Global Journal of Environmental Science and Management
(GJESM)

Homepage: https://www.gjesm.net/

ORIGINAL RESEARCH PAPER

Dissolved organic matter and its correlation with phytoplankton abundance for monitoring surface water quality

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ARTICLE INFO

Article History:
Received 04 April 2021
Revised 17 July 2021
Accepted 20 August 2021

Keywords:
Correlation
Dissolved organic matter
Fluorescence spectroscopy
Phytoplankton

ABSTRACT

BACKGROUND AND OBJECTIVES: Dissolved organic matter has a fundamental role in supporting phytoplankton abundance and growth in aquatic environments. However, these organisms produce dissolved organic matter with varied quantities or characteristics depending on the nutrient availability and the species composition. Therefore, this study aims to assess the characteristic of dissolved organic matter on surface water and its correlation with phytoplankton abundance for monitoring water quality.
METHODS: The sample was obtained at four Kali Surabaya river stations for further dissolved organic matter analysis and phytoplankton species analysis. The analysis was presented through bulk parameters of total organic, ultraviolet at 254 nm wavelength, specific ultraviolet absorbance value, and fluorescence spectroscopy using excitation-emission matrices with fluorescence regional integration analysis.
FINDINGS: The results showed the bulk parameters of dissolved organic matter at all stations were significantly different, as Station 1 and 2 were higher, while 3 and 4 had a lower concentration. Furthermore, the fluorescence spectroscopy identified four components of dissolved organic matter at all stations, namely aromatic proteins-like, humic acid-like, soluble microbial by-products-like, and fulvic acid-like, which is the unit of fluorescence spectra in arbitrary unit. Also, stations 1 and 2 were grouped in the high percentage fluorescence regional integration of humic substance (fulvic acid-like and humic acid-like), while 3 and 4 were classified in the high percentage fluorescence regional integration of non-humic substances (aromatic proteins-like and soluble microbial by-products-like).
CONCLUSION: The main phytoplankton species, namely Plectonema sp., Pinularia sp., Nitzchia sp., Navicula sp., had the highest abundance at Stations 1, 3, and 4, respectively. A strong correlation between dissolved organic matter analysis and phytoplankton abundance led to the usage of these methods for monitoring surface water quality.

DOI: 10.22034/gjesm.2022.01.0*

NUMBER OF REFERENCES
55

NUMBER OF FIGURES
8

NUMBER OF TABLES
2

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Note: Discussion period for this manuscript open until April 1, 2022 on GJESM website at the “Show Article.

INTRODUCTION

Human, industrial and agricultural activities have significantly changed aquatic ecosystems due to high organic and inorganic wastewater discharge. This runoff has appeared in the eutrophication of rivers and tributary (Conley *et al*., 2009; Bhattacharya and Osburn, 2017) causing blooming phytoplankton and consequently, and the environmental issues (Paerl *et al*., 2008; Heisler *et al*., 2008; Biggs, 2000). It is eminent that phytoplankton community dynamics (i.e., taxonomic composition, abundance, and biomass) regard the quantity of inorganic phosphorus and nitrogen in the aquatic surrounding (Cao *et al*., 2016; Cuvin-Aralar *et al*., 2004). Furthermore, the impact of the organic pollutants contributes to the quantity or quality of dissolved organic matter in surface water. Allochthonous and autochthonous with effluent organic matter are the source of dissolved organic matter (DOM) in the surface water, since allochthonous could be generated from the upstream, midstream and the downstream. The upstream was found to be covered with perennial vegetation; the midstream is used for agriculture and covered with least forest; the downstream was mainly used for residential and utilized for different forms of agriculture (Dumago *et al*., 2018). In addition, biogeochemical cycles will affect the quality and quantity of DOM from the surrounding environment. Also, DOM has an essential role in supporting phytoplankton abundance and growth in aquatic surroundings (Kissman *et al*., 2017; Burpee *et al*., 2016) due to its usage as an organic nutrient source. It can be used by these micro-organisms as a source of nitrogen, phosphorus, and carbon when inorganic phosphorus and nitrogen are unavailable (Burpee *et al*., 2016). The primary producers were proposed as an important source that influences its composition in surface water (Biddanda and Benner, 1997). Conversely, DOM can be produced by phytoplankton (Thornton, 2014), with varied characteristics and quantity which are mostly dependent on nutrient availability (Myklestad, 1995), composition of phytoplankton type (Biddanda and Benner, 1997), and bacterial interaction (Ramanan *et al*., 2016). According to previous studies, various types of DOM have been found and released by different taxonomic groups of phytoplankton (Fukuzaki *et al*., 2014; Romera-Castillo *et al*., 2010). Phytoplankton production, microbial metabolism, residue from microbial degradation after their death and other processes, release protein-like materials as one of DOM components (Liu *et al*., 2019; Mangal *et al*., 2016). The fluorescence spectroscopy fingerprints, identified the signals of protein-like and humic-like materials released from extracellular Microcystis aeruginosa (Ziegmann *et al*., 2010). In addition, the DOM which is closely related to the phytoplankton community dynamics, mainly consist of humic-like and protein-like materials (Suksomjit *et al*., 2009; Zhang *et al*., 2014) and exhibits their blooming (Altman and Paerl, 2012; Hounshell *et al*., 2017). The qualitative and quantitative methods for characterizing organic matter analysis have been implemented to clarify the types of DOM transformation through the treatment process or in source water and their following removal. For example, using the bulk parameters of dissolved organic carbon (DOC) concentration, UV/vis at 254 nm wavelength to measure the aromaticity degree of organic matter and specific ultraviolet absorbance (SUVA) (Edzwald *et al*., 1985; Lai *et al*., 2015; Hidayah *et al*., 2017), high-performance size exclusion chromatography (HPSEC) with ultraviolet detector (UVD) or an on-line organic carbon detector (OCD) (Jiao *et al*., 2014; Lai *et al*., 2015), fluorescence spectroscopy as well as fluorescence excitation-emission matrices (FEEM) (Hidayah *et al*., 2017; Ho *et al*., 2019). These procedures have been previously applied in observing the contribution of phytoplankton degradation to DOM as chromophoric by using fluorescent spectroscopy (Zhang *et al*., 2009), to characterize DOM excreted by phytoplankton (Chari *et al*., 2013), and to reveal its relationship with the community (Liu *et al*., 2021). The use of bulk parameters and fluorescent spectroscopy methods, simultaneously for characterizing organic matter considering the phytoplankton abundance, have been rarely observed. Therefore, resulting in poor implementation of optimal water quality control measures. Furthermore, using these techniques to characterize organic matter and its correlation with phytoplankton abundance for monitoring surface water quality seems to urgently need implementation. Hence, this study aims to assess the characteristic of dissolved organic matter on surface water, as well as its correlation with phytoplankton abundance using the bulk parameters and fluorescence spectroscopy to monitor surface water quality. This study was conducted in the Kali Surabaya River, Surabaya, Indonesia, in 2021.

MATERIALS AND METHODS

Data collection

This study used water from the Kali Surabaya River in Surabaya city, a surface water source for public supply. The position of station 1 to station 4 is as shown in Table 1 and Fig. 1. The sample was collected twice per week from January to March 2021, and the DOM analysis, as well as phytoplankton abundance was measured through the bulk parameters and fluorescence spectroscopy. The parameters include TOC, UV_{254}, SUVA value, while fluorescence spectroscopy identified aromatic proteins-like (AP-like), humic acid-like (HA-like), soluble microbial products-like (SMPs-like), and fulvic acid-like (FA-like).

Table 1: The study sampling location characteristics

No.	Sampling station	Coordinate	Climate	Environmental condition
1	Rolag Telu dam	7°26'40" S 112°27'25" E	- Tropical - Sunny weather - Temperature 29°C	- Downstream of the Brantas river - Stagnant water - No residential
2	Wringin Anom district	7°24'21" S 112°30'27" E	- Tropical - Sunny weather - Temperature 29°C	- Agricultural land - There are residential - There are domestic activities (bathing, washing, latrine)
3	Cangkir district	7°22'04" S 112°37'47" E	- Tropical - Sunny weather - Temperature 29°C	- Industrial area - Densely populated - Temporary dump site
4	Karang Pilang drinking water company inlet	7°20'54" S 112°40'51" E	- Tropical - Sunny weather - Temperature 29°C	- Industrial area - There are residential - There are domestic activities (bathing, washing, latrine)

Fig. 1: Geographic location of the study area in the Kali Surabaya River, Indonesia

As this study targeted on dissolved organic matter in source water, 0.45 m filter paper was used to filter the collected source water (Millipore Corporation, USA) to eliminate suspended particles before analysis the parameters. Furthermore, the ultraviolet absorbance at 254 nm (UV_{254}) and total organic carbon (TOC) concentration of the water was measured for common physicochemical characteristics based on Standard Methods procedures (APHA *et al*., 2012).

TOC was quantified using TOC-500 Model (Shimadzu, Japan), while UV_{254} was detected by UV/vis spectrophotometer Model U-2001 (Hitachi, Japan). The SUVA value showed the dissolved organics were contained in hydrophilic fraction as calculated from measurements of UV_{254} and DOC samples. Perkin Elmer LS-55 spectrometer with excitation-emission wavelength pair was used to measure the fluorescence in the source water. Moreover, the excitation-emission matrix (EEM) were resulted for each sample by skimming overexcitation (Ex) wavelengths between 230 and 400 nm at an interval of 10 nm with emission (Em) wavelengths between 300 and 547.5 nm at 0.5 nm interval (Murphy *et al*., 2013; Hidayah *et al*., 2017). Counting of fluorescence regional integration (FRI) analysis was used to provide the cumulative fluorescence reaction of organic matter with identical characteristic in selected regions by integration beneath EEMs (Chen *et al*., 2003). The phytoplankton sampling was conducted using a plankton net mesh size 60 mm as much as 100 liters. Meanwhile, its identification was carried out in the laboratory using a binocular microscope with 10 x 10 magnification (AmScope B100B-MS). Also, the abundance was calculated using Sedgewick-Rafter Counting Chamber for three replications (Marienfeld GmbH).

Analytical framework

The Kolmogorov-Smirnov, one-way Analysis of Variance (ANOVA), and Pearson correlations were applied utilizing SPSS Statistics 17.0 software (IBM, Armonk, NY, USA). The Kolmogorov-Smirnov test opposed the empirical cumulative distribution function of bulk parameters data and the results of FRI analysis with the distribution expected when the data were standard. When the observed difference is adequately significant, the test will reject the hypothesis of bulk parameters data, the results of FRI analysis data, and phytoplankton abundance normality. However, when the p-value of this test is less than 5%, it can be concluded that the bulk parameters data, the results of FRI analysis data, and phytoplankton abundance are non-normal. The one-way ANOVA was applied to determine whether any statistically significant differences between the means of bulk parameters and the results of FRI data. It was also used to determine at least two groups of the parameters data as the results of FRI analysis were different. In addition, The Pearson correlation coefficients measured the strength of the linear relationship variables among TOC, UV_{254}, SUVA value, AP-like, FA-like, SMPs-like, HA-like, and phytoplankton abundance.

RESULTS AND DISCUSSION

The bulk parameters of dissolved organic matter in the river segment.

The distribution data for the bulk parameters of dissolved organic matter in the river segment as tested by Kolmogorov–Smirnov showed the TOC concentration ($P > 0.15$), UV_{254} concentration ($P > 0.15$), and SUVA value ($P > 0.15$) was normal. Furthermore, the normal distribution data was performed using ANOVA testing to know the differences in mean concentrations of TOC and $UV_{254,}$ as well as SUVA value. ANOVA with the Tukey 95% confidence interval also determined whether there were statistically significant or non-significance differences. The results indicated statistically significant differences in the mean concentration of the bulk parameters among the river segment with a p-value of 0.011, 0.001, and 0.004 in TOC, UV_{254}, and SUVA values, respectively. Moreover, enough evidence was provided, which concluded that the average of the bulk organic matter parameters at all stations was significantly different. The Tukey analysis classified the bulk parameters concentration at each station into two main groups. Station 1 and 2 were grouped in the high concentration, while 3 and 4 were classified in the bulk parameters' low concentration, which means the former had averages significantly different from the latter. The average TOC concentration for stations 1 and 2 was about a value 10.1-11.7 mg/L, while 3 and 4 were between 9.8-10.9 mg/L. The average UV_{254} concentration for stations 1 and 2 was in the range of 10.1-11.7 mg/L, while 3 and 4 were in between 9.8-10.9 mg/L. The average UV_{254} concentration for stations 1 and 2 was in the range of

0.65-0.8/cm, while 3 and 4 were 0.39-0.65/cm. The average SUVA concentration of stations 1 and 2 was 5.3-6.4 L/mg/m, while 3 and 4 were 4.0-5.3 L/mg/m. Furthermore, statistical box plot analysis presented the pattern for the bulk parameters of dissolved organic matter in the surface water. Figs. 2, 3, and 4 show a box plot of the average concentration of TOC, UV_{254}, and average SUVA value respectively. Fig. 2 shows that the highest average TOC concentration occurred at Station 2 with a varying range. In comparison, the lowest average TOC concentration with a low range occurred at station 4. In addition, the results showed the average concentration from the highest to the lowest was found at stations 2, 1, 3, and 4. The surface water used in this study contained 7.36 – 15.50 mg/L TOC concentration, which was typically associated with the DOC range. River water has a typical concentration about 2 to 10 mg/L of dissolved organic carbon, which was much higher than groundwater and seawater. Variation in average concentrations of TOC indicated various physical or ecological drivers, chemical processes, spatial changes, which can significantly affect on organic matters dynamics (Maie et al., 2006). The organic matter compositional changes could be induced by biophysical controls, such as changes in composition, which likely result in bioavailability, photoreactivity, nutrient cycling, or chelating capacity and can affect carbon fluxes consequentially ecological drivers not accounted for (Jaffe, 2008). In addition, the hydrology dynamics of surface runoff contributed to the surface water stream (Hood et al., 2006).

Fig. 3 describes the concentration of UV_{254}, which corresponded to the organic compounds with an aromatic structure, double bonds of C=C (Matilainen *et al*., 2011). In this study, the concentration of UV_{254} for surface water was 0.148 – 1.524/cm, which was within the typical range of river (0.085 – 0.4/cm) (Edzwald *et al*., 1985). The results showed that the average highest aromatic compound was detected at Station 1, while Station 4 had the average lowest concentration. Therefore, Station 1 contained higher humic matter with conjugated C=C double structural bonds than the others. Meanwhile, Station 4 contained lower humic matter than the others. As well known, organic compounds of humic matter contain unsaturated carbon bonds (double or triple) or aromatic rings in their molecular structure. Hence, it absorbs an amount of UV light through the water sample (Her *et al*., 2002).

Fig. 4. Shows the hydrophobicity of organic matter characteristic or specific UV-absorbance (SUVA) value. The results revealed a value between 1.45 – 9.36 L/mg/m. However, it was mostly higher

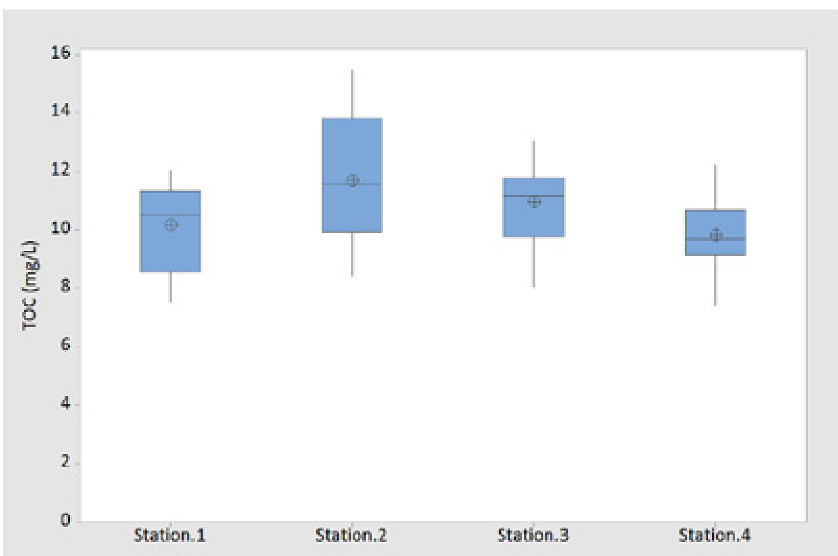


Fig. 2: The average TOC concentration in the river segment at various stations.

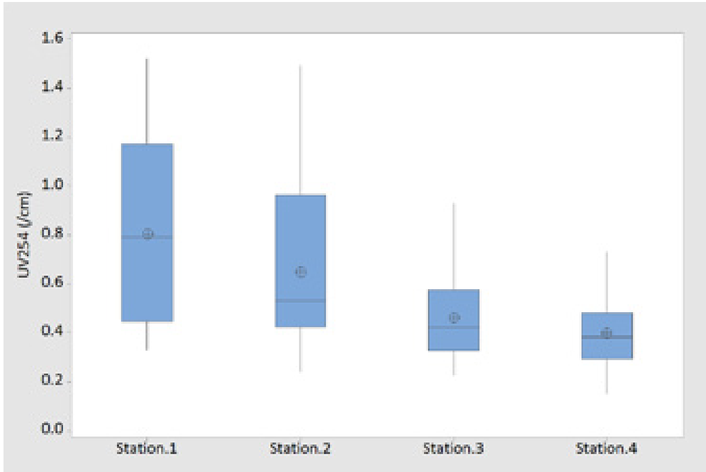


Fig. 3: The average UV_{254} concentration in the river segment at various station

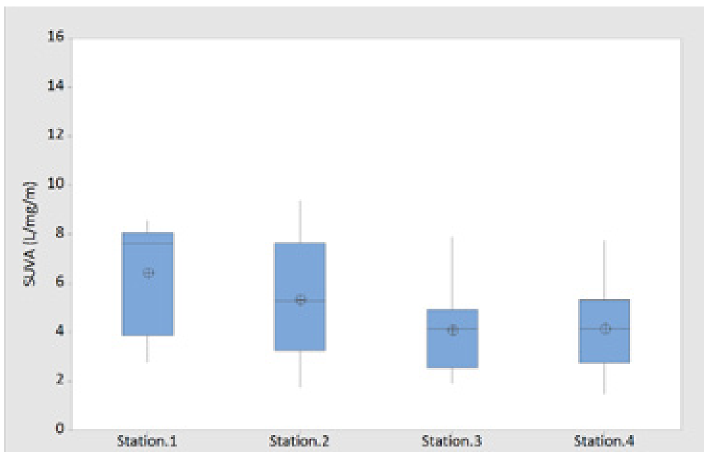


Fig. 4: The average SUVA value in the river segment at various station4

than 4 along the river segment, which means that the organic matter is mainly consists of humic, hydrophobic, and high molar mass organic material. According to Edzwald and Tobiason (2011), SUVA is a parameter of the organic matter composition in water. Source water with SUVA values ≥ 4 indicated that natural organic matter composed mainly of humic or hydrophobic matter, while those < 2 contained mainly non-humic or hydrophilic natural organic matter. The results were consistent with the high concentration of UV_{254} (0.148 – 1.524/cm). The values typically ranged from 1.0 to 6.0 L/mg/m for surface water. However, values greater than 6.0 were revealed for interstitial waters dominated by a solid terrestrial signature (Jaffe *et al*., 2008). According to previous studies, these higher values can be as a result of the absorption at 254 nm from colloids, iron, or other components in the sample (Weishaar *et al*., 2003; Hudson *et al*., 2007). Combining the bulk parameters of TOC, $UV_{254,}$ and SUVA value led to characterize the organic matter in the river. Station 2 was mainly composed of the highest TOC with lower aromatic and hydrophobic than 1, and vice versa. Also, station 4 was mainly composed of lower bulk parameters than 3. Therefore, 2 contained more aliphatic organic matter that does not absorb at 254 nm than the others. The lower SUVA value among all stations indicated the mixtures of aquatic humics, hydrophobic and hydrophilic, and molecular weights of organic matter.

Characteristic of fluorescence dissolved organic matter in the river segment through volumetric fluorescence distribution.

Fig. 5. Illustrates the fluorescence excitation-emission matrices (FEEMs) for dissolved organic matter in the river segment at a different station, taken on the first week sampling time. Dissolved organic carbon was classified into four regions based on its excitation/emission wavelengths (Ex/Em), namely Region 1 indicated the aromatic proteins-like (AP-like), such as tyrosine and tryptophan, at Ex/Em <250 nm/<350 nm. Region 2 identified the fulvic acid-like (FA-like) substances at Ex/Em <250 nm/>380 nm, Region 3 was corresponded to the soluble microbial by products-like (SMPs-like) substances at Ex/Em 250-280 nm /<380 nm, while Region 4 was identified as the humic acid-like (HA-like) substances with Ex/Em >280 nm/>380 nm (Chen *et al*., 2003).

This study shows that the fluorescence component from FEEM analysis has consistent results with previous studies (Her *et al*., 2003; Yao *et al*., 2016; Moradi *et al*., 2018; Hidayah *et al*., 2020). Generally, HA-like and FA-like correlated with aromatic compounds. They mainly exist as carboxylic and phenolic functional groups in natural dissolved organic matter. These fluorescence structures are mostly present as a significant percentage of humic substances, which typically represent over 50% of natural organic matter (Shon *et al*., 2012). In addition, source water may contain protein-like materials which microbial activities can generate. The amount, characteristics, and properties of dissolved organic matter in the aquatic system depend on their origin and environmental biochemical cycles. Sources of organic matter are classified as allochthonous (generated from a terrestrial watershed) and autochthonous (produced by organism activities,

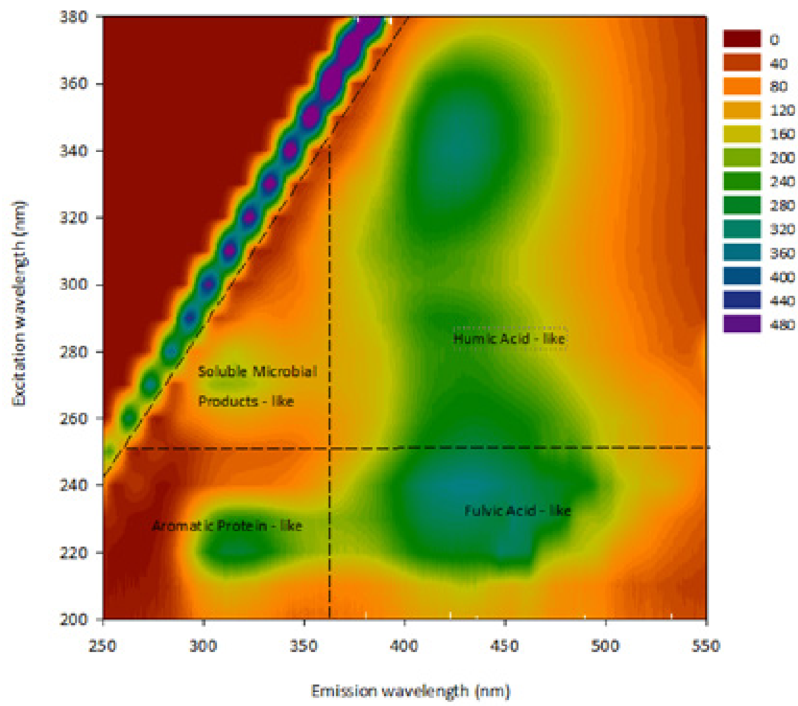


Fig. 5: Spectrum of fluorescence spectrometer analysis in the river segment

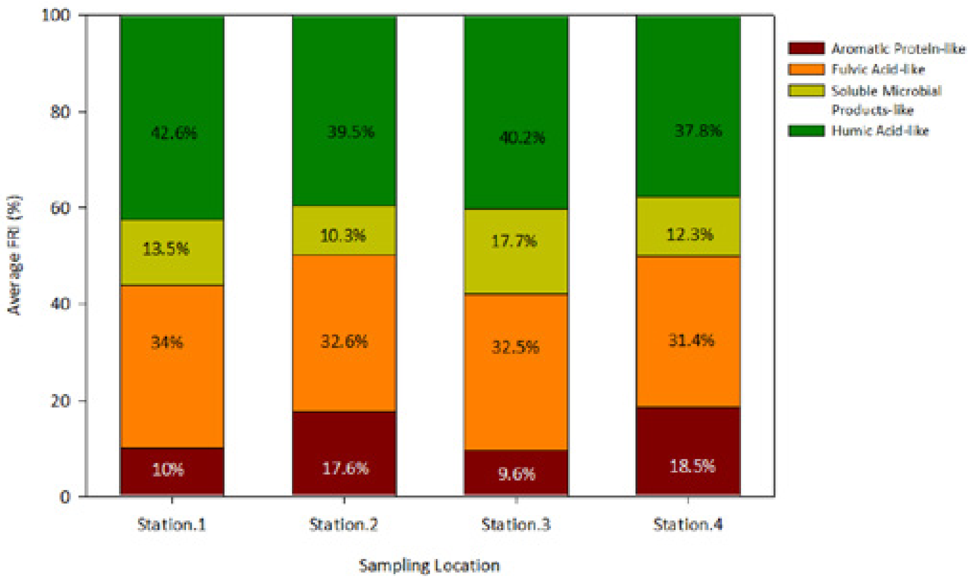


Fig. 6: FRI distribution of fractionated organic matter from the various river segment

such as phytoplankton activities) (Chari *et al*., 2013; Zhang *et al*., 2009; Haraguchi *et al*., 2019). Terrestrial watershed is mainly composed of humic substances such as fulvic and humic acids as well as humin, which are primarily hydrophobic and rich in aromatic carbon. The autochthonous source material is microbially derived organic, such as algal-derived and effluent organic matter (Kelso and Baker, 2020). Fig. 6 showed the percentage fluorescence response, which was calculated by Fluorescence Regional Integration (FRI) method. The percentage of fluorescence distribution indicated the four fractions quantity of fluorescence organic matter. This study classified the fraction into humic and non-humic substances-like. The first was represented by Region 2 (FA-like) and 4 (HA-like), while the second one by Region 1 (AP-like) and 3 (SMPs-like). Firstly, the results showed the highest total percentage of FRI in Region 2 and 4 was at Station 1 (76.6%), and the lowest total percentage for humic substances-like was at Station 4 (69.2%). Both components are classified as humic substances and are mainly composed of aromatic compounds with high to medium molecular weight (Watson *et al*., 2018; Hua *et al*., 2020). Their total percentage FRI showed a consistent UV_{254} concentration and SUVA value. Furthermore, Station 1 had the highest bulk parameters, while 4 had the lowest. Secondly, the highest total percentage FRI of Region 1 and 3 (30.8%) was identified at Station 4, with the lowest at 1 (23.4%). This indicated that Station 4 contained abundant proteins substances and microbial-like fluorescence than the others and followed the lowest SUVA value of Station 4 with the highest for Station 1. Region 1 and 3 correlated with high molecular weight protein-like, which had chemical properties related to aromatic amino acids, tryptophan or tyrosine-like (Yamashita *et al*., 2008; Hua *et al*., 2020) and low molecular weight microbial humic-like as well as less conjugated double bond organic matter (Nguyen *et al*., 2013; Hua *et al*., 2020).

The distribution data for the fluorescence of dissolved organic matter in the river segment was tested by Kolmogorov–Smirnov and the bulk parameters. The results showed distribution data for percentage FRI of Region 1 (AP-like), 2 (FA-like), 3 (SMPs-like), and Region 4 (HA-like) with $P > 0.000$, 0.007, 0.000 and 0.013 respectively were normal. Furthermore, Analysis of Variance (ANOVA) testing was carried out to determine the differences in mean percentage FRI for each region. The statistical analysis ANOVA One-Way with the Tukey 95% confidence interval also determined whether statistically significant or non-significance differences in percentage FRI of AP-like, FA-like, SMPs-like, and HA-like among all stations. The results showed statistically significant differences in the mean percentage FRI of all fluorescence organic fractions at all stations with p = 0.000, 0.007, 0.000, and 0.013 in AP-like, FA-like, SMPs-like, HA-like, respectively. The results provided enough evidence to conclude that the mean percentage FRI of all fluorescence organic fractions at all stations was significantly different. Moreover, the Tukey analysis classified their percentage FRI at each station into two main groups. Station 1 and 2 were grouped in the high percentage FRI of humic substance-like (FA-like and HA-like), while 3 and 4 were classified in the low percentage. This means the former had an average percentage FRI of FA-like and HA-like, which were significantly different from the latter. In addition, stations 3 and 4 were grouped in the high percentage FRI of non-humic substance-like (AP-like and SMPs-like), while stations 3 and 4 were grouped in the low percentage. This showed both had average percentage FRI of AP-like and SMPs-like, which were significantly different from stations 1 and 2. Moreover, statistical box plot analysis presented the pattern of the fluorescence organic matter in the river segments. Fig. 7a to 7d presented box plot with average percentage FRI of the organic matter. Firstly, a comparison among all fluoresces organic compounds showed the average FRI of HA-like was much higher and much lower for SMPs-like than the others. However, HA-like, located at Region 4 of the fluorescence spectra, had the most extensive range of excitation and emission wavelengths. Therefore, the humic acid substances-like region had the most extensive volume distribution of FRI when compared to others (Chen *et al*., 2003). Meanwhile, SMPs-

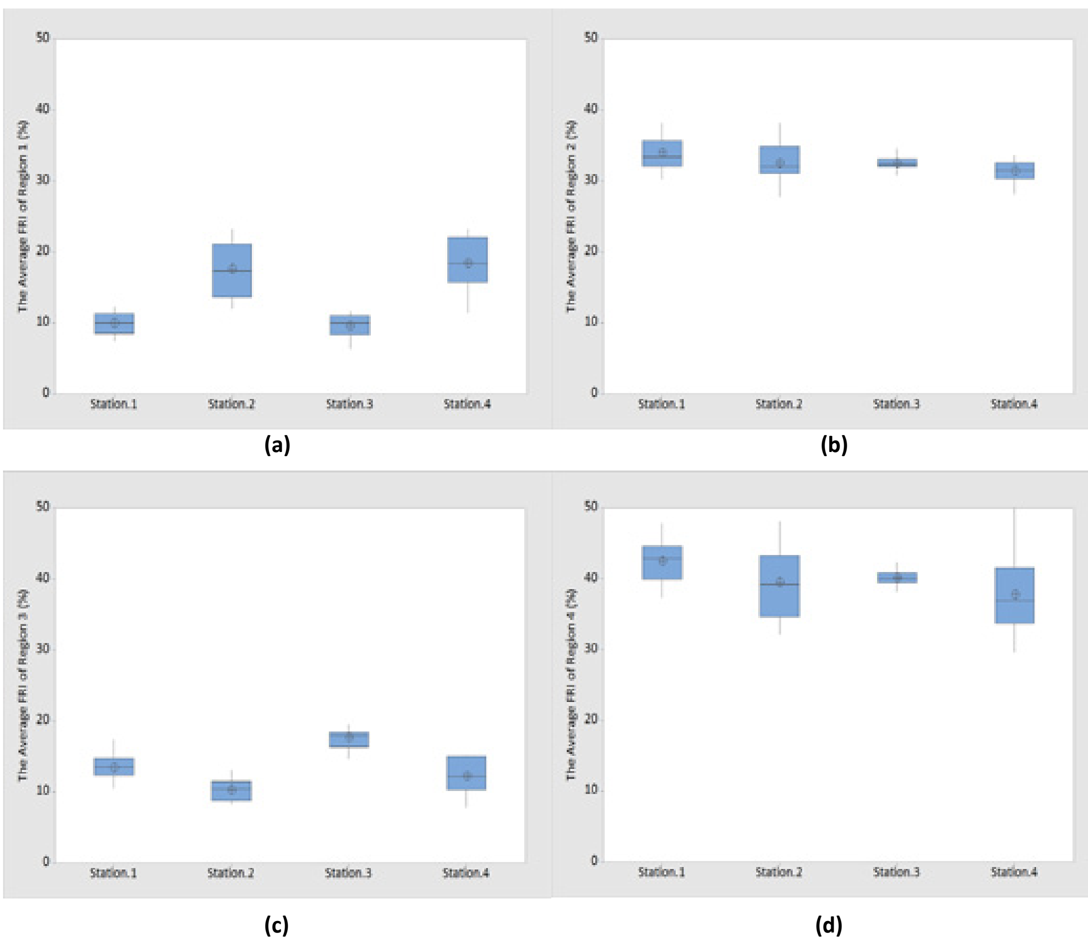
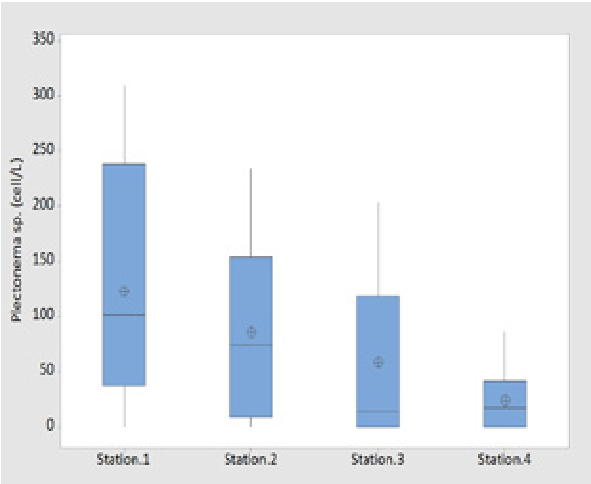
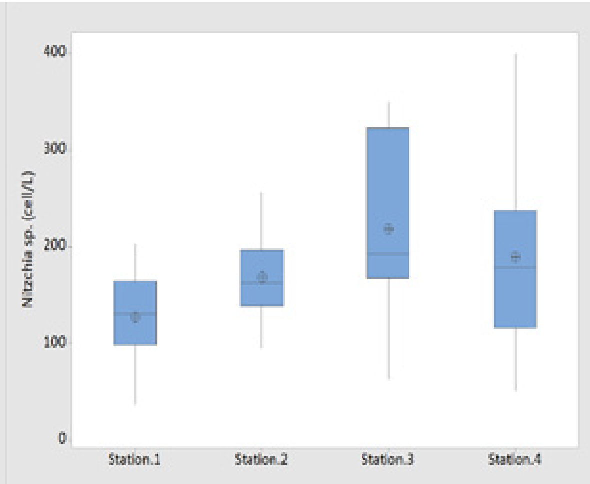


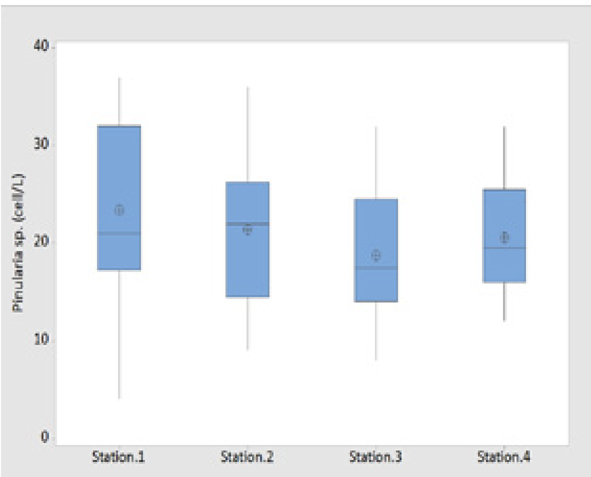
Fig. 7: The average percentage FRI of fluorescence organic matter in the river segment at various stations



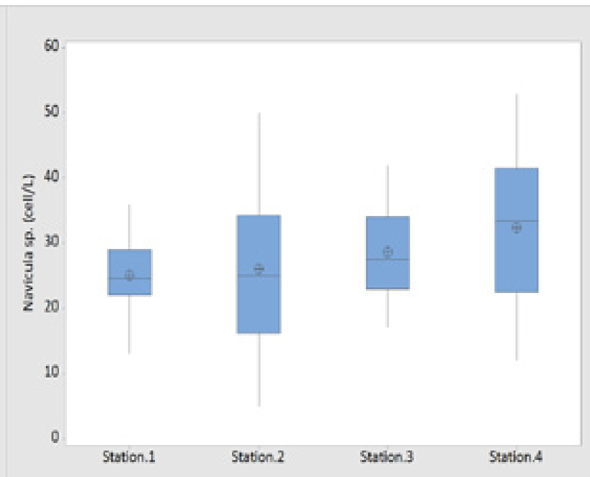
(a)



(b)



(c)



(d)

Fig. 8: The average of phytoplankton abundance in the river segment at various station

like or Region 3 comprised a dominant percentage of the fluorescence in wastewater treatment plant effluent (Chen *et al*., 2003) and was closely related to the phytoplankton activities (Liu *et al*., 2021; Hua *et al*., 2020). Second, the average percentage FRI of the organic matter indicated different quantities and quality at each station. The non-humic substances-like fluorescence as presented by AP-like, SMPs-like, with statistical analysis, had a higher percentage FRI at stations 3 and 4 than the others. It was likely that Station 3 and 4 had a higher percentage of extracellular biological organic matter fraction than the other river segments. The fraction was supposed to contain soluble microbial products of amino acids and carbohydrates. Tryptophan and tyrosine which are aromatic amino acids, were confirmed as biological activity products in natural systems and exhibited a distribution of fluorescence response similar to AP-like and SMPs-like of this study (Coble 2007; Determann *et al*., 1998). The humic substances-like fluorescence as presented by FA-like and HA-like had a higher percentage FRI at stations 1 and 2 than others and were tested by ANOVA One-Way. Combining the bulk parameters of TOC, UV_{254}, SUVA value, and fluorescence spectroscopy convinced the characteristic of organic matter in the river. Station 1 and 2 had high UV_{254} concentration, SUVA value, high percentage FRI of FA-like and HA-like substances. It was conjectured that stations 1 and 2 were mainly composed of aromatic, hydrophobic, humic substances organic matter, which may be generated from terrestrial systems.

Station 3 and 4 had lower UV concentration and SUVA values, with a high percentage FRI of AP-like and SMPs-like than the others. There was a lower SUVA value among all stations indicates in the mixtures of aquatic humics, hydrophobic and hydrophilic, as well as molecular weights of organic matter. This showed that Station 3 and 4 comprised more autochthonous and sources of organic matter from anthropogenic activities. The river ecosystem, which source is terrestrial, autochthonous, and anthropogenic, provided hotspots for storing, transporting, and transforming organic matter. The sources proportions were primarily and terrestrially derived with increased autochthonous inputs from macrophytes. In addition, the sources of dissolved organic matter are a mixture of terrestrial, autochthonous, or primarily from wastewater effluent (Kelso and Baker, 2020).

Contribution of phytoplankton abundance to fluorescence dissolved organic matter in the river segment.

This study discovered four main phytoplankton species with various abundance in the river segments, namely Plectonema sp., Nitzchia sp., Navicula sp., and Pinularia sp. The distribution data of the phytoplankton abundance in this segment was tested by Kolmogorov–Smirnov. The results showed a usual distribution data for Plectonema sp., Nitzchia sp., Navicular sp., and Pinularia sp. as abundance $P > 0.000$, 0.007, 0.000, and 0.013, respectively. Furthermore, ANOVA testing was carried out to determine the differences in the mean phytoplankton abundance of the river segments. The statistical analysis ANOVA One-Way with the Tukey 95% confidence interval determined whether there were statistically significant or non-significance differences in the abundance of the species among all stations. According to the results, there were statistically significant differences in the mean abundance of phytoplankton at all stations with p-value = 0.006 and 0.01 in Plectonema sp. and Nitzchia sp. abundance, respectively. Meanwhile, the analysis generated p-value = 0.156 and 0.412 for Navicula sp. and Pinularia sp. abundance, respectively, therefore, classified as only one group of phytoplankton abundance. This showed that there were non-significantly differences in both species abundance among all stations. The Tukey analysis classified Plectonema sp. and Nitzchia sp. abundance at each station into two main groups. Station 1 and 2 were grouped in the high Plectonema sp. and low Nitzchia sp. abundance, while Station 3 and 4 were classified in the low Plectonema sp. and high Nitzchia sp. abundance. Furthermore, the statistical box plot analysis presented the pattern of the phytoplankton abundance in the river segments.

Fig. 8a to 8d present box plots of their average abundance. Firstly, a comparison among the species at all stations conjectured that Nitzchia sp. had a higher abundance, and Pinularia sp. was lower than the others. Meanwhile, Plectonema sp. had the highest at Station 1 and the lowest at 4. Nitzchia sp. had a higher abundance at Station 3 and lower at 1. Moreover, Navicula sp. had the highest abundance at Station 4 and the lowest at Station 1. Pinnularia sp. gave the highest at Station 1, with the lowest at Station 3. This phytoplankton abundance was strongly influenced by migration, which can occur due to population density and physical environmental conditions, such as changes in temperature and currents (Basu and Mackey, 2018). Secondly, Station 1 was likely to contain a similar abundance in Plectonema sp. and Nitzchia sp., and the same for Navicula sp. and Pinularia sp. Stations 2 and 3 showed that the abundance of Nitzchia sp. was primarily dominant than others. However, Navicular sp. was similar to Pinularia sp. Station 4 identified a similar abundance of Plectonema sp., Navicular sp., and Pinularia sp. There is competition in several phytoplankton species that use the same resource lacking in availability, or even regardless of sufficient availability, and competition still occurs when they take advantage of the resource, with one attacking the other or vice versa (Burson *et al*., 2018).

The relationship among the bulk parameters, organic fluorescence parameters, and phytoplankton abundance

The degree correlation between the bulk parameters, fluorescence organic matter, and

Table 2: The degree correlation among the bulk parameters, fluorescence organic matter, and phytoplankton abundance*

Parameters	TOC	UV_{254}	SUVA	AP-like	FA-like	SMPs-like	HA-like	Navicula sp.	Plectonema sp.	Pinnularia sp.
UV_{254}	0.085 0.502									
SUVA	-0.044 0.729	0.887 0.000								
AP-like	-0.287 0.022	-0.440 0.000	-0.373 0.002							
FA-like	0.254 0.042	0.105 0.411	0.047 0.710	-0.249 0.048						
SMPs-like	-0.038 0.764	-0.198 0.116	-0.228 0.070	0.638 0.000	-0.085 0.505					
HA-like	-0.035 0.786	0.344 0.005	0.344 0.005	-0.674 0.000	-0.022 0.862	-0.348 0.005				
Navicula sp.	-0.109 0.392	-0.331 0.007	-0.289 0.021	0.193 0.126	-0.102 0.422	0.090 0.480	-0.082 0.521			
Plectonema sp.	0.271 0.030	0.137 0.281	0.131 0.303	-0.346 0.005	0.293 0.019	-0.057 0.652	0.110 0.386	0.166 0.189		
Pinnularia sp.	-0.097 0.448	-0.239 0.058	-0.292 0.470	-0.245 0.051	0.142 0.263	-0.268 0.032	0.220 0.080	0.137 0.279	0.320 0.010	
Nitzchia sp.	-0.243 0.053	-0.283 0.023	-0.203 0.108	0.160 0.205	-0.203 0.107	0.176 0.164	0.070 0.585	0.168 0.184	0.035 0.785	0.174 0.170

*Cell Contents description; Pearson correlation (the first row of the number of correlation between parameters); P-value (the second row of the number of correlation between parameters)

phytoplankton abundance was examined, as shown in Table 2. Correlation analysis was carried out using TOC and UV_{254} concentrations, SUVA value with percentage FRI of AP-like, FA-like, SMPs-like, or HA-like, as well as the abundance of Plectonema sp., Nitzchia sp., Navicula sp., and Pinularia sp. Firstly, based on the correlations of the bulk parameters, TOC concentration was positively higher with Region 1 (AP-like) and Region 2 (FA-like). In addition, UV_{254} concentration and SUVA value were significantly correlated with Region 1(AP-like) and Region 4 (HA-like). The results showed fluorescence spectroscopy, which fractionated AP-like, FA-like, SMPs-like, and HA-like could be used to identify the quantity and quality of organic matter in the source water.

This result was expected since TOC measured all organic carbon, including humic and non-humic substances, as presented by AP-like and FA-like. Secondly, a strong positive correlation between UV_{254} concentration and SUVA value indicated that higher aromatic conjugated double bond corresponded to higher molecular weight organic, more hydrophobic, and content of humic substances. These results are consistent with the Pearson correlation between bulk parameters of UV_{254} correlation, SUVA, and fluorescence organic matters of AP-like and HA-like. Furthermore, it was conjectured that fluorescence spectroscopy could be used to assess the properties of organic matter existing in the source water. Thirdly, the results showed that TOC had a stronger correlation with AP-like than HA-like. This was probably because the humic structure may incorporate protein-like-fluorophores due to weak interactions based on x-x or van der Waals forces between the dissolved organic matter components. Previous studies indicated that proteins and humic supramolecules containing specific structures attained from phenol or aniline might contribute to the fluorescence. Fourth, this study discovered a strong correlation between DOM and phytoplankton abundance. Plectonema sp. correlated with TOC, AP-like, and FA-like, while Navicula sp. and Nitzchia sp. correlated with UV_{254}, and Pinularia sp. with SMPs-like. The existence of phytoplankton was likely to enhance the quantity and characteristics of DOM in the aquatic environment. The production of marine-like fluorophores accompanied phytoplankton degradation as a significant source of autochthonous DOM (Wada *et al*., 2007). In addition, higher molecular weight compounds such as protein (tryptophan)-like fluorescence were presented in exudates when phytoplankton grows (Chari *et al*., 2013). The combination of the bulk parameters (TOC, UV_{254}, and SUVA value), fluorescence spectroscopy, and phytoplankton abundance convinced the quality of organic matter in the surface water. However, it could be eventually used to monitor the water's quality.

CONCLUSION

This study showed that the quality and quantity of DOM at all stations were significantly different, as classified into two groups with higher bulk parameters at stations 1 and 2 and a lower concentration at 3 and 4. The average TOC concentration for stations 1 and 2 was about a value 10.1-11.7 mg/L, while 3 and 4 were in between 9.8-10.9 mg/L. The average UV_{254} concentration for stations 1 and 2 was in the range of 10.1-11.7 mg/L, while 3 and 4 were between 9.8-10.9 mg/L. The average UV_{254} concentration for stations 1 and 2 was 0.65-0.8/cm, while 3 and 4 were 0.39-0.65/cm. The average SUVA concentration of stations 1 and 2 was in the range 5.3-6.4 L/mg/m, while 3 and 4 were 4.0-5.3 L/mg/m. In addition, fluorescence spectroscopy with FRI analysis showed stations 1 and 2 were grouped in the high percentage FRI of humic substance-like (FA-like and HA-like) about 74.35%. It was conjectured that stations 1 and 2 were mainly composed of aromatic, hydrophobic, humic substances organic matter, which may be generated from terrestrial systems, while stations 3 and 4 were classified in high percentages non-humic substances-like (AP-like and SMPs-like) about 29.05%. This showed that Station 3 and 4 comprised more autochthonous and sources of organic matter from anthropogenic activities. According to phytoplankton abundance, Station 1 had a high abundance of Plectonema sp. (238.5 cell/L) and Pinularia sp. (32 cell/L), while stations 2 and 3 mainly consisted of Nitzchia sp. (197.5 cell/L and 322.75 cell/L), and Navicula sp. (41.5 cell/L) was dominant at Station 4. The Pearson correlation showed a strong relationship between DOM and phytoplankton abundance. Therefore, Plectonema sp. was in correlation with TOC (0.271), AP-like (-0.346), and FA-like (0.293), while Navicula sp. and Nitzchia sp. correlated with UV_{254} (-0.331 and -0.283), and Pinularia sp. correlated with SMPs-like (-0.268). This study conjectured that the bulk parameters of DOM, fluorescence spectroscopy, and phytoplankton abundance could be used to assess the characteristic of DOM, while the combination of these methods could be used to monitor the surface water quality. Future work should be conducted on the laboratory scale for phytoplankton observation in order to identify the characteristic of organic matter that a kind of phytoplankton species has released. Therefore, it could be used to predict the amount of DOM derived by phytoplankton, DOM derived in the aquatic, and DOM from the terrestrial watershed.

AUTHOR CONTRIBUTIONS

O.H. Cahyonugroho performed the experimental design, analyzed the data, and prepared the manuscript text as well as the literature review. S. Hariyanto and G. Supriyanto interpreted the data and helped in manuscript preparation.

ACKNOWLEDGEMENT

This study was supported by the Directorate of Research and Community Service, Deputy for Strengthening Research and Development of the Ministry of Research and Technology/ National Research and Innovation Agency through Postgraduate Research – Doctoral Dissertation Research Scheme for multi-year, with Contract No. [489/UN3.15/PT/2021].

CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. Also, ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and submission, as well as redundancy, have been entirely witnessed by the authors.

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ABBREVIATIONS

%	Percent
/cm	Per centimeter
ANOVA	Analysis of variance
AP-like	Aromatic proteins-like
C=C	Carbon chain double bonds
cell/L	The number of phytoplankton cell per liter
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
Em	Emission wavelength
Ex	Excitation wavelength
FA-like	Fulvic acid-like
FEEM	Fluorescence spectroscopy using excitation-emission matrices
FRI	Fluorescence regional integration
HA-like	Humic acid-like
L/mg/m	Liter per miligrams per meter
mg/L	Miligrams per liter
mm	Milimeter
µm	Micrometer
Navicula sp.	Navicula species
Nitzchia sp.	Nitzchia species
NOM	Natural organic matter
nm	Nanometer
OCD	Organic carbon detector
P >	Probability value more than
P =	Probability value equal
Pinnularia sp.	Pinnularia species
Plectonema sp.	Plectonema species
P-value	Probability value
SMPs-like	Soluble microbial by products-like
SUVA	Specific ultraviolet absorbance
TOC	Total organic carbon
UV_{254}	Ultraviolet at 254 nm wavelength
UVD	Ultraviolet detector
UV/vis	Ultraviolet visible

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HOW TO CITE THIS ARTICLE

Cahyonugroho, O.H.; Hariyanto, S.; Supriyanto, G., (2022). Dissolved organic matter and its correlation with phytoplankton abundance for monitoring surface water quality. Global J. Environ. Sci. Manage., 8(1): 1-16.

DOI: 10.22034/gjesm.2022.01.0*

url: http://gjesm.net/***