

All the suggestions and corrections of the Reviewer were helpful for improving the revised manuscript (MS). We agree that we do not present a large number of data for the water column, this study focuses on CDOM surface waters. Despite the small size of this data set we believe that it is nevertheless a good time series evaluation of surface CDOM properties in a weakly riverine-dominated coastal zone where biological production is a dominant source of CDOM. Indeed these data allow us to point out several potential processes affecting CDOM content in surface waters. Moreover we assume, regarding water depth (60 m), that during mixing events, samples collected in surface waters are representative of the entire water column (as confirmed by CTD data). However, we agree that analysis of how the characteristics of CDOM changed across the gradient sampled would be very interesting but will be the focus of another full study.

We would like to underline that, following Reviewer 1 comments, Rhone River data and results obtained from a kinetic irradiation experiment of the Rhone River sample acquired at Arles station on 7 February 2009 were introduced in the revised MS.

#### *General points*

1. *How valid it is to include data from excitation wavelengths below 240 nm. What were the absorption values at these wavelengths? I am guessing that they might be quit high at times depending on the nitrate concentrations (especially in association with mixing events). This might make it necessary to correct the data for inner filter effects.*

Answer: Data from excitation wavelengths below 240 nm were presented in our EEMs in order to visualize and show the two emission fluorescent peaks of protein-like components (peaks T and B). This is why for a thorough identification of protein-like components we have used these short excitation wavelengths. Concerning the quantification of all fluorescent peaks determined in this study, any emission fluorescent data in response to an excitation below 240 nm were taken into account in order to avoid introducing quantification biases resulting from the response of others components that absorb in short wavelengths such as nitrates. It is worth noting that nitrate concentrations in the surface waters of the Bay of Marseilles are usually very low ( $0 < [\text{NO}_3^-] < 1 \mu\text{M}$  during the study period in surface waters). Thus any correction data for inner filter effects was necessary for our samples. It should be noted that many publications have already reported fluorescence data for excitation wavelengths below 240 nm (Baker and Curry, 2004; Baker et al., 2004; Saadi et al., 2006; Gone et al., 2009).

2. *For the majority of the sampling dates it would seem that the surface 5 m was mixed and that the 2 and 5 m samples were basically replicates. Maybe you could just report the averages instead of always referring to.*

Answer: Concerning some parameters, it is true that 2 and 5 m samples would seem basically replicates especially during mixing events. Nevertheless in other conditions (i.e., Rhone

intrusion, photodegradation process during estival period) significant differences may appear within two depths. Thus to compare all data sets in the same way, we decided to not average the data at 2 and 5 m depth during mixing events.

- I don't agree with the "purity" paragraph with regards to discussing the shapes of the emission spectra in Figure 6. We know that the fluorescence signal is (to some extent and with some assumptions) the sum of different fluorescence signals present. Why not look at these samples in other way. For example by subtracting the normalized spectra from each other. Wouldn't this reveal the fluorescence spectrum of the additional material that is present? Finally, it is not clear to me why these samples were specifically chosen. It seems that they represent contrasting hydrological conditions but this should be stated more clearly and maybe labeled on the figures as e.g. "Rhône intrusion", "well mixed" etc...*

Answer: Yes, we agree with the Reviewer: the term purity is not appropriate in this work. In the revised MS, we systematically removed it.

After following Reviewer's suggestion (subtracting the normalized spectra from each other), it appears no additional insight is gained than from normalized spectra. Thus, we think that it will be preferable to present the figure 6 as previously, with addition of normalized spectra of the peak C from the Rhône River. This spectrum strengthens the discussion of FDOM origin, since it shows a broader emission spectrum compared to the marine samples. Indeed, in the revised MS, we discuss all of the different fluorescence signals that comprised the global fluorescent signal of the peaks C and M.

The "purity" paragraph was rephrased in the revised MS:

"Shape of normalized emission spectra can provide information on the nature as well as biogeochemical processes affecting DOM. Normalized emission spectra of peaks C and M were both determined (Fig. 6) for the 3 same marine samples illustrating contrasted hydrological conditions (23/06/2008, 23/09/2008 and 25/11/2008) shown in Fig. 4. Additional normalized emission spectra of the peak C corresponding at T0, dark control and T2 of the Rhône River sample irradiation experiment were also plotted on the Fig. 6. Both humic-like peaks (C and M) in marine samples present the same pattern at both depths, with the broadest emission spectra observed on photobleached water samples (23 September 2008) followed by the Rhone River intrusion ones (23 June 2008) while the narrowest were determined during mixing event (25 November 2008). For the Rhone River, emission spectra of the peak C were likely broader than the SOFCOM ones. Interestingly, after irradiation (i.e. T2 irradiation sample) corresponding emission spectra were widening towards longer wavelengths."

Effectively, these samples were specifically chosen because they represent contrasting hydrological conditions from which all fluorescence peaks, observed in this study, could be summarized. To improve the clarity of the figure 6, these contrasted hydrological conditions were added in the caption and graphs were colored.

Figure 6 of the revised MS

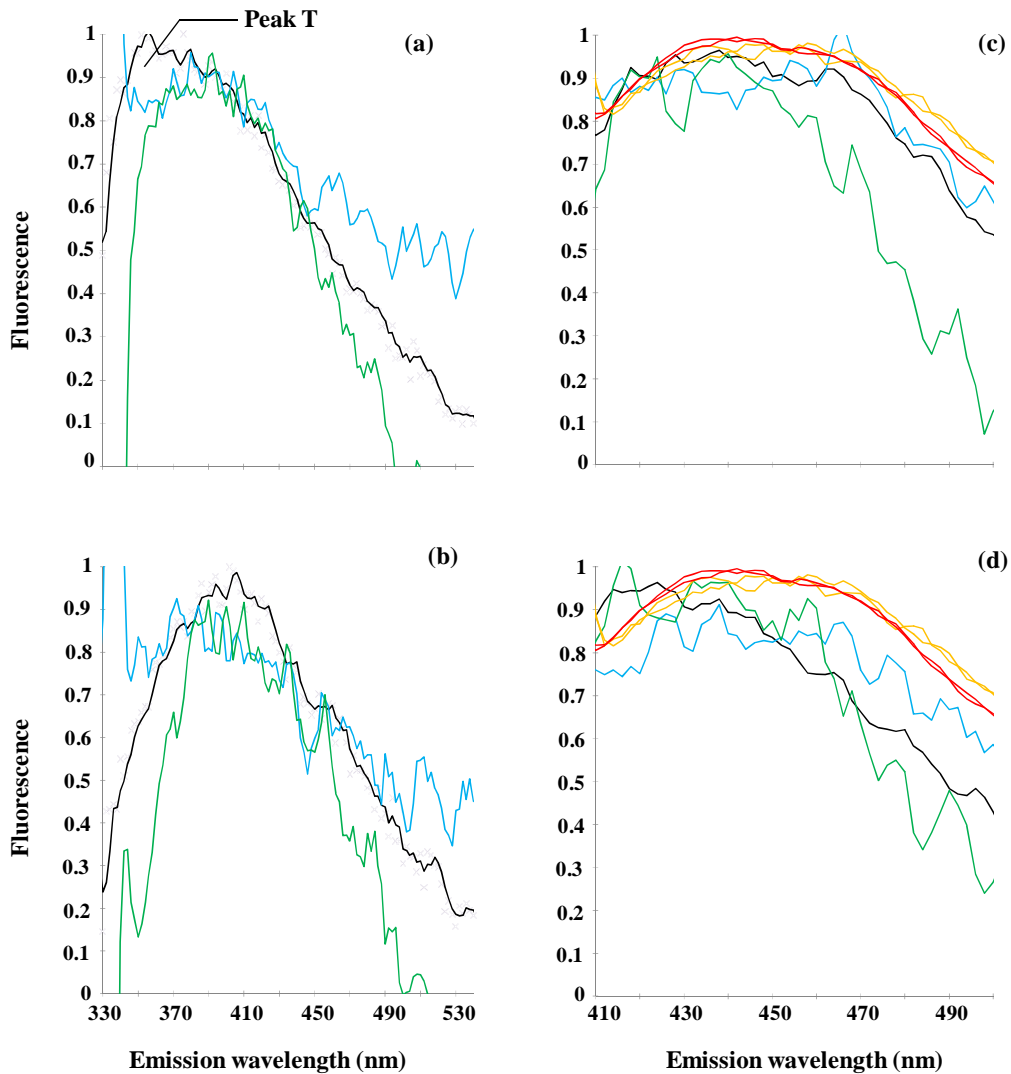


Fig. 6. Normalized emission fluorescence spectra of peak M (a and b) at  $Ex(\lambda) = 300$  nm and peak C (c and d) at  $Ex(\lambda) = 350$  nm acquired, at SOFCOM station, on 23 June 2008 (Rhône plume intrusion samples, black line), 23 September 2008 (photobleached samples, blue line) and 25 November 2008 (well mixed sample, green line) at 2 m (upper panel) and 5 m (bottom panel) depths. Normalized emission spectra of peak C determined from T0, dark control (red lines) and T2 (duplicate, orange lines) of the irradiation experiment performed on Rhône River sample collected on 7 February 2009 at 2 m depth were also plotted on both panels c and d. These emission spectra were normalized to the maximum emission intensity in the range 380-400 nm for the peak M and 430-450 nm for the peak C. These spectra were smoothed by a moving average order 3 which imposes a red shifted of 5 nm.

Effectively, these samples were specifically chosen because they represent contrasting hydrological conditions from which all the fluorescence peaks, observed in this study, could be summarize. For improve the clarity of the figure 6, these contrasted hydrological conditions were added in the caption and graphs were colored.

4. *The comparison of their data to the Determann et al results on the excitation and emission characteristics of the remains of bacteria and algae cultures is interesting although a little difficult to follow due to the figure being a unclear. There is a lot of data plotted on the same graphs. Again a different labeling of the graphs might make it easier to follow. They also have to be cautions with over interpreting the results. They may fit with the expected succession in phytoplankton and bacteria, but there is still a large difference between the conditions that Determann et al measured and these samples. And as no bacteria data was collected they cannot really be sure what after chlorophyll peak effect is. Maybe there are some other studies from the region where bacterial counts have been done which can help with this extrapolation?*

Answer: We agree with the Reviewer comment, so the graphs presented in the Fig. 7 were colored to improve figure's clarity and some cautions were added in the revised MS:

- p 5694, line 17, after "Determann et al. (1998)": "Whereas it is important to note that the following results were extrapolated from in vitro experiments to in-situ observations and thus should be taken, to some extent with caution."
- P 5694, line 29, the sentence: "Therefore, such results illustrate clearly...on 10 July 2008 at 5 m depth", was slightly modified as following: "Therefore, such results seem to illustrate a dominant phytoplankton origin.... on 10 July 2008 at 5 m depth".
- P 5695, line 3, "Establishing" was replaced by "Assessing".

To strengthen the fact that results fit with the expected succession in phytoplankton and bacteria, while no bacteria data was collected, we added the study of Lemée et al. (2002) as a reference. Indeed, this work report results concerning bacterial production and biomass on an annual basis in the upper layer of the Northwestern Mediterranean Sea (DYFAMED station) and pointed out the net heterotrophic character of this system, i.e., bacterial development after algal bloom events.

Thus p 5695, line 11, a sentence was added in the revised MS: "This ecological succession (phytoplankton, bacteria) was in a good agreement with the work of Lemée et al. (2002) which highlights the net heterotrophic character of bacteria in the upper layer of the Northwestern Mediterranean Sea."

#### *Specific comments*

1. *P.5677. Line 7. Delete « with ».*

Answer: This correction was made in the revised MS

2. *P. 5678. Line 6. The Coble and Nelson references are not appropriate here. There are other works that have looked at these processes specifically.*

Answer: The Coble and Nelson references were replaced in the revised MS by Mague et al. 1980, Jumars et al. 1989 and Nagata 2000.

3. P. 5678. Line 7. Opsahls paper states 0.7-2.4 %.

Answer: The percentages range of terrestrial DOM that composed total DOM in the ocean was rectified in the revised MS.

4. P. 5678. Line 9. Rephrase “changing local bacterial carbon demand”

Answer: According to this comment we rephrase “changing local bacterial carbon demand” by “fueling alternative labile carbon source to sustain local carbon demand in addition to autochthonous carbon source derived from phytoplankton and heterotrophic microbial food web”.

5. Paragraph starting on line 20 p. 5678 does not read well and should be re-phrased. I don't agree with EEMs now being a standard approach. The community is still trying to work out what the signals represent and how to standardize the methods used.

Answer: According to this comment, the initial sentence: “In the 1990's, excitation emission matrix (EEM) spectroscopy became the standard tool for characterizing fluorescence properties of CDOM” has been replaced in the revised MS by: “In the past 20 years, CDOM fluorescence properties have been widely studied owing to excitation-emission matrices (EEMs)”.

6. P. 5679, line 7-10. How does this compare with what you say earlier about Opsahl and Benner 1997 where terrestrial DOC can represent up to 2.4 % in the Atlantic?

Answer: We compared the importance of the yearly fluvial carbon input versus the standing carbon crop both in the world ocean (0.024 % in Smith and Hollibaugh 1993) and in the Mediterranean Sea (0.08–0.3% in Sempéré et al., 2000) underlying that in the Mediterranean Sea the yearly carbon input is significantly higher than in the world ocean relatively to DOM pool. On page 5677 lines 7-10 we give a general pattern about the importance of the terrestrial DOM pool in the oceanic DOM pool (0.7–2.4% in Opsahl and Benner 1997). The terrestrial DOM pool is, at our knowledge, unknown for the Mediterranean Sea.

7. P. 5679, line 19. Replace “showed” with shown.

Answer: This correction was made in the revised MS

8. P. 5679, line 20. Replace “depend” with depends.

Answer: This correction was made in the revised MS

9. P. 5680, line 7. Delete “for the first time”.

Answer: This correction was made in the revised MS

10. P. 5680, line 9. “dynamics in the Northwestern Med...”.

Answer: This correction was made in the revised MS

11. P. 5682, line 13. This reference 2004 is incorrect. The Blough & Del Vecchio book chapter in Hansell&Carlson DOM book (2002), reviewed these fitting techniques. So you could reference this or the original work, also cited in the chapter.

Answer: Following Reviewer’s suggestion, the reference of the original work (Stedmon et al., 2000) was cited.

12. P. 5682. Line 13-14. “Here,  $S_{CDOM}$  was determined by applying a nonlinear fit of log-linearized absorption data in the spectral range 350-500 nm ( $R^2 > 0.99$ )...” I don’t understand this. It sounds like the log of the absorption spectra was modeled with a nonlinear fit. Either a nonlinear fit was used on the original data, fitting the exponential to the spectrum, or a linear regression was applied to the log transformed absorption data.

Answer: We agree with the Reviewer. There was a mistake in this sentence: “log-linearized absorption data”. Here  $S_{CDOM}$  was well determined after applying a non-linear exponential regression to original  $a_{CDOM}(\lambda)$  data measured. That is why we have decided to correct this sentence in the revised MS by: “Here,  $S_{CDOM}$  was determined after applying a non-linear exponential regression to original  $a_{CDOM}(\lambda)$  data measured on the range 350-500. All the correlation coefficients ( $R^2$ ) calculated from these exponential fits were always higher than 0.99.”

13. P. 5683, line 15-29. Why aren’t exactly the same wavelengths as suggested by the two studies by Zsolnay et al. and Huget et al. for the indices? The wavelengths seem to have been shifted a little for your study. How does this affect the results? I understand why you use ex 255 instead of 254 nm, for example for the HIX, but why haven’t you used the integrals of the two emission regions?

Answer: Concerning HIX, we have adequately used the integrals of the two emission regions (H and L) to determine it. The only difference is that, due to technical settings (i.e., emission increment of 2 nm and excitation increment of 5 nm), the excitation wavelength was 255 nm instead of 254 nm and the emission ranges were 300-346 nm instead of 300-345 nm (L) and 434-480 nm instead of 435-480 nm (H). This very slight difference has no influence on the values of the indices. In the revised MS, we have included this information in brackets.

In the revised MS, BIX index was calculated the same manner than the one of Huget et al. for both SOFCOM and Rhone River datasets in order to allow intercomparison. We want to draw attention to that the BIX index determined on SOFCOM data set does not change its interpretation. Indeed all the BIX values were  $> 0.8$ , attesting of a biological or aquatic bacterial origin of CDOM, and the dates on which maximum and minimum BIX were observed remained unchanged.

14. *Methods section: you have forgotten to mention how you measured chlorophyll.*

Answer: Actually, it was mentioned in the section “Materials and methods”, part “Study site and sample collection” p. 5681, lines 15-18. “Since February 2008 chlorophyll was measured with a WETStar Chl-a fluorometer (WET Labs Inc.) mounted on the CTD.”

15. *P. 5686, line 8. Replace “reformation” with re-establishment.*

Answer: This correction was made in the revised MS

16. *P5686, line 20. Chl is more an indicator of phytoplankton biomass than primary productivity.*

Answer: Yes absolutely, that is why “Primary production” was replaced by “Phytoplankton biomass”.

17. *P. 5687, line 4. Delete “indeed”.*

Answer: This correction was made in the revised MS

18. *At CDOM absorption results. In the paragraph at the bottom of p 5688 you start mentioning that CDOM behaves conservatively, and then towards the end of the paragraph start discussing the production of CDOM. This seems conflicting. Another point to consider is, how far does a data value have to deviate from the regression line to be considered as significantly different?*

Answer: Here we present a general trend: overall aCDOM data have an apparent conservative behavior, with some “outliers” observed at several sampling dates during contrasted hydrological conditions from which CDOM absorption data were significantly different and thus illustrated a production or a loss of CDOM. Moreover, following Reviewer 3 suggestion we have performed a linear regression with all the data available at 2 m depth (i.e. 13 values from SOFCOM station completed by 2 values acquired close to Rhone estuary in the Rhone River plume) by using a model. We noticed the similarity of the equation of the model ( $a_{\text{CDOM}}(350) = -0.029 \text{ salinity} + 1.199$ ,  $n=15$ ,  $R^2=0.96$ ) with the one determined before ( $a_{\text{CDOM}}(350) = -0.028 \text{ salinity} + 1.201$ ,  $n=6$ ,  $R^2=0.98$ ). We have thus improved Fig. 3 in the revised MS, using this more rigorous method to establish the mixing line. In addition, the confidence interval at 95% was also plotted on the figure to show (“outliers”) which data are significantly above and below the mixing line.

Fig. 3. of the revised MS

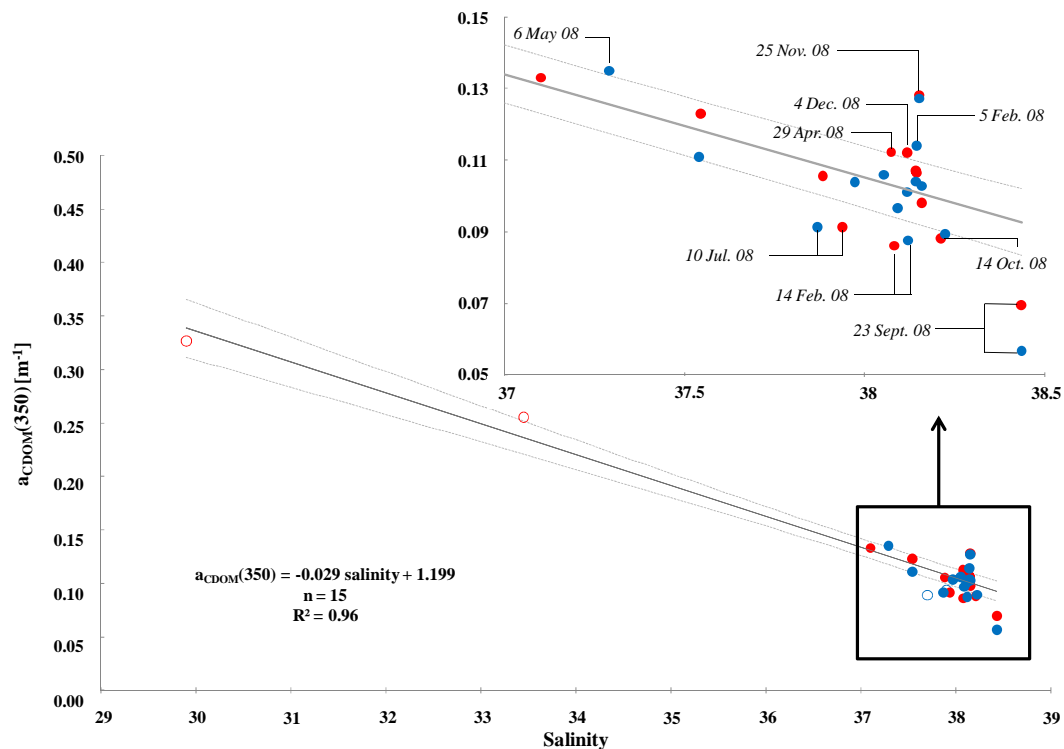


Fig. 3. Relationship between salinity and CDOM absorption at 350 nm (in m<sup>-1</sup>) acquired at SOFCOM station at 2 m (red circle, n = 13) and 5 m (blue circle, n = 14) depths. Data from Rhône plume acquired in May 08 during CHACCRA cruise at 2 m (red open circle, n = 2) and 5 m (blue open circle, n = 2) were also plotted. The mixing line (black line) with its confidence interval at 95% (dashed line) was established using all SOFCOM station data at 2 m depth (n=13) plus Rhône estuary stations (CHACCRA cruise data) at 2 m depth as well (n = 2).

19. P. 5689, line 22. *I can not see the T peak in the EEM from 23 of September. I can see it labeled but can not see the peak.*

Answer: Yes, we agree with the Reviewer. We only suspect the presence of the T peak at this date because the second excitation peak at 340 nm was not well defined compared to the first one at 225 nm. That is why in the MS we evoke its presence with caution at p. 5689, line 23 "... and possibly a slight signal of the peak T..". To show clearly on the figure that its presence is solely suspected we have completed the label "T" position on its two excitation maxima, with a "?" on the Fig. 4 in the revised MS.

20. P. 5690, line 2-3. *Aren't these two statements one and the same?*

Answer: Not exactly, in fact it is one statement completed with its consequence. To clarify this we have replaced this sentence in the revised MS by: "This result indicates that fluorescent CDOM character in the surface is mainly driven by other processes than water



mixing and thus highlights the dissimilar trends in CDOM absorption and fluorescence properties.”

21. *P. 5690, line 4 and rest of the paragraph. You should not use the words purity or pure when discussing the fluorescence peaks. It implies that we know chemically what it is and this we do not.*

Answer: Yes we agree with the Reviewer. The use of the words pure or purity is not appropriate here. These words were systematically deleted in the revised MS. Discuss about the degree of complexity of the compounds mixture that shaped the peaks in regard to their emission spectra is more rigorous. (see also answer of the general comment 3).

22. *P. 5693, line 5-9. Have you considered the fact that the presence of tryptophan can quench the fluorescence of tyrosine (See Lakowicz 2006, Fluorescence book)?*

Answer: Yes, we have kept in mind the quenching process of tyrosine by tryptophan to explain the lack of peak B. Our explanation concerning the lack of peak B is based on the results of the work of Mayer et al. (1999), which takes into account the quenching process of tyrosine by tryptophan concerning the protein state. We agree that the quenching effect was not clearly exposed in the MS that is why in the revised MS we rephrase these lines as following: “These observations are in accordance with our results: presence of peak T and absence of peak B in coastal waters (except on 23 June 2008). According to Lakowicz (2006) and Mayer et al. (1999) tyrosine fluorescence is quenched by tryptophan in folded proteins. This implies that the tryptophan observed at SOFCOM station is probably bounded in proteins rather than in free dissolved form.”

23. *Please refrain from referring to the fluorescence peaks as fluorophores. We have no idea what they are, or if they are single fluorophores that are responsible.*

Answer: Yes, we agree. In the revised MS we replaced fluorophores by “peaks”

24. *BIX and HIX data should be presented as part of the Results section and then discussed in the Discussion. As is the reader is introduced to them as part of the discussion.*

Answer: Yes, we agree with this comment and in consequence we had a supplementary paragraph in the “Results” section:

“Two indices, the humification index (HIX) and the biological index (BIX) for marine (SOFCOM station) and freshwater (Arles station) samples are presented on Table 4. At SOFCOM station, HIX average value ( $0.84 \pm 0.38$  and  $0.90 \pm 0.35$  QSU at 2 m and 5 m depths, respectively) was low and variable while BIX average value ( $1.10 \pm 0.17$  and  $1.09 \pm 0.05$  QSU at 2 m and 5 m depths, respectively) was high and constant at both depths. These results suggest a predominantly autochthonous origin of DOM in surface marine waters. BIX maximum values were observed at 2 m depth on 23 June 2008 and 25 November 2008 while the lowest one was observed on 7 July 2008 at 2 m depth. This date corresponded also at the

maximum values observed at both depths for HIX. At Arles station, Rhône River CDOM likely contains higher molecular compounds compared to marine CDOM. Indeed, high HIX average values ( $4.90 \pm 1.60$  QSU) and low BIX average value ( $0.74 \pm 0.05$  QSU) suggest a predominantly allochthonous origin of DOM. The high variability of HIX (CV = 33%), which is the ratio of H/L, for these freshwater samples came from the variability concerning the presence of complex high molecular weight components (i.e., H, CV = 41%), while low molecular weight components part remained more steady (i.e., L, CV = 13%). The Rhône River irradiation experiment shows a strong decrease of HIX at T1 and T2 while corresponding BIX remained constant compared at T0 and dark control. This result underlines the photosensitivity feature of terrestrial DOM compared to autochthonous DOM.”

25. *Tables. Suggestion: Drop table 1 as the information it provides is basically apparent in the other tables. Could also consider combining tables 2 and 3.*

Answer: Table 1 was built up in order to recapitulate parameters that were available concerning each end-member to help the reader. But it is true that this information could be provided in the other tables. So we decided to remove Table 1 in the revised MS. Since additional data concerning fluorescence properties of Rhone River CDOM are also presented in Tables 2 and 3, combining these tables in one will condense the information. That is why we decided to keep both tables in the revised MS.

## References

Baker, A., Curry, M., 2004. Fluorescence of leachates from three contrasting landfills. *Water Research* 38, 2605-2613.

Baker, A., Ward, D., Lieten, S.H., Periera, R., Simpson, E.C., Slater, M., 2004. Measurement of protein-like fluorescence in river and wastewater using a handheld spectrophotometer. *Water Research* 38, 2934-2938.

Saadi, I., Borisover, M., Armon, R., Laor, Y., 2006. Monitoring of effluent DOM biodegradation using fluorescence, UV and DOC measurements. *Chemosphere* 63, 530-539.

Gone, D.L., Seidel, J.-L., Batiot, C., Bamory, K., Ligban, R., Biemi, J., 2009. Using fluorescence spectroscopy EEM to evaluate the efficiency of organic matter removal during coagulation–flocculation of a tropical surface water (Agbo reservoir). *Journal of Hazardous Materials* 172, 693-699.

LEMEE, R., E. ROCHELLE-NEWALL, F. VANWAMBEKE, M.-D. PIZAY, P. RINALDI, AND J.-P. GATUSO. 2002. Seasonal variation of bacterial production, respiration and growth efficiency in the open NW Mediterranean Sea. *Aquat. Microb. Ecol.* 29: 227-237.

Lakowicz, J.R.: Principles of fluorescence spectroscopy. 3rd edition Springer Science; New York: 2006. pp. 158–204.

Jumars, P. A., Penry, D. L., Baross, J. A., Perry, M. J. and Frost, B. W.: Closing the microbial loop: dissolved carbon pathway to heterotrophic bacteria from incomplete ingestion, digestion and absorption in animals. *Deep-sea Res.* 36, 483-495, 1989.

Mague, T. H., Friberg, E., Hughes, D. J., and Morris, I.: Extracellular release of carbon by marine phytoplankton: A physiological approach. *Limnol. Oceanogr.*, 25: 262-279, 1980.

Nagata, T.: Production mechanisms of Dissolved Organic Matter. In *Microbial Ecology of the Oceans*, edited by DL Kirchman, 5 : 121-152, 2000.