**Sterols biomarkers settling material and sediments from contrasting areas of the Rio de la Plata basin**

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**Abstract:**

To complete

Keywords: Sterols, Sewage markers, Settling material, Rio de la Plata.

**Introduction**

To complete.

**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°43.329'S - 58°10.301'O) and a more pristine site ︡~200 km upstream on the Uruguay River, the Ñandubaysal bay (N, 33°05.270'S - 58°21.374'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 1.5m during 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 particle flux was computed as:

Sedimentation rate was calculated as:

The average settling material density (2.65 g.cm-3) was taken from previous work in the same sampling sites.

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 N station and averaged for each sediment trap deployment period (wholesale electricity market administration company: [www.cammesa.com](http://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](http://www.bdhi.hidricosargentina.gov.ar); Menendez, 2002).

Lipids were extracted ultrasonically with acetone:dichloromethane:petroleum ether (1:2:2). The extract was dried over anhydrous sodium sulfate and lipid content was determined gravimetrically. Deuterated sterols (deuterocholesterol and deuterositosterol, Steraloids, Inc., Newport, RI, steraloids.com) were added as internal standards. In order to avoid the interference of fatty acids, lipids were saponified with 1M 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 N,O-Bis(trimethylsilyl)trifluoroacetamide and trimethylchlorosilane (BSTFA:TMCS, 10:1v/v; AppliChem GmbH, Darmstadt, Germany, [www.applichem.com](http://www.applichem.com); Sigma-Aldrich, St. Louis, MO, USA, [www.sigmaaldrich.com](http://www.sigmaaldrich.com)) for 3 hours at 60ºC. The resulting trimethylsilyl derivatives were concentrated to dryness under nitrogen and resuspended in toluene prior analysis.



**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 a northern site in the Uruguay River (N).

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

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. Compounds were identified by comparison with authentic standards of 14 steroids (Steraloids, Sigma-Aldrich), literature data and interpretation of mass spectrometric fragmentation patterns. Quantification was performed using a 4-points calibration curve (0,2-50 μg ml-1) with authentic standards (Table 1). Peak areas were corrected according internal standard recoveries. Commercially standards were not available for some compounds (cholestenol, campestanol, 24-Ethylcoprostanol and γ-Sitosterol) which were quantified based on response factors of structurally related sterols.

**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) analysed in this work.

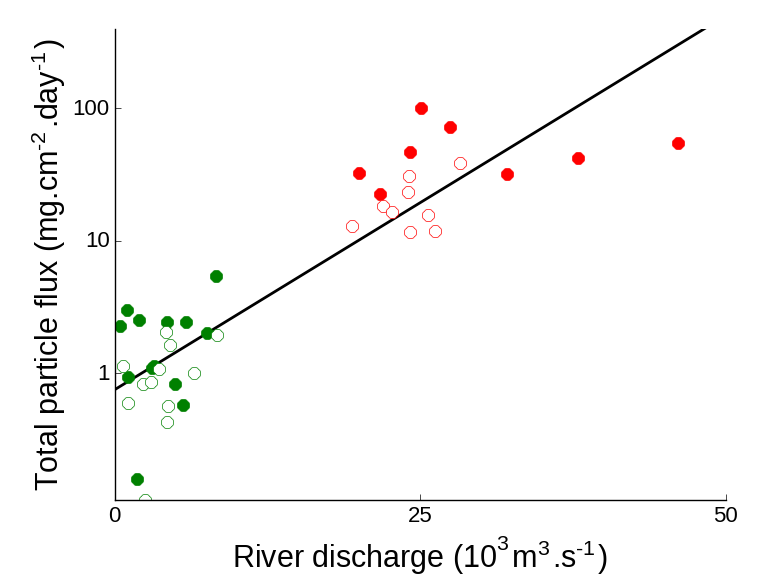
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| --- | --- | --- | --- | --- | --- | --- | --- |
| **Trivial name** | **IUPAC Name** | **Formula** | **MW** | **Rt** | **Target ion** | **Confirmatory ions** | |
| Coprostane | 5β -Cholestane | C27H48 | 372.67 | 30.80 | 217 | 357 | 372 |
| Coprostanol | (3β,5β)-Cholestan-3-ol | C27H48O | 388.67 | 35.57 | 370 | 355 | 215 |
| Epicoprostanol | (3α,5β)-Cholestan-3-ol | C27H48O | 388.67 | 36.08 | 370 | 215 | 355 |
| Cholestanol | (3β,5α)-Cholestan-3-ol | C27H48O | 388.67 | 36.16 | 215 | 355 | 370 |
| Coprostanone | (5α)-Cholestan-3-one | C27H46O | 386.65 | 37.13 | 386 | 231 | 370 |
| Deuterocholesterol | (3β)-Cholest-5-en-3-ol-25,26,26,26,27,27,27-D7 | C27H46O | 393.70 | 37.31 | 129 | 336 | 375 |
| Cholesterol | (3β)-Cholest-5-en-3-ol | C27H46O | 386.65 | 37.48 | 329 | 129 | 368 |
| Dehydrocholesterol | (3β)-Cholesta-5,7-dien-3-ol | C27H44O | 384.64 | 37.73 | 215 | 445 | 355 |
| Brassicasterol | (3β,22E)-Ergosta-5,22-dien-3-ol | C28H46O | 398.66 | 38.19 | 456 | 129 | 366 |
| Desmosterol | (3β)-Cholesta-5,24-dien-3-ol | C27H44O | 384.64 | 38.36 | 129 | 343 | 253 |
| Ergosterol | (3β)-Ergosta-5,7,22-trien-3-ol | C28H44O | 396.65 | 39.17 | 343 | 337 | 468 |
| Campestanol | (3β,5α,24R)-Ergostan-3-ol | C28H50O | 402.70 | 39.75 | 343 | 129 | 384 |
| Campesterol | (3β,24R)-Ergost-5-en-3-ol | C28H48O | 400.68 | 39.92 | 343 | 129 | 382 |
| 24-Ethylcoprostanol | (3β,5β,24S)-Stigmastan-3-ol | C29H52O | 416.72 | 40.19 | 398 | 215 | 383 |
| Stigmasterol | (3β,22E)-Stigmasta-5,22-dien-3-ol | C29H48O | 412.69 | 40.55 | 129 | 255 | 484 |
| γ-Sitosterol | (3β,24S)-Stigmast-5-en-3-ol | C29H50O | 414.71 | 41.82 | 129 | 473 | 488 |
| Deutero-β-Sitosterol | (3β)-Stigmast-5-en-3-ol-25,26,26,26,27,27,27 -D7 | C29H50O | 421.75 | 42.00 | 129 | 364 | 403 |
| β-Sitosterol | (3β)-Stigmast-5-en-3-ol | C29H50O | 414.71 | 42.20 | 129 | 488 | 473 |
| Stigmastanol | (3β)-Stigmastan-3-ol | C29H52O | 416.72 | 42.59 | 215 | 473 | 488 |

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 0,2-50 μg ml-1 . LODs values averaged……., ranging from () to (). 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 (R2>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). Detailed information on method performance is provided in S1.

Statistical analysis was carried on with Python scripting language, using SciPy, NumPy, MatPlotlib and pandas libraries. Multivariate analyses was executed in *R* language, using ggplot2 and ggbiplot packages. Data were expressed as mean ± SD. Relative standard deviation (RSD: [data – mean].100.SD-1) 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)-1. 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−Xy−1, where X = mean and y = S.D.). Forward stepwise multiple regression (*P-to-enter*<0·05) was used to identify the variables that best accounted for the observed temporal variation in sterol vertical flux.

**Results**

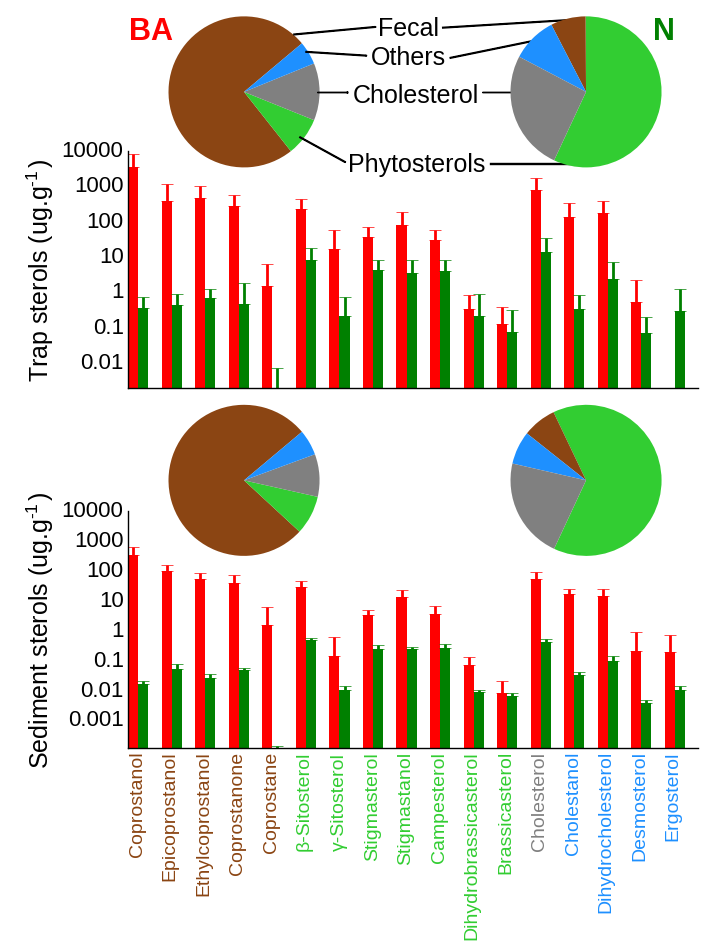
Total particle flux presented clear spatial variation with average values 10 times higher at BA relative to N (34±24 vs. 3.5±1.1 mg.cm2.day-1, respectively). This difference between BA and N was reflected in sedimentation rate (4.7±3.3 cm.year-1 vs. 1.3±0.78 cm.year-1). The settling material flux was largely dependent on river discharge which was 6-46 times higher at BA (19465-46088m3.s-1) relative to N (420-8410 m3.s-1), fitting an exponential curve (R2 = 0.78, *p*<0.0001; Fig. 2).



**Fig. 2.** Relationship between river discharge and total particle flux at Buenos Aires (red circles) and North (green circles) sites during warm (September to March, solid circles) and cold months (April to August, empty circles). Note the logarithmic scale on the y-axis.

Total sterol concentration in settling material was highly variable (RSD: 113%) and also exhibited a marked geographical difference, with 2-3 orders of magnitude higher values at BA relative to N (7140±7905 vs. 41±47 μg.g-1 dry weight). Total sterols in sediments were 10-20 times lower than in settling material, were less variable (RSD: 10-61%) but also presented a 2-3 orders of magnitude difference between BA and N (708±454 vs. 1.9±0.18 μg.g-1).

Sterol composition, both in settling material and sediments, showed contrasting differences between BA and N (Fig. 3, S2). At BA, the settling material composition was dominated by faecal sterols (75±5.4% of total sterols), mostly coprostanol (52±11%), followed by cholesterol (12±2.9%) and phytosterols (8.3±3.6%), mainly represented by β–sitosterol (4.4±1.9%). At N, phytosterols were the main constituents, making up 57±13%, basically through β–sitosterol (19±5.4%), stigmasterol (15±7.9%) and campesterol (13±11%), followed by cholesterol (26±12%) and only 7.5±7.0% of faecal sterols, mainly ethylcoprostanol (3.9±4.7%) and, to a lesser extent, coprostanol (1.3±1.3%). The change in percentage composition with total sterol concentration also showed geographical differences. At BA, as total sterol concentration increased, coprostanol proportion raised (r = 0.55; *p*<0.005) while stigmasterol and campesterol (r = -0.56 and 0.64; *p*<0.005) decreased and remaining sterol proportions were not correlated. At N, there was a strong significant correlation of total sterols with cholesterol proportion (r = 68; *p*<0.0001) and an inverse relationship with ethylcoprostanol and stigmasterol (r = -0.39 and -0.43 respectively; *p*<0.05). The sediment sterol profile, on a percentage basis, was similar to that of settling material, with some minor differences: higher epicoprostanol, stigmastanol and cholestanol proportions (16±4.5, 2.6±1.5 and 2.8±1.1%, respectively, *p*<0.05) and lower cholesterol (9.6±3.9%, *p*<0.05) at BA; higher epicoprostanol, β-sitosterol and stigmastanol proportions at N (2.7±1.2, 25±3.0 and 12±1.9%, respectively, *p*<0.05).



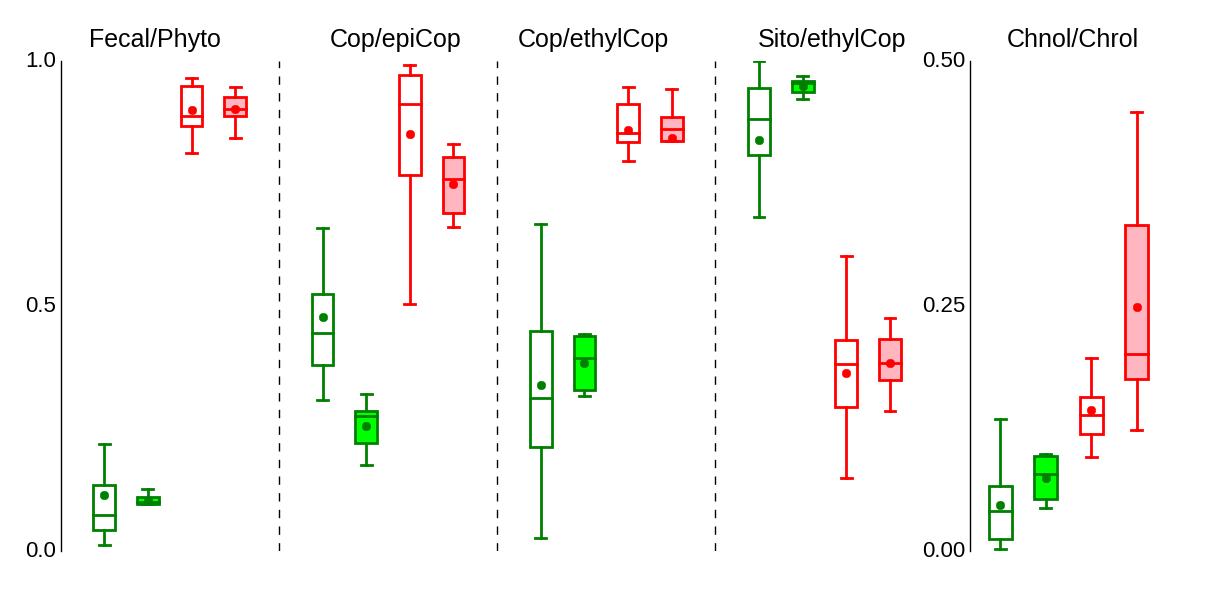
**Fig. 3.** Sterol composition of settling material (top panel) and sediments (bottom panel) at Buenos Aires (BA, red, left pie chart) and North (N, dark green, right pie chart). Pie charts show proportions of cholesterol, fecal sterols, phytosterols and other sterols. Bar graphs show individual sterols concentrations, in a dry weight basis (note the logarithmic scale). Colours of sterols labels correspond to the pie charts grouping (brown for faecal sterols, green for phytosterols, grey for cholesterol and blue for the remaining sterols analysed in this study).

Vertical flux of total sterol was highly variable and averaged 116±168 mg.cm-2.year-1 at BA, with coprostanol accounting up to 60% of it (69±108 mg.cm-2.year-1, Table 2). At N, sterol flux was four times lower, 29±44 μg.cm-2.year-1 and cholesterol and β–sitosterol were the sterols with the highest fluxes. The accumulation efficiency, used to evaluate the preservation of sterols from settling material to superficial sediments, was 2-7 times higher at BA compared with N. The general pattern of accumulation efficiency of individual sterols was rather similar for both sampling sites. Epicoprostanol was the better preserved sterol (BA: 40, N: 5.9%), followed by phytosterols (BA: 9.8-14, N: 2.9-3.4), cholestanol (BA:10, N: 6.1) and coprostanone (BA: 10, N: 3.7). Cholesterol was the lest preserved sterol (BA: 4.6, N: 1.6).

**Table 2.** Accumulation efficiency of sterols from settling material in superficial sediments (%) and vertical flux (expressed between parentheses as mg.cm-2.year-1 for BA and as μg.cm-2.year-1 for N). Minor sterols (<1% of total sterols) were excluded from calculations.

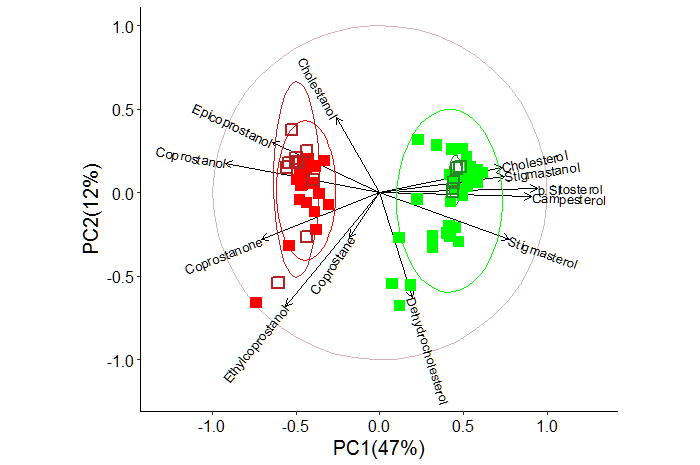
|  |  |  |
| --- | --- | --- |
|  | **Site** | |
| **Compound** | **BA** | **N** |
| Epicoprostanol | 40  (3.6±4.1) | 5.9  (0.36±0.55) |
| Stigmastanol | 14  (1.3±1.6) | 3.1  (2.8±4.2) |
| Campesterol | 12  (0.42±0.57) | 3.4  (3.3±4.4) |
| Cholestanol | 10  (2.0±4.3) | 6.1  (0.30±0.68) |
| Coprostanone | 10  (5.8±9.4) | 3.7  (0.49±1.4) |
| Stigmasterol | 10  (0.40±0.36) | 3.3  (3.2±4.3) |
| β-Sitosterol | 9.8  (4.2±7.5) | 2.9  (6.6±9.9) |
| 24-Ethylcoprostanol | 7.6  (9.8±15) | 2.5  (0.45±0.54) |
| Coprostanol | 6.5  (69±108) | 2.2  (0.30±0.42) |
| Dehydrocholesterol | 6.2  (3.2±4.6) | 1.7  (1.8±4.6) |
| Cholesterol | 4.6  (16±26) | 1.6  (9.7±19) |
| **Total** | **7.4**  **(116±168)** | **3.6**  **(29±44)** |

In order to discriminate different organic matter sources and to assess different pathways of sterol degradation in settling material, several sterol ratios were evaluated (Fig. 4). All the ratios presented highly significant differences between BA and N (*t*-test; *p*<0.0001). The ratio between faecal sterols and phytosterols was 8 times higher at BA than at N (0.90±0.044 vs 0.12±0.10). The β–sitosterol/24-ethylcoprostanol index, also used to evaluate the contribution of faecal and plant sterols (Nash et al., 2005) was 2 times higher at N (0.36±0.15 vs. 0.84±0.17). The coprostanol/epicoprostanol and the cholestanol/cholesterol ratios, used to assess the degradation of the sterol signal (Fattore et al., 1996, Chalaux et al., 1995), were 2-3 times higher at BA (0.85±0.15 vs 0.48±0.15 and 0.14±0.036 vs 0.046±0.041, respectively). The relationship between coprostanol and 24-ethylcoprostanol, useful to distinguish between different faecal soures of sterols (Leeming et al., 1996) was 2 times higher at BA (0.86±0.064 vs 0.35±0.19).



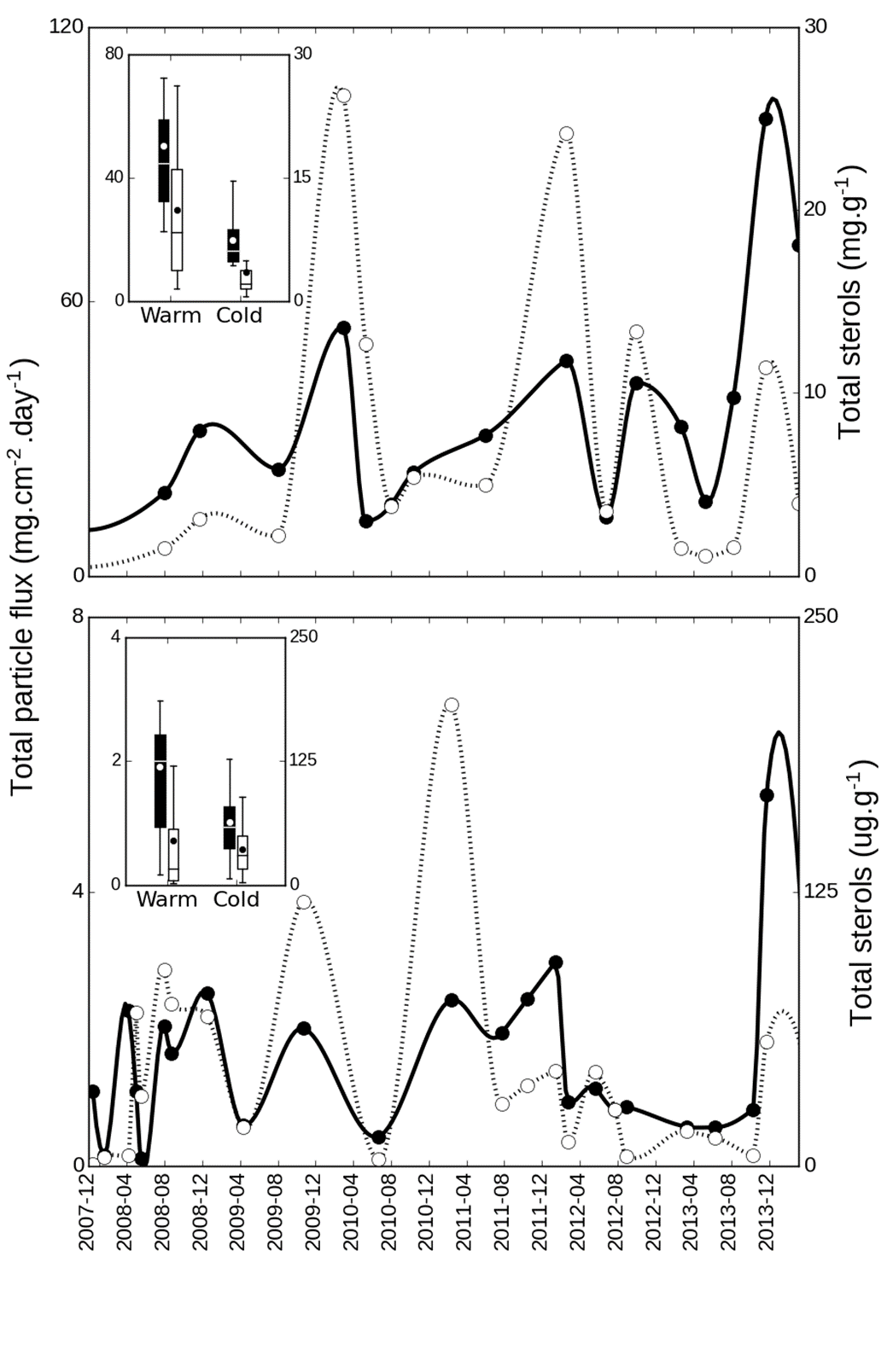
**Fig. 4.** Box plots of different sterol ratios from North (green) and Buenos Aires (red) in settling material (hollow boxes) and sediment (filled boxes). Fecal/Phyto: Fecal sterols/phytosterols, Copr/epiCop: Coprostanol/Epicoprostanol, Cop/ethylCop: Coprostanol/24-Ethylcoprostanol, Sito/ethylCop: β-sitosterol/24-Ethylcoprostanol, Chnol/Chrol: Cholestanol/Cholesterol All ratios were significantly different between North and Buenos Aires (p<0.0001).

To simultaneously evaluate the contribution of the different sterol to overall variability in settling material and sediments, multiple regression and multivariate analysis (PCA) was performed (sterols with average concentrations lower than 0.5% were excluded from analysis, Fig.). This model explain 59% of total variability, mainly through principal component 1 (47%), which is loaded in the negative side with coprostanol and epicoprostanol and in the positive side with cholesterol and plant sterols.



**Fig. 5.** Principal component analysis of sterol composition of settling particles (solid squares) and sediments (empty squares) from Buenos Aires (red) and North (green). Confidence ellipses were set to 90% of normal probability.

In addition to the spatial differences there were clear temporal variations in particle flux and sterol concentrations. Despite the large variability, there is a significant temporal pattern at BA of higher particle fluxes during warm months (September to March, 50±25 mg.cm2.day-1) relative to cold ones (April to August, 20±9.4 mg.cm2.day-1, *p*<0.005; Fig. 6). A similar seasonal variation (1.9±1.2 vs. 1.0±0.60 mg.cm2.day-1 at warm and cold months, *p*<0.05) was observed at N. Total sterol concentration at BA were significantly correlated with total particle flux (r = 0.64, *p*<0.05) and follows its temporal variation, raising during warm months (11163±9599 μg.g-1) and decreasing significantly during cold ones (3564±3711 μg.g-1; *p*<0.05, Fig. 6). At N, sterols were also significantly correlated with particle flux (r = 0.60, *p*<0.05), but there was no difference between warm and cold months (45±61 vs. 36±28 μg.g-1 respectively). The sterol composition, on a percentage basis, showed little temporal variation except for coprostanol and epicoprostanol at BA. While coprostanol proportion was higher at warm months than at cold ones (59±9.5 vs 45±8.7; *p*<0.01) and was correlated with total particle flux (*r*: 0.38; *p*<0.05), its epimer showed an opposite trend, increasing at cold months (2.6±2.0 vs 15± 9.2; *p*<0.005) and inversely correlating to total particle flux (*r*: -0.70; *p*<0.005).



**Fig. 6.** Temporal variation of total particles 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 shows 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).

**Discussion**

The intense discharge of one of the largest sewer outfall worldwide at BA results in an extraordinarily high sedimentation rate of 4.7±3.3 cm.year-1, in agreement with previous measurements at this area (5.5 ± 2.1 cm.year-1, Colombo et al., 2007). This value is much higher than sedimentation rates reported for nearby areas of this turbid estuary (0.3 -1.3 cm.year-1; Di Gregorio et al., 2007; Bonachea et al., 2010), suggesting than most particles captured by sediment traps at BA are highly organic detritus derived from urban-industrial discharges. This is supported by the high lipid content previously measured in BA settling material (11±7.4 mg.g-1), which was 27-times higher than at the Parana river (0.41±0.28 mg.g-1), which contributes the most to the solid load of the Rio de la Plata estuary (Speranza et al., 2013). The sedimentation rate at N was comparable to values reported by Colombo et al. 2015, which also showed a high variability (1.0±0.88 cm.year-1, range: 0.27-2.7 cm.year-1). In contrast to BA settling material composed of anthropogenic detritus over the background particle load derived from Parana River, the settling material at N came basically from the much smaller solid discharge of the Uruguay River (Moreira et al. 2013 from Jaime and Menendez 2002). The correlation between total particle flux and river discharge has been previously observed at the Uruguay River and reflects the enhanced transport of eroded material as river flow increases (Colombo et al. 2015).

The tendency of sterols to associate with particulate matter due to their high hydrophobicity is enhanced by the high organic content of settling particles at BA (total organic carbon: 7.7±5.5%; Colombo et al. 2007), resulting in very high sterol concentrations at this site (~7 mg.g-1). Previous studies dealing with sterols in settling particles were mostly based in ocean waters, relatively deep and clear, which had average concentrations 1-4 orders of magnitude lower compared to this shallow and turbid freshwater environment (Colombo et al. 1996; Parrish et al., 2000; Takada et al., 1994; Burns et al., 2008). In fact, total sterol concentrations in BA settling material are comparable to values reported for sewage sludge from wastewater treatment plants (2-9 mg.g-1, Venkatesan and Kaplan 1990; Kelly 1995; Nguyen et al., 1995). The sterol composition of settling material at BA has a clear fecal signature, resembling the composition of human faeces (fecal sterols: 85%, phytosterols: 8.8%, cholesterol: 5.2%, others: 1.2%; Leeming et al. 1996), reflecting the massive discharges of crude sewage at this site. Moreover, the coprostanol proportion of BA settling material felt in the 50-80% range found in sewage sludges and effluents (Venkatesan and Kaplan 1990). Nevertheless, the presence of smaller proportions of epicoprostanol, originated from microbial degradation of coprostanol evidence an incipient degradation of the fecal material. Taking into account that the length of the sewer network (7000 km, main sewer: 35km, Roberts and Villegas, 2016) implies a relatively large residence time of fecal material in this system before reaching the river, a preliminary degradation is likely to occur in sewer pipeline rather than in the very shallow (3-4 m) water column. The presence of a relatively important proportion of ethylcoprostanol is usually associated with non-human (herbivore) fecal pollution since it is the product of microbial hydrogenation of β–sitosterol, the main sterol in terrestrial vegetation. However, human feces can include significant amounts of ethylcoprostanol (Leeming et al. 1996) so the mere presence of this sterol cannot be unambiguously attributed to herbivore fecal contribution. The significance of coprostanone 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). Phytosterols in BA settling material were present in low proportions reflecting the minor contribution of vegetal inputs at this site. Despite the relative abundance of cholesterol, its utility as biomarker is rather limited since it is present in sewage material but also in multiple organic matter sources, such as algae, zooplankton and benthic fauna (Mudge et al. 1999; Creuzberg and von Elert 2009).

At N, both the sterol concentration and composition were in clear contrast with BA. The ~170-times smaller total sterol concentrations are comparable to values reported in particulate matter from tropical and subtropical riverine environments (1-184 μg.g-1; Saliot et al., 2001; Li et al 1995; Jeng and Kao 2002) reflecting natural organic matter inputs. Overall, the sterol profile presented a predominant vegetal signature. Despite being found in some algae species, the three major phytosterols at N, β-sitosterol, stigmasterol and campesterol, are strongly associated with land plants (Huang and Meinschein 1979, Volkman 2005), to such an extent that they have used as biomarkers of paper mill pollution (Lahdelma and Oikari 2006). The fecal sterols signal differs both quantitatively and qualitatively from the typical sewage signature observed at BA. The presence of ethylcoprostanol as the main fecal sterol at N probably reflects the contribution of cattle fecal matter from the neighboring livestock establishments.

The difference between settling material and sediments in terms of sterol concentrations and composition reflects the tendency of sterol to degrade at the water-sediment interfase, especially under oxic conditions (Sun and Wakeham 1998). This degradation is manifest in the increase of epicoprostanol, stigmastanol and cholestenol proportions from settling particles to sediment, due to the microbial reduction of stenols to stanols that takes places at the oxic-anoxic boundary (Wakeham 1989). Nevertheless, sediments at BA still had remarkably high sterol concentrations, especially of fecal sterols. Coprostanol concentration is among the highest reported for surficial sediments severely impacted by sewage discharges (Table 3). Most of the highest coprostanol levels were 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-1,) and cholesterol (0.48-5.1 μg.g-1), evidencing that these sterols derive mainly from the urban discharges at BA. Interestingly, the concentrations of phytosterols reported by Venturini et al. were only slightly lower to those of BA for stigmasterol and campesterol (0.30-3.14 and 0.13-2.13 μg.g-1, respectively) but not for β–sitosterol, which was 6-70 times lower (0.43-5.3 μg.g-1). This suggests that while terrestrial runoff is the main source of stigmasterol and campesterol at BA sediments, sewage discharge contributes significantly to β–sitosterol levels. These is in agreement with previous report of high concentrations of β–sitosterol in sewage effluents of domestic origin (e.g. flush of kitchen vegetable oils; Furtula et al., 2011).

At N, the low sterol concentration of sediments, comparable to other scarcely polluted riverine sites (Waterson and Canuel, 2008; Fernendes et al. 1999; Adnan et al. 2012) and its composition, dominated by land phytosterols and cholesterol, indicate that plant-derived terrestrial inputs is the main organic matter source as observed in the settling material. The marginal impact of sewage pollution at N sediments is evidenced by the low coprostanol concentrations, which are well below the threshold values reported as indicative of sewage pollution (0.1-0.7 μg.g-1; Grimalt et al. 1990; Leeming et al. 1997; Rada et al. 2015).

**Table 3.** Coprostanol concentration (μg.g-1) from highly polluted surficial sediments throughout the world.

|  |  |  |  |
| --- | --- | --- | --- |
| Sampling site | Environment | Concentration | Reference |
| Yucatan Cenotes, Mexico | Underground river | <1-1690\* | 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 and Albaiges, 1990 |
| Iguaçu and Barigui Rivers, Brazil | River | <1-375 | Puerari et al. 2012 |
| Bilbao Estuary, Spain | Estuary | 2.2-293 | Gonzalez-Oreja and 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 1995 |
| 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 et al. 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 |

\*: Sum of fecal sterols.

The accumulation efficiencies, obtained from the difference between sterol deposition (trap fluxes) and sediment concentrations allow an evaluation of the early diagenesis of these compounds. The higher accumulation efficiencies at BA compared with N reflect the differences in the oxic-anoxic transition of the sediments and the greater preservation of organic matter at sites with faster burial (Hedges and Keil, 1995). At BA, the high sedimentation rate rapidly moves the sterols to anoxic black-colored sediment, less diagenetically active, in which the sterols are well preserved. On the contrary, at N the oxic layer is thicker resulting in a greater aerobic degradation of sterols. The high epicoprostanol accumulation in sediments, especially at BA, is more probably associated to *in-situ* microbial epimerization of coprostanol rather than to an enhanced preservation during deposition. Since coprostanone and coprostanol belong to the same metabolic pathway and can readily interconvert (Grimalt et al., 1990; Bull et al., 2002), the preferential coprostanone preservation in sediments might be related to its higher resistance to biodegradation (Wakeham 1989; Chaler et al., 2001). The higher accumulation efficiency of plant sterols was previously observed by Colombo et al. (1997) and was attributable to the 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 β–sitosterol have 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. The low cholesterol preservation results from the intense breakdown of this sterol, mostly through biodegradation (Galeron et al., 2015). This explain the high accumulation efficiency of cholestanol, which results from *in situ* microbial reduction of cholesterol rather than from preservation of settling cholestanol.

Many sterol ratios (more than 15 in the reviewed literature) has been routinely used to assess the contribution of different sources of organic as well as degradation processes (Venkatesan and Kaplan, 1990; Grimault et al., 1990; Jeng and Han, 1994; Quemeneur and Marty, 1994; Takada et al., 1994; Chalaux et al., 1995; Fattore et al., 1996). Since many of them provide redundant information, only five have been applied to settling material in this work. BA and N represented the endmembers of fecal/phytosterol ratios in this basin showing the ample variation of the contribution of sewage-derived material over the background inputs of terrestrial land plant runoff. The high coprostanol/epicoprostanol ratio reflects the fresh degraded sewage inputs discharged at BA, in contrast to the weak and extensively degraded fecal signature of N, with a low ratio (~50% of coprostanol converted to epicoprostanol) typical of aged fecal material (Mudge and Duce, 2005). Coprostanol degradation continues after particle deposition, resulting in lower coprostanol/epicoprostanol ratios in sediments relative to settling material. As previously noted, the fecal sterol profile differs qualitatively between both contrasting sites as demonstrate the coprostanol/ethylcoprostanol ratio. While at BA, the high ratio corresponds to the abundance of coprostanol in human feces, at N the low values evidence the input of herbivore mammal feces, rich in ethylcoprostanol. In fact, these values fall in the extremes of this relationship according to Leeming et al. (1997), with BA ratio surpassing the 0.73 threshold for exclusively human fecal pollution and N ratio below the 0.38 threshold for solely herbivore fecal pollution. However, the overwhelming abundance of coprostanol at BA may lead to erroneously neglect the non-human fecal pollution at this site. At BA, the sitosterol/ethylcoprostanol ratio, used to assess herbivore fecal pollution, was below the threshold of 1.0 (equivalent to 0.5 for the equation used in this work) proposed as typical of cow feces runoff (Nash et al. 2005). Is important to note that beside cattle, the fecal contribution of other animals with high ethylcoprostanol proportions in their feces, such as pigs and poultry also affect this ratio (Leeming et al. 1996). This reveals a small non-human contribution to the overall fecal pollution at BA, mostly determined by human inputs. At N, this ratio was above the limit suggested by Nash et al. (2005) as indicative of non-fecal polluted plant decay inputs, denoting the minimum impact of fecal contamination at this site. The cholestanol/cholesterol ratio is useful to assess the microbial reduction of stenols to 5α-stanols that typically takes places in anoxic conditions (Reeves 2005; Nishimura and Koyama, 1977). The high values of this ratio indicate prevailing reductive conditions in the sewage effluent, which favors sterol preservation. On the contrary, oxidative conditions at N favors the sterol degradation over their hydrogenation, resulting in proportionally low amounts of cholestenol (Nishimura and Koyama, 1977). This microbial degradation of cholesterol intensifies at the sediment surface further increasing this ratio in sediments.

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