Dear Dr. Mark Yunker,

Associate Editor, Organic Geochemistry

We are pleased to resubmit the revised version of the manuscript “Early diagenetic alterations of sterols biomarkers during particle settling and burial in polluted and pristine areas of the Rio de la Plata basin” by E. D. Speranza, M. Colombo, C.N. Skorupka and J. C. Colombo (Ms. OG-3426).

We acknowledge you and both referees for your thoroughly revision which help us to clarify several concepts, to correct some mistakes and to format the manuscript according the journal style guidelines. The responses to each comment are listed below (line numbers provided in the responses correspond to the revised version of the manuscript).

Sincerely yours,

Dr. Eric D. Speranza

([esperanza@fcnym.unlp.edu.ar](mailto:esperanza@fcnym.unlp.edu.ar))

Laboratorio de Química Ambiental y Biogeoquímica, FCNyM, UNLP

Av. Calchaquí 6200, Florencio Varela (1888), Buenos Aires, Argentina

(Corresponding author)

Lic. Manuel Colombo

Lic. Carlos N. Skorupka

Dr. Juan C. Colombo

Associate editor’s comments:

* Abstract, Line 22. The ± uncertainties are defined in the Methods, but they also need to be defined at first mention in the Abstract.

**Response:** The ± uncertainties have been defined as “mean ± standard deviation” in the abstract.

* Line 120: The abbreviation for longitude should be W for west.

**Response:** Corrected.

* Line 124. There should be a space between numbers and units (i.e. 1.5 m). Please correct throughout.

**Response:** Done.

* Line 146. Specify the number of deuterium substituents for each standard. (A reference to the exact name in Table 1 should also be given. Note that the formulae in Table 1 need to be corrected to show 7 deuterium atoms.)

**Response:** Done.

* Line 189 and following. Provide references or suppliers for all libraries and packages. Also, more detail is needed on the principal component analysis method and program source.

**Response:** The URL for all the Python libraries and R packages have been supplied.

* Line 224 and 266. Five significant figures are given for the BA river discharges and four and five for sterol concentrations. Three figures are likely exceed that which could be supported by the discharge and concentration measurements. Please round off to a maximum of three figures throughout (i.e. only one decimal for numbers larger than 10).

**Response:** Values were rounded to two or three significant figures throughout the text.

* Line 253. … of sterols to degrade at the sediment-water interface, …

**Response:** Corrected.

* Lines 225 and 265. Correlations are given as R2 and r, for the coefficient of determination and correlation coefficient, respectively. Please be consistent. (Correlation is most commonly presented as the coefficient of determination with the abbreviation r2.) (Note that superscripts do not copy properly into the Elsevier system.)

**Response:** All correlations were expressed as r2. The superscripts were avoided except for numbers in exponential notation.

* Figure captions. The definition of N as the Uruguay River in the text and Fig. 1 changes to North in Fig. 3 to 7 captions. Use one definition for N throughout.

**Response:** Done.

Reviewer #1 comments:

The manuscript of Speranza and co-authors describe a comparative study of sterols composition in suspended particles and sediments as well as the flux from water to sediments between contaminated and pristine sites in an estuarine region. Overall, the research is well designed, the analytical approach is adequate and the data discussion is consistent, which make the manuscript worthy publishing.

The authors should address some minor points, as highlighted below:

* pg6, line 97: the outfall flux was set as 3.8 m3/day, which is a quite low value. For instance, an outfall in Rio de Janeiro city has a flux of 8 m3/s... please, confirm if the value for the BA outfall is correct?

**Response:** Indeed, the exponent was omitted. The value was corrected according to the reference (50 m3/s) and expressed in m3/day, in order to be consistent with previous values.

* pg8, line 132: what value for density was used? Did the authors measure the densities of the particles collected in the two sites? Based on the distinct nature of the particles collected in each site, their densities are probably quite different, and if so the calculated fluxes would be significantly affected.

**Response:** Since fluxes were calculated as particle mass settling over surface unit (see equation in material and methods section), they are independent from density. Nevertheless, sedimentation rates derived from fluxes clearly depends on density. At BA density measurements ranged between 2.2 and 2.7, therefore the typical value for sediments, 2.65 g/cm3, has been used (Colombo et al. 2007). At N the sedimentation rate was also estimated using this value. Anyway, the 4 orders of magnitude difference between BA and N in terms of flux would largely exceeds any variation in density among sites. Furthermore, calculation of coprostanol burdens in top sediment layers, using the abovementioned density, were made only for BA, as it was irrelevant for a little polluted site such as N.

* pg 11, line 194: the authors mentioned the consideration of the A/A+B ratio of sterols. However, in the Results and Discussion section, only ratios calculated as A/B were presented.

**Response:** All ratios presented in the Results and Discussion section were calculated as A/(A+B) and has been named with this format in the text.

* pg12, line 215: the authors should list the compounds grouped as 'fecal sterols'. I could not find such definition throughout the text.

**Response:** A definition has been added to the Materials and Method section.

* pg 21, lines 396-397: it was mentioned that warm-blooded animals other than humans have high ethylcoprostanol concentration that could affect the ratio sitosterol/ethylcoprostanol. In this sense, values around 1 would be typical of cow fecal material. Perhaps, the right statement should be that other animal have LOW ethylcoprostanol related to sitosterol when compared to humans. This would explain the ratio of 0.36 ± 0.15 found for BA and the conclusion that this outfall delivers basically human feces.

**Response:** According Nash et al. 2005, the value of the sitosterol/ethylcoprostanol ratio for cattle feces is typically lower than 1. At BA, this ratio is below this threshold, indicative of cow feces pollution. The paragraph has been modified to clarify the idea that a small non-human fecal pollution cannot be disregarded.

* pg 21, lines 407-413: the discussion about the changes is sterols ratios and their relation to hydrogenation/degration reactions in the suspended particles and sediments could be rethinked, as the differences reported for the selected ratios seems not significant

**Response:** Despite the difference between BA and N is pretty small (around 0.1) this was highly significant according t-test (p > 0.0001) due to the very low dispersion of data (relative standard deviation were below 5% for both sites). A minor typo error in the BA value has been corrected (0.85 ± 0.036 instead of 0.85 ± 0.043).

* pg 22, first paragraph: the discussion about PCA results is quite limited. Perhaps, it should be placed earlier in the discussion, in order to support the sterols source assignments.

**Response:** The PCA analysis has been further discussed. As suggested, the paragraph has been expanded and moved to the “Sterol composition” subsection where this multivariate analysis serve to integrate the discussion of the difference in terms of individual sterols between BA and N in settling material and sediments.

* pg 25, first paragraph: it was not clear the significance of the discussion abouth the change in the efficiency of sterol preservation in the restricted and a large area influenced by the outfall discharges.

**Response:** The paragraph has been modified to clarify the discussion about the observed coprostanol in sediments and the expected discharge according available data.

* Conclusion: it should be rethought, because in its current format it is more an abstract than a description of the relevance of the findings of this study.

**Response:** The conclusion was thoroughly reworked, changing the previous descriptive approach by a more integrative summary focused in the main findings of this work. Special emphasis was taken to underline the contrast between a severely polluted metropolitan area and a relatively pristine site in terms of sterol biogeochemical dynamics, as well as the magnitude of sewage pollution observed at BA. Additionally, the importance of settling material in aquatic lipids dynamic was emphasized.

Reviewer #2 comments:

This is an interesting manuscript that discuss diagenetic alterations of sterols biomarkers in polluted and pristine áreas of the Rio de La Plata. I think that materials and method are not fully informative and need to be improved for publication. I suggest some alterations, as listed below:

Introduction

* Include current references (above 2015).

**Response:** The introduction has been thoroughly revised and an exhaustive literature search has been conducted in order to provide newer references. Nevertheless, several relatively old reference are conserved since they are landmark papers or they could not be substituted with recent literature.

* L107: It is missing the end point.

**Response:** Corrected.

Materials and method

* Did you optimize the method for lipid extraction? If not, include the reference you used.

**Response:** The reference has been added to the text.

* Did you check the extraction efficiency?

**Response:** The extraction efficiency was evaluated through the recovery analysis. Individual recoveries were high, ranging between 82 and 110%.

* What is the volume of BSTFA used? Please specify.

**Response:** The BSTFA volume used has been specified in the text.

* Information about the solvent purity is missing.

**Response:** Added to the text.

* Is it possible to operate the mass spectrometer in scan mode and SIM mode simultaneously?

**Response:** The mass spectrometer used for sterol analyses, a Perkin Elmer Clarus 500 allow to perform multiple simultaneous mass functions, including a full scan and selective ion scanning. This functionality is particularly useful to check the identity of each compound, otherwise only identified by the retention time and a couple of characteristic ions.

* L175: Which standards were used?

**Response:** The standard used have been detailed in the text.

Results and Discussion

* L232: (RSD: 113-114%) I did not understand what it means.

**Response:** The relative standard deviation (RSD), used to measure data dispersion, was defined in the Material and Methods section as the ratio of the standard deviation to the mean. Since these values, corresponding to BA and N respectively, were almost identical, they have been replaced by a common average in the text.

* PCA analysis was not discussed in the text. It can be improved.

**Response:** The PCA discussion has been corrected and expanded. It has been moved to the “Sterol composition” subsection, in order to summarize the contribution of individual sterols to the settling material and sediments composition.