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# ASSOCIATION BETWEEN *Phytoplankton* BIOLOGY INDEXES AND FLUORESCENCE DISSOLVED ORGANIC CARBON INDEXES FOR MONITORING WATER QUALITY

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## ABSTRACT

Fluorescence dissolved organic compound indexes, such as Biological Index, Humification Index, and Fluorescence Index has been developed to identify the characteristic of organic compound properties in the water, as well as use *phytoplankton* biology index to monitor surface water quality. This study aimed to identify characteristic organic compounds and analyze the correlation between the biological *phytoplankton* index and the fluorescence dissolved organic carbon indexes in monitoring surface water quality. The results show a difference in the fluorescence of dissolved organic compounds along the surface water ecosystem during the dry and wet seasons. The upstream contains more allochthonous organic compounds, while the downstream contains more autochthonous organic compounds. According to the biological index value, the autochthonous matter is higher in the dry season, and the allochthonous humic compound is higher in the wet season, as identified by the humification index value. However, the uniformity index of *phytoplankton* and fluorescence dissolved organic compound indexes exhibited a lack of correlation, probably due to the fluorescence index applied particular intensity, which could not represent the *phytoplankton* properties.

**Keywords:** Biological index, Humification index, Fluorescence index, *Phytoplankton* index, and Water Quality.

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## INTRODUCTION

The river ecosystem is a habitat for aquatic organisms, and the surrounding environment influences its existence. In the water, *phytoplankton* plays a crucial role as a primary producer that can turn inorganic materials into organic ones through photosynthesis.[1] For example, there is the runoff of organic compounds from land due to biota activities in the waters that produce dissolved organic compounds or other by-products.[2] A dissolved organic compound is an essential source of nutrients, which organisms in the water need.[3-4] Characterizing organic compounds based on the fluorescence properties of organic compounds is the fastest, most accurate method. It does not affect the quality of the sample because the sample does not require treatment.[5] This method can identify organic compounds based on the characteristics of organic fluorophores that can be captured at wavelengths with specific excitations and emissions.[6-7] Recently, the fluorescence of dissolved organic compounds has been used to determine the quality of raw water related to the content of organic compounds that can trigger the formation of carcinogenic compounds.[8-9] Many studies have developed fluorescence and index spectroscopy methods to identify specific organic compounds based on humification characteristics known as the Humification Index (HIX)[10], based on the properties of organic compounds produced from aquatic biota or autochthonous or known as the Biological Index (BIX)[11], and based on the properties of aquatic biota. An organic compound is derived from the runoff of organic compounds from terrestrial or known as the Fluorescence Index (FIX).[12] However, few studies still use the fluorescence of dissolved organic compounds to determine water quality related to the presence of *phytoplankton*. By calculating the biological index of *phytoplankton* in the water, it will be known how the level of diversity, uniformity, and dominance of the *phytoplankton* that live in these waters will be.[13] In connection with the load of organic compound in the waters, the presence of *phytoplankton* will affect the


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quality and quantity of organic compound in the waters, in addition to the influence of runoff of organic compound from human activities in the form of domestic waste, industrial waste, and organic compound produced from the activities of biota in the waters.[14-15] Therefore, the purpose of this study is to identify characteristic organic compounds and analyze the correlation between the biological *phytoplankton* index and the fluorescence dissolved organic carbon indexes in monitoring surface water quality.

## EXPERIMENTAL

### Material and Methods

Kali Surabaya river was selected as the observation river in this study because Kali Surabaya River has been used for raw water in clean water consumption, even for drinking water. Sampling was collected in four segments of the Kali Surabaya River at Station 1, Station 2, Station 3, and Station 4 and sampling was collected twice a week during the rainy season (January-February 2021) and the dry season (September-October 2021).

### General Procedure and Analyze

The sample was measured for dissolved organic compound properties based on the fluorescence spectroscopy method and *phytoplankton* analysis using a plankton net mesh size of 60 µm as much as 100 liters. Applying Perkin Elmer LS-55 fluorescence spectroscopy at excitation wavelengths (Xw) between 230 and 400 nm at intervals of 10 nm and emission wavelengths (Mw) between 300 and 547.5 nm at intervals of 0.5 nm.[5,16] Fluorescence dissolved organic carbon applied fluorescence intensity at emission wavelength and particular excitation to know fluorescence dissolved organic carbon indexes. The number of fluorescence intensities at particular excitation and emission wavelength was used to determine Fluorescence Index (FIX), Biological Index (BIX), and Humification Index (HIX). FIX calculates the fluorescence intensity at Xw/Mw 370/450 nm divided by Xw/Mw 370/500 nm.[12] BIX presents the fluorescence intensity at Xw/Mw 310/380 nm divided by Xw/Mw 310/430 nm.[11] HIX measures the fluorescence intensity at Xw/Mw 254/435-480 nm divided by Xw/Mw 254/300-345 nm.[10] Additionally, a binocular microscope with a 10 x 10 magnification was used in the laboratory to identify *phytoplankton* (AmScope B100B-MS). Three replications of the Sedgewick-Rafter Counting Chamber were used to determine the *phytoplankton* abundance (Marienfeld GmbH).

## RESULTS AND DISCUSSION

### Characteristic Organic Compound

This study applied fluorescence excitation-emission matrices (FEEMs) to identify characteristic organic compounds based on their fluorophore properties. The identification of organic compounds based on their fluorophores properties, as shown in Fig.-1. According to Fig.-1, The results show the four regions of organic fraction, that is, aromatic proteins at Mw <350 nm and Xw <250 nm, the second region is fulvic acid-like at Mw >350 nm and Xw <250 nm, the third region is soluble microbial by-products at Mw <380 nm and Xw = 250 – 280 nm, and the fourth region is humic acid-like at Mw >380 nm and Xw >280 nm.[5,16] The identification found two main organic fractions in the water, fulvic acid-like, and humic acid-like. Humic acid-like and fulvic acid-like are humic materials with differences in the variation in molecular weight, number of functional groups (carboxyl and phenolic OH), and length of polymerization. Humic materials are essential to organic matter, having chelation properties, high carbon content, high molecular weight, mineralization, buffering, clay mineral interactions, and cation exchange capacity. Along with water quality, fluorescence intensity at a particular wavelength could indicate the properties of organic compounds in water through fluorescence spectroscopic indexes, including biological index (BIX), fluorescence index (FIX), and Humification index (HIX), as shown in Fig.-2, Fig.-3, and Fig.-4. Briefly, information for those figures is that Station 1 is represented by sample numbers 1 to 16, Station 2 is 17 to 32, Station 3 is 33 to 48, and Station 4 is 49 to 64. FIX indicates the founts of dissolved organic compounds in water, whether it is produced from microbial activities or algal, such as from metabolite products of microbial secretions (autochthonous matters), or it came from the terrestrial watershed, such as transport to the river, materials include leaves that fall or are washed into the surface water and tree that topple into the stream FIX has been identified in range less than 1.4 indicated as a terrestrial dissolved organic compound. In contrast, FIX higher than 1.8 refers to algal-microbial dissolved organic compounds.[12]

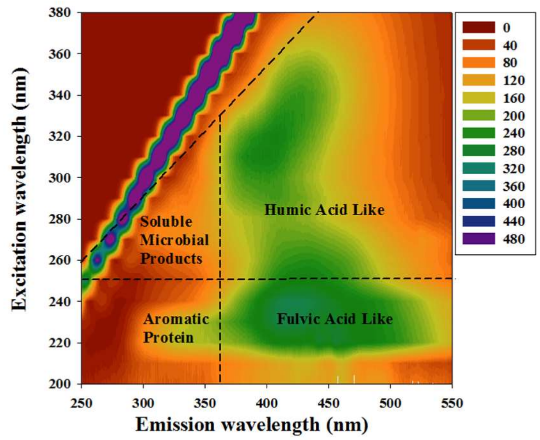


Fig.-1: Fluorescence Intensity Contour as Measured by Fluorescence Excitation-Emission Matrices

According to Fig.-2, firstly, FIX in Station 1 and Station 2 is much lower than in Station 3 and Station 4, which is in the range of 0.3 – 1.3 (dry season); 0.5 – 2.5 (wet season), and 1.4 – 3.2 (dry season); 1 – 3.5 (wet season), respectively.

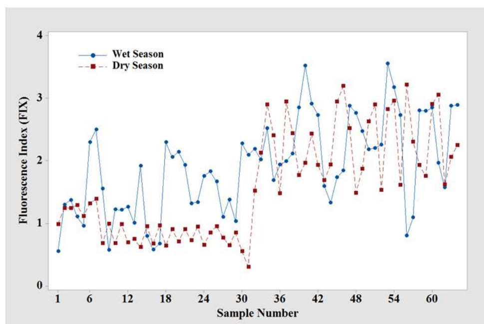


Fig.-2: Fluorescence Index (FIX) Value at Station 1 to Station 4 Kali Surabaya During Dry and Wet Seasons

An allochthonous organic compound is more dominant upstream, and the downstream contains the more autochthonous organic compound. It is probably due to the impact of industrial and domestic wastewater discharged into the river because some industries have been located after Station 2. Wastewater may contain organic, produced from industrial activities, and contribute to organic loading in the river.[14] Therefore, microbial activities were probably conducted in the ecosystem of the aquifer and released more autochthonous compounds.[17]

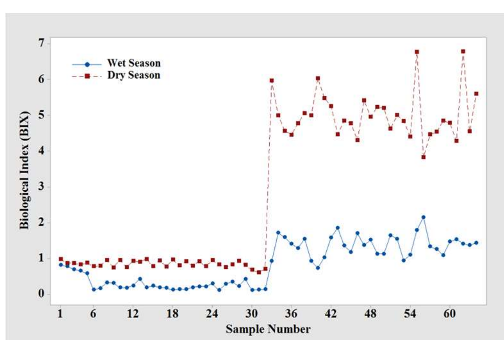


Fig.-3: Biological Index (BIX) Value at Station 1 to Station 4 Kali Surabaya During Dry and Wet Seasons

Secondly, FIX in the wet season is more supreme than in the dry season, although the trends do not perform exactly. It is probably due to the effect of the combination of organic materials from the soil and autochthonous activities.[8] According to Fig.-3, BIX indicates the opposite trends over FIX, as the BIX in the dry season is more supreme than in the wet season. BIX present the autochthonous or allochthonous, which is less than 0.8 contributing to fewer autochthonous components of a dissolved organic compound.

At the same time, BIX is higher than 0.8, describing the existence of autochthonous dissolved organic compounds and newly generated sources. BIX value at Station 1 and Station 2 is in the range of 0.8–1.1 (dry season); 0.2-0.8 (wet season), while Station 3 and Station 4 exhibits BIX values higher than 4 in the dry season and about 1.1–2.2 in the wet season. This BIX value indicates the difference in organic compound properties in the river that Station 3 and Station 4 are more autochthonous than Station 1 and Station 2. BIX value is much higher in the dry season, probably due to photodegradation in aquatic environments that can affect organic compounds' cycling and change their fate and bioavailability. According to earlier studies, an organic compound in aquatic environments can undergo photodegradation, which reduces larger molecules to smaller ones, before being removed through the volatilization of carbon gases by microbial consumption or converting labile compounds into refractory material with a higher molecular weight.[11,18] According to Fig.-4, HIX indicates that the humic compounds that have higher HIX exhibit increasing Humification, or HIX is higher than 10. HIX indicates similar trends over FIX, as the HIX in the wet season is higher than in the dry season. HIX presents the humic compounds, which is higher than 8 and contributes to more humic compounds of a dissolved organic compound, while HIX is lower than 8, which describes the existence of fewer humic compounds. HIX value in the river indicates less humic compounds in all stations during the wet and dry seasons, except station 1 and station 2 in the wet season. It is probably due to the dissolved organic compound exported from temporal ecosystems to surface waters. It is well known that dissolved organic compound is generated as it penetrates the soil's organic horizon, washes thru vegetation, precipitation moves thru the atmosphere, and permeates downward thru mineral soil horizons. Organic forms of nitrogen, carbon, phosphorus, and sulfur contribute to vast dissolved organic compounds, which depend on a watershed slope, dividers to organic soil solution permeation of the mineral soil, and preceding soil moisture. In addition, the balance between production and loss from solution, as well as the possibility of hydrological transport, determine how much dissolved organic compound from terrestrial founts is distributed to surface waters.[19-20] In order to compare with the *phytoplankton* biology index, this study applied the Uniformity Biology Index (UNX) of *phytoplankton*. UNX indicates the distribution of *phytoplankton* in a community. The uniformity index (E) value ranges from 0 to 1. The smaller the value of E indicates, the smaller the uniformity of the *phytoplankton* population, meaning that the distribution of the number of individuals in each genus is not the same, and there is a tendency for a genus dominates the population.

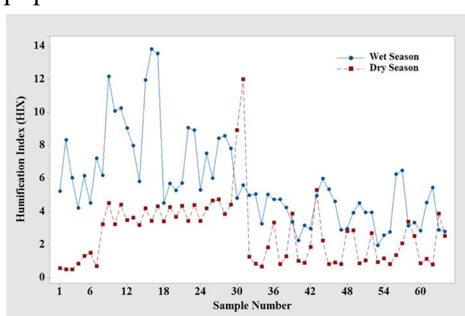


Fig.-4: Humification Index (HIX) Value at Station 1 to Station 4 Kali Surabaya During Dry and Wet Seasons

According to Fig.-5, In the dry season, the average uniformity index grade ranges from 0.6726 – 0.7316. Meanwhile, in the rainy season, the average uniformity index grade ranges from 0.5294 to 0.6066. The range of uniformity index grades for the two seasons shows a grade below one ($E < 1$).

There is a difference in UNX values in the rainy and dry seasons. UNX in the dry season shows a higher value when compared to the rainy season. This is because the river flow in the dry season looks calm, so the distribution of the *phytoplankton* population is more uniform compared to the rainy season when the river current looks strong. In addition, there is a dominance of several types of *phytoplankton* in each season. The UNX value also shows various values that fluctuate throughout the observation location. This indicates that the distribution of the *phytoplankton* population is low or that the individual diversity of each species is much different because of the value of $E < 1$. In addition, it shows a low structural uniformity between species.

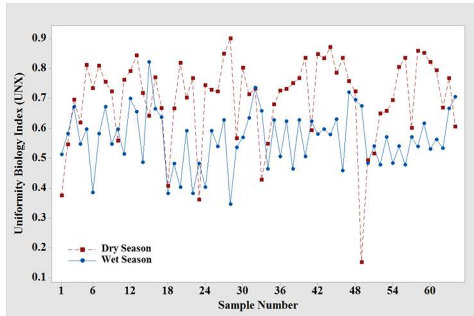


Fig.-5: Uniformity Biology Index (UNX) Value at Station 1 to Station 4 Kali Surabaya During Dry and Wet Seasons

**Correlation Between Fluorescence Dissolved Organic Compound and *Phytoplankton* Biology Index**

Pearson correlation is applied to determine the correlation between fluorescence dissolved organic compound indexes and *phytoplankton* biology index. Correlation analysis was applied using FIX, BIX, HIX, and UNX values, as shown in Table-1.

Table-1: Correlation Between FIX, BIX, HIX and UNX

| | BIX | FIX | HIX |
|---|---|---|---|
| FIX | 0.400 | | |
| HIX | -0.574 | -0.486 | |
| UNX | 0.263 | -0.105 | -0.181 |

First, the Pearson correlation analysis shows a moderate correlation between fluorescence-dissolved organic compounds, and Humification shows a negative correlation with biological and fluorescence index. It is consistent with Fig.-2, Fig.-3, and Fig.-4, since the biology and fluorescence index indicate autochthonous compounds, while the Humification index refers to allochthonous compounds. Second, as represented by the uniformity index, the *phytoplankton* biology index exhibits a low correlation with a fluorescence-dissolved organic compound. It is probably because fluorescence-dissolved organic compounds selected the particular fluorescence intensity to specify the properties of dissolved organic compounds, as described by previous studies.[10-12] The selected fluorescence intensity might not be able to represent the *phytoplankton*'s dissolved organic properties, especially related to the uniformity of *phytoplankton*.

## CONCLUSION

This study concludes that raw water contains two main organic fractions: fulvic acid-like and humic acid-like. According to the fluorescence intensity as defined by the fluorescence dissolved organic compounds index, including FIX, HIX, and BIX. This study found FIX in Station 1 and Station 2 is much lower than in Station 3 and Station 4, which is in the range of 0.3 – 1.3 (dry season); 0.5 – 2.5 (wet season), and 1.4 – 3.2 (dry season); 1 – 3.5 (wet season), respectively. An allochthonous organic compound is more dominant in the upstream, and the downstream contains more autochthonous organic compounds. BIX value indicates the opposite trends over FIX, that BIX value at Station 1 and Station 2 is about the range 0.8 – 1.1 (dry season); 0.2 – 0.8 (wet season), while Station 3 and Station 4 exhibits BIX value higher than 4 in the dry season and about 1.1 – 2.2 in the wet season. HIX value indicates similar trends over FIX, as the wet season has a higher HIX than the dry season. The Pearson correlation shows a moderate correlation among fluorescence dissolved organic compounds index. However, the uniformity index exhibits a low correlation with a fluorescence-dissolved organic compound. Further studies must continue to reveal a novelty method for implementing fluorescence and *phytoplankton* indexes in monitoring water quality.

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## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

## AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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