

Dissolved Trace Metal–Organic Complexes in the Lot–Garonne River System Determined Using the C18 Sep-Pak System

E. LEMAIRE, G. BLANC^{*}, J. SCHÄFER, A. COYNEL and
H. ETCHEBER

*Equipe Traceurs Géochimiques et Minéralogiques, TGM-UMR-CNRS 5805, Université Bordeaux I,
Avenue des Facultés, F-33405 Talence, France*

(Received 16 September 2004; accepted 18 January 2005)

Abstract. An 11-month observation of dissolved and particulate organic matter, chlorophyll *a* (Chl *a*), C18 Sep-Pak extractable hydrophobic dissolved organic matter (hDOM) fraction and associated dissolved trace metals (Cd, Cu, V, Co, Ni, Mo, U) was performed in the Lot–Garonne River system. This system includes the Riou Mort, the Lot River and the downstream reaches of the Garonne River and represents the fluvial transport path of trace metals between the major point source of polymetallic pollution, located in the Riou Mort watershed and the Gironde estuary. Spatial and temporal variations of dissolved and particulate organic carbon and Chl *a* reflect the presence of different types of organic matter and their relation with the hDOM fraction. Maximum Chl *a*/POC ratios (up to 0.03), indicate intense phytoplankton production from March to May. In the Lot River (Temple), DOC and POC concentrations were clearly higher and mean Chl *a* concentration (2.8 mg g⁻¹) was about three times higher than those of the other sites. High Chl *a*/POC ratios suggest high phytoplankton activity with maxima in spring and late summer. In the Riou Mort River, very high POC concentrations of up to 40% (mean: 20%) occurred, whereas Chl *a* concentrations were relatively low indicating low phytoplankton activity. High, strongly variable DOC and POC concentrations suggest important natural (Carboniferous soils, forests) or anthropogenic (e.g., former coal mines, waste areas, agriculture, sewage) carbon sources within the small Riou Mort watershed. Despite high DOC concentrations in the Riou Mort River, hDOM metal fractions were generally lower than those at the other sites. The general order of decreasing binding strength between metals and the organic hydrophobic phase (Cu, U > Co, Ni > V, Mo > Cd) at all four sites was in good agreement with the Irving–William series of transition element affinity towards organic ligands. Accordingly, the role of the hydrophobic phase in dissolved Cd transport appeared to be negligible, whereas the hDOM–Cu fraction strongly contributed to dissolved Cu transport.

Key words: C18, dissolved organic matter, hydrophobic fraction, trace metals

^{*}Author for correspondence. E-mail: g.blanc@epoc.u-bordeaux1.fr

1. Introduction

The Lot–Garonne–Gironde fluvio-estuarine continuum is affected by historic polymetallic pollution (e.g., Cd, Zn, Cu, Pb, Hg), mainly due to former zinc ore treatment in the Riou Mort drainage basin (e.g., Boutier, 1981; Blanc et al., 1999; Schäfer et al., 2002; Audry et al., 2004a). Along the main transport path, i.e., from the Riou Mort to the Gironde estuary's mouth, diffuse and point sources, mass balances, transport mechanisms and some aspects of bioaccumulation have been identified and quantified (e.g., Blanc et al., 1999; Audry et al., 2004b; Robert et al., 2004; Baudrimont et al., 2005). Trace metals are mainly transported in the particulate phase, but undergo complex transformation and redistribution in the Gironde estuary modifying their transport phases, their chemical speciation and, thus, their mobility and their bio availability (e.g., Boutier et al., 2002; Robert et al., 2004). Trace element bioaccumulation in parts of the food chain such as oysters, cockles, clams and fish is well documented (e.g., Boutier, 1981; Baudrimont et al., 2005) and work has been done on particulate trace element speciation (Audry, 2003). However, very little is known on binding forms and speciation of dissolved trace elements (Cd, Zn, Cu, Pb, U . . .) in the Lot–Garonne–Gironde continuum. In natural waters, complexation of major and trace elements by organic and inorganic ligands plays an important role, as it affects their reactivity, mobility and bio-availability (e.g., Buffle, 1988; Christensen et al., 1996). Additionally, biota play an active role in trace element complexation and transport (Mackey and O'Sullivan, 1990; Gonzalez-Davila et al., 1995; Hunter et al., 1997; Jansen et al., 2003).

Isolation of dissolved organic metal complexes by C18 phase was introduced by Mills and Quinn (1981) for estuarine waters. Although recovery of dissolved organic matter by this method is incomplete, the C18 Sep-Pak technique is commonly employed for the isolation of dissolved organic complexes in marine and estuarine waters (e.g., Donat, 1986; Paulson et al., 1994; Yoon et al., 1999; El Sayed and Aminot, 2000). In contrast, to our knowledge, this technique has rarely been applied to continental aquatic systems, maybe due to rapid compositional and seasonal changes of chemical and biological characteristics (Mills et al., 1989; Paulson et al., 1994). Additionally, C18 separation of dissolved organic matter may be more efficient in seawater due to “salting out” of hydrophobic organic compounds (Turner, 2003).

The objective of the present study was to quantify and characterise the dissolved organic fraction of selected trace metals along the Lot–Garonne River continuum throughout 11 months (~one hydrological year). For this, we analysed concentrations of dissolved organic carbon (DOC), particulate organic carbon (POC), chlorophyll *a* (Chl *a*) and the dissolved trace metals Cd, Cu, V, Co, Ni, Mo and U. The hydrophobic fractions of DOC and dissolved trace metals, retained by C18 SEP-Pak phases were determined.

The role of seasonal, hydrological and spatial components on trace metal complexation by hydrophobic ligands is discussed.

2. Material and Methods

2.1. STUDY AREA

The Garonne River is the dominant tributary of the Gironde estuary in terms of freshwater; suspended particulate matter (SPM) and trace metals originated from the polymetallic pollution of the Lot–Garonne system i.e., the main transport path of Cd and Zn into the Gironde estuary (e.g., Schäfer et al., 2002; Figure 1). The Garonne watershed ($\sim 56,000 \text{ km}^2$) consists of various sub-basins with contrasted properties (e.g., lithology, geomorphology, land use), producing different types of organic matter (e.g., Veyssy et al., 1999). Compared to other European basins of comparable size, the Garonne basin is only weakly industrialised and urbanised (e.g., Abril et al., 2002). Except for its upstream reaches, discharge of the major part ($\sim 150 \text{ km}$ length) of the Garonne River is controlled by only one dam at Malauze (Figure 1), resulting in frequent, pronounced floods and droughts (e.g., Schäfer et al., 2002). Therefore the present study was designed to cover contrasting hydrologic situation typically occurring within a hydrological year. The 11-month survey was conducted at four representative sites called Riou Mort (RM), Temple (T), Port Ste Marie (PSM) and La Réole (LR).

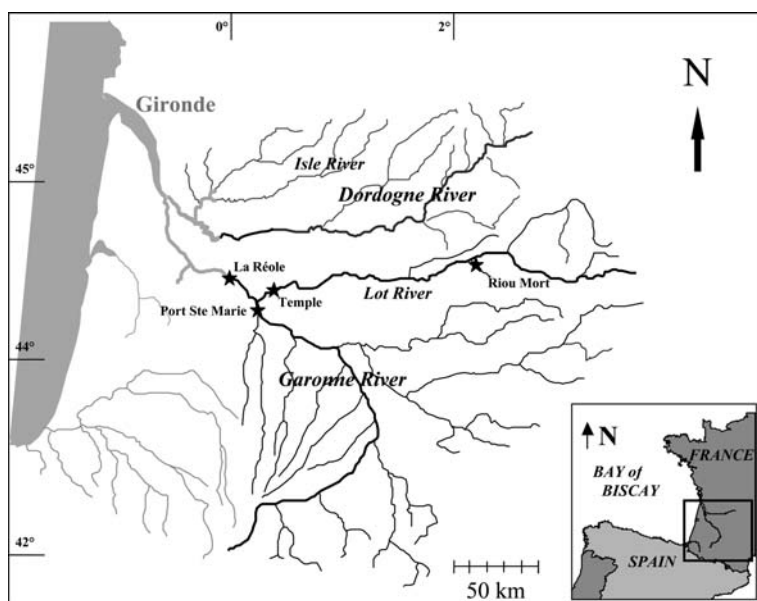


Figure 1. Map of the study area and sampling sites.

The most downstream site (La Réole) is located at the upstream limit of dynamic tide and represents the major fluvial entry of the Gironde estuary (Figure 1). The sampling sites and techniques were similar to those of our permanent long-term high-resolution observation network described elsewhere (e.g., Blanc et al., 1999; Audry et al., 2004a; Coynel et al., 2004).

2.2. SAMPLING

2.2.1. Dissolved Phase

River water was sampled using 100 mL polypropylene bottles, previously acid-cleaned (3 days in 10% HNO₃, Baker Normapur) and thoroughly rinsed with the river water of the site. The water samples were immediately filtered through 0.2 µm Nucleopore[®] polycarbonate filters in a glove box (dust free N₂-atmosphere, laboratory van) to avoid sample alteration and contamination. Filtrates were collected in acid-cleaned polypropylene bottles after thoroughly rinsing them with an aliquot of the filtrate. Acidified samples (HNO₃; Baker Suprapur; 1:1000) for total dissolved trace metal analysis and non-acidified aliquots for C18 separation were stored at 4 °C at dark until analysis and Sep-Pak extraction, respectively.

River water for DOC analysis was sampled using pre-cleaned (RBS-detergent, thoroughly rinsed with ultra-pure deionised water; Millipore[®]) and pre-combusted (500 °C; 6 h) glass bottles. The sample was filtered through pre-combusted and pre-weighed glass-fibre filters (Whatman GF/F; 0.7 µm), collected in a pre-cleaned and pre-combusted glass bottle and acidified (H₃PO₄; 1:200; Baker, Normapur).

2.2.2. Particulate Phase

An aliquot of river water was filtered through pre-combusted (500 °C; 6 h) and pre-weighed glass-fiber filters (Whatman GF/F; 0.7 µm) for POC and SPM measurements. The filters were dried (45 °C; 48 h) and stored at room temperature and at dark until analysis. The samples for pigment analysis were gently filtered (0.2–0.3 atm) through glass-fibre filters (Whatman GF/F; 0.7 µm) and immediately frozen. Representative samples of suspended particulate matter were recovered by pumping/centrifugation of up to 200 L of river water using a Wesfalia separator (12,000 g) as described in Schäfer and Blanc, (2002).

2.3. SAMPLE ANALYSES

2.3.1. SPM Determination

Precise volumes of homogenised river water were filtered through dry (45 °C; 48 h) pre-weighed filters (Whatman GF/F, 0.7 µm). The filters were again dried to constant weight and re-weighed.

2.3.2. C18 Separation

The separation of trace metal binding hydrophobic organic complexes was performed immediately after the return to the laboratory using the C18 Sep-Pak technique described by Mills and Quinn (1981). This commonly applied method is characterised by very good reproducibility and low blank values for both, trace metals and DOC analysis (see below). The C18 Sep-Pak column was conditioned by successively passing 5 mL HNO₃ (5%), 5 mL methanol and 10 mL Milli-Q[®] water. A pH adjusted (pH 2.8 ± 0.2) aliquot of 50 mL of the filtered sample was passed through the C18 Sep-Pak cartridge at a flow rate of 2.5 mL min⁻¹. After the passage through the column, the non-retained part of the sample was recovered for mass balance. The organic phase retained on the column was eluted by 5 mL methanol and recovered separately. We modified the method of Mills and Quinn (1981) by evaporation to dryness of the methanol extract (64.7 °C, boiling point of methanol), followed by dissolution in 10 mL of HNO₃ (Baker, ultrapure, 1%). This step removed methanol traces from the C18 separated organic fraction for interference-free DOC analysis. For each sample separation at least two methodological replicates were performed.

2.3.3. DOC Analysis

The analysis of DOC in samples of filtered river water, the non-retained and the C18 retained fractions was performed by the high temperature catalytic oxidation method (HTCO) using a total organic carbon (TOC) analyser (Shimadzu, TOC 5000) as described by Cauwet (1994). Detection limits (3 sigma of the blank values) varied from 0.3 to 0.4 mg L⁻¹ and methodological blank values obtained for the C18 retained fraction was 0.445 mg L⁻¹ ($n = 35$), i.e., comparable to detection limits. Reproducibility was generally better than 5% (r.s.d.) for both, DOC analysis and multiple replicates of C18 separation. Calibration was done by dilution of a potassium phthalate solution. Details on the applied method and its analytical quality evaluated in an intercalibration between four European laboratories are given elsewhere (Abril et al., 2002).

2.3.4. POC Analysis

Dry glass fibre filters containing precisely weighed masses of SPM for particulate organic carbon analysis were acidified by HCl (37%) to remove carbonates. The samples were then analysed using a carbon/sulphur analyser (LECO, CS-125) as described by Cauwet et al. (1990). Quality was checked by measuring certified reference materials (e.g., LECO 501–503). Accuracy was generally better than 5% deviation from certified values and precision was better than 5% (r.s.d.).

2.3.5. Pigment Analysis

In the present work Chl *a* was applied to indicate phytoplankton biomass. Identification and quantification of pigments was performed using liquid chromatography as described by Lemaire et al., (2002). Frozen filters were extracted by 100% acetone until a clear extract was obtained. Extracts were then methylated and dried, dissolved in a precise volume of elution solvent (Methanol, Acetonitrile, buffered water, 50:45:5 vol %) and injected. The separation and determination of pigments was achieved on a Thermo Quest analyser using a UV6000 diode-array spectrophotometer, after calibration using authentic standard material provided by the International Agency for ¹⁴C Determination.

2.3.6. Trace Metal Analysis

Representative sub-samples of SPM (i.e., 30 mg of dry, powdered and homogenised material) were digested in closed Teflon bombs (Savillex[®]) on a heating plate (2 h at 110 °C) using 750 µL HCl (12 N, suprapur) and 250 µL HNO₃ (14 N, suprapur) with 2 mL HF (26 N, suprapur). After complete cooling, the digested solution was evaporated to dryness. Each sample was brought to 5 mL using 150 µL HNO₃ and double-deionised water (MilliQ[®]). Each batch of samples included method blanks and digestion of international certified reference materials (e.g., CRM 320, SL-1, PACS). Dissolved and particulate heavy metal concentrations were measured using ICP-MS (Elan 5000, Perkin–Elmer). The applied analytical methods were further quality checked by analysis of international certified reference waters (SLRS-3, SLRS-4 and LGC6019 River Water). Accuracy was within 5% of the certified values and the analytical error (r.s.d.) generally better than 5% for concentrations 10 times higher than detection limits (e.g., Schäfer et al., 2002; Audry et al., 2004b). Methodological blank values obtained for the C18 separation were generally lower or similar to ICP-MS detection limits (3 sigma of analytical blanks).

3. Results and Discussion

3.1. EVALUATION OF THE C18 SEPARATION

The C18 phase has been commonly applied for the separation of different dissolved organic phases (Mills and Quinn, 1981; Mackey, 1983; Elbaz-Poulichet et al., 1994; El Sayed and Aminot, 2000), despite yield problems reported by different authors (Donat, 1986; Plavšić; and Branica, 1986; Yoon et al., 1999). Indeed, our results confirmed that the C18 SEP-Pak method separates only a fraction of DOC (Table I). However, low trace metal blanks, excellent reproducibility permitting the comparison of results obtained for

Table I. Total concentrations of POC (%), DOC (mg L⁻¹), Chl *a* (μg g⁻¹) and dissolved metals (μg L⁻¹) and C18-separated metal fractions (μg L⁻¹).

Site		Total Mean (range)	C18 fraction Mean (range)
Riou Mort	POC	22.4 (8.3–40.0)	–
	Chl <i>a</i>	726 (56–2770)	–
	DOC	9.20 (6.71–17.00)	2.03 (<0.10–5.96)
	V	0.53 (0.30–0.84)	0.03 (<0.03–0.05)
	Co	2.89 (1.07–9.75)	0.09 (<0.03–0.25)
	Ni	24.5 (5.32–114)	0.29 (<0.20–0.96)
	Cu	3.16 (2.12–4.85)	0.64 (0.40–0.92)
	Mo	1.47 (0.63–2.49)	0.08 (<0.03–0.24)
	Cd	22.2 (3.58–56.4)	0.06 (<0.01–0.23)
	U	0.99 (0.36–2.84)	0.17 (0.08–0.23)
Temple	POC	16.1 (4.7–31.0)	–
	Chl <i>a</i>	2630 (19–6400)	–
	DOC	4.25 (2.00–8.87)	0.92 (<0.10–1.84)
	V	0.76 (0.51–1.09)	0.04 (<0.03–0.09)
	Co	0.15 (0.08–0.18)	0.04 (<0.03–0.04)
	Ni	0.53 (0.38–0.84)	<0.20
	Cu	1.19 (0.39–4.87)	0.29 (<0.09–0.42)
	Mo	0.24 (0.10–0.57)	<0.03
	Cd	0.02 (<0.01–0.08)	<0.01
	U	0.48 (0.28–0.84)	0.14 (0.08–0.18)
Port Ste Marie	POC	5.8 (1.7–17.5)	–
	Chl <i>a</i>	822 (13–4320)	–
	DOC	2.86 (1.40–5.62)	0.55 (<0.10–1.41)
	V	0.77 (0.44–1.37)	0.03 (<0.03–0.05)
	Co	0.13 (0.08–0.18)	0.03 (<0.03–0.04)
	Ni	0.39 (0.24–0.72)	<0.20
	Cu	0.85 (0.52–1.46)	0.29 (<0.09–0.55)
	Mo	0.41 (0.21–0.77)	<0.03
	Cd	0.02 (0.01–0.04)	<0.01
	U	0.72 (0.33–0.97)	0.17 (0.04–0.27)
La Réole	POC	5.6 (1.6–13.8)	–
	Chl <i>a</i>	590 (12–2100)	–
	DOC	2.90 (1.01–7.88)	0.77 (<0.10–2.62)
	V	0.84 (0.48–1.30)	0.04 (<0.03–0.06)
	Co	0.14 (0.10–0.19)	0.03 (<0.03–0.04)
	Ni	0.50 (0.28–1.24)	<0.20
	Cu	0.98 (0.70–1.60)	0.36 (0.10–0.69)

Table I. (Continued).

Site	Total Mean (range)	C18 fraction Mean (range)
Mo	0.44 (0.24–0.76)	0.03 (<0.03–0.04)
Cd	0.03 (0.01–0.09)	<0.01
U	0.81 (0.63–1.17)	0.22 (0.14–0.30)

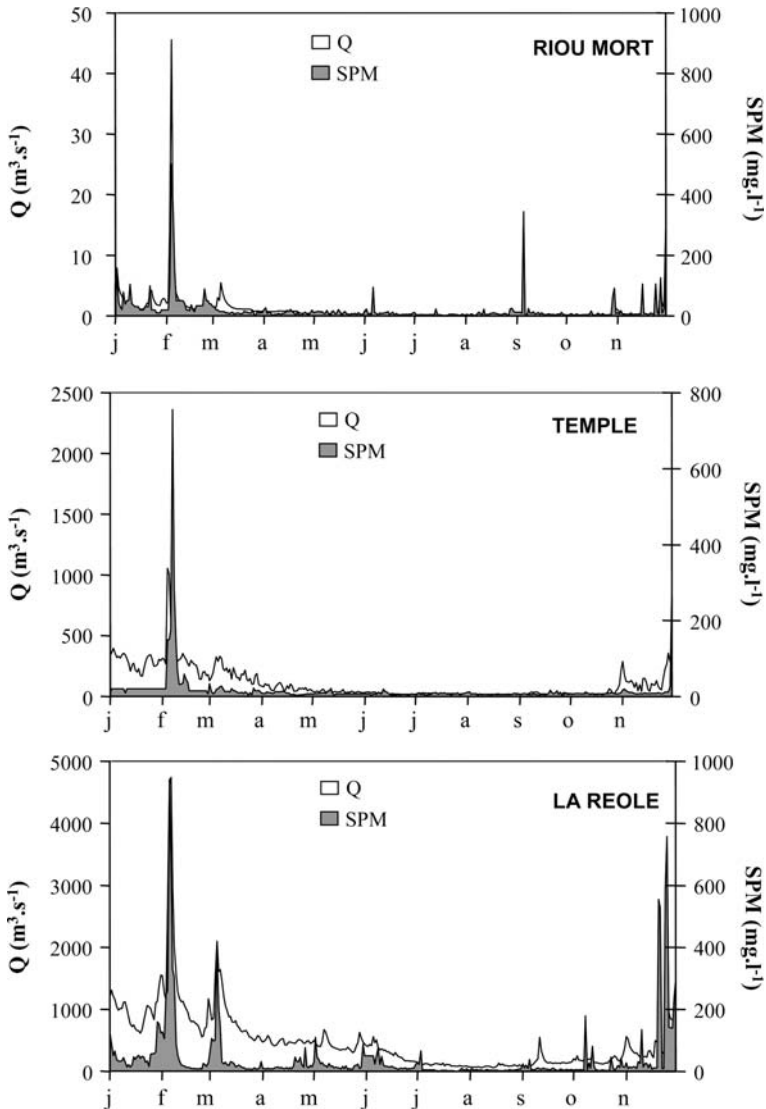


Figure 2. Discharge and SPM concentrations in the Riou Mort, the Lot and the Garonne Rivers in 2003.

different sites and hydrological situations (Table I; Figure 2), and the lack of alternative methods largely justify the application of C18 in the present study. Mass balances obtained by comparing the sum of DOC and trace metal in both the C18-retained, and the non-retained fraction with the total content in the untreated sample were generally well-equilibrated (<10% difference).

The quantification of trace metals associated with dissolved hydrophobic ligands (hDOM–metal), separated by the C18 Sep-Pak technique, was chosen to approach dissolved metal speciation in the Lot–Garonne system throughout the observation period. For this, hDOM–metal fractions were expressed in % of the total dissolved concentration.

3.2. SEASONAL VARIATIONS IN THE LOT-GARONNE RIVER SYSTEM: 11-MONTHS OBSERVATION

3.2.1. Discharge and SPM Concentrations

The instantaneous data on discharge (data from DIREN) and related SPM concentrations observed at the four sites along the Lot–Garonne continuum (Figure 1) at 11 points of time throughout 2003 were in the range of those of our long-term high-resolution observation (Figure 2; Audry et al., 2004b). The Riou Mort River (mean annual discharge $1.95 \text{ m}^3 \text{ s}^{-1}$;) drains a small mountainous watershed including highly erosive geological zones (e.g., Permian) and ore treatment waste areas and, consequently, shows highly variable discharge and related SPM concentrations (unpublished data; Coynel et al., 2004). These variations are then attenuated in the Lot River. At the outlet of the Lot River watershed (Temple), mean annual discharge and SPM concentrations were relatively low ($\text{SPM} = 16 \text{ mg L}^{-1}$ and discharge = $107 \text{ m}^3 \text{ s}^{-1}$) and showed only minor variations in 2003, reflecting a dry year (e.g., Lapaquellerie et al., 2000; Schäfer et al., 2001). Indeed, discharge in the Lot and Garonne Rivers was very low from January to November 2003, except for one short and intense flood in February and a small peak in June (Figure 2). The evolution and absolute values of discharge and SPM concentrations were rather similar at PSM and LR, i.e., upstream and downstream from the Lot River confluence, suggesting little contribution of the Lot River on the Garonne River discharge in 2003.

3.2.2. Particulate Organic Carbon and Related Chl *a*

Particulate organic carbon is derived from multiple sources, such as phytoplankton, litter, soil, urban areas and industry. Minimum POC (%) contents typically occur during floods and are interpreted as derived mainly from soil (Meybeck, 1993; Veyssy et al., 1999). In flood conditions, high SPM

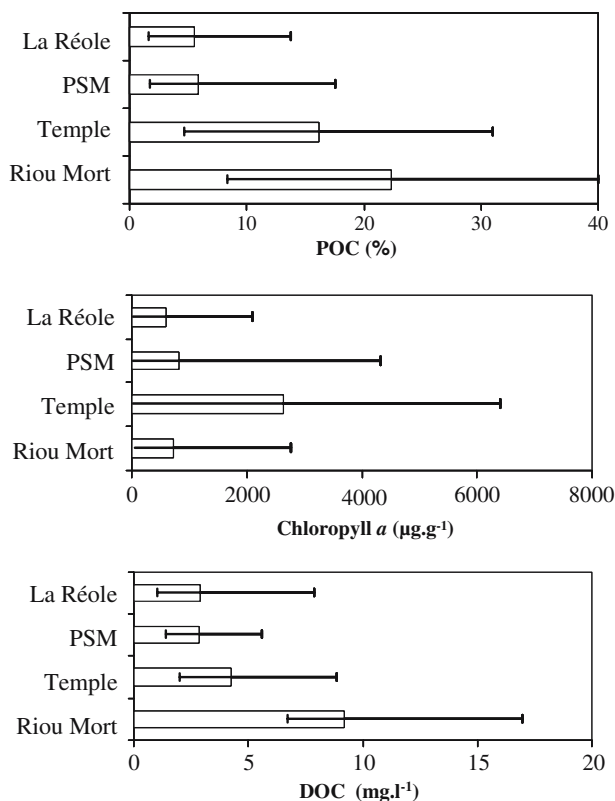


Figure 3. Mean values and ranges of POC, Chl *a* and DOC concentrations for the year 2003.

concentrations (up to 5000 mg L⁻¹ in the Garonne River) result in extremely low light penetration depths, inhibiting autochthonous POC production.

The ratios between Chl *a* and POC concentrations, typically observed in phytoplankton cultures, range from 0.01 to 0.03 (Harris, 1986). Therefore, the Chl *a*/POC ratios observed in SPM from the different sites may indicate periods of algal blooms, when phytoplankton is the main constituent of SPM (Harris, 1986; Sullivan et al., 2001). Mean and minimum POC concentrations and their seasonal evolution were similar for both sites on the Garonne River (PSM, LR) showing mean values of ~5% and minimum values of ~2% (Figure 3). Maximum POC values of around 15% occurred in summer (July). Like POC, chlorophyll *a* concentrations at PSM and LR show similar mean values (~800 µg g⁻¹) and comparable seasonal evolutions (Figure 3). Maximum Chl *a*/POC ratios occurred in spring with values of up to ~0.03, indicating very high phytoplankton production from March to May.

In the Lot River (Temple), POC concentrations were clearly higher (mean: ~15%), reaching up to 30% in summer (Figure 3). Mean chlorophyll *a*

concentration ($2800 \mu\text{g g}^{-1}$) was about three times higher than those of the other sites. Maximum values of up to $6000 \mu\text{g g}^{-1}$ occurred in spring and summer during low discharge. As Temple site is located near a hydroelectric dam, current velocities and SPM concentrations are relatively low, which probably enhances phytoplankton activity. This is supported by the Chl *a*/POC ratios that, from March to September, were mostly within the range of typical phytoplankton values, suggesting high phytoplankton activity with maxima in spring and late summer (Figure 4). The POC and Chl *a* concentrations measured in this study confirm previously reported data for the Garonne River (Etcheber, 1986; Veyssy et al., 1999; Lemaire et al., 2002) and were coherent with data obtained for the previous years by the long-term observation of the Lot–Garonne River system (e.g., Schäfer et al., 2002). Comparison of Chl *a*/POC ratios at the different sites, suggests that spring bloom occurred earlier in the Garonne River than in the Lot River (Figure 4). Additionally, the Chl *a*/POC ratios at La Réole reflect the mixing of material derived from the Lot and the upstream Garonne Rivers (Figure 4).

Very high POC concentrations of up to 40% (mean: 20%) were measured in the Riou Mort River, although Chl *a* concentrations in the Riou Mort were relatively low and similar to those in the Garonne River (Figure 3). The resulting Chl *a*/POC ratios observed in SPM from the Riou Mort were generally much lower than 0.01, suggesting low contribution of phytoplankton (Figure 4). The Chl *a*/POC ratio close to 0.01 in May was attributed to a minor algal bloom in the Riou Mort River. In this case, maximum phytoplankton activity occurred later in the Riou Mort River than

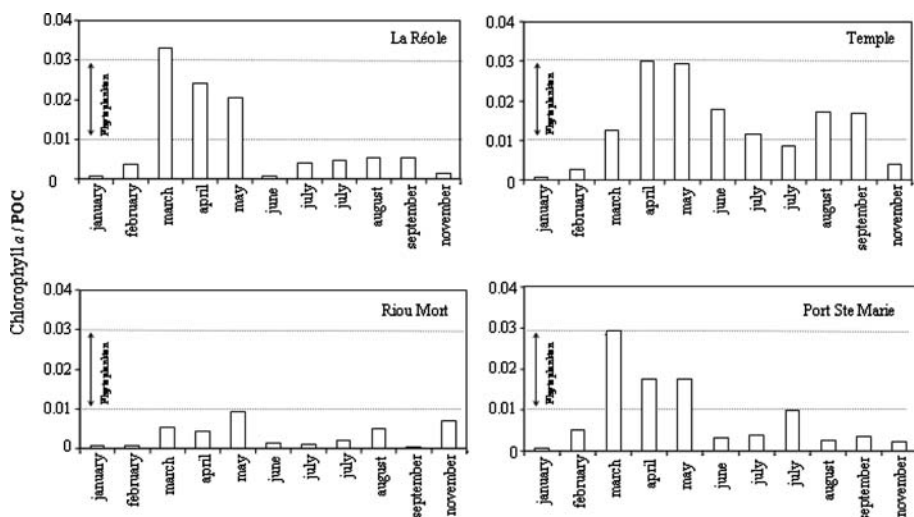


Figure 4. Chlorophyll *a*/POC ratios during the observation period. The dotted lines show the typical range for Chl *a*/POC ratios in phytoplankton cultures (Harris, 1986).

at the other sites. Low Chl *a*/POC ratios and high, strongly variable POC concentrations in the Riou Mort River, suggest the influence of important organic carbon sources, independent from autochthonic phytoplankton or usual soil erosion. Indeed, the Riou Mort watershed presents various potential carbon sources of natural (Carboniferous soils, forests) or anthropogenic origin (e.g., former coal mines, waste areas from mining and ore treatment, agriculture, urban sewage plant). However, there seems to be no clear relation between discharge and POC, suggesting dominance of direct anthropogenic POC inputs.

3.2.3. Dissolved Organic Carbon

Mean DOC concentrations in the Lot–Garonne continuum were higher in the Lot River at Temple ($\sim 4 \text{ mg L}^{-1}$) than in the Garonne River (PSM and LR; $\sim 3 \text{ mg L}^{-1}$) throughout the year (Figure 3). Thus, mean DOC concentrations in the Lot and Garonne Rivers are similar to typical values of temperate river systems (4 mg L^{-1} ; Ludwig et al., 1996).

Both sites on the Garonne River (PSM, LR) show similar evolution of DOC concentrations, reaching maximum values ($\sim 6 \text{ mg L}^{-1}$; Table I) during flood. This coincidence of high DOC and high discharge may be due to massive destruction/degradation of phytoplankton cells due to high SPM concentrations and/or the leaching of organic rich soil by superficial runoff. As discharge of the Garonne River is highly variable and floods may occur in nearly all seasons (Schäfer et al., 2002), the increase in DOC during floods might be partly due to abrupt extinction of algal populations (Etcheber, personal communication). However, as observed in other freshwater environments (Buffle, 1988; Ludwig et al., 1996; Butturini and Sabater, 2000), DOC input by runoff appears to be the dominating process, suggesting that during this period DOC in the Garonne River was dominated by allochthonous inputs.

In the Lot River, maximum DOC concentrations of 5 mg L^{-1} , 6.5 mg L^{-1} and 8.9 mg L^{-1} were measured in February, April and July, respectively. Similar to the Garonne River, high DOC concentrations in February (flood) were mainly attributed to leaching of agricultural soils by runoff. However, the other two situations were characterised by high phytoplankton activity and low discharge (Figure 2), suggesting that DOC at Temple is derived from at least two different sources: soil and phytoplankton (see above). The highest DOC concentrations generally occurred in the Riou Mort River, ranging from 7 mg L^{-1} (January–May) to 17 mg L^{-1} (in July; Table I). However, the generally low phytoplankton activity (see above) and low discharge, suggest dominance of other DOC sources as (i) urban sewage plants and/or (ii) leaching of soils on Carboniferous rocks.

3.2.4. C18-separated Hydrophobic Fraction

Typical hDOM fractions of DOC in the Garonne River at LR were 30–40% of the total DOC concentration, except for July (63%; Table I; Figure 5). At PSM, hDOM fractions were lower and more variable. The seasonal evolution of hDOM at LR, PSM shows maximum values in summer (Figure 5). Similar to total DOC, at Temple the hDOM fractions were in most cases higher and more variable than those in the Garonne River, without distinct seasonal trend. The hDOM fractions in the Riou Mort were lower than those in the Lot and the Garonne Rivers (~ 25 – 35% ; Figure 5). However, only the C18 Sep-Pak columns used for Riou Mort samples showed a light brown colour after elution, suggesting incomplete recovery of the hDOM fraction. This observation was interpreted as (i) incomplete elution and/or (ii) retention of filter passing ($<0.2 \mu\text{m}$) colloids retained by the C18 siliceous phase (nominal porosity 125 \AA , WATERS product-description). River DOC is generally composed by fulvic (50–70%) and humic (5–10%) compounds originating from soil but contains also a significant proportion of amino-acids and polysaccharides ($\leq 10\%$; e.g., Ertel et al., 1986; Chester, 1990). Nevertheless, as DOC sources in this small watershed are multiple and different from those of the other rivers (see above), this particular behaviour of the Riou Mort samples cannot be attributed to a distinct DOC compound.

3.2.5. Trace Metals Associated to the C18 Separated Hydrophobic Fraction

In the Riou Mort River, mainly the very high dissolved Cd ($22.2 \pm 3.6 \mu\text{g L}^{-1}$) and Ni ($24.5 \pm 5.3 \mu\text{g L}^{-1}$) concentrations, compared to world average values (0.05 and $0.5 \mu\text{g L}^{-1}$, respectively (Martin and Whitfield, 1983), reflected the still important polymetallic pollution of this small system. In contrast, the other sites showed dissolved heavy metal concentrations close to world average values (Martin and Whitfield, 1983).

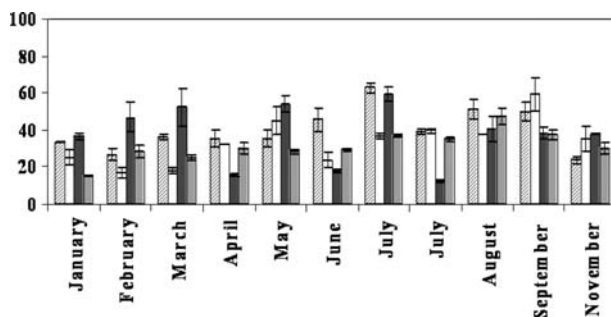


Figure 5. Fraction of DOC (%) separated by C18 Sep-Pak column. (striped bars – La Réole, white bars – Port Ste Marie, black bars – Temple, grey bars – Riou Mort).

In all samples, hDOM fractions of Cu represented the highest contribution (%) to total dissolved concentrations, followed by U (Figure 6). Cobalt, Ni, V and Mo showed lower values without clear predominance. However, hDOM–Co appeared to be more important than hDOM–Ni, hDOM–Mo and hDOM–V in most cases. During the observation, hDOM–Cd mean values were lower than those of the other metals (Figure 6). At all sites hDOM–Mo fractions were similar and represented about 2–3% of the total dissolved Mo concentration.

In the Riou Mort River, hDOM fractions of Cu, U, V, Co and Ni were generally lower than those at the other sites. For example, mean hDOM–Cu and hDOM–U fractions accounted for 22 and 24% of total dissolved Cu and U concentrations, respectively (Figure 6). For the other metals, hDOM complexes presented less than 5% of the dissolved concentration and mean hDOM–Cd never exceeded 1%. At Temple mean hDOM complexes of Cu, U, and Co were more important than at the other sites, with maximum values in spring reaching up to 70, 44 and 25% for Cu, U and Co, respectively. In contrast, hDOM–Cd was close or inferior to detection limits for T, PSM and LR. Relative contributions of hDOM complexes of V (~5%) and Ni (~10%) at Temple were similar to those in the Garonne River (Figure 6). Both sites on the Garonne River (PSM and LR) showed comparable hDOM fractions for Cu, U, V, Ni, Co and Mo, although the values at La Réole appeared to be influenced by hDOM–metals derived from the Lot River (Figure 6).

The 11-month observation showed no important seasonal variations for hDOM–metal fractions in the Lot–Garonne River system. For example, the mean hDOM–Cu fraction represented 25–40% of the total dissolved Cu concentrations, with variations of less than 25% (r.s.d.). The generally high hDOM–Cu fraction observed in the Lot–Garonne system was in good

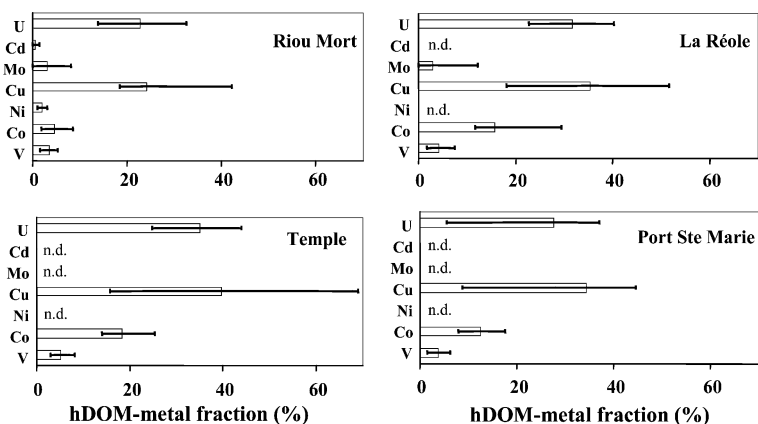


Figure 6. Mean percentage of hDOM–metal fractions for the seven studied metals (black bars represent minimum and maximum values; n.d. – not detected).

agreement with the commonly reported strong affinity of Cu to organic phases, especially humic acids (e.g., Mantoura et al., 1978; Chester, 1990; Benedetti et al., 1996). In contrast, dissolved Cd showed very low or even non-detectable hDOM fractions, suggesting (i) little abundance of organic Cd-complexes and thus very low affinity between Cd and present dissolved organic ligands or (ii) association of Cd with hydrophilic organic complexes not retained by C18. Accordingly, for the studied metals the following order of relative trace metal affinity towards the hydrophobic (C18-separated) dissolved organic fraction was observed: Cu, U > Co, Ni > V, Mo > Cd. In general, this order of decreasing binding strength between metals and the organic hydrophobic phase was in accordance with the Irving–Williams series of transition element affinity (Hg > Cu > Ni > Zn > Co > Mn > Cd) for organic ligands (e.g., humic acids, Chester, 1990; proteins, Pasternak et al., 2001; phosphonates, Nowack, 2003). The spatial and temporal variations in hDOM fractions suggest that C18 separated hDOM fractions reflect to some degree the presence of dissolved organic macromolecules of diverse nature (e.g., size, structure, conformation) or origins. Applying similar hDOM–Cu fractions as observed in 2003 to dissolved trace metal fluxes in the Lot–Garonne River observed in previous years, (e.g., Audry et al., 2004b), the role of hDOM associated trace metals in dissolved trace metal transport can be evaluated. Cadmium, the first order pollutant in the studied river system, is mainly transported in the particulate phase (80–90%; e.g., Blanc et al., 1999; Audry et al., 2004a). Additionally, Cd showed the weakest affinity for the hydrophobic fraction (<1%), suggesting that the contribution of hDOM–Cd to total Cd transport is negligible. In contrast, other hDOM associated trace metals and especially hDOM–Cu may play an important role in dissolved trace metal transport. For example, in 1999 and 2000, dissolved Cu fluxes were estimated 9.6 and 7 t a⁻¹ at Temple and 45.3 and 26.3 t a⁻¹ at La Réole, respectively (Audry et al., 2004b). Applying similar hDOM–Cu fractions as observed in 2003 (30–40%), we estimated annual dissolved Cu fluxes at Temple to be ~2.9–3.8 t a⁻¹ in 1999 and 2.1–2.8 t a⁻¹ in 2000. Accordingly, at La Réole, Cu transport by the dissolved hydrophobic fraction is estimated to be 13.6–18.1 and 7.9–10.5 t a⁻¹ in 1999 and 2000, respectively.

4. Conclusion

The present study provides first information on the dissolved, C18-extracted (hydrophobic) organic fraction and associated trace metals in the Lot–Garonne River system. Although the C18 Sep-Pak method separates only a fraction of DOC, low trace metal blanks and excellent reproducibility permitted the comparison of results obtained for different sites and hydrological situations. Spatial and temporal (monthly) variations of dissolved and particulate organic

carbon and Chl *a* reflected the presence of different types of organic matter and their relation with the hDOM fraction. The general order of decreasing binding strength between metals and the organic hydrophobic phase (Cu, U > Co, Ni > V, Mo > Cd) was in good agreement with the Irving–William series of transition element affinity towards organic ligands. Consequently, in the studied system the role of the hydrophobic phase in dissolved Cd transport appeared to be negligible, whereas the hDOM-Cu fraction strongly contributed to dissolved Cu transport. The separation capacity of C18-phases, combined with a more detailed characterisation of both the hDOM and the non-retained phases (e.g., by three-dimensional excitation-emission-matrix-spectroscopy, 3D-EEM; and/or size fractionation by ultrafiltration; (Parlanti et al., 2002; Vacher et al., 2003) will certainly improve knowledge on DOM-metal associations in natural aquatic systems.

Acknowledgments

This study was conducted in the “T. G. M.” Team of UMR CNRS 5805 EPOC, Bordeaux 1 University and is a scientific contribution of the “GIS ECOBAG” (Groupement d’Intérêt Scientifique: Ecologie du Bassin Adour-Garonne). We greatly acknowledge C. Bossy, G. Lavaux and J. P. Lissalde for their important contribution to field and laboratory work. This work was supported by grants from ‘l’Agence de l’Eau Adour-Garonne’, ‘le Ministère Français de la Recherche, the INSUE- « ECODYN » programme and ‘le FEDER-Aquitaine’.

References

- Abril G., Nogueira M., Etcheber H., Cabeçadas G., Lemaire E. and Brogueira M. J. (2002) Behaviour of organic carbon in nine contrasting European estuaries. *Estuar. Coast. Shelf Sci.* **54**, 241–262.
- Audry S. (2003) *Bilan géochimique du transport des éléments traces métalliques dans le système fluvial anthropisé Lot–Garonne–Gironde*. Ph.D. thesis, Bordeaux I University, France, 413 pp.
- Audry S., Blanc G. and Schäfer J. (2004a) Cadmium fluxes in the Lot–Garonne River system (France) – temporal variability and empiric model. *Sci. Tot. Environ.* **319**, 197–213.
- Audry S., Schäfer J., Blanc G., Bossy C. and Lavaux G. (2004b) Anthropogenic components of Heavy metal budgets (Cd, Zn, Cu and Pb) in the Lot–Garonne fluvial system (France). *Appl. Geochem.* **19**, 769–786.
- Baudrimont M., Schäfer J., Marie V., Maury-Brachet R., Bossy C., Boudou A. and Blanc G. (2005) Cadmium contamination of three bivalve species (oysters, cockles and clams) in the Nord-Médoc salt marshes (Gironde estuary, France). *Sci. Tot. Environ.* **337**, 265–280.
- Benedetti M. F., Van Riemsdijk W. H., Koopal L. K., Kinniburgh D. G., Gooddy D. C. and Milne C. J. (1996) Metal ion binding by natural organic matter: From the model to the field. *Geochim. Cosmochim. Acta* **60**, 2503–2513.
- Blanc G., Lapaquellerie Y., Maillet N. and Anschutz P. (1999) A cadmium budget for the Lot–Garonne fluvial system (France). *Hydrobiologia* **410**, 331–341.

- Boutier B. (1981) Synthèse des résultats de la surveillance des micropolluants dans la matière vivante. Ministère de l'environnement. *Bulletin du Réseau National d'Observation*. **17**, 115–174.
- Boutier B., Chiffolleau J. F., Gonzalez J. L., Lazure P., Auger D. and Truquet I. (2002) Influence of the Gironde estuary outputs on cadmium concentrations in the coastal waters: consequences on the Marennes-Oléron bay (France). *Oceanol. Acta* **23**, 745–757.
- Buffle J. (1988) *Complexation Reactions in Aquatic Systems: An Analytical Approach*, Ellis Horwood Ltd, Chichester, UK, 692 pp.
- Butturini A. and Sabater, F. (2000) Seasonal variability of dissolved organic carbon in a Mediterranean stream. *Biogeochemistry* **51**, 303–321.
- Cauwet G., Gadel F., De Souza Sierra M. M., Donard O. and Ewald, M. (1990) Contribution of the Rhône River inputs to the northwestern Mediterranean Sea. *Cont. Shelf Res.* **10**, 1025–1037.
- Cauwet G. (1994) HTCO method for dissolved organic carbon analysis in seawater: influence of catalyst on blank estimation. *Mar. Chem.* **47**, 55–64.
- Chester R. (1990) *Marine Geochemistry*, Unwin Hyman ed., London, 698 pp.
- Christensen J. B., Jensen D. L. and Christensen T. H. (1996) Effect of dissolved organic carbon on the mobility of cadmium, nickel and zinc in leachate polluted groundwater. *Water Res.* **30**, 3037–3049.
- Coyne A., Schäfer J., Hurtrez J. E., Dumas J., Etcheber H. and Blanc G. (2004) Sampling frequency and accuracy of SPM flux estimates in two contrasted drainage basins. *Sci. Tot. Environ.* **330**, 233–247.
- Donat J. R. (1986) An evaluation of a C18 solid phase extraction technique for isolating metal-organic complexes from central North Pacific Ocean waters. *Mar. Chem.* **18**, 85–99.
- El Sayed M. A. and Aminot A. (2000) C18 Sep-pak extractable dissolved organic copper related to hydrochemistry in the North-west Mediterranean. *Estuar. Coast. Shelf Sci.* **50**, 835–842.
- Elbaz-Poulichet F., Cauwet G., Guan D. M., Faguet D., Barlow R. and Mantoura R. F. C. (1994) C18 Sep-pak extractable trace metals in waters from the Gulf of Lions. *Mar. Chem.* **46**, 67–75.
- Ertel J. R., Hedges J. I., Devol A. H., Richey J. E. and Ribeiro, M. (1986) Dissolved humic substances of the Amazon River system. *Limnol. Oceanogr.* **31**, 739–754.
- Etcheber H. (1986) *Biogéochimie de la matière organique en milieu estuarien: Comportement, bilan, propriétés. Cas de la Gironde*. Ph.D. thesis, Bordeaux I University, France, 379 pp.
- Gonzalez-Davila M., Santana-Casiano J. M., Perez-Peña J. and Millero F. J. (1995) Binding of Cu(II) to the surface and exudates of the alga *Dunaliella tertiolecta* in seawater. *Environ. Sci. Technol.* **29**(2), 289–301.
- Harris G. P. (1986) *Phytoplankton Ecology. Structure, Function & Fluctuation*. Chapman and Hall, London, 384 pp.
- Hunter K. A., Kim J. P. and Croot P. L. (1997) Biological roles of trace metals in natural waters. *Environ. Monit. Assess.* **44**, 103–147.
- Jansen B., Nierop K. G. J. and Verstraten J. M. (2003) Mobility of Fe (II), Fe(III) and Al in acidic forest soils mediated by dissolved organic matter: influence of solution pH and metal/organic carbon ratios. *Geoderma* **113**, 323–340.
- Lemaire E., Abril G., De Wit R. and Etcheber H. (2002) Distribution of phytoplankton pigment in nine European estuaries and implications in an estuarine typology. *Biogeochemistry* **59**, 5–23.
- Ludwig W., Probst J. L. and Kempe S. (1996) Predicting the oceanic input of organic carbon by continental erosion. *Global Biogeochem. Cycles* **10**, 23–41.
- Mackey D. J. (1983) Metal-organic complexes in seawater – An investigation of naturally occurring complexes of Cu, Zn, Fe, Mg, Ni, Cr, Mn and Cd using High-performance liquid chromatography with atomic fluorescence detection. *Mar. Chem.* **13**, 169–180.

- Mackey D. J. and O'Sullivan J. E. (1990) Metal–organic interactions in sea water: an ecosystem experiment. *Anal. Chim. Acta* **232**, 161–170.
- Mantoura R. F. C., Dickson A. and Riley J. P. (1978) The complexation of metals with humic materials in natural waters. *Estuar. Coast. Shelf Sci.* **6**, 387–408.
- Martin J. M. and Whitfield M. (1983) The significance of the river input of chemical elements to the oceans. In *Trace Metals in Sea Water* (eds. C. S. Wong *et al.*), pp. 265–296. Plenum, New York.
- Meybeck M. (1993) Riverine transport of atmospheric carbon: sources, global typology and budget. *Water Air Soil Poll.* **70**, 443–463.
- Mills G. L., Douglas G. S. and Quinn J. G. (1989) Dissolved organic copper isolated by C18 reverse-phase extraction in an anoxic basin located in the Pettaquamscutt river estuary. *Mar. Chem.* **26**, 277–288.
- Mills G. L. and Quinn J. G. (1981) Isolation of dissolved organic matter and copper–organic complexes from estuarine samples using reverse-phase liquid chromatography. *Mar. Chem.* **10**, 93–102.
- Nowack B. (2003) Environmental chemistry of phosphonates. *Water Res.* **37**, 2533–2546.
- Parlanti E., Morin B. and Vacher L. (2002) Combined 3D-spectrofluorometry, high performance liquid chromatography and capillary electrophoresis for the characterisation of dissolved organic matter in natural waters. *Org. Geochem.* **33**, 221–236.
- Pasternak A., Kaplan J., Lear J. D. and Degrado W. F. (2001) Proton and metal ion-dependent assembly of a model di-iron protein. *Protein Sci.* **10**, 958–969.
- Paulson A. J., Curl H. C. and Gendron J. F. (1994) Partitioning of Cu in estuarine waters, I. Partitioning in a poisoned system. *Mar. Chem.* **45**, 67–80.
- Plavšić, M. and Branica M. (1986) Voltametric investigation of copper (II) interaction with DOM isolated on Sep-pak C18 cartridges. *Ocean Sci. Eng.* **11**, 99–113.
- Robert S., Blanc G., Schäfer J., Lavaux G. and Abril G. (2004) Metal mobilization in the fluid mud of the Gironde estuary (France) in low discharge regime. *Mar. Chem.* **87**, 1–13.
- Schäfer J. and Blanc G. (2002) Relationship between ore deposits in river catchments and geochemistry of suspended particulate matter from six rivers in southwest France. *Sci. Tot. Environ.* **298**, 103–118.
- Schäfer J., Blanc G., Lapaquellerie Y., Maillet N., Maneux E. and Etcheber H. (2002): Ten-year-observation of the Gironde fluvial system: fluxes of suspended matter, particulate organic carbon and cadmium. *Mar. Chem.* **79**, 229–242.
- Sullivan B. E., Prah F. G., Small L. F. and Covert P. A. (2001) Seasonality of phytoplankton production in the Columbia River: a natural or anthropogenic pattern? *Geochim. Cosmochim. Acta* **65**, 1125–1139.
- Turner A. (2003) Salting out of chemicals in estuaries: implications for contaminant partitioning and modelling. *Sci. Tot. Environ.* **314–316**, 599–612.
- Vacher L., Schäfer J., Blanc G. and Parlanti E. (2003) Modifications of the properties of dissolved organic matter along the Gironde and Seine estuaries. *21st International Meeting on Organic Geochemistry, 8–12 September 2003, Krakow, Poland.*
- Veyssy E., Etcheber H., Lin R. G., Buat-Ménard P. and Maneux, E. (1999) Seasonal variations and origins of particulate organic carbon in the lower Garonne River at La Réole (SW-France). *Hydrobiologia* **391**, 113–126.
- Yoon Y.-Y., Martin J.-M. and Cotté M.-H. (1999) Dissolved trace metals in the western Mediterranean sea: total concentration and fraction isolated by C18 Sep-pak technique. *Mar. Chem.* **66**, 129–148.