

SPATIAL AND TEMPORAL VARIABILITY OF ORGANIC MATTER FROM AN URBAN LAKE

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Abstract

Fluorescence spectroscopy was shown to characterize aquatic organic matter and to determine its impact on water quality from various sources. Past studies proposed this technique as an excellent tool for water quality continuous monitoring due to its advantages: rapid measurements, high sensitivity and selectivity, requires small quantities of sample with little or no sample preparation. Fluorescence spectroscopy was used to monitor different types of water systems; however, no complex study was undertaken to determine the spatial and temporal variability of urban lakes. These lakes are highly susceptible to contamination with surface runoff, illegal wastewaters discharge and effluent discharge, which lead to increased eutrophication and to a decrease of the ecological health status. This study aimed to develop a preliminary database with fluorescence spectra of samples collected from Morii Lake (44°27'20"N, 26°01'31"E, Bucharest, Romania) over a period of 6 months, in 2017. Results showed a reduction, from spring to summer, in the fluorescence intensity of the organic matter microbial fraction. The values increased towards autumn. The humic fraction of the organic matter presented an opposite trend, depending on the quantity of precipitation, which lead to increased surface runoff release in the summer. Fluorescence indices, such as the humification index, the biological index, the F450/500 ratio and the T/C ratio, calculated based on the fluorescence intensity at specific wavelength ranges, were used to provide a complex characterization of the organic matter from Morii Lake samples. These parameters, together with standard water quality parameters, were further used to develop a prediction model for Morii Lake water quality. Although a good correlation coefficient was obtained between the estimated and measured values, a larger database is required in order to obtain a solid prediction tool.

Keywords: Fluorescence spectroscopy, spatial and temporal variability, urban lake, organic matter

1. INTRODUCTION

In recent decades, advanced techniques have been developed to characterize water quality and the component present in all aquatic systems: organic matter (Baker et al., 2015, Coble, 1996). A new technique, fluorescence spectroscopy, which has been successfully applied in biology, medicine or chemistry, has become a promising approach to assessing aquatic organic compounds and organic pollutants. Fluorescence spectroscopy can be used to determine the concentration and composition of organic matter.

Fluorescence spectroscopy is a rapid, high sensitivity method that does not require the use of toxic substance or any complicated sample handling procedures before analysis. Concentration and chemical composition of dissolved organic matter determine the intensity and form of fluorescence spectra. Thus, fluorescence spectroscopy can provide spectral fingerprints of different types of aquatic systems (rivers, lakes, seas) influenced by the geophysical and morphological characteristics of the area.

Fluorescence occurs in certain types of molecular species when the absorption of light from an external source results in light emission. These processes are depicted in the Jablonski diagram, shown in Figure 1. When a molecule in its ground singlet state, S_0 , absorbs light, the light energy is transferred to the electronically excited states: singlet states, S_1 or S_2 . Afterwards, the molecule is subjected to internal conversion or vibrational relaxation, which implies the transition from an upper electronically excited state to a lower one. In the final stage, emission occurs when the molecule returns to the ground state, S_0 , emitting light at a greater wavelength, according to the difference in energy between the two electronic states. This process is known as fluorescence (Carstea, 2010).

A molecule that can emit fluorescence is maintained in the excited state for a finite period, and during this interval the molecule may undergo multiple interactions with the molecular environment, conformational changes and energy losses. Fluorescent molecules are called fluorophores, and their lifetime is given by the time between the excited state and the return to the ground state. The fluorescence intensity is proportional to the number of fluorophores in the solution.

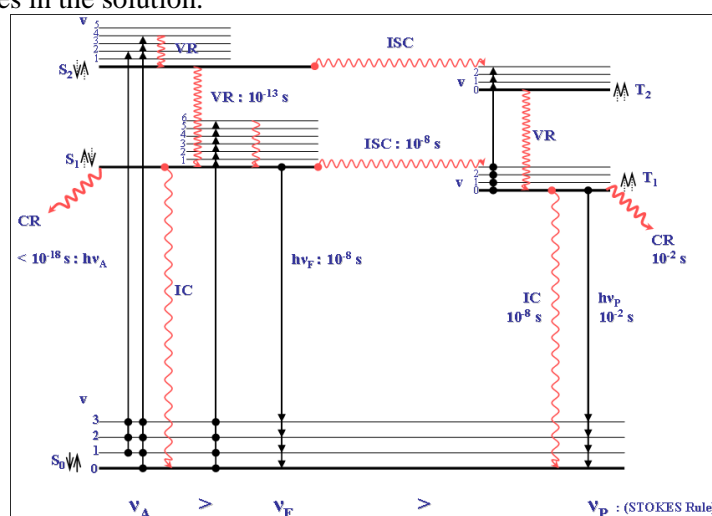


Figure 1 Jablonski diagram presenting the processes of absorption (A), fluorescence (F) and phosphorescence (P); ISC – intersystem crossing, VR – vibrational relaxation, CR – chemical reaction, IC – internal conversion. (Carstea, 2010)

From the heterogenic mixture of organic matter, only a few compounds are generally detected by fluorescence: proteins, pigments, phenols derived from lignin, humic substances and hydrocarbons. Protein fluorescence is given by aromatic amino acids: phenylalanine, tyrosine and tryptophan, indicating the activity of bacterial communities in water (Baker et al., 2015). The fluorescence of humic substances indicates the presence of substances resulting from the remineralization of organic matter from soils, water and sediments (Coble et al., 2014). The presence of hydrocarbons in fluorescence spectra indicates a petroleum contamination of the sample (Cristescu et al., 2009; Patra D. and Mishra A.K., 2002). Phenols derived from lignin come from anthropogenic sources (industrial and domestic) or natural sources.

Previous studies have determined the presence of 5 major fluorescence peaks in the water sample spectra: peak T ($\lambda_{excitație}/\lambda_{emisie}$ ~225 (~280)/~350 nm), peak B ($\lambda_{excitație}/\lambda_{emisie}$ ~225 (~280)/~305 nm), peak A ($\lambda_{excitație}/\lambda_{emisie}$ ~225/400-500 nm), peak M ($\lambda_{excitație}/\lambda_{emisie}$ ~310-320/380-420 nm), and peak C ($\lambda_{excitație}/\lambda_{emisie}$ ~300-350/400-500 nm) (Coble et al., 2014). Peaks T and B are associated with living or dead cell matter and their exudates and indicate microbial activity in aquatic systems as well as matter derived from anthropogenic activities (Bridgeman et al., 2013; Yu et al., 2014). Amino acid standards (tryptophan and tyrosine) emit fluorescence in the optical range of peaks T and C, therefore they are generically named as protein-like (more accurately attributed to tryptophan or tyrosine). Peaks A, C and M occur in water samples with organic matter from sources dominated by precursor terrestrial matter, but have been attributed to humic substances because humic standards have these three fluorescence peaks (Hudson et al., 2007). A generalized analysis of these groups of compounds, proteic and humic, is not possible because the chemical nature of organic matter varies

with the environment. Furthermore, hydrocarbons and lignin-derived phenols can emit fluorescence in both the B and T peak areas, attributed to the proteins, as well as in the A, C and M peak areas attributed to humic substances.

2. METHODOLOGY

The fluorescence spectra are obtained by analyzing the intensity emitted by the wavelength - in which case the spectra are called emission spectra. The excitation-emission matrices are contour maps made from a series of emission spectra recorded in an excitation range. EEM technique is frequently applied due to the ease with which fluorescence maxima can be interpreted as well as the various mathematical data processing capabilities.

The spectra were recorded with the Edinburgh Instruments FLS920 spectrofluorimeter using the following parameters for the excitation-emission matrices: excitation range 250 - 370 nm, 30 nm step, emission range 270 - 500 nm, 1 nm step, integration time 0.2 s. Three fluorescence matrices were recorded for each sample, the fluorescence maxima being the mean of the three measurements.

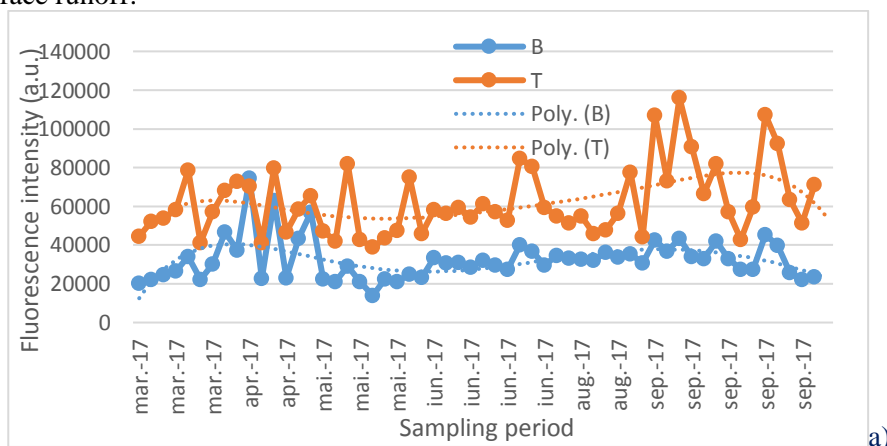
The statistical analysis was performed using Minitab 18. For factor analysis, the Varimax rotation method was applied to the 3-factor solution by the scree-plot criterion, and the prediction model was obtained by regression. The statistical analysis included fluorescence, chemical oxygen demand (COD), pH, dissolved oxygen (DO), nitrite, ammonium, phosphorus, phosphate. The samples with missing values for any of the parameters were removed from the analysis. Disparate values were also eliminated. Thus, a total of 56 measurements were included in factorial and prediction analysis. The data for the factor analysis included samples of lakes from Bucharest, Târgu Jiu and Orșova.

Morii Lake (Bucharest), Ciurel Lake (Targu Jiu) and Portile de Fier I Lake (Orsova) were selected for their large area and location within an urban environment. Morii Lake, with an area of 246 Ha, is an anthropogenic lake on Dambovita River and is located in a sector with mixed functions (collective buildings, abandoned industrial spaces, private houses, abandoned land). Ciurel Lake, with an area of 56 ha, is an arranged lake on Jiu River and has residential and industrial building on the right bank and a green area on the left side. Portile de Fier I lake is the largest lake in Romania, with 25,300 ha and is located on the Danube River. The lake is surrounded by buildings and forests. Samples were collected in sterile bottles and stored at 4⁰ C.

3. RESULTS AND DISCUSSION

3.1. Case study - Lake Morii

Samples were taken from Morii Lake for a period of 6 months starting March 2017 to September 2017. A fluorescence intensity decrease for B and T peaks (Figure 2a) was observed at summer samples compared to samples collected in the spring, a decrease that could have been caused by the higher rainfall recorded during this period in the area (https://www.meteoblue.com/en/weather/forecast/archive/bucuresti_romania_6691781). Samples collected between June and September show a slight increase in the intensity of T and B peaks, the highest values being observed in September, when the lowest quantities of rainfall have been recorded. A different trend has been observed at the peaks attributed to humic substances. Peaks A, C and M show a slight increase until June, followed by a decrease in intensity and a marked increase in September. In this case, the intensity variations of the peaks attributed to humic substances may be caused by the discharge of surface runoff.



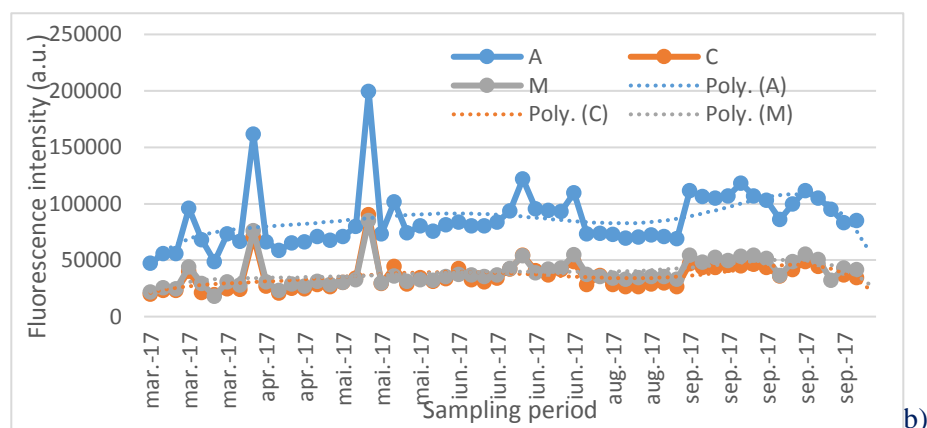


Figure 2 The temporal and spatial evolution of the organic matter fluorescence for the samples taken from Morii Lake; a) T and B peaks attributed to proteins; b) A, C and M peaks attributed to humic substances

The emission wavelength of peak C indicates the hydrophobic or hydrophilic character of organic matter in aquatic systems. Wavelengths ranging from 400 to 420 nm are characteristic to a hydrophobic organic matter, and those in the 430-450 nm range indicate tendency towards a hydrophilic organic matter. It can be seen from our data (Figure 3) that most water samples contain a mixture of hydrophobic and hydrophilic matter. However, some samples show a tendency towards a hydrophobic organic matter, with few samples being more hydrophilic.

Previous studies have shown that hydrophobic matter is mainly composed of humic acids, fulvic acids and humins. Also, the hydrophobic matter is rich in aromatic carbon, phenolic structures and twin-bonded compounds. While hydrophilic matter contains predominantly aliphatic carbon and nitrogen-based compounds, such as carboxylic acids, carbohydrates and proteins (Michael-Kordatou et al., 2015). No temporal or spatial tendency of peak C emission wavelength was observed.

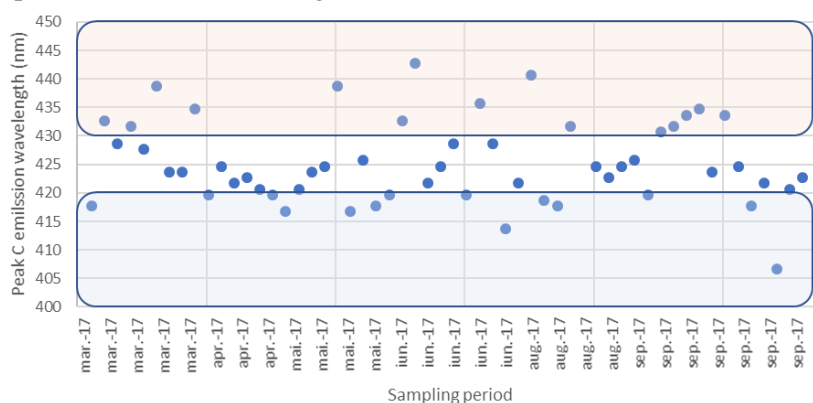


Figure 3 The temporal and spatial evolution of peak C emission wavelength.

The characteristics of organic matter can be studied rigorously by calculating the relative proportions of microbial and humic fractions in aquatic systems using fluorescence indices: humification index (HIX), biological index (BIX) and index F_{450} / F_{500} (Huguet et al., 2009).

The humification index can be calculated using the following formula:

$$HIX = \frac{\sum(F_{435} - F_{480})}{\sum(F_{300} - F_{345})}$$

where $F_{300} - F_{345}$ and $F_{435} - F_{480}$ represent the fluorescence intensity in the indicated wavelength range at 254 nm. The second fluorescence index, BIX, is calculated as the ratio between the fluorescence intensity at 380 nm and 430 nm with excitation at 310 nm. The F_{450} / F_{500} index is the ratio of the fluorescence intensity between 450 nm and 500 nm emission wavelengths at an excitation wavelength of 370 nm. Huguet et al. (2009) proposed a classification of the samples using these three indices to determine the bacterial or humic character of organic matter.

HIX indicates the degree of humification of organic matter and the presence of complex molecules such as high molecular weight aromatic substances. Our results show that the samples, with the exception of 2

samples recorded in May 2017, contain mainly organic matter of bacterial origin (Figure 4a). The 2 samples with HIX values above 4 have an important autochthonous bacterial component, but also a slight humic character. These results could indicate a greater impact of surface runoff in sampling areas, for the two samples compared to other areas, generated by the increased rainfall frequency of this period.

BIX is characteristic to an autochthonous biological activity in water samples. According to the classification provided by Huguet et al. (2009), most samples fall within the range of 0.8-1, values characteristic of a strong autochthonous component (Figure 4b). A single sample shows the BIX value below 0.8, indicating an intermediate autochthonous component. Samples with BIX values above 1 indicate the presence of organic matter of bacterial or biological origin.

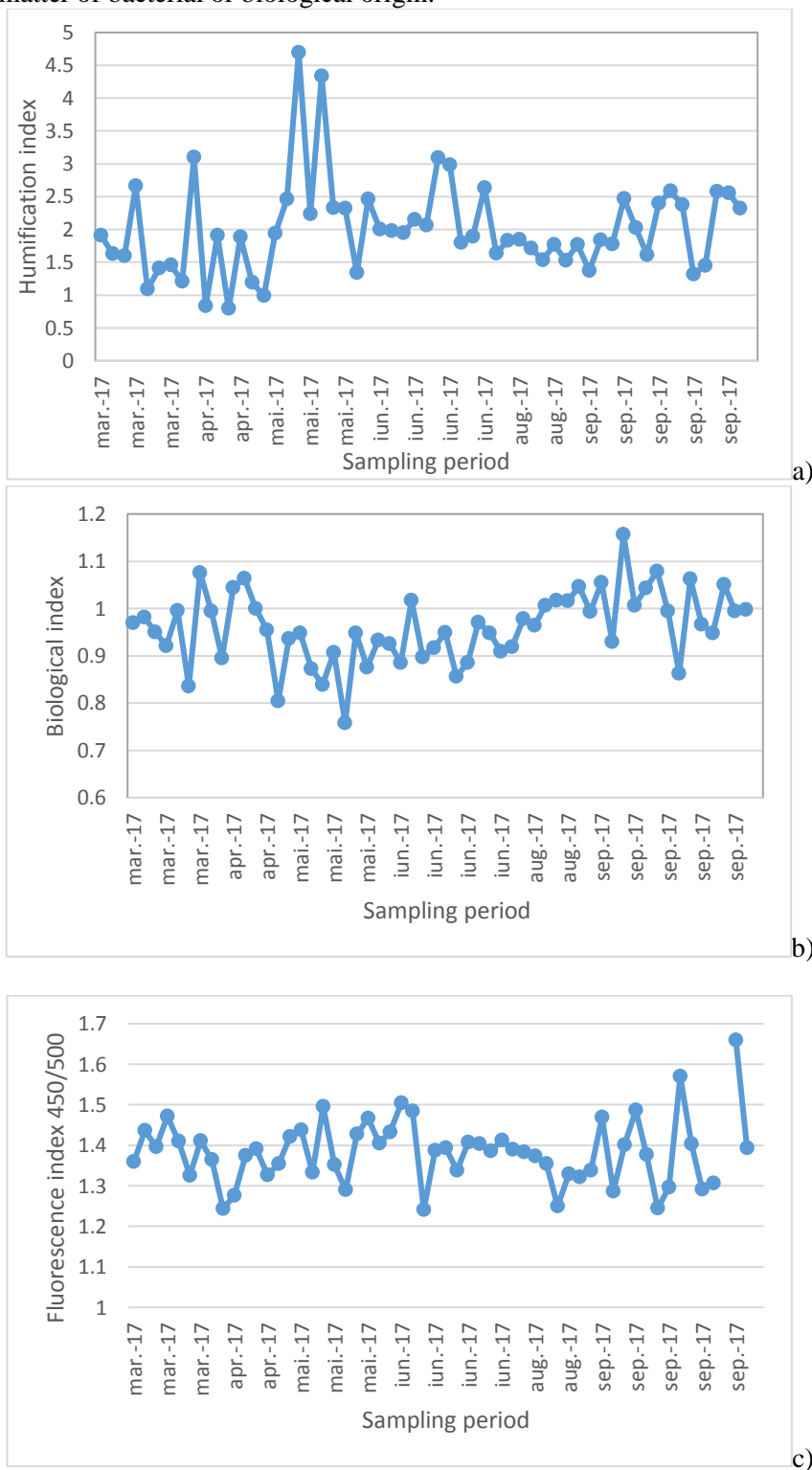


Figure 4 Fluorescence indices for the samples collected from Morii Lake: a) Humification index - HIX; b) biological index - BIX; c) fluorescence index F450 / 500

The fluorescence index F450 / 500 offers, in some cases, a differentiation between sources of organic matter, microbial or terrestrial. Index values close to 1.9 indicate a microbial origin of organic matter, and values close to 1.3 indicate the presence of organic matter from soil or terrestrial sources (McKnight et al., 2001). The highest values of the index were observed in September, indicating a slight tendency towards organic matter of microbial origin (Figure 4c). However, no sample shows values of the index close to the maximum threshold, suggesting that the organic matter in most samples is composed of a mixture of compounds of terrestrial or microbial origin. The other samples present organic matter from soil or terrestrial sources, indicating a potential impact from surface runoff. As with HIX and BIX indices, the F450 / 500 did not show a seasonal trend.

Finally, to determine the proportion of microbial matter to humic matter we used the ratio between the fluorescence intensity of the T and C maxima. The highest values were recorded in the first period of the experiment (March and April), but most of the samples presented values between 1 and 2. These results show that organic matter is predominantly microbial. In the samples collected in May, a decrease in T / C ratio values was observed, indicating a higher proportion of humic matter compared to the other months. Increased humic content may be caused by the high rainfall in May (17 events, compared with 10 rainfalls in April and 11 events in June).

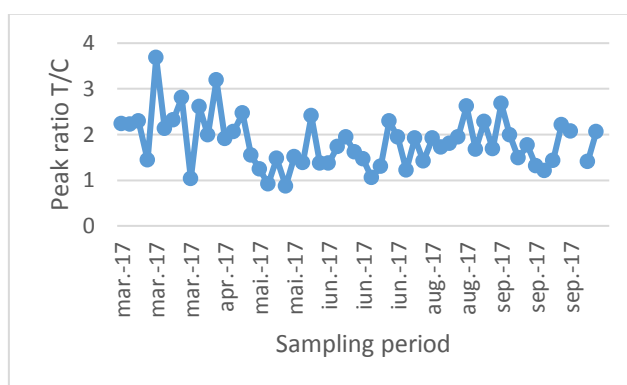


Figure 5 The ratio of the fluorescence intensities of the T and C maxima

3.2. Correlation of water quality parameters and realization of the prediction model

In order to determine the degree of correlation between water quality parameters, factor analysis was applied. The results showed that a larger database is needed to achieve conclusive results. Although the parameters analyzed fell into three factors, they are responsible for only 61% of the data set variability (Table 1). Factor 1 includes temperature and peaks B and T. This factor contributes with 24% to total variability. Factor 2 is given by CCO, phosphorus, nitrite and by pH with a negative correlation coefficient, and represents 19% of total variability. The last factor, Factor 3, contains ammonium, phosphate and, with negative correlation, the dissolved oxygen. Factor 3 contributes with approximately 18% to total variability.

Table 1. Factorial analysis by Varimax rotation.

Variable	Factor 1	Factor 2	Factor 3	Communality
Temperature	0.608	-0.437	0.372	0.699
Peak B	0.884	0.105	0.034	0.794
Peak T	0.888	0.085	0.156	0.820
pH	0.287	-0.710	-0.293	0.672
DO	-0.132	-0.104	-0.762	0.609
Nitrites	-0.472	0.518	-0.208	0.535
Ammonium	-0.045	0.004	0.762	0.583
Phosphorus	0.360	0.647	-0.101	0.558
Phosphate	0.193	-0.023	0.536	0.325
COD	0.092	0.713	0.068	0.522
% Variability	24.4	19.2	17.6	61.2

In the second stage of the statistical analysis, the regression equation was obtained, using as an example the COD versus the other parameters included in the factor analysis. Due to the low data set variability and the insufficient number of values, the regression model made for these parameters is a weak predictor. For a precise model, the standard regression error (S) must be ≤ 2.5 to obtain a 95% predictive interval sufficiently narrow. In our study, S has the value of 6.67 and the coefficient of determination is 43%, which shows that the precision of the model should be increased. However, the regression model has a $p < 0.05$ value, which shows that the value the determination coefficient is significantly different from zero.

Using the data from 56 samples, the following regression equation was obtained:

$$\text{CCO} = 24.3 - 0.949 * \text{Temperature} - 0.000053 * \text{B} + 0.000222 * \text{T} + 0.50 * \text{pH} - 0.886 * \text{OD} + 3.6 * \text{Nitrite} + 10.50 * \text{Ammonium} + 0.464 * \text{Phosphorus} - 3.59 * \text{Phosphate}$$

Between the measured and the estimated values using the regression equation, a correlation coefficient $r = 0.66$ was obtained (Figure 6). Using a larger database to determine the regression equation would allow for a better estimation of water quality parameters. Also, greater sample variability could help to achieve a better correlation between the measured and estimated values.

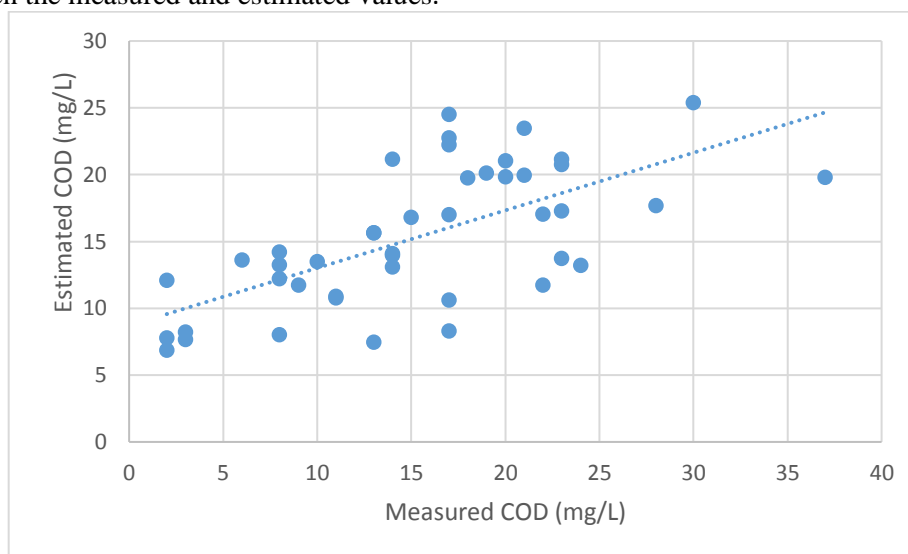


Figure 6 Measured and estimated COD values

4. CONCLUSIONS

The water quality in Morii Lake has been monitored using standard methods such as pH, dissolved oxygen, COD, etc. and the fluorescence spectroscopy method to determine the temporal variability of organic matter. The data provided by these parameters were used to obtain the regression equation in order to estimate the water quality values. A correlation coefficient of $r = 0.66$ was obtained between estimated and COD measured values. However, a larger and varied database would help determine a stronger predictive model.

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