Characterization of Chromophoric Dissolved Organic Matter (CDOM) in Rainwater Using Fluorescence Spectrophotometry

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Abstract The fluorescence excitation–emission matrix of Chromophoric dissolved organic matter (CDOM) samples from rainwater collected at Rameswaram, Tamilnadu, India are analysed. Total five peaks were observed for humic/marine and protein likes substances respectively. The peak A and C intensities varies form 1.98 ± 0.28 and 0.97 ± 0.11 QSU respectively represents humic like substances. The peak B and T intensities varies from 3.94 ± 0.75 and 7.42 ± 1.43 QSU showed association of protein like substances whereas peak M intensities varies from 1.92 ± 0.37 QSU indicates marine contribution.

Among the fluorophores, the following sequence were observed as T > B > A > M > C which indicates dominance of Tryptophan like substances in rainwater. The average peak T/C ratios was observed as 7.88 ± 2.2 indicates microbial contamination by Tryptophan-like substances with the high biological activity and low volatility.

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Chromophoric organic matter (CDOM), an optically active component of DOM, plays an important role in carbon cycling and affects ocean color (Hong et al. 2005; Coble 2007). It is present in fresh or saltwater primarily due to the release of tannins (polyphenols that bind to proteins and other large molecules) or lignins (polymers of phenolic acids) by decaying plant material. CDOM may also be characterized as byproducts from the decomposition of animals. Water color may range from pale yellow to brown as a result of varying concentrations and sources of CDOM. The presence of significant quantities of highly CDOM in atmospheric waters has profound ramifications with respect to a wide variety of fundamental processes in atmospheric chemistry because of its impact on solar radiative transfer and its involvement in the oxidizing and acid generating capacity of the troposphere. Additionally, if CDOM constituents are surface-active they will have a direct impact on droplet population and consequently cloud albedo by lowering the surface tension of atmospheric waters. CDOM, which is the fraction of DOM that absorbs light over a broad range of ultraviolet (UV) and visible wavelengths, is essentially controlled by in situ biological production, terrestrial inputs (sources), photochemical degradation, microbial consumption (sinks), as well as deep ocean circulation (Coble 2007; Para et al. 2010).

Rameswaram is a town in Ramanathapuram district in the Indian state of Tamil Nadu. It is located on Pamban Island also known as Rameswaram Island separated from mainland India by the Pamban bridge channel. It is bordered by both the Gulf of Mannar and Bay of Bengal on the southern and the northern sides, respectively. Rameswaram has dry tropical climate, with average annual rainfall 94 cm, mostly from North East monsoon from October to January. Temperature is around 30 to 35°C. In this study, three-dimensional excitation emission spectrometry was applied to characterization of CDOM in rainwater collected during September–December 2010 at Rameswaram, Tamilnadu, India. The fluorescence properties and origin of the fluorescent substances in the rainwater samples were investigated and concentration has been evaluated to gain insight into their structural nature and CDOM types.

Materials and Methods

Rainwater samples were collected at Rameswaram (09°17′12.3″N, 79°19′5.6″E) on event basis during September–December (2010) using one litre polyethylene bottle kept under funnels (diameter 14 cm). The glassware was thoroughly washed in deionized water to prevent contamination. They were placed at a height of 1-m to counter the contamination by droplet splashes. These polyethylene bottles and funnels were deployed for sampling immediately after rain began and retrieved soon after the rain stopped. A total of 27 samples were collected and had sufficient volume for chemical analysis, which represents most of the rain events during the study period.

Fluorescence characteristics of the rainwater samples were investigated by a Fluorescence spectrophotometer (F-4500, Hitachi, Japan) equipped with a Xenon flash lamp, using 10 mm quartz cells. Fluorescence measurements were made by making emission scans from 200 to 550 nm, at excitation wavelengths every 10 nm from 200 to 550 nm, with a 10 nm slit width, a PMT voltage of 700 V and scanning speed of 1,200 nm min^{-1}. Quinine sulfate in 0.1 N H_2SO_4 was selected as the reference standard because it absorbs UV light and has high quantum fluorescent yield. Maximum fluorescence yield can be achieved if diluted in weak acids. It has an excitation wavelength of 350 nm and emission wavelength of 450 nm, similar to many CDOM compounds. The standard solutions and samples were adjusted to get similar results at the same excitation wavelength (Scapini et al. 2010). The minimum detection limit of quinine sulfate solution is 0.4 ppb in fluorescence spectrophotometer. The relative fluorescent intensities of the samples were expressed in terms of standard quinine sulfate units (QSU) where 78 ± 1.1 intensity unit are equivalent to one QSU (1QSU = 1 μg/l = 1 ppb in 0.1 N H_2SO_4) (Muller et al. 2008; Coble 1996; Coble et al. 1998; Ghervase et al. 2010; Para et al. 2010). The resulting map represents a fingerprint specific for rainwater. The visual method indicates that the fluorescence of rainwater samples through EEM is given by the CDOM is composed of two major categories of fluorophores: humic-like and protein-like substances. Humic-like fluorescence is attributed to the presence of humic, and marine humic-like acids, accounting for 40%–60% of the organic matter, while the protein-like fluorescence describes peaks that are ascribed to amino acids, mainly Tryptophan and Tyrosine like substances.

Results and Discussion

Excitation/emission maxima of fluorescence in rainwater, sources, % contribution of each peak are presented in Table 1 (Coble 1996). The classification of fluorophores were made according to literature reported elsewhere (Mopper and Schultz 1993; Coble 1996; Coble et al. 1998; Blough and Del Vecchio 2002; Santos et al. 2009). Total

Table 1 Exication/emission maxima of fluorescence in rainwater at Rameswaram

| Peaks	Sources	QSU	% of each peak	Total % of humic and protein like substances
Peak A (Ex$_{<260}$/Em$_{380–460}$)	UV humic-like, less aromatic	1.98 ± 0.28 (1.65–2.92)	12	(A + C+M) = 30%
Peak C (Ex$_{320–360}$/Em$_{420–460}$)	Visible humic-like more aromatic	0.97 ± 0.11 (0.72–1.40)	6	
Peak M (Ex$_{290–310}$/Em$_{370–410}$)	Marine-humic like	1.92 ± 0.37 (1.59–3.46)	12	
Peak B (Ex$_{260}$/Em$_{280}$)	Tyrosine-like substances	3.94 ± 0.75 (3.07–5.93)	25	(B + T) = 70%
Peak T (Ex$_{250–300}$/Em$_{305–355}$)	Protein-like Tryptophan	7.42 ± 1.43 (3.93–11.6)	45	
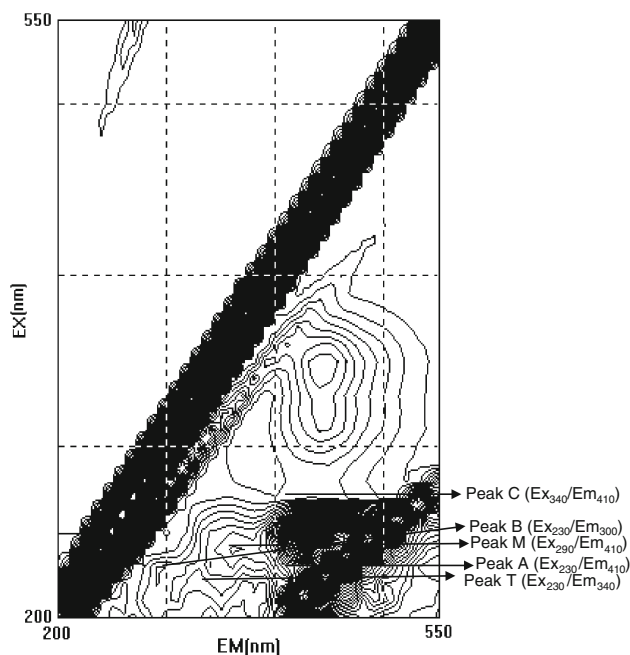
Fig. 1 Typical excitation–emission contour of rainwater at Rameswaram

five peaks were observed as UV humic-like (A) (Ex_{230}/Em_{410}), visible humic like more aromatic, (C) (Ex_{340}/Em_{410}), Tyrosine like substances (B) (Ex_{230}/Em_{300}), Tryptophan like substance (T) (Ex_{230}/Em_{340}), and Marine like substance (M) (Ex_{290}/Em_{410}) respectively. The EEM spectra of rainwater at Rameswaram are depicted in Fig. 1. Among the fluorophores, the following sequence were observed as $T > B > A > M > C$ which indicates dominance of Tryptophan like substances in rainwater. Considering the contribution humic/marine $(A + C + M)$ and protein like substances $(B + T)$, a dominance of protein like substances (70%) was observed as compared to humic/marine like (30%).

The fluorescence intensities for peak A (Ex_{230}/Em_{410}) UV humic like less aromatic substances varied from 1.98 ± 0.28 (1.65–2.92) QSU indicate presence of humic like substances in rainwater may be from aerosol in the atmosphere which is scavenged by rainwater both within and below clouds and are likely to be the main contributors in rainwater whereas fluorescence intensities for peak C (Ex_{340}/Em_{410}) UV humic like more aromatic varied from 0.97 ± 0.11 (0.72–1.40) QSU (Kiss et al. 2003; Cavalli et al. 2004; Muller et al. 2008). A study reported at Birmingham showed humic like substances in the range of 37–995 (average 209) a.u. (arbitrary unit) (Muller et al. 2008).

The fluorescence intensities for peak M (Ex_{290}/Em_{410}) marine like substances varied from 1.92 ± 0.37 (1.59–3.46)

QSU for rainwater indicate that site is influenced by coastal/marine sources prevailing in the region.

The fluorescence intensities for peak B (Ex_{230}/Em_{300}) Tyrosine like substances varied from 3.94 ± 0.75 (3.07–5.93) QSU for rainwater indicates their involvement in cloud formation due to presence of biogenic and proteinaceous matter in cloud waters (Szyrmer and Zawadzki 1997). The mean fluorescence intensities of Tyrosine like substances reported at Birmingham was 469 a.u. (Muller et al. 2008).

The fluorescence intensities for peak T (Ex_{230}/Em_{350}) Tryptophan like substances varied from 7.42 ± 1.43 (3.93–11.63) QSU for rainwater. The common source includes plant matter, bacteria, yeast, spores and pollen associated with terrestrial areas. The bacterial strains can alter the physical and chemical properties of clouds and rain via microbiological degradation and by influencing the removal of particulate and gaseous compounds (Amato et al. 2005).Considering the accountability of dissolved organic carbon in fog water indicating the presence of biogenic and proteinaceous substances in cloud water reported elsewhere, both the substances directly absorb sunlight and bring out changes in organic compound which may influence the composition of particles and rainwater (Zhang and Anastasio 2003). The mean fluorescence intensities of Tryptophan like substances reported at Birmingham was 265 a.u. (Muller et al. 2008).

In order to assess the contribution of terrestrial and marine source in rainwater at Rameswaram, humic and marine (Peak A/M) ratios were calculated and were observed as 1.05 ± 0.16 which is equal to 1 (Table 2). The ratios <1 indicates that rainwater samples holds compounds with more unsaturated bond systems, where more than two π orbital overlap leading to an increase in absorption values. The results of Aveiro, Portugal showed A/M ratios in the range of 1.3–2.72 during autumn, winter and cold season respectively (Santos et al. 2009). Similarly, at Wilmington, North Carolina, reported A/M ratio in the range of 1.07–1.29 indicates that photodegradation is more active for Peak-A type than Peak-M type fluorophores (Keiber et al. 2006). Similarly, the (Peak T/C) ratio was also calculated for the balance of tryptophan and humic like substances at maximum intensities of Tryptophan (Ex/Em range 270–280/320–350 nm) and humic-like substances (Ex/Em range 330–350/420–480 nm). The average values of peak T/C ratio was observed as 7.88 ± 2.2 indicates microbial contamination by Tryptophan-like substances in rainwater samples with the high biological activity and low volatility (Baker et al. 2008).

Humification index (HIX) usually estimates the degree of maturation of dissolve organic matter by following equation reported elsewhere (Zsolnay et al. 1999).

Table 2 Humification and biological indexes for rainwater at Rameswaram

Location	Type	HIX	BIX	Peak A:M	Peak T:C
Rameswaram	Rainwater	0.47 ± 0.11	1.30 ± 0.50	1.05 ± 0.16	7.88 ± 2.2
		(0.35–0.89)	(1.05–1.63)	(0.52–1.30)	(3.7–18.9)

$$HIX = Ex_{254}/Em_{435-480}/Ex_{254}/Em_{300-345}$$

when excited with 254 nm wavelength. Although the chosen excitation wavelength does not give the maximum fluorescence intensity of any fluorophores band, it gives qualitative details about the water samples and the degree of DOM maturation. Organic matter of aquatic ecosystems is divided into two groups-allochthonous and autochthonous. Allochthonous organic matter is a mixture of organic matter of humic nature and terrigenous origin, the sources of which are products of incomplete decomposition of plant and animal remains. Autochthonous organic matter forms in the aquatic ecosystems as a result of photosynthesis and the destruction of detritus (dead bacteria, phytoplankton, and animal bodies). The values of HIX (10–16) showed presence of strongly humic organic material (terrestrial origin), whereas low values (<4) represent autochtonous organic material (Huguet et al. 2009). The rainwater samples collected from Rameswaram showed HIX values varied from 0.35 to 0.89 indicates presence of authochtonous organic material (Table 2).

Biological index (BIX) can give information about the organic matter source and can be used for determination of the presence of the marine humic-like peak (peak M), and reflects autochthonous biological activity.

$$BIX = Ex_{310}/Em_{380}/Ex_{310}/Em_{430}$$

High values of BIX (>1) correspond to a biological origin and lowest values (<1) illustrate low abundance of organic matter of biological origin (Huguet et al. 2009). The rainwater samples collected Rameswaram showed BIX values varied from 1.05 to1.63 indicates biological origin (Table 2).

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