	—	—	—	—	—	0.16	0.1	—	—
Sebuthylazine	—	0.03	—	—	—	—	—	—	—	—	0.64	—	—	—	—	—	—	0.16	0.05	0.75	—	—
Cyanazine	0.35	0.72	—	—	0.54	0.6	0.13	—	—	0.26	—	0.02	—	—	—	—	—	0.03	0.72	0.7	—	0.34
Hexazinone	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	—
Sub-total *s*-triazines (1)	1.48	1.06	—	—	1.32	0.65	1.28	—	1.20	0.31	1.79	0.02	0.06	0.02	—	0.06	1.05	0.24	1.48	1.66	—	0,42
Methabenzthiazuron	—	0.01	—	—	—	—	—	—	0.16	0.04	—	—	—	—	—	—	—	0.05	0.03	T	—	—
Metobromuron	0.43	0.05	—	—	—	—	—	—	—	—	0.13	—	—	—	—	—	—	—	0.49	0.1	—	—
Isoproturon	—	0.01	—	—	0.80	—	0.46	0.35	—	—	—	0.42	—	0.13	—	—	0.29	—	0.06	2.3	—	—
Linuron	0.48	0.64	—	—	0.35	—	—	0.4	0.48	0.3	0.25	0,25	0.09	—	—	—	0.16	—	0.49	1.06	—	—
Sub-total sub. ureas (2)	0.91	0.7	—	—	1.14	—	0.46	0.75	0.63	0.35	0.37	0.67	0.09	0,13	—	—	0.45	0,05	1.06	3.46	—	—
Metolachlor	0.07	0.11	—	—	0.68	0.08	0.40	—	0.18	—	0.26	—	—	0.13	—	—	—	0.04	0.21	0.02	—	—
Metazachlor	0.17	0.4	—	0.01	—	—	—	—	—	—	—	—	—	—	—	—	0.91	0.04	0.19	0.05	—	0.26
Sub-total anilides (3)	0.24	0.14	—	0.01	0.68	0.08	0.40	—	0,18	—	0.26	—	—	0,13	—	—	0.91	0.08	0.40	0.07	—	0.26
Total (1) + (2) + (3)	2.63	1.9	—	0.01	3.15	0.72	2.14	0.75	2.02	0.65	2.43	0.69	0.15	0.28	—	0.06	2.41	0.37	2.95	5.18	—	0.68

[a] —, under the detection limit; T, traces of herbicide (lower than 0.01 μg g^{-1}).

tributary was the Save River (A3) with 0.28 μg g^{-1} and the two sampling sites (G1, G4) on the Garonne River showed a lower level of herbicides compared with the other sites. Excepting dams, six herbicides and DEA were significantly detected. DEA, methabenzthiazuron and metazachlor were only located in tributaries and dams. On the basis of abundance in the coarse fraction, substituted ureas dominated (54%) followed by *s*-triazines (39%) and anilides (7%), showing a different profile to that obtained with the fine fractions. On the basis of frequency, anilides dominated (50%), followed by substituted ureas (36%) and *s*-triazines (35%), just like the fine fraction.

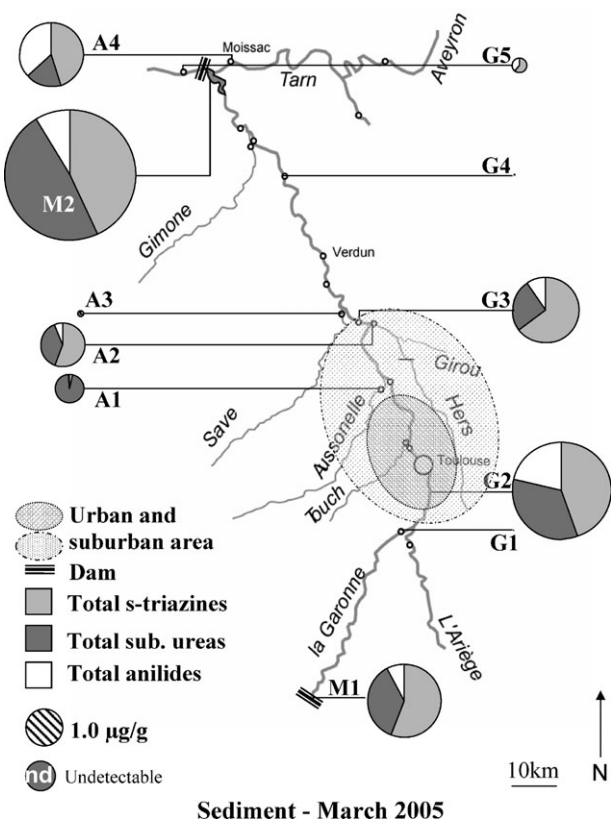

Fig. 2 Herbicide concentrations observed in the bottom sediment, grouped by family, during the sampling period of March 2005.

4.1.1.2 Water. Unfiltered water samples from the 11 sites were analysed (Table 5, Fig. 3). Only four stations exhibited a herbicide concentration higher than the detection limit (0.01 μg L^{-1}). Average surface water herbicide concentrations for the 4 sampling sites concerned was close to 1.17 μg L^{-1}. For the three urban sites, simazine, atrazine and terbuthylazine for the *s*-triazine family and methabenzthiazuron and isoproturon for substituted ureas, were present at detectable

Table 5 Herbicide concentration in the unfiltered water (expressed in μg L^{-1}). For M1, G1, A3, G4, A4, M2 and G5 stations, all the concentrations were under the detection limits[a]

	Stations			
Herbicides	G2	A1	A2	G3
DEA	—	—	—	—
Simazine	—	0.03	0.05	0.01
Atrazine	—	0.12	0.09	0.05
Terbuthylazine	—	0.04	0.05	0.01
Sebuthylazine	—	—	—	—
Cyanazine	—	—	0.04	0.01
Hexazinone	—	—	—	—
Sub-total *s*-triazines (1)	—	0.19	0.22	0.08
Methabenzthiazuron	T	1.18	1.95	0.35
Metobromuron	—	—	—	—
Isoproturon	0.06	0.18	—	0.15
Linuron	—		0,17	—
Sub-total sub. ureas (2)	0.06	1.36	2.12	0.51
Metolachlor	—	—	—	—
Metazachlor	—	—	0.05	0.07
Sub-total anilides (3)	—	—	0.05	0.07
Total (1) + (2) + (3)	0.06	1.54	2.39	0.65

[a] —, under the detection limit; T, traces of herbicide (lower than 0.01 μg g^{-1}).

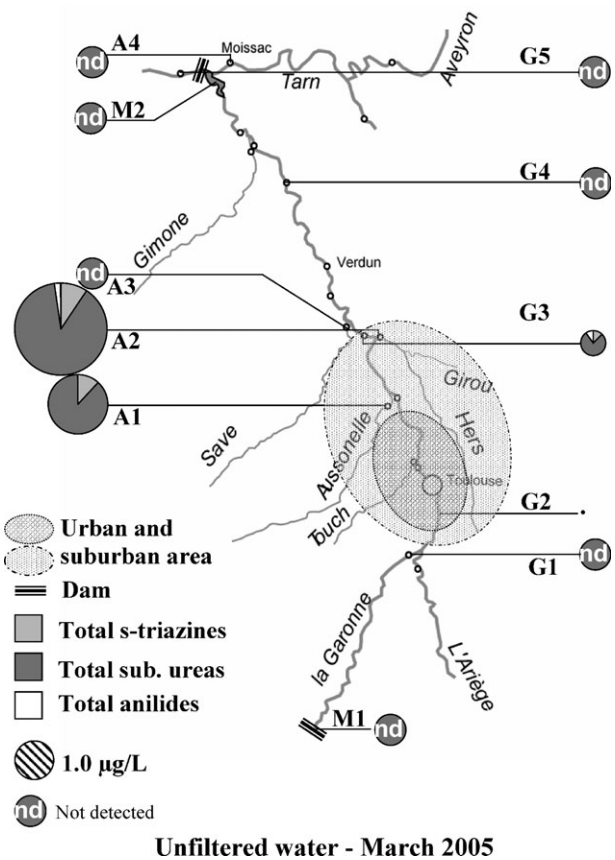

Fig. 3 Herbicide concentrations observed in unfiltered water, by family and by sampling site.

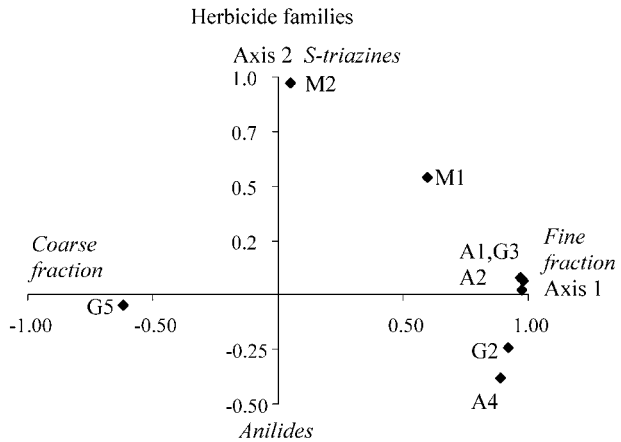

Fig. 4 PCA of herbicide concentration in the different fractions. M1: Mancies reservoir, M2: Malause reservoir, G2: Empalot, G3: Grenade, G5. Lamagistère, A1: Aussonnelle River, A2: Hers River, A4: Tarn River.

concentrations and the incriminated sites showed the same profile, except A1 for cyanazine and metazachlor and A2 for isoproturon. Cyanazine and metazachlor are present to a lesser extent.

Three surface water samples (A1, A2, G3) had a total herbicide concentration higher than the French recommendation for drinking water, which follows European directives (EEC, 1980) (0.5 µg L^{-1}): maximum concentration of 0.1 µg L^{-1} for each molecule and 0.5 µg L^{-1} for all the cumulated pesticide concentrations. Among water samples, some of them had a total herbicide concentration higher than the allowed threshold set by the World Health Organization given for each molecule.

4.1.2 Statistical analysis of herbicide distribution into the different fractions. Statistical analysis of data was run with the SPSS 12.0 software (Microsoft Corporation, Mountain View, California, USA). Principal component analysis (PCA) was performed to reveal the various sources of herbicide contamination. This analysis allows investigating the implications of different parameters, either bound to the sampling site or bound to the studied molecule parameters in the repartition of those molecules along the Garonne River (Fig. 4). The principal component analysis was performed considering the concentration of each herbicide by family and by fraction at all the sampling sites, to illustrate the relationship between sites and the sources of pollution. Some sites, presenting a very low concentration (traces) of herbicides, were excluded (Portet G1, Bourret G4 and Save River A3). The first axis (45.6%) represents the granulometric distribution, *i.e.* the relative proportion of each fraction without consideration for the participating herbicides. The second axis (29.5%) segregated the herbicides on the basis of their physiological targets *i.e.* photosystem II for *s*-triazines and substituted ureas and the growth regulation enzymes for anilides. G3 (Garonne downstream from Toulouse), A2 and A1 sites are well correlated and probably impacted by Toulouse suburbs. Conversely, M1 and M2 represented dams which are not correlated with this Toulouse site group. The two weir sampling sites, G2 (site across Toulouse city) and Tarn River (A4), are linked by these hydrological similarities, inducing sedimentation similarities. The G5 sampling site (Lamagistère) is located downstream from the Malause reservoir and showed detectable concentrations only in the coarse fraction.

4.2 Discussion

4.2.1 Herbicide concentration in water and sediment. Considering previous and preliminary reports,[16–18] contamination of the Garonne water and tributaries observed in this work presented average values lower than those described in the literature.[4,5,13] According to those references about herbicide contamination and numerous others, such as Konstantinou *et al.*, [37] which reviewed 20 European rivers, atrazine, terbuthylazine, simazine and metolachlor contamination of water in the Garonne River is comparable to the Loire, Segre, Thames or Guadiana Rivers (0.001 and 0.437 µg L^{-1}).[37] In the Garonne River, Dupas *et al.* observed higher concentrations of pesticides in 1989 than those observed in this study, except for 4 stations (G2, G3, A1 and A2), which exhibited the same level of pesticide (0.1 to 1 µg L^{-1}), particularly for DEA, atrazine, simazine, and isoproturon.

Regarding the sediments, studies of herbicide contamination are scarce and were conducted on small basins.[30,36] Müller *et al.*[30] observed the concentration of 22 organochlorinated, organophosphorus substituted ureas and *s*-triazines in

11 Australian agricultural drains (sugarcane, horticulture and cotton cultures). In these studies, the herbicide contaminations (substituted ureas and *s*-triazines) present ranged in values from 0.001 to 0.08 μg g^{-1}. Gao *et al.*[36] investigated *s*-triazines and aminotriazoles contamination of a pond located in southern Germany. They found a maximum herbicide content of 0.012 μg g^{-1}. The lowest contaminated sites presented in our study are in agreement with the lowest contaminated sites observed in the study mentioned above (0.01 μg g^{-1}). However, maximum values observed in G2, G3, A1, A2 and A4 were 10 to 100 times as high as maximum values observed elsewhere for sediment in smaller basins. In the Garonne basin, in regard to *s*-triazine concentrations, these results suggested a continued presence of *s*-triazines, even if these molecules were not allowed in use since 2003. In this way, sediments could constitute a possible source of storage of these molecules.

In addition, the analysis of pesticides in sediments of the Garonne River showed that these organic pollutants were more concentrated in the fine fractions (FF) than in the coarse ones (CF), as previously observed by Karickhoff *et al.*[11] The observed ratio FF : CF (3 : 1) is comparable to that given by Gao *et al.* (3.5 : 1).[30] Three molecules (cyanazine, isoproturon, linuron) represented more than 80% of the total herbicide content in the coarse fraction. Anilide herbicides were more concentrated in the fine fraction (17%) than in the coarse one (7%), showing that the repartition of those molecules in the sediment is complex. As previously described by Karickhoff *et al.*[11] two parameters control this repartition: lipophilicity and specific surface. The diversity of fraction components (on the one hand silts and clays for the fine fraction and on the other hand sands and in some cases plant remains for the coarse fraction) is complemented by the physicochemical properties of the studied herbicides (1 to 3.5 for *K*ow and 1 to 33 000 μg L^{-1} for solubility).

4.2.2 Spatial distribution of herbicide contamination. Fig. 2 and 3 show that the water contamination was not detectable for sites where herbicidal contamination in sediment could be found for the studied molecules.

The main values for water contamination were observed near the urban area of Toulouse, representing only 2% of the studied sector (Ibarra *et al.*).[27] Critically contaminated water samples, according to the law enforced in the European Union,[38] originated from the two tributaries (A1 and A2) situated in this suburban area. If these results contradict the generally accepted idea that pesticides originate mainly from rural areas, they are in agreement with different studies outlining this paradoxical influence of cities.[2–5] Such a significant presence of *s*-triazine, urea and anilides has been previously reported in France.[4] In addition, studies about the amenity use involved in direct urban impact are generally not taken into account by local authorities. In the present study, the lotic water contamination was strongly linked to the sediment contamination.

Upstream from Toulouse (G1), the Garonne sediment presented negligible herbicide concentration. Tributaries draining suburban areas like the Aussonnelle (A1) and Hers (A2) Rivers, presented high herbicide levels (2.14 and 2.02 μg g^{-1} for the fine fraction), whereas the Save River (A3) drainage basin is widely used for agricultural activity (wheat, corn and sunflower) and presented a much lower (0.15 μg g^{-1}) herbicide concentration. Downstream from the Toulouse area, the two tributaries of the Save (A3) and Tarn (A4) Rivers showed a large difference in found herbicide concentrations. The Save River (A3) profile is bound to the small variety of crops and characterised by the presence of the metabolite DEA. The Tarn River (A4) showed contamination due to the orchard plantations and the inherent associated pollution risks.[39–42] The observed pesticide signature (simazine in association with metazachlor) could be in agreement with tree treatments.[43]

Mancies (M1) and Malause (M2) were among the most contaminated points. Reservoir sediments contributed mostly to the herbicide content, according to the observations of Doggett and Rhodes.[44] They showed that, for diazinon (an organophosphorus insecticide), the concentrations in the reservoir were higher than along the upstream part of the river. This can be explained by the differences in the sedimentation process, owing to the differences in water velocities. In the Garonne basin, two hydraulic obstacles were discriminated: weirs (G2, A4) and dams (M1, M2). Weirs were characterized by an overflowing regime, inducing the elimination of the organic part of the coarse fraction. The effects of hydraulic conditions and sedimentation similarities are illustrated by the PCA (Fig. 4), in which sites G2 and A4 are close.

For dams, the draw off water evacuation induced a coarse fraction accumulation all along the reservoir. However, Mancies (M1) and Malause (M2) were not mutually correlated by the PCA (Fig. 4), since they have two different hydraulic regimes. Malause (M2) is situated at the confluence of the Garonne River and its main tributary, the Tarn River. A comparable disturbance is absent along the Mancies site (M1). The difference between the two dams is due to the higher contamination of the fine fraction in M1 than in M2. M2 is under the influence of the sedimentation of primary production of the Tarn River. The PCA showed the exclusion of Lamagistère (G5). This sample, with a high contribution from plant remains, presented a C/N value of 17 for the coarse fraction and a value of 7 for the fine one. The other coarse and fine fractions presented C/N values of, respectively, about 14 ± 2 and 6 ± 1 (data not shown). This high C/N value is due to plant fragments taken from aquatic flowering plants (*Potamogeton perfoliatus* L.). The coarse fraction presented much higher herbicide concentrations than the fine ones. The herbicide fine fraction concentrations were undetectable.

Conclusion

The methods of extraction, purification and multi-residues analysis herein suggested is applicable to a broad spectrum of molecules contaminating sediment, pesticides or persistent organic pollutants and with good recoveries (higher than 80%). The method is flexible according to the needs (mechanistic or monitoring) and the sample preparation.

Studies are currently being conducted to understand the complex relationships between sediment contributors and herbicides, in particular in the case of reservoirs and hydraulic regimes, leading to their distribution by fraction. For this first

analysis of herbicides contamination of the Garonne basin, water contamination by herbicides in the mid-Garonne River was found to be minor, except at three points (A1, A2 and G3). The analytical method presented herein allowed the characterisation of different profiles of contamination: idled zones with the presence of numerous derivatives in the sediment and a lack of them in the water-column, including dams, weirs, urban zones with a concomitant notable contamination of water and sediment, and rural zones less contaminated by parent molecules and where metabolites can be found. For both sediment and water, the highest concentrations of herbicides occurred in urban and suburban zones, accrediting an important impact of these contaminants in non-agricultural areas, which has to be confirmed by an extended study with more specific herbicides, such as glyphosate and diuron.

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