Characterization of water quality and fluorescence measurements of dissolved organic matter in Cabuyao river and its tributaries using excitation-emission matrix spectroscopy

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Abstract. Laguna lake is an important natural resource that provides agricultural and other uses such as transportation, recreation, and irrigation. Due to rapid changes in the land use near the west region of Laguna lake, surface water quality measurements are necessary. Using excitation-emission fluorescence spectroscopy, the optical characteristics of dissolved organic matter (DOM) in Cabuyao river were assessed. There are six sampling sites based on inflow and outflow of water from Cabuyao river and its tributaries. 3D excitation-emission matrices (EEMs) using Surfer v16 was utilized in the assessment of protein-like and humic-like fluorescence peaks. It was observed that the differences in the EEMs provided DOM composition, and its origin. A fluorescence index of approximately 1.18 showed that most of the samples are terrestrial in origin. In-situ water quality measurements such as dissolved oxygen, temperature, pH, and electrical conductivity. Hence, this study provides preliminary information on the possible use of fluorescence EEMs in providing DOM composition in natural waters.

Keywords: Cabuyao river, Laguna lake, Water quality, Fluorescence excitation-emission spectroscopy, Dissolved organic matter

1. Introduction
Laguna Lake is known as one of the largest lakes in Asia, with an approximate area of 900 km$^2$, an average depth of and length of the shoreline of Laguna lake are 2.8 m and 220 km, respectively. Water flows from Laguna lake to two bodies of water, Pasig river, considered a dead river, and Manila bay, a saltwater marine system [1, 2]. Since Laguna is in the subtropical monsoon zone, rainy season and the dry season are clearly divided. December until the following May is considered the local dry season. Generally, the river flow rate is relatively low and water quality becomes worse during these months.
accordingly [3]. It is classified as “Class C” by the Department of Environment and Natural Resources (DENR) with its intended beneficial use for fisheries, agriculture, irrigation, livestock watering, and recreational purposes [4]. With the regular monitoring of the Laguna Lake Development Authority (LLDA) and some recent studies on physico-chemical and biological parameters [1, 5, 6], most of the rivers and its tributaries are deteriorating. The main causes of water pollution of Laguna lake are surface resources such as discharge of domestic and industrial wastes [7]. The collected data from these standard parameters are accurate and precise, however, the results may take at least days to arrive. With the decreasing water quality of the lake specifically on its rivers and tributaries, another method of monitoring with faster result is necessary.

In rivers, lakes and man-made reservoirs, dissolved organic matter (DOM) are commonly found. It consists of complex mixtures of organic molecules such as carbohydrates, proteins, lignins, organic acids, and various humic substances [8]. These components are derived from different sources such as agriculture or sewage or pollutants discharged from petroleum products and industrial effluents [9, 10]. Fluorescence studies have been carried out in river waters with excitation-emission matrices (EEM) [11-13] and traditional methods [14]. The measurement of river water fluorescence is a promising measuring tool in determining DOM composition. Hence, this preliminary study was conducted to measure excitation-emission fluorescence spectra of DOM on surface waters collected along Cabuyao river and its tributaries which flows to Laguna lake.

2. Materials and Methods

2.1. Sampling site description

Cabuyao River is one of the major rivers that affects Laguna lake water quality. It has two tributaries that collects discharges from major industrial and domestic wastes. The sampling sites are divided into six (6) regions (Figure 1):

- (St 1) Discharged from Cabuyao river (14° 17' 46.1256", 121° 7' 41.0736”)
- (St 2) Discharged from tributary 1 (14° 17' 46.986", 121° 7' 40.2996”)
- (St 3) Confluence of Cabuyao river and tributary 1(14° 17' 52.7532", 121° 7' 41.808”)
- (St 4) Out flow going to Laguna lake (14° 17' 52.7532", 121° 7' 48.18”)
- (St 5) Discharged from tributary 2 (14° 18’ 37.544", 121° 7' 23.3724")
- (St 6) Out flow from tributary 2 and Laguna lake (14° 18' 38.124", 121° 7' 32.736")

Built-up is prevailing in these river systems with aquatic plants, such as water lilies and terrestrial trees located near the sampling sites. Small fish pens are also found in the opening of the rivers to Laguna lake.

![Figure 1](image-url). Map of the Sampling Stations selected from Cabuyao River and its tributaries [15]

2.2. Physico-chemical parameters

Using a chartered boat from the Talim Island Ferry Terminal, surface water collection of the different sampling sites was conducted on July 29, 2019 from 9:00 am to 1:00 pm. Following standard methods for transport and handling of water/wastewater samples, river water samples were collected and
transported in the laboratory for excitation-emission fluorescence measurements. In-situ measurements of pH, electrical conductivity (EC), temperature, and dissolved oxygen (DO) were carried out using Hach HQd/intelliCAL Rugged Field.

2.3. Fluorescence measurements

The water samples were immediately filtered using 0.47 µm pore size filter prepared in a 10-mm UV-Vis cuvette. Figure 2 shows the schematic diagram and the experimental set-up of the Ocean optics fluorescence excitation-emission spectroscopy located at the Environment And RemoTe sensing research (EARTH) laboratory, De La Salle University Manila. In our set-up, a xenon lamp source (180-2000 nm) were controlled using scanning monochromator (MonoScan 2000) before it hits the sample. The output source of the MonoScan 2000 were connected to an optical fibre splitter, one cable goes to Ocean optics USB 4000 (Spectrometer 1), and the other cable is connected to the cuvette holder. Ocean optics 2000+ XR1-ES (Spectrometer 2) measured the emission peaks of the sample using Ocean View application (Figure 2). The excitation wavelength ranges from 250-450 nm and the emission wavelength ranges from 250-600 nm. Fluorescence emission spectrum was obtained in every 5 nm-interval of the excitation wavelength. All fluorescence measurements were conducted at room temperatures (~25°C). Fifty (50) deionized water samples were average and used as blank samples. The corrected fluorescence data were examined.

Figure 2. The (a) schematic diagram and (b) experimental set-up of the excitation-emission spectroscopy

Fluorescence indices and aquatic fluorescence peaks such as Tryptophan-like (Peak T) and humic-like peaks, Humic-A (peak A), and Humic-C (peak C) were measured. Table 1 shows the major fluorescence in aquatic samples. The fluorescence DOM components peaks are designated based on Coble (1998) on the characterization of marine and terrestrial DOM using excitation-emission matrix spectroscopy. One of the basic and most widely used measurement is fluorescence index (FI), which provides information about the source of the samples or degree of degradation of DOM. Fluorescence DOM measurements are commonly calculated using 3D excitation-emission matrices which provides multiple spectra of increasing excitation ranges from UV to blue region.
Table 1. Summary of fluorescence indices and commonly observed natural fluorescence peaks of aquatic DOM [11-13]

| Index/Component | Parameters | Peak Name | Probable sources* | Description |
|-----------------|------------|-----------|-------------------|-------------|
| Fluorescence index | emission (em) 470 nm/ em at 520 nm at excitation (ex) 370 nm | - | - | Water samples as microbial (~1.8) or terrestrial (~1.2) origins. |
| Biological Index | em 380 nm/ em 420 nm and 435 nm at ex 310 nm | - | - | Indicates proportion of newly produced DOM |
| Tryptophan-like | Ex 270-275 nm and em 304-312 nm | T | T, A, M | Amino acids, free or bound in proteins |
| Ultraviolet A (UV A) humic-like | Ex 370-430 nm | A | T, A, M | Low molecular weight, associated with biological activity, found in wastewater, wetland, and agricultural environments |
| Ultraviolet C (UV C) humic-like | Ex 320-360 nm and em 420-460 nm | C | T | High molecular weight, highly present in wetlands and forested environments |

* T – Terrestrial plant or soil organic matter; A – Autochthonous production; M – Microbial processing

The EEMs contain information on the composition, origin, and processing of DOM. These indices use ratios of fluorescence intensity in different regions to provide possible sources and relative contribution of recently produced DOM.

3. Results and Discussion

3.1. Physico-chemical and fluorescence indices analysis

Surface water samples in Cabuyao River and its tributaries showed different physico-chemical properties. The pH values of water samples were all nearly neutral. These data represent initial physico-chemical measurements on the present status of the water quality in Cabuyao river-Laguna lake and its tributaries. The data presented also coincides with the quarterly data from LLDA on their water quality monitoring. There are changes in the EC values due to the continuous discharge of ionic matter during the flow of river water and its temperature dependence. High EC values were found in Tributary 2, however, lower values were observed in Cabuyao river.

Table 2. Water Quality and Fluorescence indices Measurements in the six sampling stations

| St | pH | Dissolved Oxygen (mg/L) | Electrical conductivity (µS/cm) | Fluorescence | Biological |
|----|----|-------------------------|-------------------------------|--------------|------------|
| 1  | 7.83 | 8.71 | 501 | 1.02 | 0.83 |
| 2  | 8.94 | 4.91 | 980 | 0.92 | 0.67 |
| 3  | 7.62 | 6.95 | 685 | 1.04 | 0.53 |
| 4  | 7.63 | 6.10 | 623 | 1.17 | 0.99 |
| 5  | 7.85 | 5.54 | 829 | 0.93 | 0.11 |
| 6  | 7.79 | 7.27 | 869 | 1.18 | 0.95 |

On the other hand, Dissolved Oxygen varies from the different influents and effluents conducted in the six sampling stations. Dissolved Oxygen (DO) values located at Tributary 1 is below 5 mg/L, which is low compared to other sampling stations and below the standard set by the DENR water quality standards. This may prevent organisms from growing, and lead to unhealthy and less biologically diverse communities [3]. Other DO values are higher based on the minimum value provided by the DENR Administrative Order No. 34, Series of 1990 on water quality criteria for
conventional and other pollutants contributing to the aesthetics and oxygen demand for fresh waters [4].

As presented in Table 2, fluorescence index interprets the possible source of the DOM. All surface water samples are observed to be terrestrial in origin with approximate value of 1.18 (Table 2). Hence, the DOM found in the surface water are affected by effluents coming from the domestic and industrial built-up near the sampling stations. However, it is observed that the two tributaries provided lower fluorescence index. These two tributaries come from a larger river and separated into two water systems. These confirms that the major source of possible DOM sources is Cabuyao river. No significant difference was found at the stations located in the opening of the tributaries flowing into the Laguna lake. The western region of the lake, where Cabuyao river and its tributaries are located, is a home for a variety of activities such as industrial/commercial, agricultural and predominantly of domestic origin (residential purposes).

The freshness of the freshly produced DOM with the older DOM can be explained using the Biological index (Table 2). It clearly shows that high production of freshly observed DOM are found in all sampling stations. Hence, detailed information on the biological activity and chemical parameters must be studied to support these results. Due to limitations on the measurement of salinity, the electrical conductivity was used since it has high correlation with salinity. BI and FI measurements were used to deduce regions of DOM composition. Figure 3 demonstrates similar trends provided in the vertical line region. These differences in the values of fresh water were attributed from the river and lake mixing with saline marine water.

![Figure 3. Correlation of the biological and fluorescence indices of the six sampling stations](image)

3.2. 3D excitation-emission matrices (EEMs)

All sampling stations were graphed using Surfer v16 application. Figure 4 shows the 3D EEMs of all sampling stations. The same fluorescence intensity scale was used in all water samples with arbitrary units (a.u.). The EEMs created for each sampling stations are unique and infers information on the DOM source, composition, and its processes. (St 3) shows the confluence of (St 1) and (St 2), higher excitation spectrum showed higher intensity based on the emission spectrum. The mixture of these two bodies of water affected (St 4). Hence, higher values of fluorescence DOM were observed in (St 4). On the other hand, higher fluorescence DOM were observed in both (St 4) and (St 6) due to the mixture of marine and brackish water from the Laguna lake.
Figure 4. 3D excitation-emission matrices of all six sampling stations.

Figure 5 shows the commonly observed aquatic fluorescence peaks of the six sampling stations. Protein-like fluorescence such as tryptophan are likely derived from a mixture of dissolved amino acids and other organic materials with similar fluorescence characteristics. However, with its great potential use in ecological applications, there are still limitations on the molecular size and structure of high DOM concentrations which contributes in the detection of fluorescence protein-like signatures.

Figure 5. Fluorescence intensity measurements of the different aquatic peaks at the different sampling stations

Also, selected fluorescence indices appear to be insufficient to represent the suspended solids contribution and requires more chemical analyses. Continuous water quality monitoring is necessary to identify trends which can be used to provide information in creating techniques for in-situ measurements.

4. Conclusion
3D excitation-emission fluorescence spectra can provide optical DOM characteristics of the water samples along Cabuyao river and its tributaries. Based on fluorescence indices and water quality
parameters, the difference obtained from these data can be attributed to the effluents from the land use built-up near the sampling stations. However, it is suggested that more water sampling collection must be done from wet to dry season to compare DOM composition and sources. Additional information on the effects of river water types and sources must be done with the use of water quality parameters and fluorescence measurements.

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