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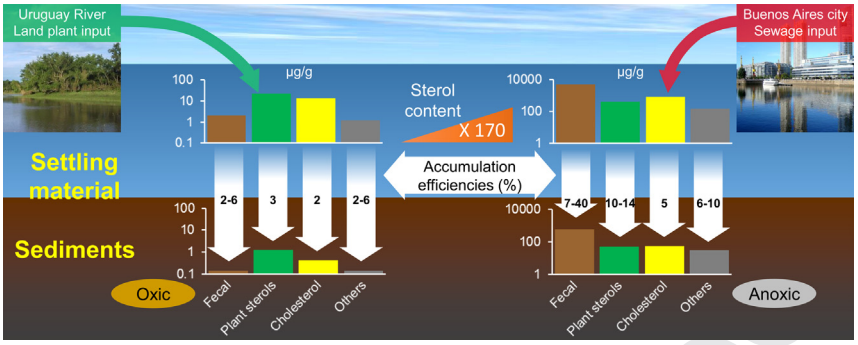
Graphical abstract

Early diagenetic alterations of sterol biomarkers during particle settling and burial in polluted and pristine areas of the Rio de la Plata Basin pp xxx–xxx

Eric Demian Speranza *, Manuel Colombo, Carlos Norberto Skorupka, Juan Carlos Colombo



Highlights

• Settling particles contained ~10–20 times more sterols than underlying sediments. • Higher runoff during rainy months significantly increased particle and sterol flux. • Buenos Aires sediments contained huge coprostanol levels, similar to sewage sludge. • A non-polluted upstream site showed dominant plant sterols. • Higher fluxes and anoxic sediments at Buenos Aires favored sterol preservation.

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Early diagenetic alterations of sterol biomarkers during particle settling and burial in polluted and pristine areas of the Rio de la Plata Basin

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ABSTRACT

Sources and diagenetic alterations of sterol markers were studied in settling material and sediments near the Buenos Aires main sewer (BA), and at a relatively non-polluted northern site at the Uruguay River (N). Vertical particle fluxes were 7-times higher at BA relative to N (34 ± 24 vs 4.6 ± 3.6 mg/cm^2/day; mean ± standard deviation), increasing during rainy months. Total sterol contents were consistently higher at BA, both in settling material (7140 ± 7905 vs 41 ± 47 μg/g at N) and sediments (708 ± 454 vs 1.9 ± 0.18 μg/g). This difference was further amplified in the vertical flux of sterols (116 ± 168 vs 0.070 ± 0.13 mg/cm^2/year). At BA, sterol composition of settling material and sediments was dominated by fecal sterols (75–77%), with extreme coprostanol concentrations (3.6 ± 4.8 vs 0.35 ± 0.28 mg/g at N) which are similar to sewage sludge. In contrast, at N the sterol profile was dominated by plant sterols (57–64%), mainly sitosterol, stigmasterol and campesterol. At BA the discharge of fresh sewage was confirmed by the high coprostanol/(coprostanol + epicoprostanol) ratio. At N, the overwhelming dominance of plant sterols over herbivore fecal sterols was reflected by the high sitosterol/(sitosterol + 24-ethylcoprostanol) ratio and the low coprostanol/(coprostanol + 24-ethylcoprostanol) ratio. The coprostanol/(coprostanol + epicoprostanol) and cholesterol/(cholesterol + cholestanol) ratios were lower in sediments than in settling material, reflecting the sterol degradation at the sediment surface. The accumulation efficiencies, calculated as the difference between trap fluxes and sediment inventories, were 2–7 times higher at BA reflecting strong vertical fluxes and enhanced preservation under anoxic conditions. During diagenetic processes, epicoprostanol (partially produced in situ), cholestanol and plant sterols were the best-preserved sterols, while cholesterol was the most labile during burial.

1. Introduction

The molecular composition of lipids from settling material and sediments provides useful information about the sources and diagenetic alteration of organic matter (Meyers and Ishiwatari, 1993; Canuel and Hardison, 2015). Sterols, present as components of cell membranes in eukaryotes and also in a few prokaryotes, are especially suited as biomarkers due to their widespread environmental occurrence, stability and structural diversity (Volkman, 2005). The source specificity of sterols ranges from some rather unspecific sterols (e.g., cholesterol) to several marker sterols associated to particular organisms, such as diatoms, dinoflagellates, plants and fungi (Puglisi et al., 2003; Volkman, 2016). A group of sterols, collectively referred as fecal sterols, have been widely used as sewage tracers. Coprostanol, formed during the biohydrogenation of the Δ^5 double bond of cholesterol by bacteria present in the gut of humans or animals, is the primary fecal sterol detected in domestic wastes (< 60% total sterols; Bull et al., 2002). Coprostanol, unlike cholesterol, is barely absorbed by the intestinal epithelium and is massively excreted with feces (Veiga et al., 2005). Although it is degraded under oxic conditions, it can resist relatively unaltered for many years in anoxic sediments (Nishimura and Koyama, 1977).

Discharge of municipal wastewater to rivers and coastal areas is a source of continuing environmental concern. Municipal discharges are a major source of organic matter and nutrients that

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may cause eutrophication, oxygen depletion, turbidity increase, acidification, and trophic structure alterations leading to habitat deterioration (deBruyn et al., 2003; Blanch et al., 2004; Kress et al., 2016). Moreover, since most sewer systems in Latin America also receive storm drainage and industry inputs, sewage contains many hazardous materials such as organic and inorganic pollutants and pathogens that jeopardize the use of receiving water for human consumption, fishing activities or recreation (Helmer and Hespanhol, 1997). Urban-industrial effluent discharges in major river systems are a key source of anthropogenic material to marine environments.

Among these major river systems worldwide, the Rio de la Plata Basin ranks fifth in terms of drainage area (2.8×10^6 km^2), covering nearly 20% of the South America surface area (Milliman and Meade, 1983). The main tributary rivers of this basin (the Parana and Uruguay rivers) discharge an average of 22,000 m^3/s of water to the Atlantic Ocean through the Rio de la Plata estuary, a large funnel-shaped, shallow estuary that receives > 82–129 $\times 10^6$ tons/year of particulate load, making it one of the most turbid estuaries in the world (Milliman and Meade, 1983). The coastal area of metropolitan Buenos Aires is strongly impacted by anthropogenic discharges resulting in high concentrations of hydrocarbons, organochlorine pesticides, PCBs and metals in sediments (Colombo et al., 1989, 2005; Tatone et al., 2009), settling material (Colombo et al., 2007c; Tatone et al., 2012) and biota (Colombo et al., 1997, 2007a, 2007b, 2011). Before the installation of a primary wastewater treatment plant in 2015, the main Buenos Aires sewer outfall discharged 2.2×10^6 m^3/day of crude domestic wastes from 6×10^6 inhabitants as well as industrial and municipal wastes 2.5 km offshore (www.aysa.com.ar; FREPLATA, 2005). The Riachuelo River, located 20 km upstream of the main sewer, also discharges sewage material and industrial waste. The combined loads of both effluents reach 4.3×10^6 m^3/day, which is comparable to the flow of the world's largest sewage outfall in Boston (Roberts and Villegas, 2016).

In this context, the analysis of settling material in river systems with high turbidity and organic matter loading is particularly relevant. Settling material represents the fresh inputs of organic matter to aquatic environments and it is thus useful to assess its sources as well as the temporal variability. Sediments integrate these signals over a wide temporal range, with a composition dominated by refractory compounds. The comparison between settling material and underlying sediments allows for a detailed evaluation of the early diagenetic behavior of organic compounds, mainly controlled by factors such as sedimentation rate, temperature and redox conditions (Colombo et al., 1996b).

In this paper, we evaluate the sources, vertical fluxes and diagenetic alterations of sterol markers during burial in two contrasting sites of the Rio de la Plata Basin: the highly impacted metropolitan area of the Rio de la Plata estuary and a relatively non-polluted northern site at the Uruguay River. Differences in terms of sterol concentration and composition, vertical fluxes, differential preservation in sediments and temporal variation are discussed.

2. Materials and methods

The sampling strategy comprised two sites with contrasting anthropogenic impact: the heavily polluted Buenos Aires metropolitan area of the Rio de la Plata estuary near the main sewer outfall (BA, 34°43.33′S 58°10.30′W) and a more pristine site ~200 km upstream on the Uruguay River, the Ñandubaysal Bay (site N, 33°05.27′S 58°21.37′W; Fig. 1). Sampling campaigns were carried out seasonally from 2007 to 2014. Settling material was collected in pre-weighed polypropylene conical Falcon tubes coupled to a fixed 10 cm diameter cylindrical sediment trap deployed at 1.5 m for 1–3 days (BA) or 30–60 days (N). Superficial sediments were collected using a stainless steel Hydro-Bios Van-Veen grab sampler. Samples were immediately refrigerated and transported to the laboratory. Tubes containing the settling material were centrifuged and weighed after discarding supernatant water. Water content was determined gravimetrically after drying in an oven at 40 °C. Total organic carbon determination was carried out on a Thermo Finnigan Flash EA 1112 elemental analyzer.

The total particle flux was computed as:

$$\text{Flux (mg/cm}^2\text{/day)} = \text{settling matter (mg)}/(\text{trap surface (78 cm}^2\text{)} \times \text{deployment time (days)}).$$

The sedimentation rate (SR) was calculated as:

$$\text{SR (cm/yr)} = (\text{Flux} \times 365)/(1000 \times \text{density (g/cm}^3\text{)})$$

The discharge of the Uruguay River was calculated as the turbinated plus compensation flow discharged daily by the Salto Grande Dam, located 240 km upstream of N station, and averaged for each sediment trap deployment period (Wholesale Electricity Market Administration Company: www.cammesa.com). The discharge of the Rio de la Plata estuary was assumed as the sum of the corresponding monthly discharges of the Uruguay River, measured 90 km upstream N station, and of the Parana River, measured near the mouth of its main channels (Paraná Guazú and Paraná de las Palmas; Base de Datos Hidrológica Integrada, bdhi.hidricosargentina.gov.ar; Jaime and Menéndez, 2002).

Lipids were extracted ultrasonically with acetone:dichlorome thane:petroleum ether (1:2:2, v:v:v), dried over anhydrous sodium sulfate and gravimetrically determined (Colombo et al., 1996a). Deuterated sterols (deuterocholesterol-D7 and deuterositosterol-D7, Steraloids, Inc., Newport, RI; Table 1) were used as internal standards. In order to avoid the interference of fatty acids, lipids (ca. 100 mg) were saponified with 1 M KOH in methanol and non-saponifiable compounds were extracted with petroleum ether:diethyl ether (4:1, v:v; Christie, 1989). The extracts were concentrated under a nitrogen stream and derivatized with 150 µl of N,O-*bis*(trimethylsilyl)trifluoroacetamide and trimethylchlorosilane (BSTFA:TMCS, 10:1, v:v; AppliChem GmbH, Darmstadt, Germany; Sigma-Aldrich, St. Louis, MO, USA) for 3 h at 60 °C. The resulting trimethylsilyl ether derivatives were taken to dryness under nitrogen and resuspended in toluene prior to analysis. All solvents used were pesticide residue analysis grade.

Samples were analyzed using a Perkin Elmer Clarus 500 GC–MS (Perkin Elmer, Waltham, MA, USA) fitted with a Quadrex 007-5MS capillary column (60 m × 0.32 mm i.d. × 0.25 µm film thickness; Quadrex Corp., Bethany, CT, USA). Helium was used as a carrier gas with a flow rate of 1.2 ml/min and the temperature of injector was set at 250 °C (split-splitless mode). The oven temperature program started at 100 °C with a ramp to 225 °C at 15 °C/min and to 300 °C at 3 °C/min with a final holding time of 10 min. The transfer line temperature was set at 200 °C and the analytes were ionized by 70 eV electron impact at 180 °C. The mass spectrometer was simultaneously operated in scan mode (60–600 Da) and selective ion monitoring. Data were acquired and processed with TurboMass 5.1 software.

Steroids with their trivial and IUPAC names, molecular weight, retention times and mass-to-charge ratios (m/z) used for quantification and confirmation are presented in Table 1. Coprostanol, epicoprostanol, coprostanone and 24-ethylcoprostanol are collectively referred to as fecal sterols. Compounds were identified by comparison with standards of 15 steroids (brassicasterol, campesterol, coprostanone, deuterocholesterol, deuterositosterol, epicoprostanol, ergosterol and sitosterol from Steraloids; Cholesterol, coprostane, coprostanol, dehydrocholesterol, desmosterol, stigmastanol and stigmasterol from Sigma-Aldrich), litera-

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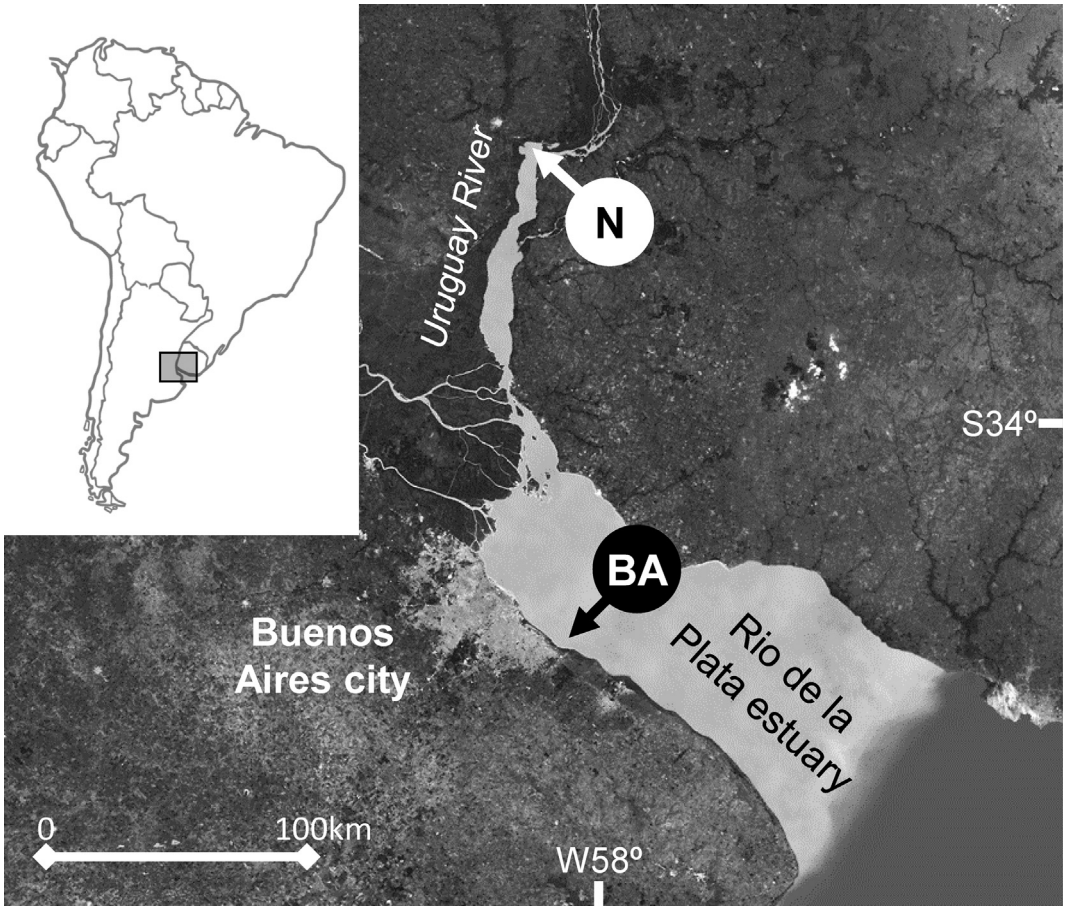

Fig. 1. Sampling stations of settling material and sediments in the metropolitan coastal area of the Rio de la Plata estuary, near Buenos Aires main sewer (BA) and at North site (N) in the Uruguay River.

Table 1
Names, formula, molecular weight (MW), retention time (Rt) and mass of ions used for quantification (target ion) and identification (confirmatory ions) of sterols and steroids (coprostane and coprostanone) analyzed in this work.

Trivial name	Systematic name[a]	Formula	MW	Rt	Target ion	Confirmatory ions	
Coprostane	5β-cholestane	$C_{27}H_{48}$	372.37	30.80	217	357	372
Coprostanol	5β-cholestan-3β-ol	$C_{27}H_{48}O$	388.37	35.57	370	355	215
Epicoprostanol	5β-cholestan-3α-ol	$C_{27}H_{48}O$	388.37	36.08	370	215	355
Cholestanol	5α-cholestan-3α-ol	$C_{27}H_{48}O$	388.37	36.16	215	355	370
Coprostanone	5β-cholestan-3-one	$C_{27}H_{46}O$	386.35	37.13	386	231	370
Deuterocholesterol	cholest-5-en-3β-ol-25,26,26,26,27,27,27-D7	$C_{27}H_{39}D_7O$	393.70	37.31	129	336	375
Cholesterol	cholest-5-en-3β-ol	$C_{27}H_{46}O$	386.35	37.48	329	129	368
Dehydrocholesterol	cholesta-5,22E-dien-3β-ol	$C_{27}H_{44}O$	384.34	37.73	215	445	355
Brassicasterol	ergosta-5,22E-dien-3β-ol	$C_{28}H_{46}O$	398.35	38.19	456	129	366
Desmosterol	cholest-5,24-dien-3β-ol	$C_{27}H_{44}O$	384.34	38.36	129	343	253
Ergosterol	ergosta-5,7,22E-trien-3β-ol	$C_{28}H_{44}O$	396.65	39.17	343	337	468
Dihydrobrassicasterol	ergost-5-en-3β-ol	$C_{28}H_{48}O$	400.37	39.75	343	129	384
Campesterol	campest-5-en-3β-ol	$C_{28}H_{48}O$	400.37	39.92	343	129	382
24-Ethylcoprostanol	24S-5β-stigmastan-3β-ol	$C_{29}H_{52}O$	416.40	40.19	398	215	383
Stigmasterol	stigmasta-5,22E-dien-3β-ol	$C_{29}H_{48}O$	412.37	40.55	129	255	484
Deuterositosterol	stigmast-5-en-3β-ol-25,26,26,26,27,27,27-D7	$C_{29}H_{43}D_7O$	421.75	42.00	129	364	403
Sitosterol	stigmast-5-en-3β-ol	$C_{29}H_{50}O$	414.39	42.20	129	488	473
Stigmastanol	stigmastan-3β-ol	$C_{29}H_{52}O$	416.40	42.59	215	473	488

[a] According to LIPID MAPS classification system (http://www.lipidmaps.org/data/classification).

ture data and interpretation of mass spectrometric fragmentation patterns. Quantification was performed using a four point calibration curve (0.20–50 μg/ml) prepared in dichloromethane from certified standards (Table 1). Peak areas were corrected according to internal standard recoveries. Commercial standards were not available for some compounds (cholestanol, dehydrobrassicasterol and 24-ethylcoprostanol) and these sterols were quantified based on response factors of structurally related sterols.

The limit of detection (LOD) of each steroid was estimated by calculating the signal to-noise ratio (S/N) of triplicate standard solutions in the range of 0.20–50 μg/ml. LODs values averaged 6.5 ± 11 ng/g, ranging from (0.31 ng/g, coprostanol) to (43 ng/g,

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ergosterol). Reproducibility was assessed by the relative standard deviation (RSD) of triplicate analysis of the same samples in different batches, and averaged 11 ± 3.8. The method was highly linear in the range of concentrations of calibration curves ($r^2 > 0.99$ for all steroids with available authentic standards). Recoveries of deuterated internal standards averaged 96% ± 1.7%. Individual recoveries, evaluated by analysis of spiked samples ranged from 82% ± 15% (ergosterol) to 110% ± 19% (desmosterol).

Statistical analysis was carried on with Python scripting language (www.python.org), using SciPy (www.scipy.org), NumPy (www.numpy.org), MatPlotlib (matplotlib.org) and Pandas (pandas.pydata.org) libraries. Multivariate analyses were executed in *R* language, using RStudio development environment (www.rstudio.com) and ggplot2 and ggbiplot packages (http://ggplot2.org/). Data are expressed as mean ± SD. Relative standard deviation (RSD: [data − mean] × 100/SD) was used to assess parameter variability. To avoid division by zero errors, the ratios between two sterols, A and B were calculated as: A/(A + B). The accumulation efficiency of sterols from settling material to sediments was estimated as the relationship between the annual vertical flux of the sterol and its corresponding one year inventory in sediments (sterol concentration in sediment × annual mineral flux). Student's *t* test was used to perform comparisons between two means as well as to evaluate the significance of correlation coefficients. Multivariate analysis was performed by principal component analysis of standardized data (x − X/y, where X = mean and y = SD).

3. Results and discussion

3.1. Total particle flux

The intense discharge of one of the largest sewers worldwide at BA adds to the natural particle load of the Rio de la Plata resulting in extraordinarily high vertical particle fluxes (34 ± 24 mg/cm^2/day) and sedimentation rates (4.7 ± 3.3 cm/year), in agreement with previous measurements in this area (5.5 ± 2.1 cm/year, density: 2.65 g/cm^3; Colombo et al., 2007c). These values are higher than sedimentation rates reported for nearby sites of this turbid estuary (0.3–1.3 cm/year; Di Gregorio et al., 2007; Bonachea et al., 2010). This suggests that most particles captured by sediment traps at BA are highly organic-rich detritus derived from urban-industrial discharges, as confirmed by the high concentration of lipids (Speranza et al., 2013) and fecal sterols of this material (see below). At N, the total particle flux was 7 times lower (4.6 ± 3.6 mg/cm^2/day), comparable to values previously reported for the Uruguay River (2.7 ± 2.3 mg/cm^2/day, range: 0.73–7.3 mg/cm^2/day; Colombo et al., 2015), resulting in a sedimentation rate of 0.64 ± 0.49 cm/year. In contrast to BA, where the settling material is composed mostly of anthropogenic detritus over the background particle load from the Parana River, the settling material at N reflects the lower solid discharge of the Uruguay River (Moreira et al., 2013). The total particle flux was largely dependent on river discharge, which was 6–46 times higher at BA (19–46 × 10^3 m^3/s) relative to N (0.42–8.4 × 10^3 m^3/s), fitting an exponential curve ($r^2 = 0.78$, $p < 0.0001$; Fig. 2). This correlation had been previously observed at the Uruguay River and reflects the enhanced transport of eroded material as river flow increases (Colombo et al., 2015).

3.2. Total sterol concentrations

The total sterol concentration in settling material was highly variable (RSD: 113%) and exhibited a marked geographical difference. At BA, the tendency of hydrophobic sterols to associate with particulate matter is enhanced by the high organic content of settling particles (total organic carbon: 9.6% ± 7.4%), resulting in high sterol concentrations at this site (7.1 ± 7.9 mg/g dry weight). Previous research on sterols in settling particles primarily conducted in ocean waters, relatively deep and clear, which had average sterol concentrations 1–4 orders of magnitude lower than the Rio de la Plata estuary metropolitan area, a shallow, turbid and polluted environment (Takada et al., 1994; Colombo et al., 1996; Parrish et al., 2000; Burns et al., 2008). The range of sterol concentrations in settling material published for riverine environments is considerably lower than those measured at BA (1–184 μg/g; Li et al., 1995; Saliot et al., 2001; Jeng and Kao, 2002). In fact, the sterol concentrations at BA are comparable to values reported for sewage sludge from wastewater treatment plants (2–9 mg/g; Venkatesan and Kaplan, 1990; Kelly, 1995; Nguyen et al., 1995). At N, total sterol concentrations in settling material are 2–3 orders of magnitude lower (41 ± 47 μg/g) and comparable to aforementioned values in particulate matter from freshwater environments. Total sterols in sediments were 10–20 times lower than in settling material and were less variable (RSD: 10–61%), and also presented a 2–3 orders of magnitude difference between BA and N (708 ± 454 vs 1.9 ± 0.18 μg/g). The reduction in sterol concentration from settling material to sediments reflects the degradation of sterols at the sediment–water interface, especially under oxic conditions (Sun and Wakeham, 1998).

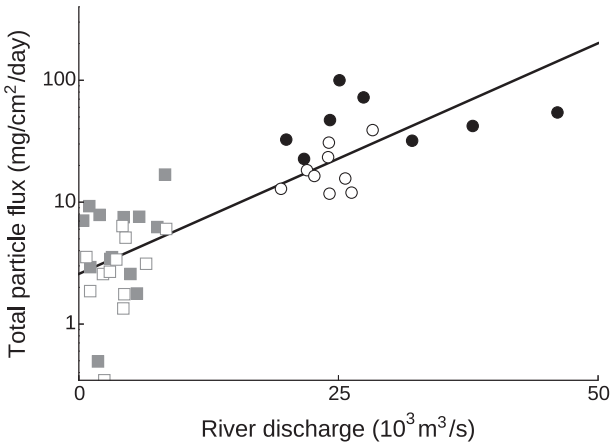

Fig. 2. Relationship between river discharge and total particle flux at Buenos Aires (black circles) and North site (gray squares) sites during warm (September to March, solid markers) and cold months (April to August, open markers). Note the logarithmic scale on the y-axis.

3.3. Temporal variation of particle flux and sterol concentrations in settling material

The large variability in the data for settling material observed for both BA and N can be explained by temporal variations between warm and cold periods. A distinctive temporal pattern of higher particle fluxes during warm and rainy months (September to March, 22 ± 2.6 °C, 127 ± 18 mm) relative to cold and dry months (April to August, 13 ± 2.5 °C, 74 ± 23 mm) was observed at BA (50 ± 25 vs 20 ± 9.4 mg/cm^2/day, $p < 0.005$; Fig. 3) and N (6.2 ± 4.0 vs 3.2 ± 1.9 mg/cm^2/day, respectively, $p < 0.05$). Total sterol concentrations at BA were significantly correlated with total particle flux ($r^2 = 0.41$, $p < 0.05$) following its temporal variation, rising during warm months (11 ± 9.6 mg/g) and decreasing significantly during cold ones (3.6 ± 3.7 mg/g; $p < 0.05$, Fig. 3). This increase of sterol flux during the rainy period is related to the wash-out of streams and effluents that discharge in this area of the Rio de la Plata, as previously observed for other organic tracers (Colombo et al., 2007c). The reinforcement of total flux and

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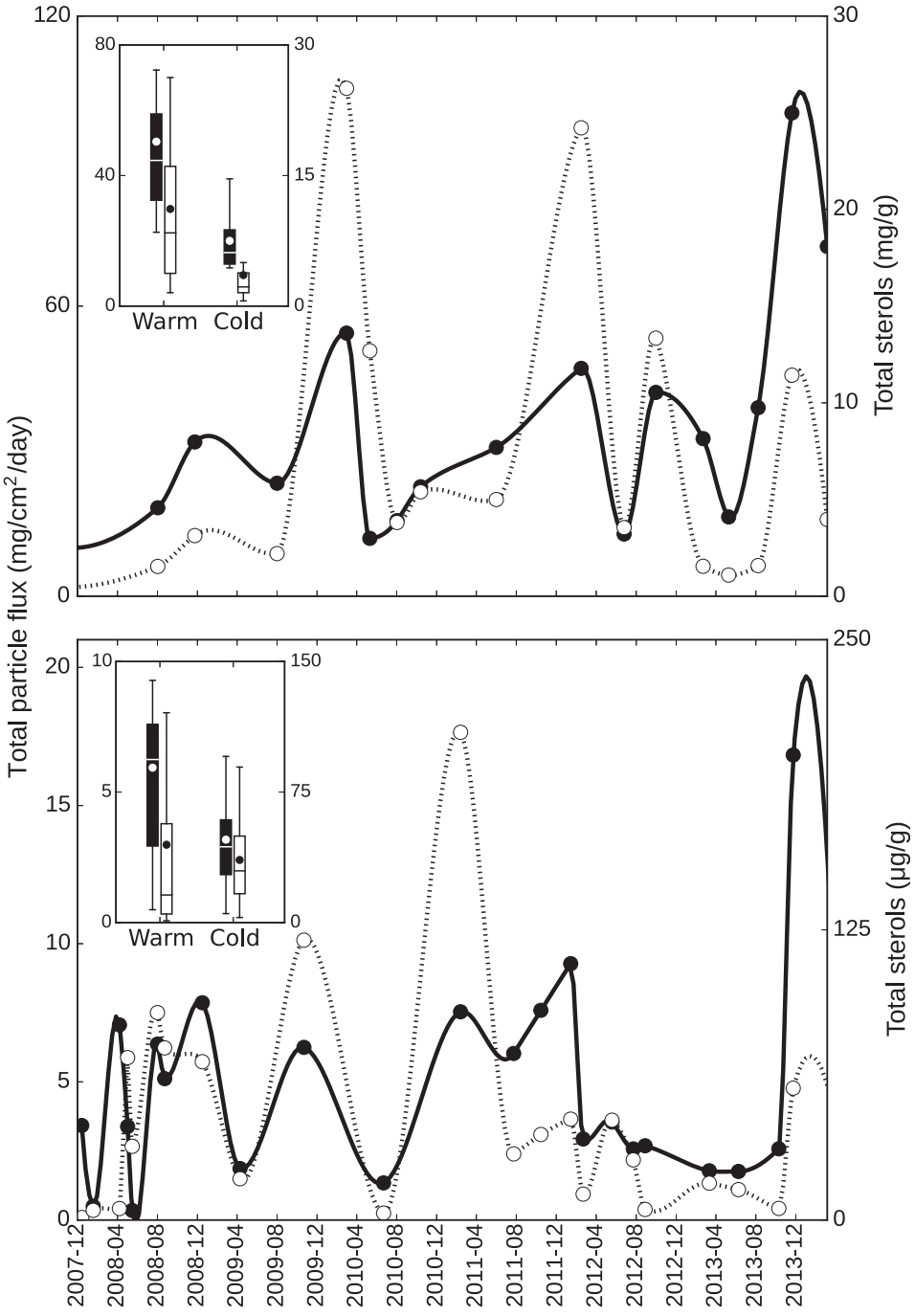

Fig. 3. Temporal variation of total particle flux (solid line, left axis) and total sterol concentration in settling material (dotted line, right axis) at Buenos Aires (top panel) and North (bottom panel). The boxplot inserts show the averages for warm months (September to March) and cold months (April to August) for total particle flux (black boxes) and total sterols (white boxes).

concentration patterns results in an order of magnitude higher sterol vertical fluxes during warm periods (220 ± 202 vs 23 ± 19 mg/cm^2/year in cold months). At N, sterols were also significantly correlated with particle flux (r^2 = 0.36, p < 0.05), but there was no significant difference between warm and cold months (45 ± 61 vs 36 ± 28 µg/g respectively) thus sterol fluxes reflect the total particle flux pattern of higher values during the warm period (87 ± 165 vs 52 ± 63 µg/cm^2/year in cold months).

3.4. Sterol composition

Compositional data are shown in Supplementary Tables S1 and S2. The sterol composition of settling material showed contrasting differences between BA and N (Fig. 4). At BA, fecal sterols predominated (75% ± 5.4% of total sterols), mostly coprostanol (52% ± 11%), followed by cholesterol (12% ± 2.9%) and phytosterols (8.3% ± 3.6%) whereas at N the contribution of plant sterols prevailed (phytosterols: 57% ± 13%, cholesterol: 26% ± 12%, fecal sterols: 7.5% ± 7.0%). The fecal signature of BA resembled the composition of human feces (fecal sterols: 85%, phytosterols: 8.8%, cholesterol: 5.2%, others: 1.2%; Leeming et al., 1996), with extremely high concentrations of coprostanol (3.6 ± 4.8 mg/g) similar to sewage sludge and effluents (1–4 mg/g, 50–80% total sterols; Venkatesan and Kaplan, 1990; Nguyen et al., 1995). The presence of epicoprostanol (9.3% ± 9.6%), derived from coprostanol biodegradation, is evidence of an incipient alteration which is likely occurring in

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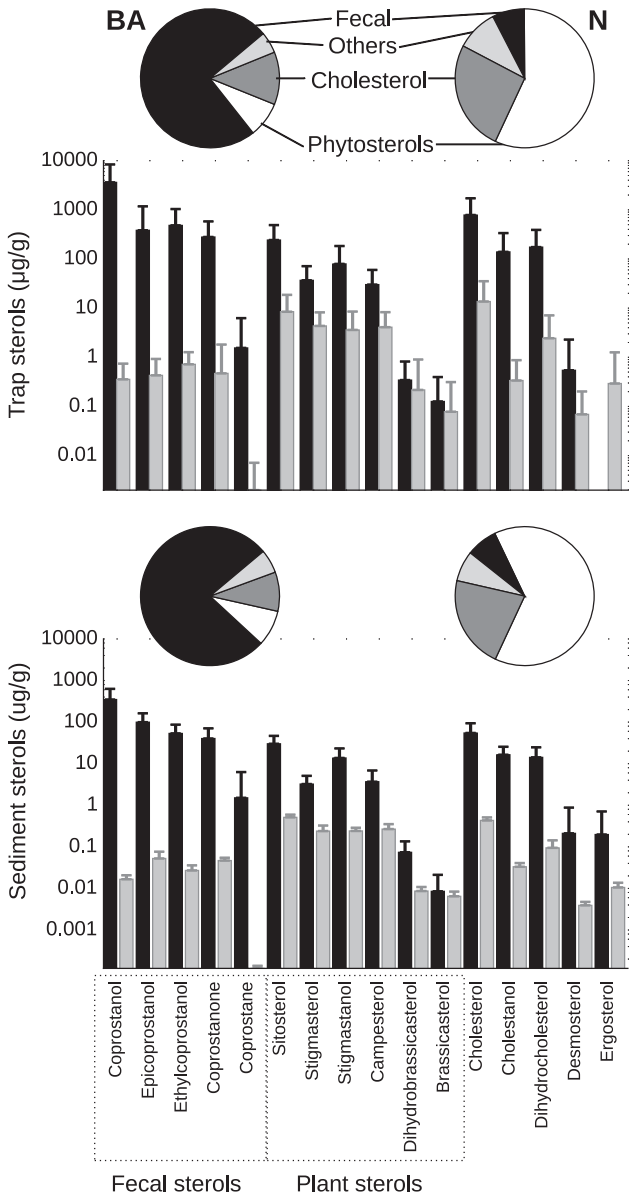

Fig. 4. Sterol composition of settling material (top panel) and sediments (bottom panel) at Buenos Aires (BA, black bars, left pie chart) and North site (N, gray bars, right pie chart). Pie charts show proportions of cholesterol, fecal sterols, phytosterols and other sterols. Bar graphs show individual sterols concentrations on a dry weight basis (note the logarithmic scale).

the long sewer pipeline (9900 km total, main sewers > 100 km, www.aysa.com.ar) rather than in the very shallow (3–5 m) water column. Despite the relative abundance of cholesterol at BA, its utility as biomarker is limited since it is present in multiple organic matter sources (Mudge et al., 1999; Martin-Creuzburg and Von Elert, 2009). A typical fecal herbivore marker, 24-ethylcoprostanol derived by hydrogenation of sitosterol form terrestrial vegetation (Bull et al., 2002), is also relatively abundant at BA (8.5% ± 4.4%), but human feces can also include significant amounts of 24-ethylcoprostanol (Leeming et al., 1996). The significance of coprostanone (5.4% ± 3.3%) is difficult to ascertain since it originates in mammalian gut as an intermediary in coprostanol microbial synthesis, but it can also be produced in sediments as a result of interconversions between this ketone and coprostanol and epicoprostanol (McCalley et al., 1981; Bull et al., 2002). The relatively low proportions of phytosterols observed at BA, mainly represented by sitosterol (4.4% ± 1.9%), reflect the minor contribution of vegetal inputs, possibly including kitchen oil and foodstuff products, at this site.

Despite being found in some algae, the three major phytosterols found in settling material from N, sitosterol (19% ± 5.4%), stigmasterol (15% ± 7.9%) and campesterol (13% ± 11%), are strongly associated with land plants (Huang and Meinschein, 1979; Volkman, 2005) and have been used as biomarkers of paper mill pollution (Lahdelma and Oikari, 2006). The fecal sterols signal at N, dominated by 24-ethylcoprostanol (3.9% ± 4.7%) followed by coprostanol (1.3% ± 1.3%), differs both quantitatively and qualitatively from the sewage signature of BA. The presence of 24-ethylcoprostanol as the main fecal sterol at N probably reflects the contribution of cattle fecal matter from the neighboring livestock establishments. The small concentrations of coprostanol cannot be unambiguously attributed to sewage pollution since small relative amounts of coprostanol can be formed by in situ hydrogenation of cholesterol in sediments not contaminated by fecal pollution (Nishimura and Koyama, 1977).

The change in percentage composition with total sterol concentration and its seasonal variation in settling material also showed geographical differences. At BA, as total sterol concentration increased, the proportion of coprostanol also increased ($r^2 = 0.30$; $p < 0.005$) while stigmasterol and campesterol ($r^2 = 0.31$ and 0.41; $p < 0.005$) decreased and the remaining sterol proportions were not correlated, confirming that the increase in particulate sterol responds basically to anthropogenic discharges. At N, there was a strong significant correlation of total sterol concentration with cholesterol proportion ($r^2 = 0.46$; $p < 0.0001$) and an inverse relationship with 24-ethylcoprostanol and stigmasterol ($r^2 = 0.15$ and 0.18 respectively; $p < 0.05$). The sterol composition, on a percentage basis, showed little temporal variation except for the inverse trend of coprostanol and epicoprostanol observed at BA. While the proportion of coprostanol tended to be higher during warm months (59 ± 9.5 vs 45 ± 8.7 in cold months; $p < 0.01$) and correlates with total particle flux ($r^2 = 0.15$; $p < 0.05$), its epimer increases during the cold period (2.6 ± 2.0 to 15 ± 9.2; $p < 0.005$) and it is inversely correlated to total particle flux ($r^2 = 0.49$; $p < 0.005$). This is in agreement with previous work in this area of Rio de la Plata estuary where the terrestrial runoff results in an enhanced discharge of organic compounds with a fresher signature during warm and rainy periods, in contrast with the less intense and more degraded signal observed during cold and dry months (Colombo et al., 2007c). Similarly, Puerari et al. (2012) observed an enhanced level of sewage degradation in the dry winter period in Brazilian rivers associated with a lower terrestrial runoff.

The sediment sterol profile was similar to that of settling material, with some minor differences related to sterol degradation at the sediment surface. At BA, this degradation is apparent in the relative increase of degradation products such as epicoprostanol, stigmastanol and cholestanol from settling particles (9.3% ± 9.6%, 1.6% ± 0.88% and 1.7% ± 1.2%) to underlying sediments (16% ± 4.5%, 2.6% ± 1.5% and 2.8% ± 1.1%, respectively, $p < 0.05$), reflecting the microbial reduction of stenols to stanols and coprostanol epimerization at the oxic–anoxic boundary (Wakeham, 1989). Despite these degradation processes, sediments at BA still have remarkably high sterol concentrations, especially of coprostanol whose concentration (349 ± 282 µg/g) is among the highest reported for surficial sediments severely impacted by sewage discharges (Table 2). The highest coprostanol values were chiefly measured in freshwater locations or in relatively enclosed seawater environments where ocean dilution is reduced. In sediments from the Uruguayan coast of the Rio de la Plata near Montevideo, Venturini et al. (2015) reported 17–400 times lower concentrations of coprostanol (0.05–21 µg/g) and cholesterol (0.48–5.1 µg/g), showing that the background levels of these sterols are quite low and that they derive mainly from local urban discharges at BA. Interestingly, at

Table 2
Coprostanol concentration (μg/g) from surficial sediments throughout the world.

Sampling site	Environment	Concentration	Reference
Highly polluted sediments			
Yucatan Cenotes, Mexico	Underground river	< 1–1690[a]	Arcega-Cabrera et al. (2014)
Northeastern Hamilton Harbour, Canada	Lake	< 1–1600	Coakley et al. (2002)
Rio de la Plata, Argentina	**River**	**59–708**	**This study**
Barcelona, Spain	Sea	< 1–390	Grimalt et al. (1990)
Iguaçu and Barigui Rivers, Brazil	River	< 1–375	Puerari et al. (2012)
Bilbao Estuary, Spain	Estuary	2.2–293	Gonzalez-[illegible]nd Saiz-Salinas (1998)
Barigui River, Brazil	River	< 1–196	Froehner et al. (2009)
Firth of Clyde, Scotland, United Kingdom	Sea	< 1–176	Kelly and Campbell (1996)
Tan-Shui Estuary Taiwan	Estuary	< 1–163	Jeng and Han (1994)
Northeastern Hamilton Harbour, Canada	Lake	< 1–147	Bachtiar et al. (1996)
Guanabara Bay, Brazil	Sea	1.4–105	Lima da Costa and Carreira (2005)
Kaoping River, Taiwan	River	< 1–58	Jeng and Han (1996)
Lake of Neuchatel, Switzerland	Lake	6.1–55	Pittet et al. (1990)
Ria Formosa, Portugal	Sea lagoon	< 1–42	Mudge and Bebbiano (1997)
Venice Lagoon, Italy	Sea lagoon	< 1–41	Sherwin et al. (1993)
Narrangansett Bay, USA	Sea	< 1–39	Le Blanc et al. (1992)
Rio de la Plata, Uruguay	River	< 1–21	Venturini et al. (2015)
Reference low-moderately polluted river sediments			
Siak River, Indonesia	River	0.050–11	Liebezeit and Wöstmann (2010)
Mississippi River, USA	River	0.10–7.5	Writer et al. (1995)
Capibaribe River, Brazil	River	0.52–7.3	Fernandes et al. (1999)
Uruguay River, Argentina	**River**	**nd–1.7**	**This study**
Santa Ana River, USA	River	nd–0.49	Noblet et al. (2004)
Langat River, Malaysia	River	0.0028–0.42	Adnan et al. (2012)

[a] Sum of fecal sterols.

BA the concentrations of phytosterols were only slightly lower than those for stigmasterol and campesterol (0.30–3.14 μg/g and 0.13–2.13 μg/g, respectively; Venturini et al., 2015), but not for sitosterol, which was 6–70 times lower (0.43–5.3 μg/g). This suggests that while sewage discharge significantly contributes sitosterol at BA sediments, terrestrial runoff is the main source of stigmasterol and campesterol. This is in agreement with previous reports of high concentrations of sitosterol in sewage effluents of domestic origin (e.g., flush of kitchen vegetable oils; Furtula et al., 2011). At N, the sediment sterol profile was dominated by terrestrial plant phytosterols and cholesterol, as observed in settling material, but with higher proportions of epicoprostanol, sitosterol and stigmastanol (2.7% ± 1.2%, 25% ± 3.0% and 12% ± 1.9%, respectively, $p < 0.05$). The marginal impact of sewage pollution at site N sediments is evidenced by low coprostanol concentrations, which are well below the threshold values reported as indicative of sewage pollution (0.1–0.7 μg/g; Grimalt et al., 1990; Leeming et al., 1997; Rada et al., 2016) and are comparable to values reported for riverine sites with low to moderate sewage pollution (Table 2).

To simultaneously evaluate the contribution of different sterols to the overall variability observed in settling material and sediments, PCA multivariate analysis was performed using the major sterols (compounds with < 0.5% abundance were excluded, Fig. 5). The first two principal components in the model explain 59% of the total variability, mainly through principal component 1 (47%), which is loaded in the negative side with fecal coprostanol, coprostanone and epicoprostanol and in the positive side with cholesterol and the plant sterols. The second component accounts for 12% of data variability and is negatively loaded with 24-ethylcoprostanol and dehydrocholesterol and positively loaded with cholestanol and epicoprostanol. Settling material from BA is clustered on the left side of the PCA, denoting fecal inputs, and is clearly discriminated from site N, plotting on the right due to the major contribution of plant sterols to the overall composition. The average sterol composition of human feces plots in the center of the BA cluster, further confirming the sewage origin of settling material at this site. The separation of sediments was similar to that of settling material, with minor differences reflecting the degradation that takes place at the water–sediment interface. BA sediments are scattered on the right, with most samples gathering near the epicoprostanol and cholestanol vectors, reflecting the degradation of coprostanol and cholesterol respectively. N sediments are more homogeneous and plotted on the upper right side of the PCA, close to cholesterol and stigmastanol vectors.

3.5. Sterol ratios

Sterol ratios have been routinely used to assess the contribution of different sources of organic matter as well as degradation processes (Jeng and Han, 1994; Takada et al., 1994; Chalaux et al., 1995; Fattore et al., 1996). All the ratios evaluated in this work presented highly significant differences between BA and N (*t*-test; $p < 0.0001$; Fig. 6). In settling material, the high coprostanol/(coprostanol + epicoprostanol) ratio at BA (0.85 ± 0.15) reflects the relatively fresh sewage inputs discharged, in contrast to the weak and highly degraded fecal signature at site N (0.48 ± 0.15). The coprostanol/(coprostanol + 24-ethylcoprostanol) ratio is 2 times higher in BA settling material relative to N (0.86 ± 0.064 vs 0.35 ± 0.19) indicating that the reduced fecal sterols at N are chiefly from the feces of herbivorous mammals. However, despite the overwhelming abundance of coprostanol at BA a small non-human contribution to the overall fecal signal cannot be disregarded. At this site, the sitosterol/(sitosterol + 24-ethylcoprostanol) index was 0.36 ± 0.15, in the range of values proposed by Nash et al. (2005) as typical for runoff of feces from herbivores such as cattle and pigs with high 24-ethylcoprostanol proportions. At N, this ratio (0.84 ± 0.17) was above the limit suggested as indicative of non-fecal polluted plant decay inputs (Nash et al., 2005), denoting minimum impact of fecal contamination at this site. The cholesterol/(cholesterol + cholestanol) ratio is useful to assess the microbial reduction of stenols to 5[illegible]anols that typically takes places under anoxic conditions (Re[illegible], 2005). At BA, the relatively low values of this ratio (0.85 ± 0.036) indicate prevailing reductive conditions in the sewage effluent, which favors sterol preservation. In contrast, oxic conditions at N favors the

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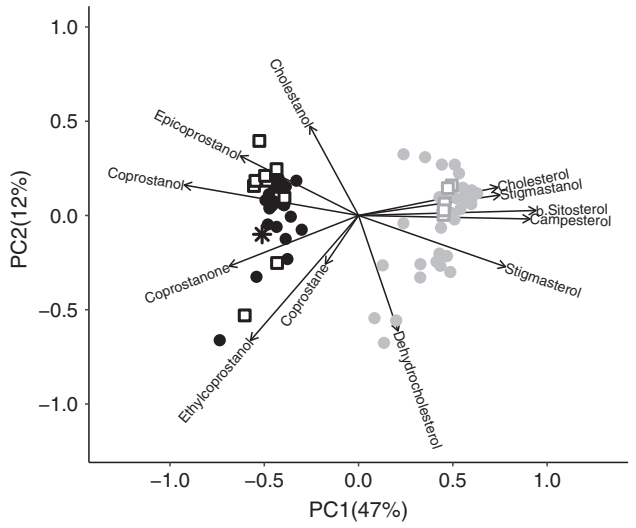

Fig. 5. Principal component analysis of sterol composition of settling particles (solid circles) and sediments (open squares) from Buenos Aires (black) and North (gray). The black asterisk corresponds to the average sterol composition of human feces according Leeming et al. (1996).

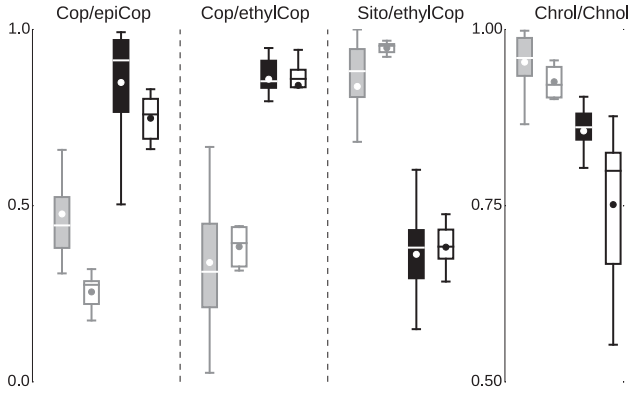

Fig. 6. Box plots of different sterol ratios from Buenos Aires (black) and North (gray) in settling material (filled boxes) and sediment (open boxes). Copr/epiCop: coprostanol/(coprostanol + epicoprostanol), Cop/ethylCop: coprostanol/(coprostanol + 24-ethylcoprostanol), Sito/ethylCop: sitosterol/(sitosterol + 24-ethylcoprostanol, Chnol/Chrol: cholesterol/(cholesterol + cholestanol) All ratios were significantly different between Buenos Aires and North site ($p < 0.0001$).

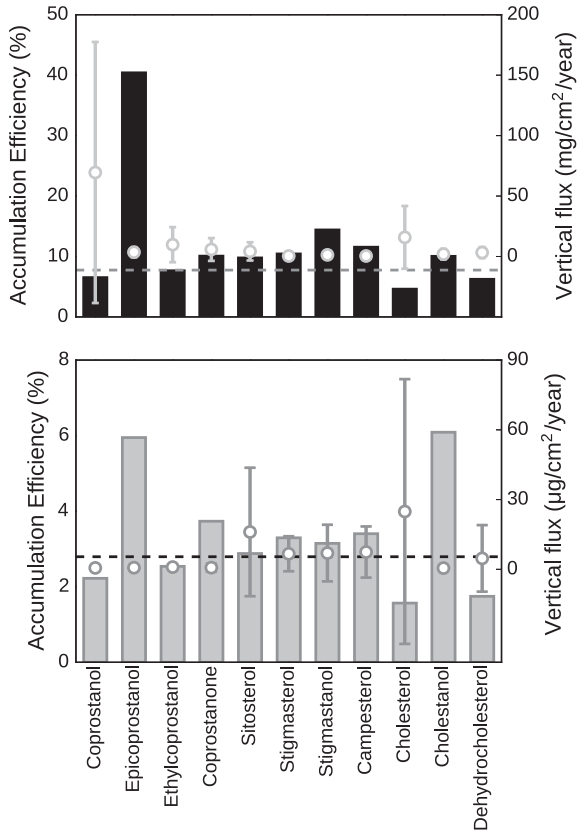

Fig. 7. Accumulation efficiencies of sterols from settling material in superficial sediments (%, bars, left axis) and vertical fluxes (points with standard error bars, right axis) for Buenos Aires (upper panel) and North (bottom panel). Horizontal dotted lines indicate accumulation efficiency of total sterols. Minor sterols (< 1% of total sterols) were excluded from the calculations.

sterol degradation over their hydrogenation (Nishimura and Koyama, 1977), resulting in proportionally low amounts of cholestanol (ratio: 0.95 ± 0.043).

In the sediments, these ratios exhibited the same geographical differences observed in the settling material, but reflected the diagenetic processes that take place at the sediment surface. The degradation of coprostanol and cholesterol, which is intensified after particle deposition, resulted in lower coprostanol/(coprostanol + epicoprostanol) ratio (BA: 0.75 ± 0.064, N: 0.26 ± 0.058) and cholesterol/(cholesterol + cholestanol) ratio (BA: 0.75 ± 0.11, N: 0.93 ± 0.025) in sediments relative to settling material.

3.6. Sterol vertical fluxes and accumulation efficiency

The vertical flux of total sterols was highly variable and averaged 116 ± 168 mg/cm^2/year at BA, with coprostanol accounting up to 60% (70 ± 108 mg/cm^2/year, Fig. 7). At N, the sterol flux was four orders of magnitude lower (0.070 ± 0.13 mg/cm^2/year) and cholesterol and sitosterol were the sterols with the highest fluxes. The accumulation efficiencies, obtained from the difference between sterol deposition based on trap fluxes and the inventories estimated from the observed sediment concentrations allow an evaluation of the early diagenetic behavior of these compounds. The accumulation efficiencies were 2–7 times higher at BA compared with N, but the general pattern of accumulation efficiency of individual sterols was rather similar at both sampling sites. The higher accumulation efficiencies at BA reflect the variation in vertical fluxes and the differences in the oxic–anoxic transition of the sediments and the greater preservation of organic matter at sites with fast burial (Hedges and Keil, 1995). At BA, the high sedimentation rate rapidly removes sterols to anoxic black-colored sediments, favoring their preservation. In contrast, at N the oxic layer is thicker resulting in a greater aerobic degradation of sterols. Epicoprostanol showed the highest accumulation efficiency, especially at BA (BA: 40%, N: 5.9%) probably due to in situ microbial epimerization of coprostanol rather than to an enhanced preservation during deposition. Coprostanone accumulated more efficiently than coprostanol (BA: 10% vs 6.5%, N: 3.7% vs 2.2%). Since coprostanone and coprostanol belong to the same metabolic pathway and can readily interconvert (Grimalt et al., 1990; Bull et al., 2002), the preferential preservation of coprostanone in sediments might be related to its higher resistance to biodegradation (Wakeham, 1989; Chaler et al., 2001). Plant sterols were in general well preserved (BA: 9.8–14%, N: 2.9–3.4%), as has been previously observed in the Saint Lawrence estuary (Colombo et al., 1997), possibly as a result of enhanced resistance of terrestrial sterols, associated with waxy higher plant material that hinder bacterial degradation (Volkman et al., 1987). Galeron et al. (2015) found that sitosterol has a low susceptibility to biodegradation and most of its

decomposition proceeds via autoxidation and photodegradation, a process that is especially intense on land where chlorophyll acts as a sensitizer. Cholesterol was the least preserved sterol (BA: 4.6%, N: 1.6%) reflecting the intense breakdown of this sterol, mostly through biodegradation (Galeron et al., 2015). This explains the high accumulation efficiency of cholestanol (BA: 10%, N: 6.1%), which originates from in situ microbial reduction of cholesterol rather than from preservation of settling cholestanol.

Despite the large spatial and temporal variability of hydrological parameters and sewage discharges, an attempt was made to compare the sediment burden of coprostanol with the expected discharge from the BA outfall. The massive vertical flux of coprostanol results in its rapid buildup in superficial sediments, which contain 24 ± 19 g/m^2 of this sterol in the top 5 cm layer. Human coprostanol excretion depends on multiple factors such as diet, water intake, lifestyle and genetic differences, with daily emission varying from < 0.2 to > 2 g/day per capita (Walker et al., 1982; Keller and Jahreis, 2004; Daughton et al., 2012). Considering an average coprostanol excretion of 1 g/day per capita and taking into account that the sewer network serves 6 × 10^6 people (www.aysa.com.ar), the expected sewer discharge of coprostanol can be roughly estimated to be 2200 tons/year. As previously discussed, coprostanol undergoes an extensive degradation at the water–sediment interface, so based on its accumulation efficiency estimated in this work (6.5%) from 2200 tons/year only 142 tons/year would be effectively preserved in sediments. Considering an average outfall plume area of 25 km^2 (Roberts and Villegas, 2016) in which most of the sewage material would accumulate, and a sedimentation rate of 4.7 cm/year, the expected coprostanol inventory for the top 5 cm layer would be 6.0 g/m^2 ([5 cm/4.7 cm/year] × [1.42 × 10^8 g/year/2.5 × 10^7 m^2]). This rough estimation, based on a homogenous coprostanol settling over the whole plume area, does not take into account the rapid coprostanol decrease usually observed with distance from sources (Venkatesan and Kaplan, 1990; Le Blanc et al., 1992; Bachtiar et al., 1996). Therefore, the expected coprostanol inventory (6.0 g/m^2) is lower than the one based on our measurements (24 ± 19 g/m^2), which considers sediments sampled close to the sewer outfall (0.5 km), where most of the coprostanol settling takes place.

4. Conclusions

The simultaneous analysis of sterols in settling material and underlying sediments allowed the identification of sources, the calculation of vertical fluxes and the evaluation of early diagenetic changes. The massive inputs of anthropogenic organic matter at the Buenos Aires (BA) area of the Rio de la Plata estuary cause remarkable alterations in the fluxes and signature of particulate sterols. Indeed, huge vertical fluxes of highly organic particles enriched in fecal sterols, i.e. coprostanol, comparable to raw sewage sludge are observed at this site. These anthropogenic discharges are further intensified during warm-rainy periods due to enhanced sewage discharge and terrestrial runoff. In contrast, at a relatively pristine northern site (N), vertical particle fluxes and particulate sterol concentrations are 3–7 orders of magnitude lower, with a composition dominated by plant sterols, i.e. sitosterol, stigmasterol and campesterol, derived from terrestrial vegetation. The sterols signature of underlying sediments reflects the early diagenetic alteration occurring at the water–sediment interface. Thus, compared to settling material, the concentrations decrease 10–20 times and the composition shows an enrichment of degradation products, i.e. cholestanol, epicoprostanol and stigmastanol. The accumulation efficiency of sterols in sediments varies according to the differential resistance of individual sterols and in situ production. Overall, the combination of higher sedimentation rates and prevailing anoxic conditions in the highly polluted BA site results in enhanced sterol preservation with a remarkably high coprostanol accumulation which is among the highest ever reported in the literature.

5. Uncited references

Canuel and Hardison (2016), Daughton (2012), Froehner and Fernandes (2009), Mudge and Lintern (1999), Reeves and Patton (2005) and Venturini et al. (2014). Q5

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.orggeochem.2017.11.013. These data include Google maps of the most important areas described in this article.

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References

Adnan, N.H., Zakaria, M.P., Juahir, H., Ali, M.M., 2012. Faecal sterols as sewage markers in the Langat River, Malaysia: integration of biomarker and multivariate statistical approaches. Journal of Environmental Sciences 24, 1600–1608.

Arcega-Cabrera, F., Velázquez-Tavera, N., Fargher, L., Derrien, M., Noreña-Barroso, E., 2014. Fecal sterols, seasonal variability, and probable sources along the ring of cenotes, Yucatan, Mexico. Journal of Contaminant Hydrology 168, 41–49.

Bachtiar, T., Coakley, J.P., Risk, M.J., 1996. Tracing sewage-contaminated sediments in Hamilton Harbour using selected geochemical indicators. Science of the Total Environment 179, 3–16.

Blanch, A.R., Belanche-Muñoz, L., Bonjoch, X., Ebdon, J., Gantzer, C., Lucena, F., Ottoson, J., Kourtis, C., Iversen, A., Kühn, I., Moce, L., Muniesa, M., Schwartzbrod, J., Skraber, S., Papageorgiou, G., Taylor, H.D., Wallis, J., Jofre, J., 2004. Tracking the origin of faecal pollution in surface water: an ongoing project within the European Union research programme. Journal of Water and Health 2, 249–260.

Bonachea, J., Bruschi, V.M., Hurtado, M.A., Forte, L.M., da Silva, M., Etcheverry, R., Cavallotto, J.F., Dantas, M.F., Pejon, O.J., Zuquette, L.V., Bezerra, M.O., Remondo, J., Rivas, V., Gómez-Arozamena, J., Fernández, G., 2010. Natural and human forcing in recent geomorphic change; case studies in the Rio de la Plata basin. Science of the Total Environment 408, 2674–2695.

Bull, I.D., Lockheart, M.J., Elhmmali, M.M., Roberts, D.J., Evershed, R.P., 2002. The origin of faeces by means of biomarker detection. Environment International 27, 647–654.

Burns, K.A., Hernes, P.J., Brinkman, D., Poulsen, A., Benner, R., 2008. Organic geochemistry dispersion and cycling of organic matter from the Sepik River outflow to the Papua New Guinea coast as determined from biomarkers. Organic Geochemistry 39, 1747–1764.

Canuel, E.A., Hardison, A.K., 2016. Sources, ages, and alteration of organic matter in estuaries. Annual Review of Marine Science 8, 409–434.

Chalaux, N., Takada, H., Bayona, J.M., 1995. Molecular markers in Tokyo Bay sediments: sources and distribution. Marine Environmental Research 40, 77–92.

Chaler, R., Simoneit, B.R., Grimalt, J.O., 2001. Bile acids and sterols in urban sewage treatment plants. Journal of Chromatography A 927, 155–160.

Christie, W.W., 1989. Gas Chromatography and Lipids: A Practical Guide. The Oily Press, Ayr.

Coakley, J.P., Skafel, M.G., Marvin, C.H., Bachtiar, T., 2002. Transport of sewage-contaminated sediment in northeastern Hamilton Harbour. Journal of Great Lakes Research 28, 77–90.

Colombo, J.C., Pelletier, E., Brochu, C., Khalil, M., 1989. Determination of hydrocarbon sources using n-alkane and polyaromatic hydrocarbon

distribution indexes. Case study: Rio de La Plata estuary, Argentina. Environmental Science and Technology 23, 888–894.
Colombo, J.C., Silverberg, N., Gearing, J.N., 1996a. Biogeochemistry of organic matter in the Laurentian Trough, I. Composition and vertical fluxes of rapidly settling particles. Marine Chemistry 51, 277–293.
Colombo, J.C., Silverberg, N., Gearing, J.N., 1996b. Lipid biogeochemistry in the Laurentian Trough: I—fatty acids, sterols and aliphatic hydrocarbons in rapidly settling particles. Organic Geochemistry 25, 211–225.
Colombo, J.C., Silverberg, N., Gearing, J.N., 1997. Lipid biogeochemistry in the Laurentian Trough—II. Changes in composition of fatty acids, sterols and aliphatic hydrocarbons during early diagenesis. Organic Geochemistry 26, 257–274.
Colombo, J.C., Capelletti, N., Lasci, J., Migoya, M.C., Speranza, E., Skorupka, C.N., 2005. Sources, vertical fluxes and accumulation of aliphatic hydrocarbons in coastal sediments of the Rio de la Plata Estuary, Argentina. Environmental Science and Technology 39, 8227–8234.
Colombo, J.C., Cappelletti, N., Migoya, M.C., Speranza, E., 2007a. Bioaccumulation of anthropogenic contaminants by detritivorous fish in the Río de la Plata Estuary: 1–aliphatic hydrocarbons. Chemosphere 68, 2128–2135.
Colombo, J.C., Cappelletti, N., Migoya, M.C., Speranza, E., 2007b. Bioaccumulation of anthropogenic contaminants by detritivorous fish in the Río de la Plata Estuary: 2–polychlorinated biphenyls. Chemosphere 69, 1253–1260.
Colombo, J.C., Cappelletti, N., Speranza, E., Migoya, M.C., Lasci, J., Skorupka, C.N., 2007c. Vertical fluxes and organic composition of settling material from the sewage impacted Buenos Aires coastal area, Argentina. Organic Geochemistry 38, 1941–1952.
Colombo, J.C., Cappelletti, N., Williamson, M., Migoya, M.C., Speranza, E., Sericano, J., Muir, D.C.G., 2011. Risk ranking of multiple-POPs in detritivorous fish from the Río de la Plata. Chemosphere 83, 882–889.
Colombo, J.C., Skorupka, C.N., Bilos, C., Tatone, L., Cappelletti, N., Migoya, M.C., Astoviza, M., Speranza, E., 2015. Seasonal and inter-annual variability of water quality in the Uruguay River, Argentina. Hydrological Sciences Journal 60, 1155–1163.
Daughton, C.G., 2012. Real-time estimation of small-area populations with human biomarkers in sewage. The Science of the Total Environment 414, 6–21.
deBruyn, A.M.H., Marcogliese, D.J., Rasmussen, J.B., 2003. The role of sewage in a large river food web. Canadian Journal of Fisheries and Aquatic Sciences 60, 1332–1344.
Di Gregorio, D.E., Fernández Niello, J.O., Huck, H., Somacal, H., Curutchet, G., 2007. ^{210}Pb dating of sediments in a heavily contaminated drainage channel to the La Plata estuary in Buenos Aires, Argentina. Applied Radiation and Isotopes 65, 126–130.
Fattore, E., Benfenati, E., Marelli, R., Cools, E., Fanelli, R., 1996. Sterols in sediment samples from Venice Lagoon, Italy. Chemosphere 33, 2383–2393.
Fernandes, M.B., Sicre, M.-A., Cardoso, J.N., Macêdo, S.J., 1999. Sedimentary 4-desmethyl sterols and *n*-alkanols in an eutrophic urban estuary, Capibaribe River, Brazil. The Science of the Total Environment 231, 1–16.
FREPLATA, 2005. Análisis Diagnóstico Transfronterizo del Río de la Plata y su Frente Marítimo. Documento Técnico. Proyecto PNUD/GEF/RLA/99/G31.
Froehner, S., Fernandes, R., 2009. Assessment of fecal sterols in Barigui River sediments in Curitiba, Brazil. Environmental Monitoring and Assessment 157, 591–600.
Furtula, V., Liu, J., Chambers, P., Osachoff, H., Kennedy, C., Harkness, J., 2011. Sewage treatment plants efficiencies in removal of sterols and sterol ratios as indicators of fecal contamination sources. Water, Air, and Soil Pollution 223, 1017–1031.
Galeron, M.-A., Amiraux, R., Charriere, B., Radakovitch, O., Raimbault, P., Garcia, N., Lagadec, V., Vaultier, F., Rontani, J.-F., 2015. Seasonal survey of the composition and degradation state of particulate organic matter in the Rhône River using lipid tracers. Biogeosciences 12, 1431–1446.
Gonzalez-Oreja, J.A., Saiz-salinas, J.I., 1998. Short-term spatio-temporal changes in urban pollution by means of faecal sterols analysis. Marine Pollution Bulletin 36, 868–875.
Grimalt, J., Fernínder, P., Bayona, J.M., Albaiges, J., 1990. Assessment of fecal sterols and ketones as indicators of urban sewage inputs to coastal waters. Environmental Science and Technology 1, 357–363.
Hedges, J.I., Keil, R.G., 1995. Sedimentary organic matter preservation: an assessment and speculative synthesis. Marine Chemistry 49, 81–115.
Helmer, R., Hespanhol, I., 1997. Water Pollution Control – A Guide to the Use of Water Quality Management Principles. F & FN Spon, London.
Huang, W.Y., Meinschein, W.G., 1979. Sterols as ecological indicators. Geochimica et Cosmochimica Acta 43, 739–745.
Jaime, P., Menéndez, A.N., 2002. Análisis del Régimen Hidrológico de los Ríos Paraná y Uruguay, Report INA-LHA 05-216-02. FREPLATA, Buenos Aires.
Jeng, W., Han, B., 1994. Sedimentary coprostanol in Kaohsiung harbour and the Tan-Shui estuary. Marine Pollution Bulletin 28, 494–499.
Jeng, W., Han, B., 1996. Coprostanol in a sediment core from the anoxic Tan-Shui Estuary, Taiwan. Estuarine, Coastal and Shelf Science 42, 727–735.
Jeng, W., Kao, S., 2002. Lipids in suspended matter from the human-disturbed Lanyang River, northeastern Taiwan. Environmental Geology 43, 138–144.
Keller, S., Jahreis, G., 2004. Determination of underivatised sterols and bile acid trimethyl silyl ether methyl esters by gas chromatography–mass spectrometry–single ion monitoring in faeces. Journal of Chromatography B 813, 199–207.
Kelly, A.G., 1995. Accumulation and persistence of pesticides and faecal sterols at the Garroch Head sewage sludge disposal site, Firth of Clyde. Environmental Pollution 88, 207–217.
Kelly, A.G., Campbell, L.A., 1996. Persistent organochlorine contaminants in the Firth of Clyde in relation to sewage sludge input. Marine Environmental Research 41, 99–132.
Kress, N., Shoham-Frider, E., Galil, B.S., 2016. Twenty two years of sewage sludge marine disposal monitoring in the Eastern Mediterranean Sea: impact on sediment quality and infauna and the response to load reduction. Marine Pollution Bulletin 110, 99–111.
Lahdelma, I., Oikari, A., 2006. Stratigraphy of wood-derived sterols in sediments historically contaminated by pulp and paper mill effluents. Journal of Paleolimnology 35, 323–334.
Le Blanc, L.A., Latimer, J.S., Ellis, J.T., Quinn, J.G., 1992. The geochemistry of coprostanol in waters and surface sediments from Narragansett Bay. Estuarine, Coastal and Shelf Science 34, 439–458.
Leeming, R., Ball, A., Ashbolt, N., Nichols, P., 1996. Using faecal sterols from humans and animals to distinguish faecal pollution in receiving waters. Water Research 30, 2893–2900.
Leeming, R., Latham, V., Rayner, M., Nichols, P., 1997. Detecting and distinguishing sources of sewage pollution in Australian inland and coastal waters and sediments. In: Eganhouse, R.P. (Ed.), Molecular Markers in Environmental Geochemistry. American Chemical Society, pp. 306–319.
Li, W., Dagaut, J., Saliot, A., 1995. The application of sterol biomarkers to the study of the sources of particulate organic matter in the Solo River system and Serayu River, Java, Indonesia. Biogeochemistry 31, 139–154.
Liebezeit, G., Wöstmann, R., 2010. Coprostanol in Siak River sediments, E Sumatra, Indonesia. Bulletin of Environmental Contamination and Toxicology 85, 585–588.
Lima da Costa, R., Carreira, R.S., 2005. A comparison between faecal sterols and coliform counts in the investigation of sewage contamination in sediments. Brazilian Journal of Oceanography 53, 157–167.
Martin-Creuzburg, D., Von Elert, E., 2009. Ecological significance of sterols in aquatic food webs. In: Arts, M.T., Brett, M., Kainz, M. (Eds.), Lipids in Aquatic Ecosystems. Springer, New York, pp. 43–64.
McCalley, D.V., Cooke, M., Nickless, G., 1981. Effect of sewage treatment on faecal sterols. Water Research 15, 1019–1025.
Meyers, P.A., Ishiwatari, R., 1993. Lacustrine organic geochemistry – an overview of indicators of organic matter sources and diagenesis in lake sediments. Organic Geochemistry 20, 867–900.
Milliman, J.D., Meade, R.H., 1983. World-wide delivery of river sediments to the oceans. The Journal of Geology 91, 1–21.
Moreira, D., Simionato, C.G., Gohin, F., Cayocca, F., Luz Clara Tejedor, M., 2013. Suspended matter mean distribution and seasonal cycle in the Río de La Plata estuary and the adjacent shelf from ocean color satellite (MODIS) and in-situ observations. Continental Shelf Research 68, 51–66.
Mudge, S.M., Bebbiano, M.J., 1997. Sewage contamination following an accidental spillage in the Ria Formosa, Portugal. Marine Pollution Bulletin 34, 163–170.
Mudge, S.M., Lintern, D.G., 1999. Comparison of sterol biomarkers for sewage with other measures in Victoria Harbour, B.C., Canada. Estuarine, Coastal and Shelf Science 48, 27–38.
Nash, D., Leeming, R., Clemow, L., Hannah, M., Halliwell, D., Allen, D., 2005. Quantitative determination of sterols and other alcohols in overland flow from grazing land and possible source materials. Water Research 39, 2964–2978.
Nguyen, D.K., Bruchet, A., Arpino, P., 1995. Determination of sterols in sewage sludge by combined *in situ* trimethylsylation/supercritical fluid extraction and GC/MS. Environmental Science and Technology 29, 1686–1690.
Nishimura, M., Koyama, T., 1977. The occurrence of stanols in various living organisms and the behavior of sterols in contemporary sediments. Geochimica et Cosmochimica Acta 41, 379–385.
Noblet, J.A., Young, D.L., Zeng, E.Y., Ensari, S., 2004. Use of fecal steroids to infer the sources of fecal indicator bacteria in the lower Santa Ana River Watershed, California: sewage is unlikely a significant source. Environmental Science and Technology 38, 6002–6008.
Parrish, C.C., Abrajano, T.A., Budge, S.M., Helleur, R.J., Hudson, E.D., 2000. Lipid and phenolic biomarkers in marine ecosystems: analysis and applications. In: Wangersky, P. (Ed.), The Handbook of Environmental Chemistry, Part D Marine Chemistry, vol. 5. Springer-Verlag, Berlin, pp. 193–223.
Pittet, A., Stettler, R., Kuebler, B., 1990. Use of coprostanol as a specific allochthonous fecal indicator in surface sediment of the Lake of Neuchâtel. Aquatic Sciences 52, 130–143.
Puerari, L., Carreira, R.S., Neto, A.C.B., Albarello, L.C., Gallotta, F.D.C., 2012. Regional assessment of sewage contamination in sediments of the Iguaçu and the Barigui Rivers (Curitiba city, Paraná, southern Brazil) using fecal steroids. Journal of the Brazilian Chemical Society 23, 2027–2034.
Puglisi, E., Nicelli, M., Capri, E., Trevisan, M., Del Re, A.A.M., 2003. Cholesterol, β-sitosterol, ergosterol, and coprostanol in agricultural soils. Journal of Environmental Quality 32, 466–471.
Rada, J.P.A., Duarte, A.C., Pato, P., Cachada, A., Carreira, R.S., 2016. Sewage contamination of sediments from two Portuguese Atlantic coastal systems, revealed by fecal sterols. Marine Pollution Bulletin 103, 319–324.
Reeves, A.D., Patton, D., 2005. Faecal sterols as indicators of sewage contamination in estuarine sediments of the Tay Estuary, Scotland: an extended baseline survey. Hydrology and Earth System Sciences 9, 81–94.
Roberts, P.J.W., Villegas, B., 2016. Modeling and design of the Buenos Aires outfalls. Journal of Hydraulic Engineering 143, 1–17.
Saliot, A., Méjanelle, L., Scribe, P., Fillaux, J., Pepe, C., 2001. Particulate organic carbon, sterols, fatty acids and pigments in the Amazon River system. Biogeochemistry 53, 79–103.

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Sherwin, M.R., Van Vleet, E.S., Fossato, V.U., Dolcit, F., 1993. Lagoonal sediments and mussels of Venice, Italy. Marine Pollution Bulletin 26, 501–507.

Speranza, E.D., Tatone, M.L., Cappelletti, N., Colombo, J.C., 2013. Cost-benefit of feeding on anthropogenic organic matter: lipid changes in a detritivorous fish (*Prochilodus lineatus*). Ichthyological Research 60, 334–342.

Sun, M., Wakeham, S.G., 1998. A study of oxic/anoxic effects on degradation of sterols at the simulated sediment–water interface of coastal sediments. Organic Geochemistry 28, 773–784.

Takada, H., Farrington, J.W., Bothner, M.H., Johnson, C.G., Tripp, B.W., 1994. Transport of sludge-derived organic pollutants to deep-sea sediments at Deep Water Dump Site 106. Environmental Science and Technology 28, 1062–1072.

Tatone, L.M., Bilos, C., Skorupka, C.N., Colombo, J.C., 2009. Vertical fluxes and accumulation of trace metals in superficial sediments of the Río de la Plata Estuary, Argentina. Bulletin of Environmental Contamination and Toxicology 83, 913–919.

Tatone, L.M., Bilos, C., Skorupka, C.N., Colombo, J.C., 2012. Trace metals in settling particles from the sewage impacted Buenos Aires coastal area in the Río de la Plata Estuary, Argentina. Bulletin of Environmental Contamination and Toxicology 90, 318–322.

Veiga, P., Juste, C., Lepercq, P., Saunier, K., Ge, P., 2005. Correlation between faecal microbial community structure and cholesterol-to-coprostanol conversion in the human gut. FEMS Microbiology Letters 242, 81–86.

Venkatesan, M.I., Kaplan, I.R., 1990. Sedimentary coprostanol as an index of sewage addition in Santa Monica Basin, southern California. Environmental Science and Technology 24, 208–214.

Venturini, N., Bíc[illegible]C., Taniguchi, S., Sasaki, S.T., García-Rodríguez, F., Brugnoli, E., Muniz, P., 2014. A multi-molecular marker assessment of organic pollution in shore sediments from the Río de la Plata Estuary, SW Atlantic. Marine Pollution Bulletin 91, 461–475.

Volkman, J.K., 2005. Sterols and other triterpenoids: source specificity and evolution of biosynthetic pathways. Organic Geochemistry 36, 139–159.

Volkman, J.K., 2016. Sterols in microalgae. In: Borowitzka, M.A., Beardall, J., Raven, J. A. (Eds.), The Physiology of Microalgae. Springer International Publishing, Switzerland, pp. 485–505.

Volkman, J.K., Farrington, J.W., Gagosian, R.B., 1987. Marine and terrigenous lipids in coastal sediments from the Peru upwelling region at 15°S: sterols and triterpene alcohols. Organic Geochemistry 11, 463–477.

Wakeham, S.G., 1989. Reduction of stenols to stanols in particulate matter at oxic–anoxic boundaries in sea water. Nature 342, 787–790.

Walker, R.W., Wun, C.K., Litsky, W., Dutka, B.J., 1982. Coprostanol as an indicator of fecal pollution. CRC Critical Reviews in Environmental Control 12, 91–112.

Writer, J.H., Leenheer, J.A., Barber, L.B., Amy, G.L., Chapra, S.C., 1995. Sewage contamination in the upper Mississippi River as measured by the fecal sterol, coprostanol. Water Research 29, 1427–1436.

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