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Supplementary material

Insights into natural organic matter and pesticide characterisation and distribution in the Rhone River

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Table S1. On-site physical and chemical characterisation of Rhone River

DOC, dissolved organic carbon; DIC, dissolved inorganic carbon; POC, particulate organic carbon; SPM suspended particulate matter. Error represents the standard deviation of duplicate samples

	Sampling Site	Temperature (°C)	pH	Electrical Conductivity (µS cm^{-1}, 25 °C)	Alkalinity (mequiv. L^{-1})	O_2 (mg L^{-1})	Saturation (%)	DOC[a] (mg L^{-1})	DIC[a] (mg L^{-1})	POC[a] (mg L^{-1})	Turbidity Formazin Nephelometric Unit (FNU)	SPM Concentration[a] (mg L^{-1})	Median SPM Size (µm)
N	Seyssel	5.9	8.30	325	1.99	10.95	89.3	2.19 ± 0.06	24.6 ± 0.1	0.26 ± 0.0	10.6	5.34	11.53
↑	Jons	6.2	8.31	418	3.23	11.19	90.1	2.89 ± 0.08	37.9 ± 0.4	0.33 ± 0.02	7.7	2.95	11.12
↑	Givors	6.5	8.25	398	3.19	12.02	96.9	4.42 ± 0.02	37.6 ± 0.5	-	26.7	20.58	16.68
↑	St Vallier	7.4	8.21	414	3.31	11.86	97.3	4.40 ± 0.04	38.3 ±0.4	1.15 ± 0.22	28.6	16.88	13.62
↑	Valence	6.9	8.21	420	3.15	11.95	96.6	3.77 ± 0.02	36.7 ± 0.4	0.92 ± 0.11	27.2	16.33	14.20
↑	Donzere	5.8	8.22	434	3.14	11.86	93.2	4.04 ± 0.26	35.9 ± 0.6	0.83	35.6	29.37	14.13
↑	Montfaucon	6.8	8.28	394	3.00	11.97	96.6	4.12 ± 0.13	34.9 ± 0.1	1.13 ± 0.10	40.5	22.59	13.47
↑	Aramon	7.2	8.35	417	3.03	11.29	92.0	3.37 ± 0.01	35.3 ± 0.4	1.07 ± 0.39	36.9	19.05	14.74
S	Arles	7.0	8.22	408	3.03	11.46	94.0	4.17 ± 0.12	35.6 ± 0.0	1.30 ± 0.09	47.5	40.94	15.09

[a], Samples were collected on site, analysed in laboratory.

Table S2. Pesticides analyzed and their limits of quantification (µg L^{-1})

AMPA	0.05
Acetochlor	0.005
Acetochlor oxalinic acid	0.05
Acetochlor ethane sulfonic acid	0.05
Alachlor	0.005
Alachlor oxalinic acid	0.05
Alachlor ethane sulfonic acid	0.05
Ametryne	0.005
Atrazine	0.005
Chlortoluron	0.005
Cyanazine	0.005
Desmetryne	0.005
Dimethenamide	0.005
Diuron	0.01
Desisopropylatrazine	0.005
Desethylatrazine	0.005
Desethylterbutylazine	0.005
Fluzilazole	0.005
Glyphosate	0.05
Hexazinon	0.005
Isoproturon	0.005
Isoproturon-CH_3	0.005
Isoproturon-$(CH_3)_2$	0.01
Linuron	0.005
Metazachlor	0.005
Metolachlor	0.005
Metolachlor oxanilic acid	0.05
Metolachlor ethane sulfonic acid	0.05
Prometryne	0.005
Propazine	0.005
Propyzamide	0.005
Simazine	0.005
Sebutylazine	0.005
Tebuconazole	0.005
Terbuthylazine	0.005
Terbutryne	0.005
Tétraconazole	0.005

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Table S3. Division of EEM fluorescence regions and their corresponding NOM groups

Region	Excitation (nm)	Emission (nm)	Description
I	220–250	280–332	Aromatic proteins I
II	220–250	332–380	Aromatic proteins II
III	220–250	380–580	Fulvic acids
IV	250–470	280–380	Microbial by-products
V	250–470	480–580	Humic acids

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Experimental S1. Pyrolysis GC-MS Characterisation of NOM

Based on a previously developed procedure,[1] the pyrolysis oven was connected to the split/splitless injector port of a Trace GC Ultra gas chromatograph coupled to a DSQ II single quadrupole mass spectrometer (Thermo Fisher Scientific, Inc). The pyrolyzer was programmed from 220−740 °C at a rate of 20 °C/ms. The GC injector was maintained at 220 °C and operated in the split mode (split ratio 1:10) with He as the carrier gas. Separation of the pyrolysis fragments was carried out on a 30 m, 0.32 mm internal diameter, 0.5 μm film thickness, Stabilwax column (Restek Corporation, Bellefonte, PA), programmed from 35−220 °C at a rate of 3 °C/min, with final hold for 20 min. The mass spectrometer was operated in electron impact mode at 70 eV and scanned from 30 to 350 amu. Bovine serum albumin (BSA) and cellulose acetate were utilised as standards for this analysis.

Experimental S2. Determination and Quantification of Common Pesticides

A Waters Ultra Performance Liquid Chromatography (UPLC) system coupled to a Waters micromass MSMS (Waters Quattro-Premier XE/Q) was used to identify and quantify several pesticides and their metabolites as reported in Amalric et al.[2] Neutral molecules were extracted using a Gilson GX 274 ASPEC solid phase extraction system and Oasis HLB (6mL - 500 mg) cartridges from Waters. The cartridges were conditioned with acetonitrile (5 mL), then with methanol (5 mL), and finally with HPLC grade deionized water (5 mL, neutral pH), all at a flow rate of 1 mL min^{-1}. The water samples (1 L, pH 6−8), as collected on-site, were spiked with atrazine-d5 solution (0.2 mL) and loaded at a flow rate of 5 mL min^{-1}. After 30 min of drying, the analytes were eluted with acetonitrile (2x4 mL) at 1 mL min^{-1}. Extracts were evaporated to 1 mL under a gentle stream of nitrogen at ambient temperature, then spiked with simazine-d10 solution before analysis.

For the extraction of ionic compounds (metabolites oxanilic acid (OXA) and ethane-sulfonic acid (ESA) of the 3 choroacetanilides), cartridges were conditioned with methanol (5 mL) followed by acetonitrile (5 mL) containing 0.2% v/v acetic acid at a flow rate of 1 mL/min. The water sample (1 L; pH 6−8), spiked with 0.2 mL of butachlor-ESA solution, was loaded at a flow rate of 5 mL min^{-1}. Butachlor-ESA was used as the extraction indicator because its recovery ratio provides information concerning the running of the automatic extractor and the extraction steps. However, it was not used to correct the results. After 30 min of drying, analytes were eluted with methanol (2x4 mL) at 1 mL min^{-1}. Extracts were evaporated under a gentle stream of nitrogen at ambient temperature to 1 mL and spiked with 0.020 mL of mecoprop-d3 solution (internal standard) before analysis.

All compounds were analyzed by a Waters Ultra Performance Liquid Chromatography (UPLC) system coupled to a Waters micromass MSMS (Waters Quattro-Premier XE/Q). Chromatographic separation for neutral and ionic compounds was done with a Waters Acquity UPLC BEH C18 column (2.1 mm x 150 mm, particle size 1.7μm). The mobile phase was a gradient of (A) water/0.05% formic

acid and (B) acetonitrile / 0.05% formic acid and (A) water/0.007% formic acid and (B) methanol / 0.007% formic acid with a flow rate of 0.4 mL/min for neutral and ionic molecules, respectively.

Due to the low weight of the glyphosate and aminomethylphosphonic acid (AMPA) molecules, a step of derivatization was needed prior to their concentration and analysis by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS, Waters Quattro-Premier XE/Q). After adjustment of the pH with potassium hydroxide (pH=9), the derivatization was performed with 9-fluorenylmethyl chloroformate (FMOC-Cl). A solid phase extraction cartridge was utilized and isotope labelled ^{13}C glyphosate and AMPA were used as internal standard for the correction of eventual matrix effects and to compensate any error occurring during analysis processing. The limits of detection and quantification were 0.025 and 0.050 µg L^{-1}, respectively.

Table S4. Specific UV absorbance at 254 nm (SUVA254) for sampling sites along Rhone River

Percent aromaticity calculated from Weishaar et al.[3] using the equation % aromaticity = 6.52*SUVA254 + 3.63

Sampling location	$SUVA_{254}$	% Aromaticity
Seyssel	1.06	10.5
Jons	1.29	12.0
Givors	2.05	17.0
St. Vallier	1.71	14.8
Valence	1.68	14.6
Donzere	1.74	15.0
Montfaucon	1.71	14.8
Aramon	1.73	14.9
Arles	1.51	13.5

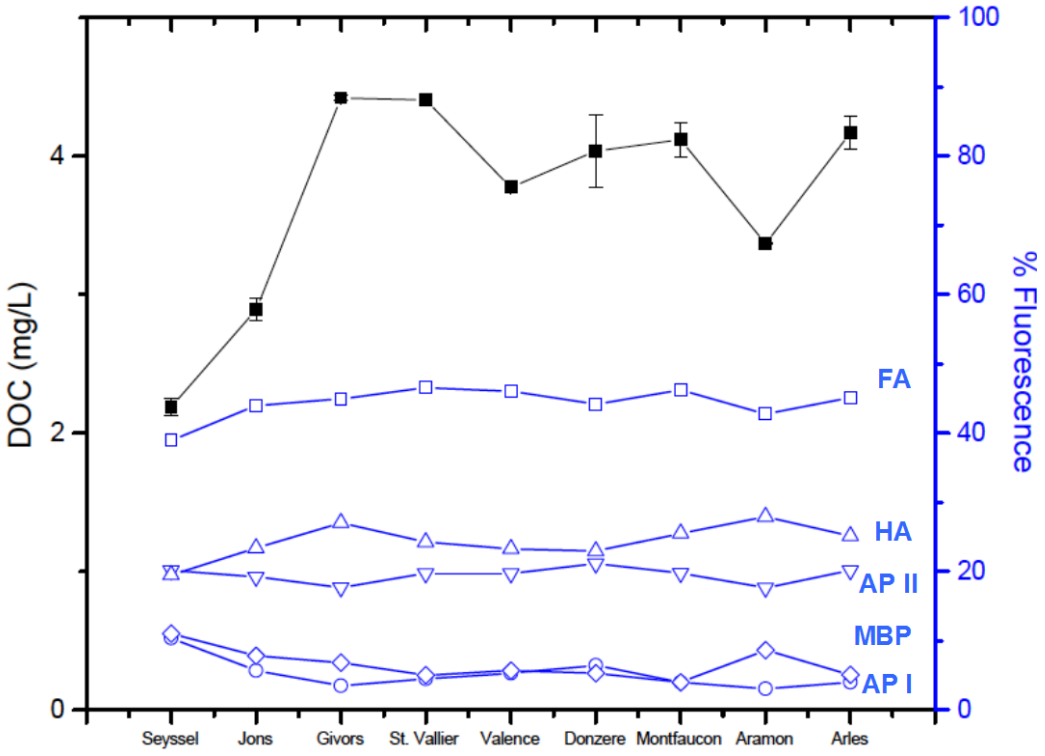

Figure S1. Dissolved organic carbon (DOC, left axis, black) and % fluorescence each NOM group in original Rhone waters (right axis, blue) as determined via fluorescence analysis of regions in the Supplementary material: Table S3. Fluorescence regions are divided into fulvic acids (FA), humic acids (HA), aromatic proteins II (AP II), microbial by-products (MBP), and aromatic proteins I (AP I).

Experimental S3. Pricipal component analysis (PCA) of pesticides and suspended matter in Rhone waters

The principal component analysis (PCA) here was performed using the R software for statistical computing (https://www.r-project.org). The pesticide, DOC, POC and SPM concentrations for each of the nine Rhone sampling sites were read into the system. The average of the Jons and St. Vallier POC concentrations was used to replace the missing POC value at Givors. The pesticide concentrations were correlated individually with the DOC, POC and SPM concentrations, and the first principal component (Comp1), a weighted average (0.596*DOC + 0.591*POC + 0.571*SPM) of all three parameters was found to explain 83% of the variability in the values of these explanatory variables. The second principal component (Comp2) contrasting the DOC and SPM, was responsible for ~10% of the variability in these parameters, while the third principal component (Comp3) constrasting the POC and the DOC and SPM also did not explain much of the variability (~7%) within the

measurements of the three parameters. Owing to the low explanatory power of Comp2 and Comp3, they have been disregarded in our discussion.

A

	DOC	POC	SPM	Comp1
Propyzamide	0.790	0.517	0.511	0.662
Isoprot	0.914	0.833	0.744	0.910
Chlortoluron	0.892	0.863	0.771	0.923
MOXA	0.354	-0.123	0.041	0.096
MESA	0.923	0.793	0.735	0.895
Metalo	0.793	0.945	0.725	0.901
AMPA	0.748	0.812	0.632	0.801
Gly	-0.157	0.237	-0.003	0.031

B

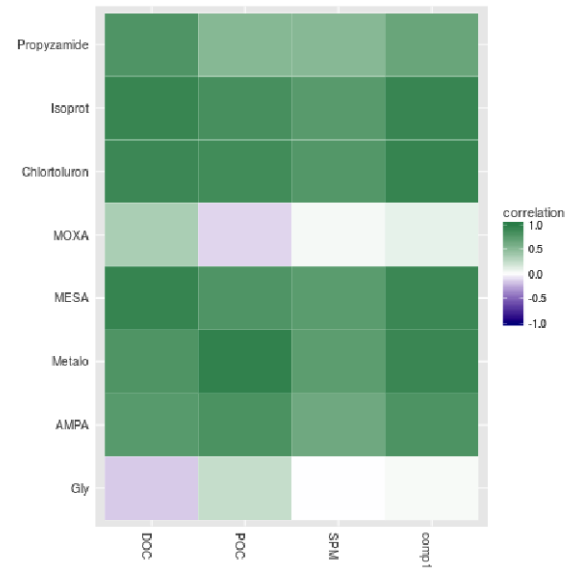

Figure S2. Principal component analysis (PCA) where the pesticide concentration along the Rhone River is correlated individually with the DOC, POC and SPM and with the linear combination (average) of these three parameters (Comp1). Strong positive correlation is represented by a value of 1, no correlation by a value of 0, and strong negative correlation by a value of -1. (A) PCA correlation matrix. (B) Heat map showing correlation between pesticides and DOC, POC, SPM, or the linear combination, Comp1. Abbreviations not yet defined are designated for the following pesticides: Isoprot (isoproturon), Metalo (metalochlor), and Gly (glyphosate).

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