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Distribution of dissolved organic matter in estuaries of the Southern Iberian Atlantic Basin: sources, behavior and export to the coastal zone

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Abstract

The Gulf of Cádiz, located in the Southern Iberian Atlantic Basin, connects the North Atlantic Ocean and the Mediterranean Sea and receives freshwater input from three main estuaries: Guadalquivir, Guadiana, and Tinto-Odiel. These estuaries differ in their hydrology, basin characteristic, and land use. One of them, Tinto-Odiel, is one of the most polluted estuaries in the world. However, little is known about the export of DOM from these estuaries to the Gulf of Cádiz. In this work, the estuaries were sampled during the dry season. Dissolved organic matter (DOM) was chemically characterized along a longitudinal gradient to better understand the reactivity and fate of carbon exported to the Gulf of Cádiz. We also performed a tidal study at the mouth of each estuary to understand how the tide affects the composition of DOM and its export to the coastal zone. Fluorescent DOM (FDOM) modeling employing multivariate parallel factor analysis and analytical pyrolysis were used to characterize DOM. DOM from Guadalquivir and Guadiana estuaries presented a predominant allochthonous origin with humic-like compounds making up to ~80 % of the total fluorescent DOM. These estuaries receive lateral inputs from surrounding watersheds and agricultural practices. Instead, in Tinto-Odiel estuary DOM was predominantly autochthonous with a higher content of protein-like material associated with the prevalence of the marine influence over the low water discharges and anthropogenic pollution from industrial activities. Tidal cycles affected the distribution of DOM and its quality with higher humic-like material during low tide and protein-like substances predominating during high tide. During the dry season, the three estuaries represented a source of DOM to the Gulf of Cádiz. Guadalquivir was the main contributor with 1.25 Kg C s^{-1} and $0.22 \times 10^3 \text{ m}^2 \text{ s}^{-1}$, for DOC and CDOM, respectively. Around 70 % of the FDOM exported from the three estuaries was of humic nature. The results obtained in this work highlight the importance of local conditions, such as the basin characteristic and estuarine biogeochemical reactivity in the composition of the DOM exported to the coastal ocean. Moreover, Py-GC-MS analysis was a useful technique, complementary to fluorescence spectroscopy, which improves DOM characterization in estuarine systems.

Nomenclature
List of acronyms
a_{254} absorption coefficient at 254 nm (m^{-1})
a_{350} absorption coefficient at 350 nm (m^{-1})
BIX index of recent autochthonous contribution
CDOM chromophoric dissolved organic matter
DOC dissolved organic carbon (μM)
DOM dissolved organic matter
EEMs Excitation-Emission matrixes
FDOM fluorescent dissolved organic matter
HIX humification index
PARAFAC parallel factor analysis
Py-GC-MS pyrolysis-gas chromatography-mass spectroscopy
S salinity
SUVA ₂₅₄ specific ultraviolet absorbance at 254 nm ($\text{mg}^{-1} \text{L m}^{-1}$)
T temperature ($^{\circ}\text{C}$)
TDLP9 Total dissolved lignin phenol (nmol L^{-1})

1. Introduction

The input of dissolved organic matter (DOM) by rivers to the oceans occurs primarily via estuaries, being a significant fraction in the global carbon budget (Raymond and Spencer, 2015). Annually, rivers transport ~ 0.25 Gt of dissolved organic carbon (DOC) from continents to the ocean (Hedges et al., 1997), yet the processes which control its distribution and fate are not completely understood (Bauer and Bianchi, 2012). Riverine DOM is considered to be recalcitrant and conservatively transported to the ocean; that is, an inverse linear relationship with salinity is often observed. However, the biogeochemical complexity of estuaries, which comprises both allochthonous and autochthonous sources of DOM (Cauwet, 2002), can contribute to a non-conservative behavior. Diverse factors (physical, chemical, biological), such as anthropogenic pollution, land use, and microbial metabolism, as well as the presence of wetlands and salt marshes, can significantly alter the composition, reactivity and optical properties of DOM before it reaches the coastal zone (e.g., Benner and Opsahl, 2001, Abril et al., 2002, Jaffé et al., 2004). These factors regulate the net efflux of DOM from land to the global ocean. Therefore, it is important to better understand the composition and biogeochemical reactivity of DOM in these land-ocean transitional systems.

The colored fraction of DOM (CDOM), and the sub-fraction that emits light as fluorescent radiation, named fluorescent DOM (FDOM), have been widely used to assess the sources and behavior of different fractions of DOM in estuaries (e.g., Rochelle-Newall and Fisher, 2002, Jaffé et al., 2004, Guo et al., 2007). FDOM can be divided into two major groups, humic-like and protein-like components, which presence and relative intensities vary according to the type, origin, and concentration of DOM (Coble, 1996). River discharge is generally the main source of humic-like components into coastal waters, whereas protein-like FDOM is associated with amino acids occurring in proteins (tryptophan, tyrosine, and phenylalanine, Determann et al., 1998) associated with biological production (Coble et al., 2014). Additionally, protein-like signals have also been interpreted as an indication of anthropogenic DOM (Hong et al., 2005). Furthermore, many other natural and anthropogenic aromatic compounds exhibit

fluorescence in the protein region (Maie et al., 2007, Hammam et al., 2013). The use of excitation-emission matrixes (EEMs) coupled with the multivariate statistical method, parallel factor analysis (PARAFAC), allows for the differentiation of a wider range of underlying FDOM components (Stedmon and Bro, 2008).

Although in estuarine systems the supply of terrestrial CDOM often dominates (Stedmon and Nelson, 2015), autochthonous sources and anthropogenic inputs from the watershed (industrial or agriculture) have also been shown to be important contributors to estuarine CDOM pools (e.g., Hong et al., 2005, Coble, 2007). Furthermore, microbial transformation (Moran et al., 2000) and photochemical removal of DOM (Vodacek et al., 1997) play an important role in altering the physicochemical characteristics of these materials. Thereby, estuaries discharging into the same coastal region may contribute with different quantity and quality of DOM (Coble, 1996).

The Gulf of Cádiz, located in the southern Iberian Atlantic Basin, is the only connection between the North Atlantic Ocean and the Mediterranean Sea and receives freshwater input from three main estuaries: Guadalquivir, Guadiana, and Tinto-Odiel. These estuaries differ in terms of their morphology and hydrology, as well as their basin vegetation and land use. Guadalquivir estuary has been identified as a net source of DOC to the adjacent coast (Ribas-Ribas et al., 2011). However, the origin of this DOC is barely known. González-Ortegón et al., (2018), suggest that these three rivers discharge large amounts of humic-like FDOM to the Gulf of Cádiz, mainly through Guadalquivir and Guadiana rivers and much less through Tinto-Odiel. However, their work was performed in the coastal zone with only one station sampled at the mouth of each estuary using an EXO FDOM sensor limited to humic substances from terrestrial sources. On the other hand, a recent study found that protein-like was the most abundant fraction of FDOM in the coastal-ocean zone of the Gulf of Cádiz. The distribution of both DOC and protein-like FDOM was related to biological activity. In contrast, the distribution of humic-like FDOM was mainly explained by water mass mixing (Amaral et al., 2020). To the best of our knowledge, there is no information about the dynamic of DOM in these three estuaries, as well as the quality and quantity of the DOM that they export to the Gulf of Cádiz. Furthermore, it is largely unknown how the basin characteristics of these rivers influence the export of DOM and whether the processes occurring during its transport change the chemical composition and biogeochemical reactivity of DOM in the estuaries.

The objective of this study was to determine the composition and behavior of DOM in Guadalquivir, Guadiana, and Tinto – Odiel estuaries. This study presents the concentration of DOC and the optical characteristics of DOM for longitudinal salinity transects and a tidal cycle study in each system. It also estimated the fluxes of DOC, CDOM, and different fractions of FDOM from these estuaries to the Gulf of Cádiz. We applied spectroscopic techniques coupled with PARAFAC (Murphy et al., 2013) and pyrolysis-gas chromatography-mass spectroscopy analysis (Py-GC-MS, Kaal, 2019) to assess DOM composition. Despite the quantitative limitations of Py-GC-MS since only estimations of relative proportions of pyrolysis products can be obtained, it has been proved to provide valuable information about DOM sources (Kaal, 2019). Additionally, few studies have applied this technique to assist in the interpretation of fluorescence spectroscopy characterization (Jiang et al., 2017).

We hypothesized that the different river basin characteristics, freshwater discharge, tidal

cycles, and each basin singularities might lead to different DOM composition and behavior between estuaries.

2. Methods and Materials

2.1. Study Area

The study was carried out in three estuaries located on the Southwest coast of the Iberian Peninsula: Guadalquivir, Guadiana, and Tinto-Odiel (Fig. 1). These rivers basins are located within the Mediterranean-climate region, a semiarid environment, and regulated by dams.

The three estuaries are affected by a tidal cycle showing a semidiurnal period and a mesotidal regime with a maximum range of ~3.3 m (spring tides) and an average of 2 m (Carro et al., 2019; Morales and Garel, 2019; Rodríguez-Ramírez et al., 2019). Guadalquivir and Guadiana rivers have similar length and dams regulate their freshwater discharge into the estuary. Guadalquivir river discharge is the highest with an annual mean of $164 \text{ m}^3 \text{ s}^{-1}$ (Rodríguez-Ramírez et al., 2019). Although Guadiana river annual discharge is generally low ($< 50 \text{ m}^3 \text{ s}^{-1}$) (Garel and Cai, 2018), it is highly variable along the year with severe droughts and episodic flood events in the lower part of the estuary (Garel et al., 2009). The Guadalquivir river basin covers an area of 57527 Km^2 . An extensive wetland area surrounds the Guadalquivir estuary and its middle zone is dominated by intertidal salt marshes (Rodríguez-Ramírez et al., 2019). However, this river is subjected to elevated anthropogenic pressure due to densely populated areas on its margins (4.2 million people), as well as the development of agriculture and aquaculture industries, and a high demand of freshwater for irrigation. Irrigated crops occupy $\sim 8100 \text{ Km}^2$ (Carrasco et al., 2010) that used $\sim 85 \%$ of the withdrawal water from this river (www.chguadalquivir.es). Guadiana is a rock-bound estuary, which consists of a single narrow channel (50 – 700 m wide) (Morales and Garel, 2019). Although salt marshes are highly reduced due to the strong anthropogenic pressure, an extensive wetland area ($\sim 1000 \text{ ha}$) surrounds its mouth (Ménanteau et al., 2005). The Tinto-Odiel estuary extends 35 km from its mouth towards the upstream tidal limit (Carro et al., 2019). This estuary is composed by two rivers, Tinto and Odiel that converge in a common marine area called Padre Santo channel, which flows to the Gulf of Cádiz. Tinto and Odiel rivers have a short length of 100 and 140 km and a discharge of $5 \text{ m}^3 \text{ s}^{-1}$ and $14 \text{ m}^3 \text{ s}^{-1}$, respectively. The fluvial basin of both rivers mainly lies on top of a volcano-sedimentary area. It flows through materials of the Iberian Pyrite Belt complex, which contains the largest polymetallic sulfide deposits in the world (Leblanc et al., 2000). Mining activity led to great pollution of its waters, which now contain high concentrations of heavy metals and extremely low pH values (< 3 , Grande et al., 2000). Consequently, the mixing of acidic waters from mines, industrial effluents, and seawater leads to significant changes in the chemical composition of the water (Carro et al., 2019).

2.2. Field Sampling

Surface water samples were collected in July 2017 onboard the R/V UCADIZ, using an auxiliary boat in the inner part of the Tinto-Odiel estuary. Since the objective of the study was to determine DOM dynamics along these estuaries and the effect of tidal cycles, two different types of sampling were carried out. A longitudinal transect along a

salinity gradient including external stations in the coastal zone and a tidal study. We divided the different samples as coastal (external stations) and estuarine samples, where we differentiated three zones within each estuary: upper estuary ($S < 2$), middle estuary ($2 < S < 30$) and lower estuary ($S > 30$) (Fig. 1). Samples for the tidal study were collected during two tidal cycles at a fixed station at the mouth of each estuary (black dots, Figure 1).

Surface water samples were taken with Niskin bottles (10 L) mounted on a rosette-sampler coupled to a CTD Seabird 25, which was able to measure temperature and salinity. During the tidal study, the water current and tidal height were measured every 5 minutes with a Nortek Aquaprobe Current Meter. The current velocity was measured every one meter from the bottom to the surface. Estuaries sections were calculated during low tide using small boats equipped with depth probe (Guadalquivir estuary) or nautical charts (Guadiana and Tinto-Odiel, <https://www.navionics.com>). In this latter case, the tidal height during low tide was corrected taking into account the tidal intensity during the days the study was conducted. Salinity and temperature in superficial water were continuously measured with a Seabird SBE - 45 thermosalinograph.

2.3. Analysis of DOC and optical properties, and estimation of dissolved lignin concentration

Following the sampling, all water samples ($n = 150$) were filtered through pre-combusted Whatman GF/F glass-fiber filters (450 °C, 4 h, nominal pore size 0.7 μm) using acid-cleaned glass filtration systems, previously rinsed with the sample. Although fine particles released by phytoplankton (0.2-0.7 μm) could have a significant impact in CDOM absorption in productive environments (Massicotte et al., 2017), in aquatic ecosystem influenced by terrestrial DOM, this fraction has not a significant effect on CDOM composition (Nimptsch et al., 2014, Massicotte et al., 2017). Moreover, unlike 0.2 μm polycarbonate filters, GF/F filters can be cleaned by combustion at high temperatures and it is possible to filter an adequate volume and number of samples without clogging. It should be noted that pre-combustion might reduce the effective pore size to 0.3 μm for GF/F (Nayar and Chou, 2003). Therefore, we opted for the use of the GF/F filters.

DOC concentrations were measured using the high-temperature catalytic combustion method with a Multi N/C 3100 Analytik Jena analyzer, and potassium hydrogen phthalate was used to calibrate the system daily. Deep seawater and low carbon reference waters (Hansell CRM Program, 42-45 μM) were measured to assess instrument variability ($n = 5$, $43 \pm 1.8 \mu\text{M}$). UV-visible absorption spectra were obtained immediately on board, from 250 nm to 800 nm using a 1 cm path length quartz cuvette in a JASCO-V750 spectrometer connected to a programmable temperature control system set at 20 °C. The absorbance values were then converted to Napierian absorption coefficients (a_λ) in m^{-1} (Green and Blough, 1994). We focused on the absorption coefficients at 254 (a_{254}), proportional to the abundance of conjugated carbon double bonds, and 350 (a_{350}), used as a quantitative indicator of CDOM (Coble, 2007). The C-specific ultraviolet absorption coefficient at 254 nm (SUVA_{254}) strongly correlated with the aromaticity percentage and was calculated by dividing the decadic absorption coefficient at 254 (in m^{-1}) by the concentration of DOC (in mg L^{-1}) (Weishaar et al., 2003). Concentrations of total dissolved lignin phenol (TDLP9, nmol L^{-1}), a proxy for terrigenous and plant-derived DOM, were estimated from CDOM

absorption coefficient using the equation proposed by Fichot et al. (2016). When a_{250} values were lower than 4 m^{-1} : $\ln(\text{TDLP9}) = 0.7672 \times a_{263} - 0.3987$ and when a_{250} values were higher than 4 m^{-1} : $\ln(\text{TDLP9}) = -2.282 \times \ln(a_{350}) - 8.209 \times \ln(a_{275}) + 11.365 \times \ln(a_{295}) + 2.909$. These equations were established with samples from a wide variety of rivers, estuaries, and coastal waters across the northern hemisphere (subtropical and temperate) and are expected to be widely applicable in terrestrially influenced coastal systems (Fichot et al., 2016).

Excitation-emission matrixes were obtained immediately onboard using a spectrofluorometer (JASCO FP-8300) connected to a Peltier Thermostatted Cell Holder with stirrer accessory (EHC-813) for temperature control (20°C) using a 1-cm quartz cuvette. The excitation wavelength ranged from 250 nm to 450 nm (5 nm steps) and emissions wavelength from 300 nm to 560 nm (1 nm steps), with a bandwidth of 5 nm, and the integration time was 0.2 s. The drEEM 0.2.0 toolbox was used to standardize the EEMs (Murphy et al., 2013). Spectra were improved by the instrument correction factors and the inner filter effect was addressed by correcting EEMs using the absorbance-based approach (Kothawala et al., 2013). Data was standardized and normalized to Raman Units (RU) based on measurements of Raman peak at 350 nm (Lawaetz and Stedmon, 2009). We used PARAFAC to identify the different fluorescent components that comprise the estuaries DOM and trace their distribution and changes in the systems. PARAFAC was performed using drEEM's toolbox (version 0.2.0; Murphy et al., 2013) for Matlab (R2015b).

Two indexes were further derived from the EEMs: 1) the humification index (HIX), calculated as the area under the emission spectra 435–480 nm divided by the peak area 300–345 nm + 435–480 nm, at excitation 254 nm. HIX values ranges from 0 to 1, with higher values indicating a higher degree of humification (Ohno, 2002); 2) the index of recent autochthonous contribution (BIX), calculated as the ratio of emission intensity at 380 nm divided by the emission intensity at 430 nm, obtained at excitation 310 nm (Huguet et al., 2009). BIX values > 1 correspond to freshly released DOM, while values < 0.6 – 0.7 correspond to a lower DOM production in the system.

2.4. Molecular characterization

A representative sample of each estuary (black dot, Figure 1) was extracted by solid phase extraction using PPL cartridges following Dittmar et al. (2008). Briefly, 2 L of each sample were immediately filtered through pre-combusted GF/F filter, acidified to pH 2 and passed through the PPL cartridges. DOM was eluted with methanol and stored at -20°C in glass ampoules until analysis. Py-GC-MS was performed using a Pyroprobe 5000 coupled to an Agilent 5975 MSD system. The samples were put into quartz tubes either by cutting and homogenizing solid particles that were added to quartz wool contained in quartz tubes or by sweeping liquid into quartz wool, which was then inserted into the tube. The pyrolysis was performed at 650°C for 20 seconds (heating rate 10°C ms^{-1}) and the GC-MS with a temperature program of $10^\circ\text{C min}^{-1}$ and operating in 1:50 split mode. The GC was equipped with an HP5-MS non-polar column and the MS was scanning in the m/z range 50–500. For additional details, refer to Kaal et al. (2017). Relative proportions were calculated for a series of products that reflect the macromolecular DOM fraction of interest, based on the peak area integration and expressed as the % of total quantified peak area.

2.5. Transport estimations

The amount of different DOM components that are exchanged with the tides at the mouth of the estuaries was estimated from simple mass balance. Applying mass balance for the evaluation of estuarine cross-sectional fluxes has been widely used by other authors (Gardner and Kjerfve, 2006; Chen et al., 2012).

The instantaneous water flow (Q_i) was calculated from the average current velocity in the water column (v_i) and the section area (A_i):

$$Q_i = A_i v_i = (A_0 + (h_i - h_0) L_0) v_i \quad (1)$$

Where h_i is the depth at a given instant and A_0 , h_0 , L_0 are the section area, depth and length of the section in low tide, respectively. In Guadalquivir and Guadiana estuaries, the increase of the section in high tide was included due to the existence of small intertidal zones on its margins that flooded during high tides and represents < 0.5 % and 2 % of the estuary, respectively. Q_i and v_i were attributed to a positive sign for ebb conditions and negative for flood conditions.

The approach that was used assumes that the estuary is vertically homogenous and that there are no changes in concentration along the sections considered. Moreover, the tidal cycle is considered to be in steady state and only horizontal advection transport is considered. Under these conditions, river flow rates (Q_R) were calculated using the following equation:

$$\int_{t_0}^{t_F} Q_i dt = Q_R (t_F - t_0) \quad (2)$$

where $t_F - t_0$ includes two consecutive tidal cycles (25 h).

Net transport with tides has also been estimated using a simple mass balance:

$$Tidal\ transport = \int_{t_0}^{t_F} Q_i C_i dt \quad (3)$$

$$River\ transport = C_R C_0 (t_F - t_0) \quad (4)$$

where C_i and C_R are the concentrations during the tidal cycle and in the river, respectively. Positive transport is associated with an output of the studied component from the estuary to the coastal area.

2.6. Statistical Analysis

We performed two ways ANOVA and post hoc Tukey multiple comparisons of means 95 % family-wise confidence level to assess spatial differences within the estuaries and between them in DOM characteristics. Linear regression analysis between DOC and DOM properties and with salinity were fitted and considered statistically significant when $p < 0.01$. All analyses were performed in R 3.5 software (R Development Core Team, 2018).

3. Results

3.1 Hydrological characteristics

In the coastal zone next to the three estuaries, salinity varied between 36.2 and 36.5 and temperature between 21.6 and 23.8 °C ($p > 0.05$). In the estuaries, salinity ranged between 0.3 and 36.4 in both Guadalquivir and Guadiana estuaries, increasing downstream (Fig. 2A and 2B, respectively). In Tinto-Odiel estuary, salinity was higher than in the other two estuaries ($p < 0.05$), and an opposite pattern was observed. Thus, salinity decreased from 38.8 to 36.4, with higher values upstream (Fig. 2C). This inverse behavior is due to the strong evaporation and low flow rate of freshwater during summer that makes this system to be regarded as a sea arm. Temperature ranged between 21.6 and 28.0 °C, increasing upstream in the three estuaries ($p > 0.05$).

The highest Q_R measured in this work (Eq. 2) was in Guadalquivir followed by Guadiana (47.1 and 5.85 $\text{m}^3 \text{s}^{-1}$, respectively). A negative value was obtained for Tinto-Odiel ($-0.13 \text{ m}^3 \text{s}^{-1}$) since, in summer, evaporation exceeds freshwater discharge in this system. Similar values have been reported for Guadalquivir ($45.04 \text{ m}^3 \text{s}^{-1}$) and Guadiana ($5.04 \text{ m}^3 \text{s}^{-1}$) by their respective Hydrographic Confederation (www.chguadalquivir.es and www.chguadiana.es), and no data was available for Tinto-Odiel estuary.

A clear tidal effect was observed (Fig. 3) with maximum tidal flow rates in Guadalquivir followed by Tinto-Odiel and Guadiana (5084, 4651, and 1493 $\text{m}^3 \text{s}^{-1}$, respectively). This may be due to the bigger estuary sections where the tidal study was performed in Tinto-Odiel ($7682 \pm 707 \text{ m}^2$) and Guadalquivir ($5936 \pm 448 \text{ m}^2$) compared to Guadiana ($2461 \pm 229 \text{ m}^2$). Throughout the tidal cycles study, salinity values fluctuated between 25.5 and 36.2 in both Guadalquivir and Guadiana estuaries, with the lowest values at low tide and the highest ones during high tide. Conversely, in Tinto-Odiel estuary, salinity barely varied 0.3 units (36.3- 36.6) with the highest values during low tide. No stratification of the water column was observed in the salinity profiles and current velocities showed typical parabolic vertical profiles (Uncles et al., 1985), decreasing towards the surface and close to the bottom (Fig S1).

3.2 Longitudinal variation in DOM composition

DOC concentrations and the optical properties of DOM are presented in Table 1. The lowest average values of DOC, CDOM (a_{254} and a_{350}), and FDOM components were observed in the coastal zone of the three transects ($p > 0.05$). Tinto-Odiel estuary did not show significant differences in DOC and CDOM between coastal and estuarine stations ($p > 0.05$).

In the estuaries, the highest DOC values were observed in the Guadalquivir ($p < 0.05$) and the lowest ones in the Tinto-Odiel. In the Guadalquivir estuary, DOC showed higher values upstream (321.0 μM) than in the lower zone (108.8 μM). However, its maximum values were observed in its middle part (Fig. 4A, max. 541.5 μM). For Guadiana estuary, instead, DOC showed a continuous decrease with increasing salinity, with values ranging from 103.3 to 404.2 μM (Fig. 4B). Lastly, in Tinto-Odiel, an increase in DOC concentrations upstream was also observed with values ranging from 97.6 to 340.6 μM (Fig. 4C). CDOM (a_{254} and a_{350}) followed the same trend as DOC in the three estuaries, decreasing with salinity in Guadalquivir and Guadiana and increasing in Tinto-Odiel. The highest a_{254} values were observed in the Guadalquivir estuary ($p < 0.05$), with maximum values in the middle zone (34.62 m^{-1} , Fig. 4A). Overall, lower values were found in Guadiana and Tinto-Odiel estuaries with maximum values upstream (21.25 m^{-1} , Fig. 4B and 14.72 m^{-1} , Fig. 4C). Absorption coefficient a_{350}

and $SUVA_{254}$ index showed similar values between estuaries ($p > 0.05$) ranging from 0.30 to 6.31 m^{-1} and 0.26 to 2.83 $mg^{-1} L m^{-1}$, respectively. Both a_{350} and $SUVA_{254}$ showed the same pattern as a_{254} and DOC in each estuary.

Fluorescence indexes HIX and BIX showed similar values and trends in both Guadalquivir and Guadiana estuaries ($p > 0.05$), with HIX increasing upstream while BIX decreased. In Tinto-Odiel estuary, maximum values of BIX (4.27) and minimum of HIX (0.21) were observed in the confluence of both Tinto and Odiel rivers.

Similar to DOC and CDOM concentration, the highest TDLP9 were found in Guadalquivir estuary ($p < 0.05$), ranging between 0.98 and 536 $nmol L^{-1}$, and one order of magnitude higher than in Guadiana and Tinto-Odiel (0.02-68.95 $nmol L^{-1}$). These values were within the range of those found in Fichot et al. (2016).

3.3 Assignments and distribution of FDOM components

Four fluorescent components were validated, and their characteristics are reported in Table S1 and Fig. S2. The components were distinguished by their excitation-emission spectra and compared with previously published data on the open-access spectral database OpenFluor by Murphy et al. (2014) (Table S1). The four fluorescent components were found in the coastal zone of the Gulf of Cádiz (Amaral et al., 2020). Component 1 (C1) and component 2 (C2) were identified as humic-like components. C1 has been reported as ubiquitous, found in almost all environments and derived from both allochthonous and autochthonous sources. C2 was referred to as terrestrial origin observed in freshwater impacted by agriculture. Component 3 (C3) and component 4 (C4) represent protein-like fluorescence containing fractions of autochthonous DOM, and they resembled the amino acids tryptophan and tyrosine, respectively (Coble, 1996). Humic-like components C1 and C2 were strongly correlated as well as the protein-like components C3 and C4 among them ($R^2 = 0.98$ and 0.69 , respectively, $n = 150$, $p < 0.01$).

Similar to the concentration of DOC and CDOM, humic-like C1 and C2 showed the highest values in Guadalquivir estuary (Fig. 4D and Table 1), followed by Guadiana (Fig. 4E) and Tinto-Odiel estuary (Fig. 4F) ($p < 0.05$). No significant differences were observed in protein-like C3 and C4 between estuaries ($p > 0.05$). Regarding the longitudinal transects, FDOM components also followed the same pattern as DOC and CDOM. Thus, in Guadalquivir estuary, the highest values were observed in the middle zone, while in Guadiana and Tinto-Odiel increased upstream; however, Tinto-Odiel estuary showed the maximum values of protein-like C3 and C4 in the confluence of both rivers and in the Pedro Santo channel.

3.4 Molecular characterization: Py-GC-MS analysis

Table S2 shows the list of products that were considered of interest and their relative contributions to the pyrolyzed sample. The identified products were grouped based on structural features and prevailing sources (Table 2). The pyrolysis chromatograms for each estuarine are presented in Fig. S2.

All samples produced a peak that corresponds to elemental sulfur or sulfur dioxide (m/z 64), which is probably indicative of inorganic sulfur from mineral particles (e.g.,

pyrite). The samples produced isooctylphthalate, which is probably indicative of plastic contamination (Roy, 2004), and N-butylbenzenesulfonamide, that is not related with DOM from natural environments and could also be from contamination, i.e., from the sample itself (industrial activities), sample preparation or from the interaction of compounds with instrument parts. Another relatively abundant product in Tinto-Odiel, was dichlorobenzoic acid, which may originate from pharmaceutical and agrochemical sources (Rappe, 1979) but also could be released by marine organisms (Repeta et al., 2004).

Lignin products (guaiacol and 4-vinyl guaiacol), which represent a DOM fraction that originates from terrestrial or subaquatic vegetation (vascular plants), were most abundant in Guadalquivir, followed by Guadiana and Tinto-Odiel estuaries. Products with nitrogen (N-compounds), such as pyrrole, pyridine, acetamide, indole, and diketodipyrrole, were enriched in the sample from Guadiana estuary. The balance between lignin from vascular plants, and N-compounds from protein, peptidoglycan and chitin from marine primary producers, was also highest in Guadalquivir (0.38) and lowest in Tinto-Odiel (0.2) and Guadiana (0.14), indicative of a larger proportion of plant-derived DOM in the former. Monocyclic aromatic hydrocarbon compounds (MAH), indicative of degraded polyphenolic material from higher plants, were higher in Guadiana and lower in Tinto-Odiel estuary. Phenols are considered to be indicative of degradation of lignin-derived DOM (van Heemst et al., 1999) and from microbial material (Martin, 1979) and were also higher in Guadalquivir, followed by Guadiana. The compounds that represent methylene chain compounds (MCC) were, by far, most abundant in Tinto-Odiel estuary and may originate from anthropogenic or natural sources (Peulvé et al., 1996). Polycyclic aromatic hydrocarbons that may represent pyrogenic organic matter (fire residues) showed the lowest proportion in Tinto-Odiel, but additional caution is required here as matrix effects can cause aromatization and perhaps contamination contribute to these compounds.

3.5 Tidal Cycles

Similar to the longitudinal transects, DOC, CDOM (a_{254} and a_{350}), $SUVA_{254}$ and HIX values were higher in Guadalquivir estuary, followed by Guadiana and Tinto-Odiel estuaries, while BIX values were higher in Tinto-Odiel estuary (Fig. 5, $p < 0.05$).

In Guadalquivir and Guadiana estuaries, DOC and CDOM (a_{254} and a_{350}), followed the typical sinusoidal shape along time, associated with tidal cycles with maximum values during low tide and minimum at high tide (Fig. 6A and 6B, respectively). In Guadalquivir estuary, DOC concentration was between 146.6 and 378.5 μM , while in Guadiana, it ranged between 79.4 and 235.1 μM . Absorption coefficients a_{254} and a_{350} fluctuated from 4.06 to 22.28 m^{-1} and from 0.64 to 5.90 m^{-1} in Guadalquivir, respectively. In Guadiana they ranged from 1.50 to 10.16 m^{-1} and from 0.39 to 3.44 m^{-1} , respectively. For Tinto-Odiel, no clear tidal variations were observed (Fig. 6C), with DOC values ranging between 100.8 and 159.2 μM , a_{254} ranged from 1.13 to 6.51 m^{-1} and a_{350} from 0.05 to 2.39 m^{-1} .

The concentration of DOC, a_{254} and a_{350} showed an inverse relationship with salinity during the tidal study in both Guadalquivir ($R^2 = 0.89, 0.90$ and 0.64 , respectively, $p < 0.01$) and Guadiana ($R^2 = 0.52, 0.73$ and 0.40 , respectively, $p < 0.01$) estuaries, as seen for the longitudinal transects. Although in Tinto-Odiel estuary, a smaller variability in

salinity was observed (Fig. 3), a_{254} and a_{350} followed a similar behavior than salinity ($R^2 = 0.31$ and 0.28 , respectively, $p < 0.01$), while DOC remains relatively constant.

Similar to DOC and CDOM, the fluorescence intensity of the four components also show a clear distribution depending on salinity during the tidal cycles in both Guadalquivir ($R^2 > 0.91$, $p < 0.01$) and Guadiana estuaries ($R^2 > 0.74$, except for C3 where $R^2 = 0.32$, $p < 0.01$) with higher concentrations of humic-like components during low tide (Fig 6D and 6E). This trend was not observed in Tinto-Odiel estuary (Fig. 6F), although a slight dependence with salinity was observed, especially in the humic-like fraction (R^2 C1 = 0.18 and C2 = 0.31 , $p < 0.01$). Fluorescent indexes, HIX and BIX, showed the lowest variation in Guadalquivir and Guadiana estuary (Fig. 6G and 6H) and the highest in Tinto-Odiel (Fig. 6I). The indexes showed opposite trends, thus, HIX decreased during high tide while BIX increased.

4 Discussion

4.1 Distribution of DOM in the estuaries

Four fluorescent components widely distributed in aquatic systems and found in the online repository OpenFluor database (Murphy et al., 2014) were validated using PARAFAC. The components explained 93 % of FDOM variability observed in the estuaries.

DOC concentrations and CDOM absorption coefficients (a_{254} and a_{350}) obtained in this study were similar to those values found in other estuarine systems (e.g., Rochelle-Newall and Fisher, 2002, Guo et al., 2007). Our results indicate that the Guadalquivir estuary has the highest concentrations of DOC, CDOM, and humic-like material, followed by Guadiana and Tinto-Odiel. The Guadalquivir river is the major river flowing into the continental shelf of the Gulf of Cádiz with the largest freshwater discharges. In contrast, Tinto-Odiel estuary has a predominantly marine influence, and the lower freshwater discharge could explain this trend during the dry season. Regarding the DOM sources, protein-like fluorescence is known to dominate in estuarine systems where autochthonous production overwhelms the diluted terrestrial signal (Osburn and Steimmon, 2011), and in systems under the influence of urban and industrial activities (Hong et al., 2005, Coble et al., 2014). During the whole study, maximum intensities of protein-like C3 and C4 were recorded in the confluence of Tinto and Odiel rivers. The contribution of protein-like components (% C3 + % C4) to the total FDOM pool in this estuary was 53 %. Also, BIX values > 1 , observed in Tinto-Odiel estuary, indicated the presence of autochthonous DOM and organic matter freshly released into the water (Huguet et al., 2009). In Guadalquivir and Guadiana estuaries, protein-like components only accounted for 28 %, being the humic fraction the dominant one with ~ 82 % of the total FDOM pool.

4.1.2 Behavior of DOM during estuarine mixing

DOM can undergo a variety of biogeochemical reactions within estuaries that determine the concentration and composition of DOM reaching the ocean. The behavior of terrestrial DOM during estuarine mixing is highly variable.

Mixing diagrams of DOM concentration along the salinity gradient were used to characterize the estuarine behavior of DOM and identify changes in its properties within estuaries. A linear behavior may reflect fewer changes in DOM properties along the estuary. In contrast, points falling below the theoretical dilution line could be associated with removal of DOM (photochemical or microbial removal) while points falling above the line indicate that *in situ* production and/or inputs from lateral sources are occurring (e.g., anthropogenic pollution, autochthonous production, inputs from tributaries) (Guo et al., 2007, Raymond and Spencer, 2015). Concentrations of DOC, CDOM (as absorption coefficients), and fluorescent components of each estuary were plotted as a function of salinity and non-linearity was examined (Table 3). Differences in the mixing behavior of DOM between estuaries were observed. In Guadiana, DOM showed linear trends with salinity, indicating a major terrestrial origin of DOM (Fig. 4B and 4E). Dilution and physical mixing explain the majority of the spatial variability observed as fresh and coastal waters mix along the Guadiana estuary. Similar behavior during estuarine mixing has been reported in other coastal systems (Rochelle-Newall and Fisher, 2002, Jaffe et al., 2004, Guo et al., 2007, Yamashita et al., 2008). However, positive deviations from the linear mixing were observed in most variables of DOM. To quantify those deviations from the theoretical dilution line we calculated the anomalies/residuals. We found an average DOC excess of $29.9 \pm 9.9 \mu\text{M}$ in stations with $S > 29$, that is mainly in the lower zone of the estuary. The average excess of CDOM, as both a_{254} and a_{350} , and humic-like components (C1+C2) in those stations was $3.5 \pm 0.8 \text{ m}^{-1}$, $0.7 \pm 0.5 \text{ m}^{-1}$ and $0.04 \pm 0.01 \text{ RU}$, respectively. These deviations could be due to DOM inputs from the $\sim 16 \text{ km}^2$ salt marshes near Guadiana mouth, in the lower zone of the estuary, where the Natural Park “Marismas de Castro Marim y Vila Real de Santo Antonio” are located (www.chguadiana.es).

In Guadalquivir estuary, a non-linear relationship between DOM variables and salinity was observed (Table 3, Fig. 4A and 4D), with the highest values (positive deviations) in the upper and middle zone coinciding with the maximum turbidity zone. This implies a possible contribution of organic matter to the dissolved phase that may be derived from different sources. In the upper zone, Guadalquivir received the freshwater inputs of a tributary (Guadaira river), however its discharge is less than 5 % of the one measured in Guadalquivir, thus a minor effect in DOM properties is expected. On the other hand, 36000 ha of rice crops are situated in the margins of Guadalquivir estuary (www.chguadalquivir.es), between 45 and 80 km from the mouth, where these maximum values were found. Noteworthy, $\sim 15 \%$ of the water withdrawal for irrigation is used in these crops (Carrasco et al., 2010). Given the shape of the curve with a progressive increase of DOM through the area where the rice fields end and an almost linear behavior towards the mouth ($R^2 = 0.98$), we can conclude that this could be the main explanation for the positive deviation observed here. Furthermore, the strong relation observed between TDLP9 with DOC ($R^2 > 0.94$), humic-like components ($R^2 > 0.98$), and protein-like components ($R^2 > 0.90$), indicated that terrestrial DOM dominates the bulk of DOM pool. This was also evident in the Py-GC-MS analysis, where DOM fractions from terrestrial sources were more abundant in Guadalquivir (e.g., lignin, phenols, Table 2), as observed in headwater streams (Kaal et al., 2016, 2017). Additionally, wetlands have been considered an important factor in the transport of large amounts of organic matter into inland waters (Wetzel, 1992), and Guadalquivir is located in one of the largest wetlands of Europe (Doñana Natural Park), where salt marshes occupy $\sim 27000 \text{ ha}$ (www.juntadeandalucia.es). *Spartina* salt marshes, found in this estuary, have shown to be a major source of CDOM in other

estuarine systems (Gardner et al., 2005, Tzortziou et al., 2008). Therefore, our results suggest the existence of two main allochthonous sources of DOM located on the banks of this estuary, rice crops and wetlands.

It should be noted that the salinity gradient in Tinto-Odiel barely exceeds two units and that this system comprises different parts (Tinto River, Odiel River, and the common marine area Padre Santo channel, Fig. 1). Therefore, we examined DOM mixing behavior independently in each part of the estuary. In general, positive linear relationships with salinity were found (Table 3) when removing maximum values of DOC in Tinto river (340 μ M) and protein-like components in the confluence of both rivers and the Pedro Santo channel (> 0.4 RU, Fig. 4F). These stations are located near the Industrial and Chemical Complex of Huelva which comprises a marine loading dock and embraces fertilizer, metal, oil, and paint factories that could have an influence in the composition of DOM in this system (Fig. 1). There is also the water treatment plant from Huelva city (150000 pop.) and 1200 ha of phosphogypsum (PG) residue rafts from the fertilizer industry that were stockpiled on the salt marshes of the Tinto river (Pérez-López et al., 2011). PG shows emission peaks at 300 nm and 342 nm after excitation in the UV (Hammas et al., 2013) but identifying its characteristic peak using PARAFAC was not possible here since it fluoresces in the same region than the protein-like components (Table 1S). However, we found that fluorescence intensity at Ex/Em 260 nm/342 nm (“peak picking” technique, Coble, 1996) was one and two order of magnitude higher in the samples located downstream the PG deposit (0.14 and 0.25 RU) than in the rest of the samples (< 0.06 RU). Nevertheless, further analysis will be necessary to confirm the origin of this elevated fluorescent signal. Tinto-Odiel estuary is one of the most polluted estuaries in the world due to the mining of the massive sulfide deposits in its basin and the industrial activities occurring in its margins (Davis et al., 2000). This could explain the presence of relatively elevated chemical pollutants in pyrolyzates of DOM in this estuary (Table 2).

On the other hand, the positive trend observed between DOM variables and salinity in Tinto-Odiel estuary is because evaporation exceeds riverine inputs during the dry season and makes this system to behave as an inverse estuary. This is often the case in Mediterranean estuaries during the dry season (Catalá et al., 2013). In addition to its inverse nature and low freshwater discharge, there is an autochthonous production of DOM, as well as anthropogenic sources of pollution. Unlike Guadalquivir and Guadiana estuaries, DOC and protein-like components showed a low correlation with TDLP9 in Tinto-Odiel ($R^2 < 0.3$, $p < 0.01$). Hence, the main sources of these components were probably not from terrestrial origin. The microbial production of protein-like components has been reported in other coastal environments (Yamashita et al., 2011, Amaral et al., 2016) as well as in phytoplankton and bacterial experiments (Romera-Castillo et al., 2010, Fox et al., 2017). Usually, lower freshwater discharge, as it is the case in this estuary, results in greater potential for microbial mineralization, prevailing its byproducts over the terrestrial sources of DOM (Raymond and Spencer, 2015). Results from Py-GC-MS analysis support this statement since DOM fractions from vascular plants were lower in Tinto-Odiel while the structures likely derived from *in situ* production and anthropogenic pollutants were the most abundant (Table 2). For example, the higher proportion of MCC in Tinto-Odiel estuary (~ 25 %) than Guadalquivir and Guadiana (< 3 %) could be indicative of larger proportions of planktonic and aliphatic-rich materials (Peulvé et al., 1996, Jiang et al., 2017). We can

conclude that estuarine biological activity, together with anthropogenic contamination, might be the dominant sources of DOM in Tinto-Odiel estuary.

CDOM removal associated with photodegradation has been observed along the salinity gradient in other estuarine systems (Vodacek et al., 1997, Li et al., 2015). However, this was not the case for the estuaries studied here, in agreement with other estuarine systems (Rochelle-Newell and Fisher 2002, Guo et al., 2007), possibly due to the low transparency and a relatively short water residence time (Guo et al., 2007). Guadalquivir and Guadiana showed a high turbidity nature reaching values of 1000 and 600 FTU, respectively (data not shown), which could affect the photochemical processes (Hansell and Carlson, 2014). Also, photochemical alteration could be smaller than the terrestrial DOM inputs (Rochelle-Newell and Fisher 2002). On the other hand, the minor effect of photodegradation observed in plankton-derived material (Obernosterer and Benner, 2004) could explain the case of Tinto-Odiel estuary.

4.2 The effect of tidal cycles in DOM properties

Tidal cycles were clearly observed in DOC and CDOM distribution in both Guadalquivir and Guadiana estuaries, with higher values during low tide and lower during high tide (Fig. 6). Tidal cycles also affected DOM composition in these estuaries, indicating that a discharge increase provokes a terrigenous DOM enrichment of the estuarine water, as it has been previously observed in other tidal-influenced systems (Tzortziou et al., 2008, Zhou et al., 2019). In contrast, in Tinto-Odiel estuary, only a slight dependence with salinity was found (Fig. 6), indicating, again, the minor contribution of terrestrial DOM to this system.

The relative contribution (%) of each fluorophore was calculated to evaluate the influence of tidal cycles in FDOM variability. During the two tidal cycles, in Guadalquivir and Guadiana, humic-like components account for an average of $83 \% \pm 1.7 \%$ and $75 \% \pm 2.7 \%$ of the FDOM pool, respectively. The higher values observed during the low tides were due to a larger contribution of these compounds from the rivers. On the other hand, protein-like components increased almost twofold their contribution at high tide, since they are associated with the production of organic matter in the lower zone of the estuaries. In Tinto-Odiel estuary, humic-like components contribution ($47 \% \pm 1.7 \%$) was smaller than in the other two estuaries but similar to the protein-like fraction ($53 \% \pm 5.2 \%$). These results reinforced the hypothesis that DOM in Guadalquivir and Guadiana is mainly from terrestrial origin, while in Tinto-Odiel DOM has a major autochthonous character. Although, this elevated protein-like fluorescence could also be due to anthropogenic inputs from the industrial activities occurring in its margins. Thus, both biological production and anthropogenic pollutants could explain the highest contribution of the protein-like fraction observed in this system.

4.3 Flux of DOC, CDOM, and FDOM

Figure 7 shows the evolution of instantaneous fluxes of DOC, a_{254} , a_{350} , and the different components of FDOM during the tidal cycles. Maximum and minimum values are produced during the ebb and flood tides, respectively. The asymmetries observed in its variation are related to the evolution of each variable during the tidal cycle.

Table 4 shows the flux values estimated for both tidal (Eq. 3) and river transport (Eq. 4) in Guadalquivir and Guadiana, but only tidal transport in Tinto-Odiel since Q_R here was negligible (Eq. 2). Guadalquivir estuary showed the highest DOC, CDOM and FDOM flux estimates in both cases, even one order of magnitude higher than the other two estuaries. Besides, the highest concentration of DOM and discharge were observed in Guadalquivir, and it is generally accepted that rivers with higher discharges have greater DOM export (Raymond and Spencer, 2015). Additionally, lateral inputs of DOM in the upper and middle zone of the estuary from the rice crops and the extensive wetland coverage in Guadalquivir watershed may also contribute to explain these results, as it has been observed in other systems (Spencer et al., 2013). In Guadiana estuary, the tidal net transport of DOM was considerably higher than river transport. Salt marshes could be a source of DOC and CDOM to adjacent estuarine ecosystems (Tzortziou et al., 2008). Thus, the existence of salt marshes in the lower zone of this estuary, could explain this trend since DOC, CDOM and humic-like FDOM showed positive deviation from the linear mixing behavior in the lower zone of the estuary. Unexpectedly, Tinto-Odiel estuary showed a substantial transport of DOC, CDOM and FDOM to the coastal zone (except for C3 that is imported), even higher than Guadiana estuary. This could be due to the higher discharge observed where the tidal study was performed in Tinto-Odiel ($\pm 5000 \text{ m}^3 \text{ s}^{-1}$) in comparison with Guadiana estuary ($\pm 1500 \text{ m}^3 \text{ s}^{-1}$).

Among fluorescent components, there was a relatively higher net export of humic-like components (C1+C2) than protein-like components (C3+C4). Thus, between 68 % and 77 % of the FDOM exported from the three estuaries was of humic nature with a low contribution of autochthonous DOM, as it has been observed in riverine DOM fluxes (Stepanaukas et al., 2005). According to Sickman et al. (2010), land use and disturbance result in the export of aged components that could also explain these results, mainly in Guadalquivir and Guadiana. Noteworthy, in Tinto-Odiel, the protein-like C4 accounts for almost 50 % of the total FDOM that this system exports to the coastal zone. This may be associated with anthropogenic sources, such as the PG residue rafts from the above-mentioned fertilizer industry.

Sampling only one season is not enough to capture the full variability range over one year, and recent studies have demonstrated large seasonal variations in DOC export from rivers to the ocean (Lee and Kim, 2018, Li et al., 2019). If these data were extrapolated to an annual export, the contribution of the freshwater discharge from Guadalquivir and Guadiana ($1.67 \text{ Km}^3 \text{ yr}^{-1}$) would account for $< 0.004 \%$ to the total rivers discharge in the world ($37400 \text{ Km}^3 \text{ yr}^{-1}$, Cauwet, 2002). The total DOC load from these three rivers would be 0.06 Tg yr^{-1} , equivalent to 0.024% of the global annual riverine load of DOC (250 Tg yr^{-1}) transported to the oceans (Hedges et al., 1997), and CDOM export (derived from a_{350}) would be $1.73 \times 10^{10} \text{ m}^2 \text{ yr}^{-1}$. These values are lower than those observed for other rivers (Spencer et al., 2013, Raymond and Spencer 2015, Li et al., 2019). Noteworthy, the fluxes estimated in our work were during the dry season, in the absence of precipitations, when the lower discharge occurs. For example, during the rainy season (January-February), discharges in Guadalquivir river can reach values of $5000 \text{ m}^3 \text{ s}^{-1}$ (Vanne, 1970). Thus, sampling during the rainy season will be necessary to obtain sufficient temporal data required for accurate estimates of DOC and CDOM annual fluxes. However, our results could be a baseline for these three systems in dry conditions, where, as far as we know, no DOM composition studies and flux estimations have been done until now. Seasonal studies of DOM fluxes will be also necessary to understand its potential impact on the coastal ocean under a climate change

scenario, where rainfall increase has been predicted (IPCC, 2012).

4.3 Relation between DOM properties

Several studies have found strong correlations between DOC, CDOM, and FDOM in a variety of aquatic systems (Rochelle-Newel and Fisher, 2002, Spencer et al., 2013, Rochelle-Newel et al., 2014, Osburn et al., 2016). In this work, when all water samples are combined ($n = 150$), a positive linear relationship was also observed between DOC concentrations, CDOM absorption coefficients and FDOM components (Fig. 8). Humic-like components and a_{254} showed the strongest relationship with DOC ($R^2 > 0.91$) indicating that allochthonous materials dominated the DOC pool since CDOM is generally terrestrial in estuaries (Osburn et al., 2016). On the other hand, protein-like components and a_{350} had lower relationships with DOC ($R^2 = 0.68-0.81$). A higher contribution of protein-like components in Tinto-Odiel estuary may affect the lower relationship compared to humic-like components. Rochelle-Newel et al. (2014) suggest that variations in the ratio of terrestrially / microbial-derived DOC and CDOM will result in alterations in the relationship between CDOM and DOC. Although CDOM and FDOM are often proposed as a rapid method for the estimation of DOC, a high seasonal variability has been observed in DOC-CDOM and DOC-FDOM relationships (Rochelle-Newel et al., 2014) in estuaries mostly associated to river discharge (Osburn et al., 2016). Thereby, the strong relationship found in this work may only be useful during the dry season when the lowest river discharge occurs.

Conclusions

The application of EEM-PARAFAC, together with Py-GC-MS analysis in the assessment of DOM dynamics in the estuaries of the Southern Iberian Atlantic Basin, showed to improve the understanding of the biogeochemical processes controlling DOM. As such, while in Guadiana DOC and FDOM components decreased linearly with increasing salinity, it was not the case for Guadalquivir estuary. This result was attributed to lateral inputs in the upper and middle zone of the estuary, where a large extension of rice crops and wetlands exists. Both Guadiana and Guadalquivir estuaries showed that DOM was predominantly from allochthonous origin with a contribution of 83 % of humic-like components to the total FDOM pool. Conversely, DOM in Tinto-Odiel estuary showed that biological activity and anthropogenic pollution from industrial activities were the main sources of DOM. Py-GC-MS analysis supported these results with the highest relative proportions of products from terrestrial/coastal vegetation in Guadalquivir and Guadiana, and products from marine organisms and potential anthropogenic contaminants in Tinto-Odiel estuary. Moreover, results from TDLP9 were consistent with our data, with the highest values in Guadalquivir estuary, and strongly related with the concentration of DOC in this system where terrestrial DOM predominates ($R^2 = 0.95$, $p < 0.01$). Instead, in Tinto-Odiel estuary, where terrestrial DOM is not the main source, a weaker relation was observed ($R^2 < 0.34$, $p < 0.01$). Therefore, we trust that TDLP9 equations may be valid for our systems. The results of the tidal study indicated that these systems are a net source of DOC and CDOM, mainly of humic nature, to the Gulf of Cádiz. Guadalquivir exhibited the highest estimated DOC, CDOM, and FDOM values, followed by Tinto-Odiel and Guadiana, which together export 1.88 Kg s^{-1} of DOC, $0.55 \text{ m}^2 \text{ s}^{-1}$ of CDOM and $76.4 \text{ RU m}^3 \text{ s}^{-1}$ of humic-like substances and $24.4 \text{ RU m}^3 \text{ s}^{-1}$ of protein-like material to the Gulf of Cádiz. DOC and CDOM exports are among the lowest of the major world

rivers; however, this work was performed during the dry season, in the absence of precipitation. Climate change is expected to increase riverine freshwater, hence DOM supply. Therefore, this work could be a baseline for the export of terrestrial DOM to the Gulf of Cadiz. Overall, a robust positive linear relationship between DOC, CDOM and FDOM was found, with R^2 higher than 0.91 for a_{254} and humic-like components, which could be used for a rapid estimation of DOC in these estuaries during the dry season. Thus, spectroscopic techniques have potential to improve the estimations of DOC fluxes to the ocean either through CDOM measurements or via remote sensing techniques.

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Author's contribution

VA, CR-C and JF designed the study and performed research; MG and AGP helped with data acquisitions and processing; VA was in charge of writing the manuscript with the help and inputs of JF and CR-C. All authors revised and approved the submitted version.

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Figure. 1. Map with the samples stations in the Guadalquivir, Guadiana and Tinto-Odiel transect. Tinto-Odiel estuary comprises three parts: Tinto River, Odiel River and the common marine part named Padre Santo channel.

Figure. 2. Salinity gradient along the three estuaries. A: Guadalquivir, B: Guadiana and C: Tinto-Odiel.

Figure. 3. Depth, current velocity (V), tidal flow rate (Q) and salinity (S) during the two tidal cycles at the mouth of Guadalquivir, Guadiana and Tinto-Odiel estuaries. Q was calculated during low tide (depth (h_0) = 4.05, 6.30 and 6.78 m, respectively; section (A_0) = 5197, 2135 and 6598 m², respectively; length of the section (L_0) = 722, 387 and 927 m, respectively).

Figure. 4. Longitudinal distribution of dissolved organic carbon (DOC), CDOM absorption coefficient (a_{254} and a_{350}) and fluorescent components (C1 to C4) along the three estuaries: Guadalquivir (A and D), Guadiana (B and E) and Tinto-Odiel (C and F). Confluence of Tinto and Odiel rivers at salinity = 36.9.

Figure. 5. Boxplots showing dissolved organic carbon (A: DOC), CDOM absorption coefficient (B: a_{254} and C: a_{350}), SUVA₂₅₄ index (D), humification index (E: HIX) and the index of recent autochthonous contribution (F: BIX) for Guadalquivir (GD), Guadiana (GU) and Tinto-Odiel (TO) during the tidal study.

Figure. 6. Distribution of dissolved organic carbon (DOC), CDOM absorption coefficient (a_{254} and a_{350}) and fluorescent components (C1 to C4), and fluorescent indexes in the three estuaries during the tidal study: Guadalquivir (A, D and G), Guadiana (B, E and H) and Tinto-Odiel (C, F and I).

Figure. 7. Tidal fluxes of dissolved organic carbon (DOC), CDOM absorption coefficient (a_{254} and a_{350}) and fluorescent components (C1 to C4) in each estuary.

Figure. 8. Linear relationship between dissolved organic carbon (DOC) with CDOM absorption coefficient (a_{254} and a_{350}) and fluorescent components (C1 to C2) with a $p < 0.01$ and $n = 150$.

Highlights

- Linear mixing mainly explains DOC and DOM distribution in Guadiana estuary.
- Non-linear behavior in Guadalquivir, linked to lateral inputs from rice crops and wetlands.
- Tinto-Odiel might have more autochthonous production of DOM associated to anthropogenic pollutants from industrial activities.
- The three estuaries represent a net source of DOC and CDOM to the Gulf of Cádiz.
- About 70 % of the DOM export by these estuaries is of humic nature.
- Spectroscopic techniques as a rapid method of DOC estimation during the dry season.