



Characteristics, Sources, and Seasonal Variability of Dissolved Organic Matter (DOM) in the Ganges River, Bangladesh

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Abstract This study investigated seasonal variability of sources and fluorescence characteristics of dissolved organic matter (DOM) in the Ganges River using three-dimensional excitation-emission matrix (3DEEM) spectroscopy and parallel factor analysis (PARAFAC). 3DEEM and PARAFAC identified five fluorophores (Peak: A, C, M, T, W) and four fluorescent components, i.e., detergent, fulvic acid (M-type), fulvic acid (C-type), and protein-like, respectively, showing high fluorescence characteristics in the pre-monsoon (March-June) and monsoon (July-October) compared to the post-monsoon (November-February) period. Dissolved organic carbon (DOC) and Specific ultraviolet absorbance (SUVA₂₅₄) were in the range 1.85–4.7 mg/L and 1.23–5.51 L/mg-m, respectively. DOM components were both fresh and microbial-derived throughout the year. Local hydrological and physicochemical conditions supported the autochthonous and aromatic DOM production in post-monsoon, and richness of terrestrial-derived natural and anthropogenic DOM in the pre-monsoon and monsoon periods. The molecular weight of DOM decreased from the mid of pre-monsoon and continued till monsoon with the increase of terrestrial-derived DOM and photo-irradiation as well as microbial activity. Tryptophan-like fluorescence (TLF) showed high intensity throughout the peak-flow pre-monsoon and monsoon periods, demonstrating a high to intermediate risk of microbial contamination of the Ganges River. The seasonal variability of DOM characteristics suggests that the source and biogeochemistry of DOM in the Ganges Rivers might be sensitive to local hydrology and climate.

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Highlights

- DOC concentrations and DOM fluorescence intensity attained their highest values at the beginning of monsoon and showed high seasonal variability.
 - The compositions of DOM carried by the Ganges River varied at different times of the year, and fresh, autochthonous and less aromatic DOM dominated in the late pre-monsoon and monsoon.
 - PARAFAC model identified four fluorescent DOM components (detergent-, fulvic- (M like), fulvic- (C like) and protein-like) which showed high seasonal variability with rainfall and river discharge.
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Keywords DOM · 3DEEM · PARAFAC · fluorophore · SUVA₂₅₄ · microbial contamination

1 Introduction

Dissolved organic matter (DOM) mainly refers to the large source of carbon-based complex heterogeneous compounds found in all aquatic environments (Battin et al. 2009). DOM in aquatic environments come from mostly natural sources such as algae, plant degradation, soil organic matter (SOM), release from zooplankton and lysis of algae and bacteria cells (Aitkenhead-Peterson et al. 2003; Bertilsson and Jones 2003; Thornton 2014; Fuentes et al. 2016), and anthropogenic sources such as fluorescent whitening agents (FWAs) or detergent components and other organic pollutants from industries, agriculture, household and sewerage effluents (Chau 2006; Luo et al. 2008). The chemical characteristics of DOM vary greatly in rivers, lakes and seawater depending on the prevailing environmental conditions (Wang et al. 2020). DOM plays dynamic roles in ecological and biogeochemical systems and is responsible for influencing the toxicity and transport of organic pollutants and trace metals in aquatic systems (Aiken et al. 2011). Despite having a large carbon pool, a significant part of DOM is still uncharacterized (Kujawinski 2011).

Fluorescence spectroscopy is a sensitive and powerful technique to characterize DOM components in both marine (Coble 2007) and freshwater (Mostofa et al. 2009; Hudson et al. 2007) without any pre-concentration and treatment. Fluorescence measurements are used in Excitation Emission Matrix (EEM) Spectroscopy which ultimately produces a three-dimensional fluorescence landscape in which a particular peak position can provide information about characteristics, sources and biogeochemical cycling of DOM. Over the last two decades, fluorescence EEM spectroscopy has been successfully applied for the identification of terrestrial, marine, and anthropogenic components of DOM (Stedmon and Bro 2008; Zielinski et al. 2018). Recently, a multivariate data analysis technique named Parallel factor (PARAFAC) is extensively used to identify individual fluorescent components in the complex mixtures of fluorescence dissolved organic matter (fDOM) and to estimate the fate of DOM in the natural environments (Stedmon and Bro 2008; Stedmon and Markager 2005a, 2005b). The procedures of 3DEEM and PARAFAC analysis are much easier than high-performance liquid chromatography (HPLC) and Fourier-transform ion cyclotron (FT-ICR) mass spectrometry (MS) for the characterization of DOM and the simple technique of fluorescence spectroscopy made it worthy to use as method for this study.

The Ganges River is an important and one of the largest transboundary rivers in the world running through India and Bangladesh. This river basin is considered a densely populated region since about 400 million people in Bangladesh, India and Nepal live in this basin with an average population density of 520 persons/km² (Das and Tamminga 2012). High population density, industrial growth, unplanned agricultural practice and poor waste disposal, as well as sewage treatment systems in urban and semi-urban areas, are polluting the Ganges River both in Bangladesh and India (Haque et al. 2018). In the Indian portion of this river, about 138 main drains are linked to the Ganges River with various domestic and industrial wastewater, maintaining an average flow rate of 6.1×10^6 m³/d and an average biological oxygen demand (BOD) load of 906.3 tonnes/day in the river pathways through Uttar Pradesh, Bihar, Utrakhand and West Bengal (Kumar et al. 2015). In Bangladesh, the Ganges River is also polluted regularly by domestic, municipal sewage, industrial wastewater and agricultural run-off (Haque et al. 2020). These significant pollution loads from the river catchment and high influences of monsoon climate might result in altered characteristics of DOM in the lower Ganges River. Consequences on the Ganges River water quality due to ritual bathing at the festival in India have also been studied considering various physicochemical parameters (Tyagi et al. 2013). Seasonal water quality variation in the Ganges River has been observed using different water quality indices and pollution indices (Tareq et al. 2013; Haque et al. 2020; Zhang et al. 2014). Effects on the microbial community for various allochthonous and anthropogenic inputs into the Ganges River water have also been studied (Zhang et al. 2018; Jani et al. 2018). Studies have also been conducted on the spatial distribution of polycyclic aromatic hydrocarbon (Sharma et al. 2018), distribution of heavy metals and their seasonal abundance trend (Singh and Pandey 2014), ecological and health risk assessment for inputs of emergent contaminant such as personal care products, pharmaceuticals and artificial sweeteners (Sharma et al. 2019), documentation of plastic pollution (Nelms et al. 2020) in the Ganges River. Various types of natural DOM besides anthropogenic pollutants can also be present in the river water (Mostofa et al. 2010; Reyes and Crisosto 2016). But, to date, to the best of our knowledge, there is no systematic work on the DOM characteristics of the lower Ganges River, Bangladesh, carried out to identify sources, seasonal variations and ultimate fate of DOM in the lower Ganges, especially in Bangladesh.

DOM is one of the major components in river water, plays a vital role in water chemistry (Du et al. 2018) and already various types of research work have been done on DOM characteristics in major rivers of the world. But DOM characteristics, their sources, seasonal variations and connection with local hydrological systems of one of the longest rivers in the world, the Ganges River, are still unknown. Characteristics of the organic matter and their origins should be known to better understand the biogeochemical process of the Ganges River. The present study aimed to characterize DOM in the lower Ganges River water using optical properties, mostly 3DEEM fluorescence, UV visible spectrophotometry, and PARAFAC analysis. These measurements and analyses are used to specify DOM nature, their origin whether they come from natural or anthropogenic sources, their aromaticity, relative molecular size and decomposition state in the freshwater ecosystem. This study also identified the persistent nature of anthropogenic fluorescing pollutants through observing their presence over a year at monthly interval. Finally, seasonal variability of DOM and its relation to monsoon precipitation and river water discharges were identified at the lower Ganges in Bangladesh.

2 Materials and Methods

2.1 Study Area

The Ganges River originates from the Gangotri glacier at Gomukh (30°36' N; 79°04' E) located in the Garhwal Himalaya of Uttarakhand province in India (Vass et al. 2010). After flowing through the plains of Uttar Pradesh, Bihar, and West Bengal consecutively, this river enters Bangladesh through Chapainawabganj district by the name of Padma and flows through various south-western districts of Bangladesh before unloading into the Bay of Bengal (Fig. 1). The river basin is situated in the subtropical Asian monsoon climate area and can be characterized by dry and cold winter besides rainy and warm summer. Agricultural land, forest and residential areas are mainly located in the downstream areas. Land-use change indicates the rapid urbanizations of the whole basin areas. The mean annual water discharge in the Ganges River is about 18,700 m³/s and the mean maximum flow is about 468.7×10⁹ m³ with high flow variation within the catchment area (Sarkar et al. 2012). The Ganges basin receives about 1,000 mm of precipitation yearly and among this total rainfall, about 84% rain occurs in the monsoon, 7% in the pre-monsoon and the remainder in the post-monsoon periods. Average temperature ranges between 21.5 and 30.3 °C in summer and 6.4 and 21.1 °C in winter, with May and June being the hottest months in the lower and upper Ganges basin, respectively, and January the coldest month across the entire basin (The Himalayan Climate and Water Atlas 2015).

2.2 Sampling and Field Monitoring

Water samples were collected from the Hardinge Bridge Point (24° 03' 52.3" N, 89° 01' 52.7" E) of the Ganges River, Kushtia, Bangladesh (Fig. 1) at monthly interval. Samples were collected in 1 L prewashed polypropylene bottles along the main channel. Samples were filtered through Whatman GF/F glass fiber filters (pre-combusted at 450 °C for 4 h) immediately after sampling and preserved in cooler box at 4 °C during transport to the laboratory at the Department of Environmental Sciences, Jahangirnagar University. Water quality parameters such as pH, dissolved oxygen, Electrical conductivity, salinity, total dissolved solids were measured using a YSI 6920 V2 Sonde multiparameter which was connected with Ecowatch lite software and temperature data was collected from Weather micro-station that was connected with HOBOWare Pro software.

2.3 DOC and Optical Properties Analysis

Dissolved organic carbon (DOC) was measured using a high-temperature catalytic oxidation method (Shimadzu TOC analyzer L_{CPH/CPN}) using a non-dispersive infra-red (NDIR) detector. Fluorescence measurements were carried out in Hitachi F-4600. Intensities of fDOM were measured between 225 and 400 nm (5 nm interval) excitation (Ex) wavelength, and 250 and 550 nm emission (Em) wavelength (1 nm interval). EEMs were corrected for inner filter effect and blanks using corresponding absorbance measurements. The Raman peak value of ultra-pure Milli-Q water was used as a reference to describe fluorescence intensities at Raman units.

Filtered samples were also measured in UV Spectrophotometer (SPECORD 210 Plus, Analytikjena) concurrently keeping scanning range between 190 nm to 1100 nm wavelength and scanning speed 60 nm/min. Milli-Q (18MΩ·cm) water was used as the blank solution.

Specific ultraviolet absorbance ($SUVA_{254}$) was calculated using absorption at 254 nm and DOC values (Gao et al. 2016). Spectral slope ratio (S_R) and absorption ratio (E_{250}/E_{365}) were also calculated using the UV dataset (Helms et al. 2008). Some fluorescence-dependent indices such as fluorescence index (FI), humification index (HIX), biological index (BIX) and freshness index ($\beta:\alpha$) were also calculated to evaluate fDOM features such as distinguishing sources of natural organic matters, identification of the level of humification, autochthonous and also fresh autochthonous fDOM production, respectively. FI was calculated as the ratio of fluorescence intensities at 470 nm and 540 nm emission at 370 nm excitation according to Cory and McKnight (2005). HIX was quantified from the intensity ratio at emission 435-480 nm and 300-345 nm keeping excitation wavelength at 254 nm (Ohno 2002). BIX was computed considering excitation wavelength at 310 nm for the ratio of fluorescence intensity at emission wavelength 380 nm and 410 nm (Huguet et al. 2009). $\beta:\alpha$ was also calculated from the ratio of fluorescence intensity at emission 380 nm and 420-435 nm keeping excitation wavelength at 310 nm (Wilson and Xenopoulos 2009).

2.4 PARAFAC Modeling

Individual fluorescent components were modeled by parallel factor analysis (PARAFAC) from the EEMs using the DOMFluor Toolbox (v1.7) (Stedmon and Bro 2008). Before using the EEM dataset for PARAFAC analysis, blank data was subtracted from the original to remove Raman scattering, rayleigh spectral lines were completely removed to count exact component numbers and arbitrary units were converted into Raman fluorescence units (RU) (Mostofa

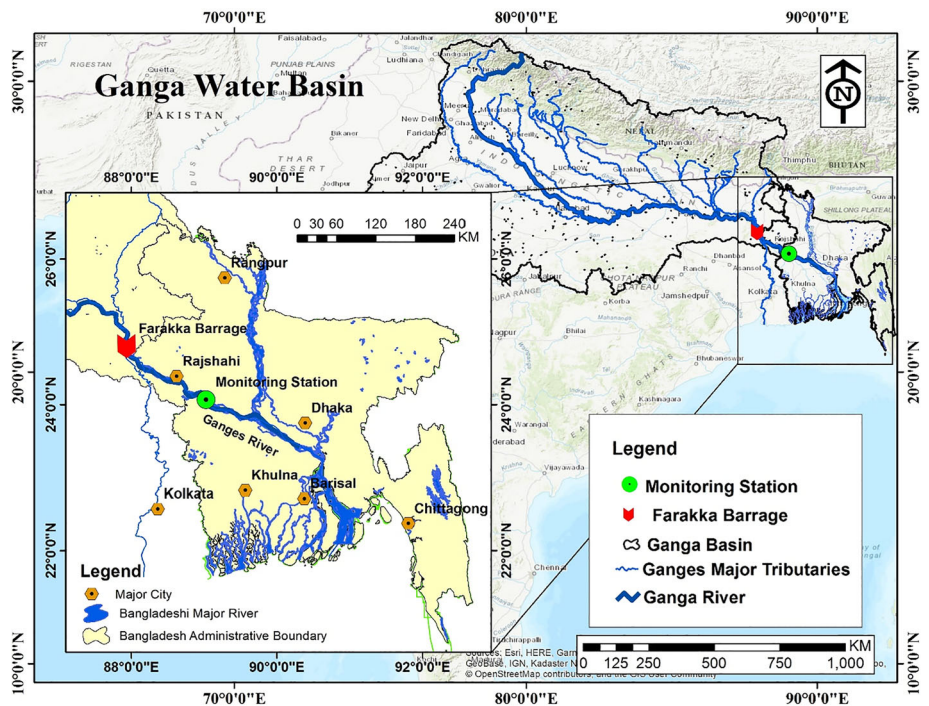


Fig. 1 Map of the study area and monitoring station at the Ganges River. The map was created using ArcGIS 10.3.1 and the topographic image modified from ArcGIS/ ESRI online

et al. 2009; Goletz et al. 2011; Stedmon and Bro 2008). Intensity (i.e., F_{\max}) values were read out in Raman Unit (RU). A total of 60 water samples from the Ganges River were used for PARAFAC analysis. Specific validation and calibration techniques were followed to identify the exact number of fluorescent components, including split-half and residual analysis. At last, validated four fluorescent components were found in the present study.

2.5 Microbial Risk Assessment

Tryptophan-like fluorescent component (TLF) of DOM was used for microbial risk assessment. The Ganges River water was categorized for microbial contamination at different risk levels, namely low, intermediate, high and very high, based on TLF according to Nowicki et al. (2019). The concentration of TLF was calculated from the fluorescence intensity-concentration relationship of the standard Tryptophan compound.

2.6 Statistical Analysis

The physicochemical parameters of the Ganges River water, DOC, rainfall and discharge, DOM indices (FI, HIX, BIX, $\beta:\alpha$, S_R , E_{250}/E_{365} , $SUVA_{254}$) and fluorescent components were tested to determine significance, the standard deviation of the dataset, and also for comparison between indices applying t-test in SPSS (v16.0) software. One-way ANOVA and Post hoc multiple comparisons (Tukey) were performed in SPSS to determine the seasonal variations of DOM indices. Pearson correlation and linear regression were also used to study the significance of the relationship between indices. The relationship between DOM indices with PARAFAC components was studied for identification and characterization of fDOM sources and their connection with hydrological systems through performing principal component analysis (PCA) in SPSS.

3 Results and Discussions

3.1 Hydrochemistry of the Ganges River

Water quality parameters of the Ganges River water showed dynamic seasonal variations. Parameters such as pH, salinity, electrical conductivity (EC), dissolved oxygen (DO) and total dissolved solids (TDS) significantly ranged in 7.8-8.48 (t-test; $t=11.95$, $p<0.05$, $\sigma=0.23$, $R=0.92$), 0.08-0.17 PSU (t-test; $t=3.804$, $p=0.003$, $\sigma=0.033$, $R=0.91$), 174.07-360.79 $\mu\text{S}/\text{cm}$ (t-test; $t=-44.983$, $p<0.05$, $\sigma=70.16$, $R=0.91$), 6.29-11.18 mg/L (t-test; $t=-3.59$, $p=0.004$, $\sigma=1.47$, $R=0.97$) and 113.14-234.44 mg/L (t-test; $t=-61.703$, $p<0.05$, $\sigma=45.6$, $R=0.91$) maintaining average values at 8.19, 0.14 PSU, 288.98 $\mu\text{S}/\text{cm}$, 8.47 mg/L and 187.83 mg/L, respectively (Table 1). Comparatively low values of these five parameters were recorded in the monsoon. Low pH indicated the inputs of slightly acidic substances from surrounding terrestrial and sewage sources during the monsoon. But the average pH value of the river water was alkaline, and thus, useful for aquatic species. Reduced DO in the monsoon indicated increase of biological activities in the river water. But the average DO showed that the Ganges River water quality was excellent and fulfilled minimum DO requirements of the environmental quality standard (EQS) to use water for fishing, livestock, industrial and recreation purposes. EC was low in the monsoon period due to the low presence of dissolved solids

Table 1 Variation in physicochemical and hydrological parameters of the Ganges River water throughout the year

Parameters	Average (Min-Max)	References
Physicochemical parameters		
Temperature (°C)	25.95 (18.4-30.07)	This study
pH	8.19 (7.8-8.48)	This study
Salinity (PSU)	0.14 (0.08-0.17)	This study
EC (µS/cm)	288.98 (174.07-360.79)	This study
DO (mg/L)	8.47 (6.29-11.18)	This study
TDS(mg/L)	187.83 (113.14-234.44)	This study
BOD(mg/L)	2.6±1.13 (1.10-4.53)	Tareq et al. (2013)
COD (mg/L)	51±24.56 (15-103)	Tareq et al. (2013)
Alkalinity (mg/L as CaCO ₃)	74 (55-82)	Tyagi et al. (2013)
Acidity (mg/L)	20.4 (15-30)	Tyagi et al. (2013)
Turbidity (FTU)	82.78±55.61(15.96-169.4)	Tareq et al. (2013)
TSS (mg/L)	17(11-23)	Tyagi et al. (2013)
NH ₄ ⁺ -N (mg/L)	0.45 (0.14-0.7)	Tyagi et al. (2013)
NO ₃ ⁻ -N (mg/L)	0.49 (0.24-0.65)	Tyagi et al. (2013)
PO ₄ ³⁻ (mg/L)	0.020 (0.011-0.047)	Tyagi et al. (2013)
Hydrological parameters		
Rainfall (mm)	138.0 (8.1-335.6)	This study
Discharge (m ³ /s)	8948.16 (969.15-40749.14)	This study

during this time. Nevertheless, high water discharge during the monsoon in the Ganges River, low TDS values indicated the possibility for the presence of more colloidal and large particles that started to degrade from the post-monsoon and increased EC values till the late of pre-monsoon. But the water temperature was significantly high in the pre-monsoon and monsoon periods, and varied in 18.4-30.07 °C with an average value at 25.95 °C (t-test; $t=2.511$, $p=0.029$, $\sigma=4.07$, $R=0.89$). Among hydrological parameters, annual rainfall and river water discharge in the sampling station ranged in 8.1-335.6 mm with a mean value at 138.0 mm (t-test; $t=3.716$, $p=0.003$, $\sigma=127.44$, $R=0.769$) and 969.15-40749.14 m³/s with an average value at 8948.16 m³/s (t-test; $t=2.307$, $p=0.028$, $\sigma=13432.26$, $R=0.631$), respectively. Hydrological parameters were gradually started to increase from pre-monsoon, were high at monsoon and gradually decreased at post-monsoon periods (Table 1).

Turbidity of the Ganges River water was found higher (15.96-169.4 FTU) than the DoE (Department of Environment) standard of Bangladesh, and was maximum in the mid of monsoon. High discharge rate and rainfall increased the turbidity values and the significant presence of silts, clays, fine inorganic and organic particles, aquatic organisms and planktons in the river water (Tareq et al. 2013). BOD and COD of the Ganges River water ranged in 1.10-4.53 mg/L and 15-103 mg/L, respectively (Tareq et al. 2013) and exceeded the permissible limit standard for drinking purposes, but were within the acceptable limit for irrigation and fish culture activities (Table 1). However, another study found comparatively higher BOD value at upstream of the Ganges River (Tyagi et al. 2013). Acidity, alkalinity, total suspended solids (TSS), NH₄⁺-N, NO₃⁻-N and PO₄³⁻ were also found at a significant amount into the Ganges River water and ranged in 15-30 mg/L, 55-82 mg/L, 11-23 mg/L (t-test; $p<0.05$, $t=2.5$, $R=0.97$), 0.14-0.7 mg/L (t-test; $p<0.05$, $t=2.5$, $R=0.94$), 0.24-0.65 mg/L and 0.011-0.047 mg/L (t-test; $p<0.05$, $t=2.5$, $R=0.98$), respectively (Tyagi et al. 2013) (Table 1). NH₄⁺-N and PO₄³⁻ might come from urinal discharge and detergent sources, respectively, into the river water (Tyagi et al. 2013).

3.2 DOC and Fluorescence Characteristics of DOM

DOC in the lower Ganges River significantly varied between 1.85 and 4.66 mg/L with mean value at 2.54 mg/L (t-test; $t=-12.142$, $p<0.05$, $\sigma=0.84$, $R=0.69$). It had maximum and minimum values at the pre-monsoon and post-monsoon, respectively (Table 2) due to variation in local hydrological conditions. DOC was related to the annual precipitation and discharge into the Ganges River. The DOC concentrations reached the highest value at the late pre-monsoon with the increase of water level and the lowest values at the late post-monsoon period with the decline of water level in the Ganges River. The nature of DOC carried by the Ganges River was different at different times of the year and the maximum contribution of labile constituents to organic matter occurs during the early rising water stage. After the entire dry post-monsoon period, long-time deposited terrestrial organic loads flushed into the river due to increased monsoon precipitations and terrestrial run-off discharge during the entire pre-monsoon and monsoon periods, and resulted in the increase of DOC into the Ganges River water. This seasonal variation in characteristics of DOC with monsoon precipitations reflected the important hydrological influences on DOC in the lower Ganges River, particularly in the high-flow periods. The discrete seasonal patterns of DOC also indicated different sources of carbon. Additional details regarding DOM chemical characteristics and composition were investigated using 3DEEM fluorescence spectroscopy to see whether it supported further clarifications.

Maximum five peak positions of fDOM were identified in the lower Ganges River water samples by three-dimensional Excitation Emission Matrix (3DEEM) fluorescence spectroscopy (Fig. 2). Identified five peak positions were Peak A (Ex/Em =255-275/390-454 nm), Peak W (Ex/Em =340-350/436-454 nm), Peak T (Ex/Em =270-280/344-354 nm), Peak M (Ex/Em =305-310/394-402 nm) and Peak C (Ex/Em =320-325/412-424 nm) (Fig. 2). Peak A, C and M mainly indicate the presence of humic acid-like and fulvic acid-like substances while Peak T and Peak W confirm the presence of protein-like and detergent-like components, respectively (Mostofa et al. 2009, 2010). Both qualitative (peak position) and quantitative (peak intensity) information of the 3DEEM model confirmed the seasonal variation of DOM characteristics

Table 2 Various index values calculated using fluorescence and absorbance data; DOC= Dissolved organic carbon, $SUVA_{254}$ = Specific ultraviolet absorbance, FI= Fluorescence index, HIX= Humification index, BIX= Biological index, S_R = Slope ratio, E_{250}/E_{365} = Molecular size, $\beta:\alpha$ = Freshness index

Month	Total fluorescent intensity (RU)	DOC (mg/L)	$SUVA_{254}$ (L/mg-m)	FI	HIX	BIX	S_R	E_{250}/E_{365}	$\beta:\alpha$
January	0.133	1.845	4.41	1.62	6.36	0.87	0.053	1.07	1.02
February	0.067	1.96	3.39	1.44	6.5	0.65	0.041	1.08	0.96
March	0.098	2.15	4.3	1.41	4.93	0.87	0.053	1.07	1.06
April	0.466	2.65	5.5	1.51	2.74	0.86	0.7	1.06	1.08
May	0.416	3.8	1.23	1.31	4.93	0.49	0.85	0.11	0.96
June	0.502	4.66	1.74	1.51	3.83	0.72	0.31	1.09	1.04
July	0.333	2.01	2.81	1.35	3.95	0.6	0.52	1.12	1.01
August	0.404	2.2	2.79	1.41	5.55	0.7	0.48	1.11	1.02
September	0.344	2.01	4.92	1.55	4.52	0.74	0.07	1.08	1.05
October	0.375	2.45	3.14	1.49	7.75	0.78	0.05	1.09	1.03
November	0.178	2.5	5.36	1.43	7.74	0.8	0.12	1.06	1.03
December	0.097	2.29	3.44	1.36	6.38	0.69	0.07	1.06	1.01

and compositions in the lower Ganges River water (Fig. 2; Fig. 3). High fluorescence intensities in the pre-monsoon and monsoon resulted from the presence of large amounts of organic loads into the Ganges River due to high discharge and rainfall during this time. Peak A and peak C were dominated during the late pre-monsoon and monsoon in presence of other peaks. The post-monsoon is mostly considered as a dry winter period in context to the Asian subcontinent when precipitation and discharge remain low in most of the rivers in this region. Monsoon precipitation-driven river discharges might be the main factor of high fluorescence intensity and compositional changes of DOM in the lower Ganges River. The lower precipitation and discharge in the post-monsoon were the reasons for the comparatively low fluorescence intensity of DOM in the Ganges River.

3.3 PARAFAC Components

The PARAFAC model identified four fluorescence components based on the 60-sample dataset from the Ganges River, comprising fulvic acid-like (C2 and C3), detergent-like (C1) and protein-like (C4) components (Fig. 4). Four identified fluorescent components contain some specific fluorophores (Mostofa et al. 2010; Salve et al. 2012; Huguet et al. 2009; Gao et al. 2016). Component 1 showed two fluorophores at the wavelength region of Ex/Em (260/454 nm) (Peak A) and (350/454 nm) (Peak W) indicating the occurrence of detergent-like substance in the Ganges River water (Fig. 4a). The Source of Component 1 was anthropogenic (commercial laundry detergents) and could be characterized as ubiquitous, high molecular weight (HMW), aromatic and photochemically degradable DOM (Cory and McKnight 2005; Mostofa et al. 2010). The intensity of Component 1 was high in the entire monsoon and low during the post-monsoon period (Fig. 5). Component 2 showed fluorescence peaks at Ex/Em (255(310)/395 nm) indicating fluorescence characteristics as marine humic-acid (M like) described by Coble (1996) (Fig. 4b). Component 2 was a microbial-originated recently

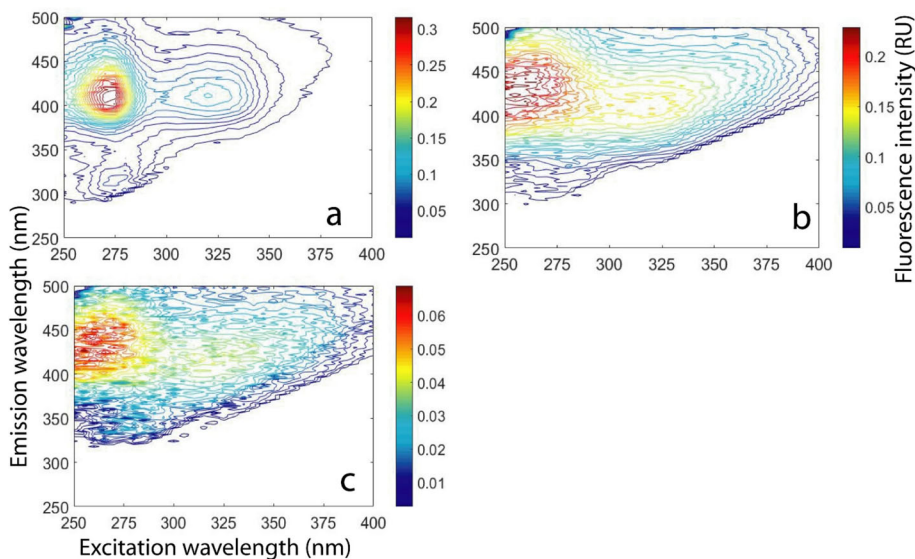


Fig. 2 Representative three-dimensional excitation-emission matrix (3DEEM) spectra plotting for the collected water samples of **a.** Pre-monsoon (May) **b.** Monsoon (October) **c.** Post-monsoon (December) seasons from the Ganges River.

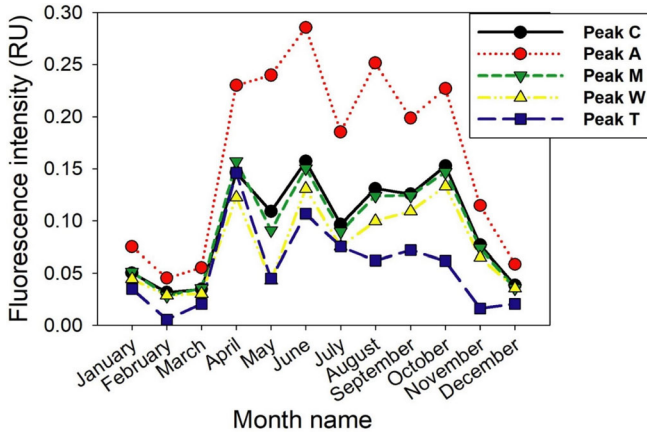


Fig. 3 Seasonal variation in fluorescence intensity (RU) of different peaks, Peak A (Ex/Em =255-275/390-454 nm), Peak W (Ex/Em =340-350/436-454 nm), Peak T (Ex/Em =270-280/344-354 nm), Peak M (Ex/Em =305-310/394-402 nm) and Peak C (Ex/Em =320-325/412-424 nm), in the three-dimensional excitation-emission matrix (3DEEM) spectra of the Ganges River water.

produced substance (autochthonous) and could be characterized as labile, low aromatic, possibly altered by microbial processes and available in wastewater (Salve et al. 2012; Huguet et al. 2009; Gao et al. 2016). Seasonal variation of component 2 showed higher dominance in monsoon compared to that of post-monsoon and fluctuating largely during the entire pre-monsoon (Fig. 5). Component 3 had Ex/Em (275 (325)/410 (414) nm) (peak A) and 325/414 nm (Peak C) wavelength pairs in the visible region, representing terrestrial-derived fulvic acid-like fluorophores according to the conventional peak picking process (Yamashita and Jaffé 2008). This terrestrial-originated fulvic acid-like component can be characterized as aromatic which is also related to DOC and not available in wastewater like Component 2 (Gao

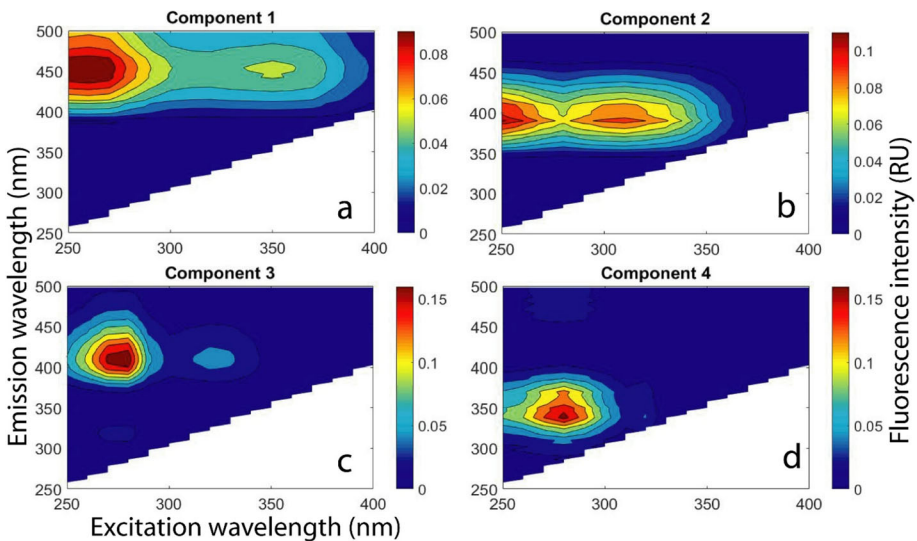
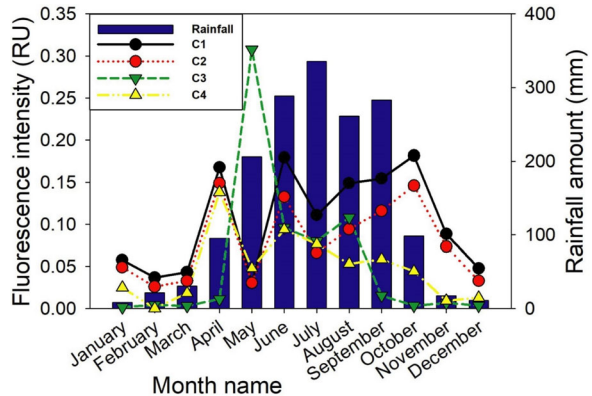


Fig. 4 Contour plots of four DOM EEM components, namely Component 1 (a), Component 2 (b), Component 3 (c), and Component 4 (d), identified by PARAFAC model.

Fig. 5 Seasonal variation in fluorescence intensity (RU) of four PARAFAC components (C1, C2, C3, C4) with monthly rainfall data of the monitoring location at the Ganges River.



et al. 2016; Goldman and Sullivan 2017). The fluorescence intensity of this component was maximum at the mid-pre-monsoon and remained comparatively high till the peak of monsoon (Fig. 5). In the late monsoon and entire post-monsoon, the intensity of this component was much low (Fig. 5). Component 4 showed fluorescence peak at Ex/Em (280/342 nm) (peak T) indicating the presence of a tryptophan-like substance in the Ganges River water (Fig. 4d). This protein-like component was autochthonous; fresh, microbial-derived, low aromatic and more labile (Dubnick et al. 2010; Goldman and Sullivan 2017). Component 4 indicated the pollution sources caused by autochthonous bacterial and anthropogenic activities (Singh et al. 2014; Nowicki et al. 2019). Fluorescence intensity of protein-like component was maximum in the late pre-monsoon (i.e., during summer) and showed a descending intensity order from the early monsoon to the end of the post-monsoon period (Fig. 5).

Total fluorescence intensity of fDOM varied between 0.067–0.502 RU with mean value at 0.28 RU (*t*-test; *t*=−59.196, *p*<0.05, σ =0.16, *R*=0.95) and was comparatively higher at the pre-monsoon and monsoon periods than post-monsoon (*p*>0.05) (Table 2). The average intensity of fDOM in pre-monsoon and monsoon periods was almost three times higher than the post-monsoon (Table 2). Fluorescence intensity remained in the low ranges throughout the entire post-monsoon due to minimal discharge (*t*-test; *t*=2.33, *p*>0.05, σ =2504.22, *R*=0.698) and precipitation (*t*-test; *t*=4.68, *p*<0.05, σ =6.12, *R*=0.04) during this time. But from the mid of pre-monsoon, intensities of fDOM started to increase significantly (*p*<0.05) and remained at the maximum ranges till the end of pre-monsoon. fDOM intensity was on the slightly lower ranges in the monsoon than pre-monsoon period (*t*-test; *t*=0.076, *p*>0.05, σ =0.164). Despite maximum annual discharge in the monsoon, comparatively high temperature, more microbial degradation and dilution effects quenched the fDOM intensity to some extent. Temperature and pH have effects on fluorescence intensity. Fluorescence intensity decreases with the increase of these two parameters (Zhang et al. 2010; Henderson et al. 2009). Fluorescence intensity can increase between 10 and 45 °C by about 1 % with a 1 °C decrease in temperature for DOM substances (Henderson et al. 2009; Baker 2005; Elliott et al. 2006). Correlation between temperature and fluorescence intensity variability was found significant (*t*-test; *p*=0.003, *R*=0.771) in this study indicating the fluctuations of intensity with temperature. Changes in pH (2–10) also affect the properties and intensities of fDOM components (Zhang et al. 2010; Henderson et al. 2009). But the correlation between fluorescence intensity and pH was found insignificant (*t*-test; *p*=0.11, *R*=−0.486) indicating no effect of pH on fluorescence properties in this study.

3.4 Sources and Variations of DOM in the Ganges River

The Fluorescence index (FI) has been widely used to identify the microbial or terrestrial sources of DOM fractions (McKnight et al. 2001; Cory et al. 2007, 2010). According to McKnight et al. (2001) and Cory and McKnight (2005), FI values ranging in ~ 1.7 -1.9 and ~ 1.3 -1.4 indicate microbial and terrestrial-derived fulvic acid-like components in water, respectively. FI, in the range 1.4-1.5 can still indicate terrestrial-originated fulvic acid-like substances in large rivers (McKnight et al. 2001). FI values of the Ganges River water were within the range 1.31-1.62 in the present study, with a mean value of 1.45 (t-test; $p < 0.05$, $\sigma = 0.09$), indicating the presence of both terrestrial and microbial-derived fulvic acid-like fDOM component (e. g., Component 2 and Component 3) (Table 2). FI had a significant positive correlation with BIX ($R = 0.697$, $p = 0.012$) and freshness index ($R = 0.789$, $p = 0.002$), and a negative relationship with C3 ($R = -0.539$, $p = 0.071$) describing the dominance of autochthonous DOM components in the Ganges River water (Table 3; Fig. 6d).

The Humification index (HIX) is mainly used as an indicator to describe the source, sorptive capacity and diagenesis of DOM (Ohno 2002). When HIX increases, the ratio of hydrogen to carbon decreases and emission spectra of fluorescing component shifts towards longer wavelengths. High (10-16) and low (< 4) HIX values describe the presence of strongly humified terrestrial-originated and aquatic microbial-derived DOM components, respectively (Parlanti et al. 2000; Salve et al. 2012). Indication of the humic character of allochthonous DOM components starts to increase from $HIX = 6$ (Parlanti et al. 2000). HIX values ranged between 2.7 and 7.75 with an average value at 5.4 (t-test; $p > 0.05$, $\sigma = 1.56$), confirming the presence of both microbial-originated and allochthonous DOM fractions in the Ganges River (Table 2). Aquatic microbial and weak humic characterized homogenous DOM were predominantly present during the entire pre-monsoon and the early of the monsoon periods ($HIX = 2.7$ -5.5, $p > 0.05$) (Table 2). But strong humic characterized terrestrial-originated homogenous DOM components were predominantly present in the Ganges River water during the rest of the time of the year ($HIX = 6.36$ -7.75, $p > 0.05$) (Table 2). Flush-out of land-derived organic matter might occur due to intense rainfall during pre-monsoon and monsoon. Favorable climatic conditions (temperature, rainfall) for aquatic microbial or biological DOM production during the pre-monsoon and monsoon resulted in the dominance of microbial-derived DOM

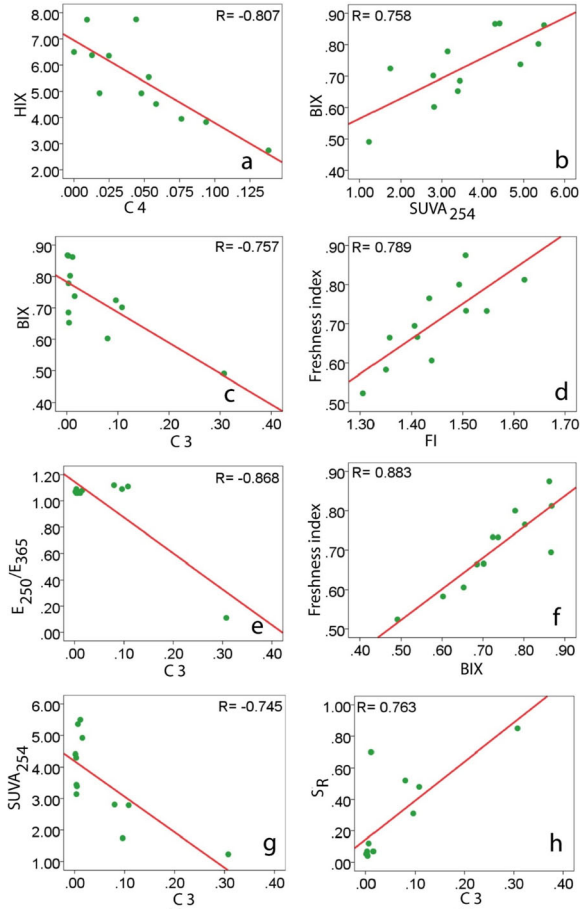
Table 3 Pearson correlation matrix of the variables (fluorescent components and calculated DOM indices)

	SUVA ₂₅₄	FI	HIX	BIX	S _R	E	$\beta:\alpha$	C1	C2	C3	C4
SUVA ₂₅₄	1										
FI	0.488	1									
HIX	0.084	0.021	1								
BIX	.758**	.697*	0.085	1							
S _R	-0.386	-0.428	-.612*	-0.48	1						
E	0.506	0.479	0.087	.624*	-.601*	1					
$\beta:\alpha$.676*	.789**	0.033	.883**	-0.291	0.523	1				
C1	0.067	0.422	-0.291	0.257	0.125	0.409	0.534	1			
C2	0.166	0.489	-0.279	0.347	0.138	0.334	.653*	.971**	1		
C3	-.745**	-0.539	-0.278	-.757**	.763**	-.868**	-.635*	-0.153	-0.177	1	
C4	-0.028	0.18	-.807**	0.06	.647*	0.017	0.323	.702*	.727**	0.192	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Fig. 6 Linear regression for most significant Pearson correlation between various variables: (a) C4-HIX, (b) SUVA₂₅₄-BIX, (c) C3-BIX, (d) FI-Freshness index, (e) C3-E₂₅₀/E₃₆₅, (f) BIX-Freshness index, (g) C3-SUVA₂₅₄, (h) C3-S_R



fractions into the Ganges River. HIX had a negative relationship with S_R ($R=-0.612$, $p=0.034$), rainfall ($R=-0.571$, $p=0.052$), and C4 ($R=-0.807$, $p=0.002$) describing that, autochthonous derived fDOM had higher molecular weight than allochthonous components and rainwater facilitated the more production of autochthonous DOM in the Ganges River (Table 3; Fig. 6a).

High BIX (>1) value specifies a large abundance of autochthonous DOM components while BIX (<1) indicates low production of DOM components from biological activities in the natural environment. Huguet et al. (2009) categorized BIX values as BIX (0.6-0.7) for a low abundance of autochthonous DOM components, BIX (0.7-8.00) for intermediate autochthonous organic components, BIX (8.00-1.00) for high autochthonous components and BIX (>1) for the aquatic bacterial origin of DOM components. BIX values of the Ganges DOM components varied between 0.49 and 0.87 (mean 0.73; t-test; $p<0.05$, $\sigma=0.1184$) and confirmed most of the types of aquatic DOM productions throughout the year (Table 2). A lower abundance of aquatic DOM fractions was observed from late pre-monsoon to early monsoon (Table 2). But the rest of the time of the year, autochthonous DOM productions were either intermediate or high (Table 2). BIX had strong positive correlation with SUVA₂₅₄ ($R=0.758$, $p=0.004$), FI ($R=0.697$, $p=0.012$), E₂₅₀/E₃₆₅ ($R=0.624$, $p=0.03$) and Freshness index ($R=0.883$, $p=0$) indicating higher aromaticity and low molecular size autochthonous DOM components

(Table 3; Fig. 6b, 6f). BIX had a strong negative correlation with C3 ($R=-0.757$, $p=0.004$) statistically proving that C3 was from allochthonous sources (Fig. 6c).

Freshness index ($\beta:\alpha$) is an indicator to describe the relative contribution of newly produced microbial DOM components and autochthonous inputs in the aquatic system (Parlanti et al. 2000; Wilson and Xenopoulos 2009). β component is composed of a spectral band of protein-like fluorophores considering both sources of much macro-algae production and autochthonous biological sources, and thus, shows the existence of recently produced biologically derived DOM compounds (Parlanti et al. 2000). The freshness index is also sensitive to photochemical processes along with microbial degradation. Photo-exposure increases the $\beta:\alpha$ values of DOM in the water while microbial degradation has the opposite function decreasing $\beta:\alpha$ values. $\beta:\alpha$ values in the Ganges River water ranged from 0.52 to 0.88 with an average value of 0.71 (t-test; $p<0.05$, $\sigma=0.102$) (Table 2). Fluctuations of $\beta:\alpha$ ratio were observed in the entire study (Table 2). Comparatively low $\beta:\alpha$ values described the prevalence of microbial activities throughout the year in the Ganges River. In the late pre-monsoon, BIX values were in the lower ranges compared to other months indicating the production of more microbial-derived autochthonous DOM components in the river water. Suddenly increased rainfall and water discharge at the late pre-monsoon gathered anthropogenic derived organic materials into the river channel which enhanced microbial activities and retarded natural autochthonous fresh DOM production. Apart from the specific enhanced pre-monsoon, both fresh and microbial-derived DOM fractions prevailed simultaneously and rationally into the Ganges River (Table 2). Freshness index had positive correlation with $SUVA_{254}$ ($R=0.676$, $p=0.016$), FI ($R=0.789$, $p=0.002$), BIX ($R=0.883$, $p=0$), E_{250}/E_{365} ($R=0.523$, $p=0.081$) and C2 ($R=0.653$, $p=0.021$), showing that autochthonous fulvic acid-like DOM components were fresh and lower in molecular size (Table 3). The freshness index had a negative correlation with C3 ($R=-0.635$, $p=0.026$) indicating the source of component C3 as a terrestrial environment (Table 3).

$SUVA_{254}$ values varied between 1.23 and 5.51 L/mg-m (mean= 3.59 L/mg-m; t-test; $p>0.05$, $\sigma=1.356$) and were comparatively on the high range in the post-monsoon and low range in the pre-monsoon and monsoon periods in the Ganges River water (t-test; $p>0.05$) (Table 2). Surface water $SUVA_{254}$ value typically ranges between 1-6 L/mg-m. $SUVA_{254}$ value reflected aromatic contents in DOM fractions (Weishaar et al. 2003) and can also be a good indicator of the molecular weight of DOM (Chowdhury 2013). Due to low photo-irradiation and more microbial activity, DOM fractions were converted from low molecular weight (LMW) to high molecular weight (HMW) and the aromaticity was increased in the post-monsoon period. During the pre-monsoon and monsoon periods, comparatively high photo-irradiation could degrade the HMW compounds to produce LMW DOM fractions and decrease the aromaticity. Moreover, due to high rainfall and water discharge in the pre-monsoon and monsoon periods, dilution effects also influenced HMW components (t-test; $p<0.05$). $SUVA_{254}$ had a significant negative correlation with DOC ($R=-0.593$, $p=0.042$) and C3 ($R=-0.745$, $p=0.005$) describing that allochthonous derived DOM components had higher organic carbon content but lower aromaticity than autochthonous DOM (Table 3; Fig. 6g). $SUVA_{254}$ had positive correlation with BIX ($R=0.758$, $p=0.004$), E_{250}/E_{365} ($R=0.506$, $p=0.093$) and Freshness index ($R=0.676$, $p=0.016$) indicating the presence of autochthonous DOM components with high aromaticity (Table 3).

DOM components showed dynamic variations in characteristics, sources and intensities in the Ganges River over the year. In the post-monsoon period, intensities of most PARAFAC components were comparatively lower than the other two seasons (t-test; $p>0.05$). Low

temperature was unfavorable for the production of autochthonous DOM (C2 component) in water which was also responsible for low DOM content besides low rainfall and discharges. But comparatively high HIX and BIX and low FI values showed that the amount of DOM from autochthonous sources was higher than from allochthonous sources. The dominance of autochthonous DOM revealed their higher aromaticity during the post-monsoon. From the mid of pre-monsoon, the fluorescent intensity of most DOM components started to increase (Table 2). Increased temperature in the pre-monsoon was also favorable for the autochthonous production in water. But availability of autochthonous DOM was less than from terrestrial-derived DOM for high flush-out of terrestrial-derived DOM and total aromaticity of organic components decreased during pre-monsoon than post-monsoon (Table 2). Low HIX and BIX values also confirmed the minimal production of DOM in the pre-monsoon. Fluorescence intensities of all PARAFAC components were also high at monsoon period in the Ganges River. Like pre-monsoon, aromaticity of DOM components was also less during monsoon than post-monsoon which revealed the abundance of terrestrial-derived natural and anthropogenic DOM (Component C1, C3 and C4) in the Ganges River water. The persistent nature of detergent residue was also responsible for higher aromaticity in the post-monsoon. Other PARAFAC components, e.g., protein-like and autochthonous fulvic acid-like substances were both fresh and decomposed in the Ganges River (Table 2).

3.5 Relative Molecular Size and Weight Distribution

The S_R and the E_{250}/E_{365} ratio are inversely related to the molecular weight and size of DOM fractions, respectively (Helms et al. 2008). The S_R value of the Ganges River ranged between 0.041 and 0.85 with an average value at 0.2764 (t-test; $t=-18.185$, $p<0.05$, $\sigma=0.29$) (Table 2). Earlier studies reported S_R values in range 0.70-2.40 in lake water (Zhang et al. 2010), 0.76-1.79 in wetlands (Helms et al. 2008) and 1.6-3.4 in ocean water (Stubbins et al. 2012). Low S_R values were observed from the late monsoon to the early period of pre-monsoon (t-test; $p<0.05$) (Table 2). Decreased S_R values are related to the microbial production of HMW aromatic DOM and loss of LMW low aromatic DOM (Hansen et al. 2016). Low irradiation, high microbial activity, water discharge and rainfall were the reasons for increasing the HMW DOM fractions throughout the entire post-monsoon in the Ganges River. From the pre-monsoon, S_R value increased and was in the high ranges till the peak of monsoon indicating the presence of large amounts of LMW and low aromatic DOM fractions in the river water (t-test; $p<0.05$) (Table 2). Photo-irradiation, water discharge and rainfall were higher in monsoon period than that of the post-monsoon which were the reasons for more photolytic degradation of HMW DOM and consequent production of LMW DOM fractions. S_R had negative relation with HIX ($R=-0.612$, $p=0.034$), E_{250}/E_{365} ($R=-0.601$, $p=0.039$) showing that allochthonous DOM such as C3 had low molecular weight but higher molecular size (Table 3). S_R had a positive relation with rainfall ($R=0.501$, $p=0.097$), C3 ($R=0.763$, $p=0.004$) and C4 ($R=0.647$, $p=0.023$) indicating the increase of terrestrial-derived DOM in water with the intensification of rainfall (Table 3; Fig. 6h).

The E_{250}/E_{365} ratio ranged between 0.11 and 1.12 maintaining average value at 1.00 (t-test; $t=0$, $p>0.05$, $\sigma=0.28$) (Table 2). This ratio did not vary greatly throughout the year except for the pre-monsoon, confirming the presence of similar molecular size DOM fractions in most of the time into the Ganges River (Table 2). In the mid of pre-monsoon, E_{250}/E_{365} ratio decreased unanticipatedly (Table 2). This sudden decrease was probably caused by the increase of rainfall that flushed out the surrounding terrestrial organic materials into the river water and

resulted in an increase of large molecular size DOM fractions. Except for this specific season, a relatively lower molecular size DOM fraction prevailed in the Ganges River. E_{250}/E_{365} had a significant negative correlation with S_R ($R=-0.601$, $p=0.039$) indicating that, components with higher molecular size were hollow and lower in molecular weight (Table 3). But E_{250}/E_{365} had a negative relation with C3 ($R=-0.868$, $p=0$) showing that molecules in terrestrial-derived DOM had comparatively lower in size (Fig. 6e).

3.6 Principal Component Analysis (PCA)

PCA based on four PARAFAC components and all calculated DOM indices showed that PC1 and PC2 (first two principal components) contributed 39.16% and 29.47% to the total variance, respectively (Fig. 7). PC1 had strong positive correlation with BIX (0.89), Freshness index (0.82), E_{250}/E_{365} (0.79) and FI (0.73), and firm negative correlation with fulvic acid (C-like) component C3 (-0.936), S_R (-0.702) and DOC (-0.531) (Fig. 7). High BIX, Freshness index and FI values show sources of autochthonous or microbial originated DOM components. Strong negative correlation with BIX, Freshness index and FI confirmed the origination of C3 from the allochthonous or terrestrial environment. C3 had also an inverse correlation with E_{250}/E_{365} which explained that, the molecular size of allochthonous DOM component (C3) was much larger than autochthonous components. Component 2 (C2) had also a positive correlation with BIX, Freshness index and FI along with the PC1 axis indicating its generation from autochthonous or biological sources. On the other hand, PC2 positively correlated with detergent-like component C1 (0.884), fulvic acid (M-like) component C2 (0.874), protein-like component C4 (0.928), and negatively correlated with HIX (-0.665) (Fig. 7). Inverse relation with HIX indicated the very low humic content in autochthonous originated fulvic acid-like component C2. Anthropogenic originated fDOM components such

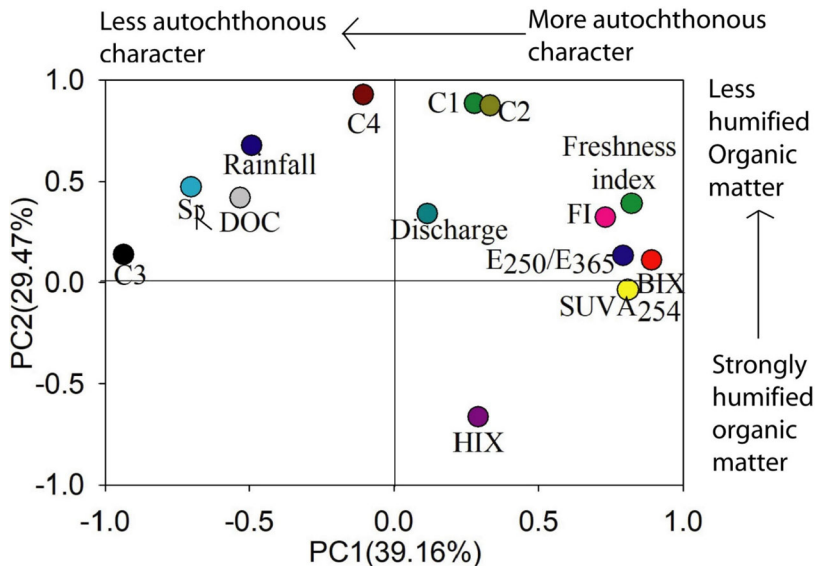


Fig. 7 Principal component analysis (PCA) using all calculated DOM indices (FI, HIX, BIX, Freshness index, S_R , E_{250}/E_{365} , $SUVA_{254}$), hydrological data (rainfall, discharge) and fluorescent DOM components (C1, C2, C3, C4)

as detergent-like component (C1) and protein-like component (C4) had also a positive correlation with rainfall (0.676) and water discharge (0.341) along the PC2 axis revealing that abundances of C1 and C4 from terrestrial sources into the water were highly dependent on these two hydrological parameters (Fig. 7). Another fDOM component, C3 had also a positive close association with DOC (0.419) and S_R (0.473) along the PC2 axis, which explained that this PARAFAC component had higher organic carbon content but lower molecular weight than other components (Fig. 7).

3.7 Microbial Contamination Risk Level of Ganges River

Tryptophan-like fluorescence (TLF) can be used as an indicator to assess the microbial contamination risk in the aquatic environment (Nowicki et al. 2019). Microbially-contaminated waters can be classified according to the world health organization (WHO) defined risk classes using tryptophan like fluorophores (Nowicki et al. 2019). Concentration of TLF varied between 0.004–1.76 ppb and was >1 ppb in pre-monsoon (*t*-test; $t=-9.44$, $p<0.05$, $\sigma=0.51$). TLF results were categorized into four types of risk classes named low, intermediate, high and very high as described in Nowicki et al. (2019). Ganges river water quality was in the high-risk category during pre-monsoon (TLF value >1) indicating the occurrence of more microbial activities in river water at this time. Ganges river water was in the intermediate-risk category in the entire monsoon due to the continuing input of terrestrial-derived anthropogenic materials. But compared to these two seasons, microbial contamination was low throughout the post-monsoon, and thus, the risk of the Ganges River water was in low rank in this specific time.

The reason behind the high microbial contamination in the pre-monsoon was the increase of rainfall and water discharge after the entire post-monsoon which transported the protein-like component C4 and terrestrial anthropogenic waste (component C1) including pit latrine waste into the river channel. Anthropogenic pollutants and protein add up with local climatic parameters in the pre-monsoon and monsoon and facilitate the microbial activity in water. This higher anthropogenic organic load from both urban and suburban areas of the riverbank might deteriorate the microbial quality of the Ganges river water. Rainfall and water discharge were also higher in the monsoon period. But due to solar irradiation during summer, the concentration of this TLP-like component might be decreased and microbial risk of river water ranked down from high to an intermediate position.

4 Conclusions

In the present study, DOC concentrations, fluorescence characteristics of DOM and their sources showed distinctive seasonal variation, alteration of the chemical composition of DOM and their relation with local hydrological parameters (rainfall, river water discharge) in the lower Ganges River, Bangladesh. 3DEEM and PARAFAC identified five fluorophores (Peak: A, C, M, T, W) and four different types of fluorescent components of DOM in the river water: detergent-like, fulvic acid (M-like), fulvic acid (C-like) and protein-like. Total fluorescent intensity of DOM was on the higher ranges (0.098–0.50 RU) in the pre-monsoon and monsoon periods compared to post-monsoon. The anthropogenic detergent-like component was present most of the month with higher intensity (0.03–0.18 RU) describing the persistent nature of anthropogenic pollutants in the Ganges River. Fulvic acid-like autochthonous DOM

components dominated in the post-monsoon period, although in the other two seasons allochthonous DOM were abundant in the river water. Both fresh and microbial-derived DOM were present throughout the year. Autochthonous DOM components had higher molecular weight and aromaticity than terrestrial-derived components. During the pre-monsoon and monsoon periods, rainfall-runoff and river water discharge washed out anthropogenic waste materials from terrestrial environment in the Ganges River. Local climatic parameter and abundance of pollutants in the pre-monsoon and monsoon resulted in microbial activity and deterioration of the water quality. Thus, Ganges River was also in the intermediate and high-risk category due to microbial contamination in the pre-monsoon and monsoon. Principal component analysis statistically confirmed all the findings in our study. All these results indicated that DOM fractions in the Ganges river showed dynamic nature in their character, sources and abundance, and were highly dependent on rainfall and water discharge and other local climatic parameters.

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Author contribution Nahin Mostofa Niloy: sampling, data collection and analysis, methodology, software, validation and draft preparation. Md. Morshedul Haque: sampling and data collection. Shafi M Tareq: Conceptualization, reviewing and editing manuscript, providing necessary laboratory supports including chemicals and instruments and supervision.

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author and first author on reasonable request.

Declaration

Conflict of interest Authors have no conflict of interest

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