Characterization of Fluorescent Dissolved Organic Matter in an Affected Pollution Raw Water Source using an Excitation-Emission Matrix and PARAFAC

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ABSTRACT

Cikapundung River is the main raw water source for 2-millions inhabitants of Bandung city but has been severely deteriorated due to organic pollution such as cattle manure, domestic, and agriculture wastes. Dissolved Organic Matter (DOM) in raw water can influence the process of water treatment. This study characterized and identified the origins of fluorescent DOM (FDOM) in Cikapundung River. Raw water samples were collected from intake outlets during dry and rainy seasons and analyzed using Fluorescence Excitation-Emission Matrix spectroscopy combined with parallel factor (PARAFAC). FDOM origins were identified by Fluorescence Index (FI) while autochthonous process contribution in water body was determined by Biological Index (BIX). Chromophoric DOM as UV absorbance at 254 nm (A254) and Chemical Oxygen Demand (COD) were also measured. The FI were 1.82 (dry season) and 1.77 (rainy season), and the BIX were 0.92 (dry season) and 0.65 (rainy season). PARAFAC identified three compounds: water contaminant-like (C1), humic-like (C2) and tryptophan-like (C3) compounds. C2 was predominantly present in the rainy season with a C3/C2 ratio of 0.33. In the dry season, C3 increased substantially with a C3/C2 of 1.60. Strong correlation between C1 and C3 (R=0.86) was evidence that contaminant-like and tryptophan-like compounds were from the same anthropogenic sources. Strong correlation with A254 may indicate these identified compounds are aromatics.

Keywords: Fluorescence Dissolved Organic Matter/ Tropical/ Raw water source/ PARAFAC

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1. INTRODUCTION

The main purpose of drinking water treatment plants is to produce drinking water that meets health standards by maximizing the removal of pollutants and pathogens. Rivers in West Java are important raw water sources for drinking water. However, the quality of these rivers have deteriorated due to contamination of organic compounds from anthropogenic activities especially from disposal of domestic and livestock wastes (EPA, 2020). The presence of DOM in raw water may disrupt the performance of drinking water treatment system by increasing the coagulant dosage and increase backwash of filter unit frequency (Jacangelo et al., 1995; Matilainen and Sillanpää, 2010; Ødegaard et al., 2010).

The authorized drinking water company in Bandung City has reported difficulties in treating the raw water during dry season (Sururi et al., 2020). This was indicated by not optimum formation of flock, and more frequent cleaning of secondary treatment units and backwash of rapid sand filter during the dry season than the rainy season. These occurred possibly due to the presence of DOM in the raw water, particularly in tropical countries when the intensity of precipitation may affect the characteristics of DOM in water bodies (Vasyukova et al., 2012). However, the presence of DOM, particularly in polluted raw water bodies and its changes along the water treatment plant (WTP) are not well understood (Ye et al., 2019). Moreover, the majority of drinking water treatment in Indonesia use chlorine-based disinfection because of their low cost and availability (Sururi et al., 2017). In particular, specific fractions of the DOM in the raw water are known as the major precursors for the formation of

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carcinogenic disinfection byproducts (DBPs) such as Trihalomethanes (THMs) when chlorine is used as a disinfecting agent (Abouleish and Wells, 2015; Jiang et al., 2017). Therefore, the raw water characteristics combined with chlorination method in the final disinfection stage can potentially produce drinking water that contains harmful THMs. Nonetheless, studies investigating the quantity and characteristics of DOM compound in tropical raw water sources including Indonesian rivers are very limited (Qadafi et al., 2020). The presence of DOM in the raw water is commonly represented as the concentrations of chemical oxygen demand (COD) and biochemical oxygen demand (BOD) including in Indonesia. However, both COD and BOD represent the lability of organic matter and thus inadequately indicate the characteristics of organic matter that may influence the performance of drinking water treatment. This suggests measuring alternative surrogate parameters of DOM that could provide complete information of DOM characteristics is needed.

Studies determining the types of DOM compounds in water body and their origins have gained growing interest worldwide. Recent studies (Hur et al., 2014; Yang et al., 2015) have used the fluorescence of DOM (FDOM) compounds to characterize chromophoric DOM (CDOM) as an alternate of CDOM, the key fraction of DOM which absorbs light over a broad range of ultraviolet-visible wavelengths (Fellman et al., 2010). Recently, FDOM was analyzed by fluorescence spectroscopy with excitation emission matrix and parallel factor analysis (PARAFAC). The composition of FDOM may suggest the origin of DOM whether from terrestrial inputs (allochthonous) or microbial activities in the water body (autochthonous). Among CDOM parameters available, UV absorbance at 254 nm (A254) has been one of the most common CDOM parameters to indicate the presence of humic and aromatic compound.

The purposes of this study were to: (i) identify the origins and FDOM compounds by PARAFAC in tropical raw water source during the dry and rainy seasons; (ii) determine the relationships between the quantity of FDOM and other surrogate parameters of organic such as COD and A254 during both seasons. The results of this study can be used as one of the main references to gain better understanding of DOM in tropical drinking water sources and determine the best strategies to produce safe drinking water.

2. METHODOLOGY

2.1 Study area

Cikapundung River is located in Bandung District West Java Province. The upper stream of Cikapundung River has been used as a raw water source to provide drinking water for almost 2 million inhabitants of Bandung Metropolitan City. The upstream area of Cikapundung River is located in Lembang District, inhabited by 197,640 people with a population density of 2,068 people/km² in 2019. The air temperature ranges from 19-32°C, and the average rainfall is 295.7 mm with the highest occurring in April (560 mm) while the lowest is in December (60 mm).

The Cikapundung River has a minimum discharge of 0.88 m³/sec and a maximum discharge of 2.11 m³/sec. Nearly 600 L/sec raw water is tapped by Bantar Awi Intake and treated at conventional drinking water treatment operated by the local water company. As seen in Figure 1, the water intake is located in natural forestry area (Djuanda National Park) with an area that occupies almost 20% of the catchment of the upper stream area (6,933.30 Ha). The catchment of the upstream area is dominated by anthropogenic activities (81% of the total catchment area) such as agriculture, plantations, cattle manure, tourism, and residential (Sururi et al., 2020). According to the Central Bureau of Statistics, the largest livestock in this area is located along this watershed (BPS KBB, 2019a), accounted by approximately 21,043 cows, 17,918 sheeps, and 526 horses (BPS KBB, 2019b). Cattle wastes were observed in the upper stream of the Cikapundung River which was originated from livestock located ±7 km (Batu Lonceng area) from the intake. The upper Cikapundung has been subjected to domestic wastes pollution since at least 20% of the total inhabitants in the watershed do not have appropriate sanitation facilities (BPS KBB, 2019b).

2.2 Water sampling

The average monthly rainfall intensity in the rainy season ranged from 443 mm/month (November 2019) to 199 mm/month (February 2020) with an average total number of rainy days of 24 days/month. Meanwhile during dry season, the average rainfall intensity and total rainy days was 48 mm/month and 6 days/month (August 2019), respectively. Given these values, raw water samples were collected as grab samples from the outlet of the intake in the dry season: August 14-23, 2019 (n=9); and two sampling periods in the rainy season: 9-17 November 2019 (n=9; period-1),
and 28-15 February 2020 (n=9; period-2). Therefore, in total, there were 27 samples/dataset for further analysis which was within the range for minimum input data required for PARAFAC (20-100 samples) as suggested by Stedmon and Bro (2008). 5L-polyethylene bottles were used for the raw water samples. These samples were then stored in a refrigerator at 4°C. Prior to analysis, the samples were filtered through a membrane of Advantech A045H047A Sterile MCE gridded filter 0.45 μm, 47mm. Other parameters such as pH and temperature were measured onsite, and the measured average pH values were 7.42 in the rainy season and 8.32 in the dry season, and temperature range between 23 and 25°C.

Figure 1. Area of study: Upper stream of Cikapundung River and its Catchment A

2.3 Identification of origin and types of DOM compounds

2.3.1 Spectral measurements

Fluorescence EEMs (FEEMs) were measured with a Shimidzu RF-5301 Spectro fluorophotometer set at emission wavelengths of 250-550 nm and excitation wavelengths range of 220-450 nm, with measurement intervals of 5 nm and 1 nm, respectively. Spectral corrections were applied for both the excitation and emission spectra. The correction procedures include: (1) reduction of inner filter effect using the absorption spectra data, and the fluorescence response of Milli-Q water blank (Murphy et al., 2010); (2) normalization of EEMs to the Raman peak area; and (3) finally removal of the Raman scatter. The correction factors obtained for the inner filter were generated based on the recorded UV-Vis absorbance which was measured at wavelengths of 220-600 nm (Murphy et al., 2010). The Rayleigh effects were then eliminated by replacing the spectra at emission wavelength between two excitation wavelengths in a range of −20 nm to +20 nm. The corresponding values were then set as missing values (Bieroza et al., 2011). The Raman peak area which resulted from these procedures were used for the normalization of the fluorescence intensity and then reported in Raman Units (RU).

2.3.2 Identification origin of DOM

The origin of DOM was identified based on the value of Fluorescence Index (FI) and a representative of algal and microbial versus terrestrial DOM sources (McKnight et al., 2001). The FI was calculated as the ratio of fluorescence intensities of 450 nm emission wavelength measured at 370 nm excitation wavelength to 500 nm at the same excitation wavelength (McKnight et al., 2001). Meanwhile the contribution of autochthonous process in the raw water was identified based on the value of Biological Index (BIX) since BIX values was an indication of the relative importance of biological or microbial DOM (Huguet et al., 2009). The value of BIX was

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**Figure 1.** Area of study: Upper stream of Cikapundung River and its Catchment A
determined as the ratio of intensity of 380 nm to 430 nm emission wavelength which was measured at 310 nm excitation wavelength.

2.3.3 Identification of DOM compound

The FDOM compounds in the raw water were determined statistically by conducting PARAFAC regardless the spectral shapes or number of the FDOM compounds. Briefly, PARAFAC model was developed based on the three key variables: excitation wavelengths, emission wavelengths, and fluorescence intensities as suggested by Stedmon and Bro (2008) using equation below:

\[ X_{ijk} = \sum_{f=1}^{F} a_{jf} b_{if} c_{kf} + e_{ijk} \quad i = 1, \ldots, I; j = 1, \ldots, J; k = 1, \ldots, K \]

Where; \( X_{ijk} \) is the intensity of fluorescence for sample \( i \)th which was measured at \( j \) emission wavelength and \( k \) excitation wavelength; \( a_{jf} \) is the \( f \)th analyte concentration in sample \( i \); \( b_{if} \) and \( c_{kf} \) are the emission and excitation spectra at wavelengths \( j \) and \( k \) respectively for the analyte \( f \); and \( e_{ijk} \) is the noise of residual and variability which was not accounted by the model.

The toolbox from drEEM (decomposition routines for Excitation Emission Matrices) in the MATLAB R2015a (MathWorks) was used for identifying FDOM compounds through PARAFAC. There were 27 EEM (comprised 352 emission and 47 excitation wavelengths for each EEM) which were decomposed into individual components. Split-half validation method was then used to evaluate the results of the PARAFAC for determining valid FDOM compounds. The spectral shapes of individual valid compound were finally compared with those shapes available in the online spectral library of auto-fluorescence (https://openfluor.lablicate.com).

2.4 Measurement of COD and \( A_{254} \)

The characteristics of DOM were identified as Chemical Oxygen Demand (COD), and \( A_{254} \). Water samples were filtered by 0.45 \( \mu \)m membrane prior to analysis. COD was analyzed based on the Standard Method protocol 5220C (close reflux method) (APHA, 2005). \( A_{254} \) were measured using a spectrophotometer (Shimadzu-1700 UV/Vis with a 1-cm quartz cell) at 254 wavelengths according to the standard method 5910 B (APHA, 2005).

2.5 Correlation analysis

The relationship between each DOM parameters (DOC and \( A_{254} \) and each of the identified compounds was determined based on the results of Pearson correlation analyses using a p-value of 0.05 to determine the significance. A correlation coefficient of \( >0.65 \) represented “good” correlation, 0.40-0.64 was “moderate” correlation, \( \leq0.39 \) represented “poor” correlation between the pair. T-test analysis was also conducted to indicate the difference of each parameter between the dry and rainy seasons. A t-test value \(<0.5\) was an indicative of statistically significant difference whereas a t-test value \(>0.5\) was interpreted that there were no significant differences between the two seasons (Awad et al., 2016). All statistical analysis was performed using SPSS 19.0 software package: IBM SPSS Statistics.

3. RESULTS AND DISCUSSION

3.1 Origins of FDOM in raw water

The measured ranges of FI and BIX in the raw water (Cikapundung River) during both seasons are summarized in Table 1.

| Seasons | FI Range | FI Mean±SD | BIX Range | BIX Mean±SD |
|---------|----------|------------|-----------|------------|
| Dry     | 1.55-3.23 | 1.82±0.09  | 0.62-1.33 | 0.92±0.21  |
| Rainy   | 1.60-1.95 | 1.77±0.10  | 0.48-0.81 | 0.65±0.12  |

It was observed that the measured FI average was 1.82 in the dry season and 1.77 in the rainy season, which was consistent with the results of t-test that show insignificant differences between the two seasons. The observed FI were comparable with those in a previous study suggesting FDOM in the raw water sources were from terrestrial and microbial activities (Tang et al., 2019). In this current study, however, the values of FI in the dry was greater than the rainy season, suggesting FDOM that originated from microbial activities was predominant. The results were consistent with the existing land use in the catchment area which is dominated by anthropogenic activities as evident by a large pile of animal waste in the upper stream. The observed results were within the range of FI values for polluted water body such as Han River (1.54-2.07) (Hur et al., 2014), and above the FI values for a natural water body such as Epulu-Congo River.
during the dry season (1.45) (Spencer et al., 2010). These suggest that the high values of FI in water body was most likely due to the contribution of wastewater discharges as reported by Ye et al. (2019).

The average value of BIX in the dry season was 0.92, greater than the rainy season (0.65) (Table 1), but the results of t-test did not indicate significance differences in the BIX values during both seasons. The results of the rainy season suggest less contribution of autochthonous DOM (Huguet et al., 2009) and terrestrial humic compounds which had entered the water body possibly through rainwater runoff was predominant (Parlanti et al., 2000). However, an increase of BIX during the dry season was likely indicating DOM with autochthonous sources of recently produced organic matter of bacterial origin (Huguet et al., 2009). These BIX values are comparable with those reported by (Hur et al., 2014) for a polluted river (0.58-1.04). Ye et al. (2019) have found that the more polluted the water body, the higher the BIX value. Therefore, the observed BIX during the dry season may indicate an increase in tryptophan compound which had possibly been the result of microorganisms decomposition activity in the cattle waste (Parlanti et al., 2000). Further study regarding the effect of land use on FI and BIX parameters in all segments of Cikapundung River is needed for strengthening the results of current study.

3.2 Type of FDOM compounds and DOM quantity in raw water

The PARAFAC have identified three main compounds in the raw water samples. The split-half validations have shown the spectral of these identified compounds overlapped the excitations and emissions loading of the three compounds in half the data set as well as the entire data set (Figure 2). Direct comparison of the measured EEMs, spectral shapes and position between each of identified FDOM compound with those in the spectral database (library/openfluor.lablicate.com) have resulted in similarities of 90-95%. The fluorescence characteristics of Compound-1 (C1) from this study were very similar to unknown compounds identified by other studies (Garcia et al., 2019; Murphy et al., 2008; Yamashita et al., 2010). Murphy et al. (2006) suggested that the fluorescence characteristic of Compound-1 as an unknown compound and resembled contaminants in water.

Previous studies (Borisover et al., 2011; D’Andrilli et al., 2019; Walker et al., 2009) have found that humic-like compounds were characterized at an excitation maximum of 370-390 nm and an emission maximum of 460-480 nm. Those characteristics closely resembled those found for compound-2 in this current study, suggesting compound-2 (C2) was likely representing humic from the terrestrial origin. The third compound (C3) has similar characteristics to the prior reported fluorescence peak for tryptophan-like compounds particularly tryptophan which were characterized at an excitation maximum of 275 nm and an emission maximum of 350 nm (Cawley et al., 2012; Osburn et al., 2011; Williams et al., 2013). The
presence of tryptophan in the raw water was an indication of water contamination by anthropogenic activities (Baker, 2001; Bieroza et al., 2010; Yang et al., 2012). The contour plots of EEM for each compound can be seen in Figure 3.

As seen in Table 2, average COD concentrations during both seasons were above the maximum limit according the national standard (PP 82/2001) for COD concentration in the raw water source (10 mg/L), confirming the raw water source is in polluted condition. The observed COD value was higher than the COD concentration in Baitapuhe River which was considered aerobic (16.86±4.72 mg/L), but lower than the COD concentrations in Xihe River (60±15.73 mg/L) which was considered as anaerobic (Yu et al., 2016). The results, therefore, indicated Cikapundung River was polluted by organic matter but has not reached anaerobic state.

Figure 3. Contour plots of EEM for three valid compounds: (A) compound-1 (C1), (B) compound-2 (C2), and (C) compound-3 (C3) in the raw water samples (sampling period-1)

Table 2 also shows higher concentrations of organic aromatic compounds \(A_{254}\) in the dry season \((0.35 \text{ cm}^{-1})\) than the rainy season \((0.20 \text{ cm}^{-1})\) was consistent with the result for C1 and C2 quantity which was also greater during the dry season than the rainy season. However, in polluted raw water, this high value of \(A_{254}\) during the dry season might be associated with the high values of tryptophan which is also considered as aromatic tryptophan-like compound (Preuße et al., 2000; Stubbins et al., 2014). Therefore, although C2 decreases in the rainy season, the aromatic nature of C1 and C3 were also measured in \(A_{254}\). The results indicate that measuring COD and CDOM \(A_{254}\) was insufficient to represent the organic presence in the raw water and may cause misinterpretation, leading to inappropriate approaches and strategies for drinking water treatment.

Table 2. The measured COD, \(A_{254}\) and Fmax of FDOM compounds in the dry and rainy seasons

| Parameter      | Dry (n=9) | Rainy (n=18) |
|----------------|-----------|--------------|
|                | Range     | Mean±SD      | Range       | Mean±SD      |
| COD (mg/L)     | 16.00-38.40 | 25.18±7.70  | 12.8-44.8   | 36.80±9.60   |
| \(A_{254}\) (cm\(^{-1}\)) | 0.21-0.48 | 0.35±0.08   | 0.16-0.25   | 0.20±0.04    |
| C1 (RU)*       | 0.13-0.19 | 0.17±0.02   | 0.01-0.02   | 0.01±0.005   |
| C2 (RU)        | 0.05-0.06 | 0.05±0.03   | 0.02-0.05   | 0.03±0.008   |
| C3 (RU)*       | 0.05-0.14 | 0.08±0.03   | 0.01-0.03   | 0.01±0.008   |
| C3/C2          | -         | 1.60        | -           | 0.33         |

* indicate there are differences between two seasons (sig<0.05)

There were seasonal variations in the concentrations of each FDOM compound in the raw water as indicated by the corresponding measured maximum intensity values (Fmax) (Table 1). Both the C2- and C3 compounds had maximum concentrations during the dry season. The tryptophan/humic ratio
(C3/C2) was 0.33 during the rainy season, and 1.60 during the dry season. The greater concentrations of the C3 than C2 during the dry season were similar with those found in a polluted river in England (Baker et al., 2003), adding evidence for the bioavailability and microorganism activities decomposing wastewater in the upper stream of Cikapundung River. The results might be explained by continuous discharges of organic pollutants from anthropogenic activities into the water body throughout the year, but lacking dilution effect of the rainwater during the dry season. This fact added evidence of the consistent results between the identified compounds through PARAFAC and greater values of FI and BIX in the dry season.

The concentration of C2 in the rainy season, on the other hand, was higher than C3 as indicated by the lowest Fmax and both FI and BIX averages during this season. Higher concentrations of humic-like compound indicate that water comprises tannin, lignin, polyphenols and melanin from plants decay (Fellman et al., 2010). The dominance of the terrestrial-derived compounds and the occurrence of rainfall during this season might suggest that soil origin-DOM entered the water body through the surface run-off. This highlights the importance of further studies on the dynamics of CDOM in conventional WTP treating raw water from Cikapundung River with differences in DOM composition during the rainy and dry seasons.

3.3 Correlations among PARAFAC components, CDOM absorption and COD

The results of correlation analysis between COD; A254 and each of identified FDOM compound during both seasons are summarized in Table 3. The results between A254 and each of FDOM compound (p<0.001, R≥0.60) demonstrate that the content of aromatic compound well correlated with A254. However, poor correlation between COD and A254 as well as between COD and each FDOM compound added evidence that the pollution level of Cikapundung River has not reached anaerobic condition. Yu et al. (2016) have reported good correlation between COD and DOM compound once the water body was in anaerobic condition. The contaminant-like (C1) and tryptophan-like (C3) compounds in the upper stream of Cikapundung River were most likely to be originated from the same anthropogenic activities as shown by strong correlation coefficient between C1 and C3 (R=0.86), as suggested by Hur and Cho (2012). The results of t-test also show that C1 significantly differed with C3 in both the dry and rainy seasons. The correlation between A254 and all compounds showed that these compounds have aromatic characteristic (Abbt-Braun et al., 2004; Du et al., 2012). Importantly, the observed strong correlation between A254 and all identified FDOM compounds suggest it would be inadequate to characterize organic compounds either based on COD or common CDOM parameter such as A254 in Cikapundung River. Further study is necessary to add evidence of the potential use of FI and BIX parameters for monitoring the quality of raw water as well as the use of EEM and PARAFAC for characterizing DOM compounds in urban raw water source such as Cikapundung River. This will provide more relevant information to determine appropriate and specific drinking water treatment strategy.

| Parameters | COD | A254 | C1 | C2 | C3 |
|------------|-----|------|----|----|----|
| COD        | 1   |      |    |    |    |
| A254       | 0.24| 1    |    |    |    |
| C1         | 0.15| 0.82*| 1  |    |    |
| C2         | 0.14| 0.69*| 0.80*| 1  |
| C3         | -0.08| 0.60*| 0.86*| 0.76*| 1  |

* Significant correlation (p-value<0.01)

4. CONCLUSION

The presence of FDOM compounds in Cikapundung River during the dry season were due to microbial activities which indicate the anthropogenically impacted DOM in Cikapundung River as shown by FI=1.82, BIX=0.92. FDOM compounds were less impacted by anthropogenic activities during rainy season with FI=1.77, BIX=0.65. Identified FDOM compounds by PARAFAC were water contaminant-like (C1), humic-like (C2) and tryptophan-like (C3). C3 was the predominant compound during the dry season (C3/C2=1.60), and the main compound during rainy season was C2 with C3/C2=0.33. A254 was well correlated with all FDOM compounds (R≥0.60, p<0.01), with the strongest correlation between C1 and A254 (R>0.82, p<0.01). C1 and C3 most likely originated from similar sources (R=0.86, p<0.01). Characterizing organic compounds solely based on COD and common CDOM parameter (A254) was insufficient to determine the quantity of organic compounds present in surface water.
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