Defining the Characteristics of Optical Sensors Dissolved Organic Matter Monitoring

A Major Qualifying Project

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Abstract

This study investigates the characteristics that can be observed at certain excitation wavelengths through the use of fluorescence spectroscopy. Characterization of samples was performed via TOC, UV-visible spectroscopy, and fluorescence spectroscopy. This research confirms that it is possible to utilize fluorescence spectroscopy with an excitation wavelength of 270 nm to determine the levels of protein-like fluorescence present in a water sample. This confirmation makes it possible to monitor water quality via a probe with a 270 nm LED light for quicker, easier analysis of water quality in the field over periods of time. Further investigation is recommended to refine the equation used to transform the fluorescence data to intensity of protein-like fluorescence.

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Introduction

The impact that human beings have on the planet is vast, and in an effort to ensure that the Earth and its resources are preserved in a sustainable manner, the Terra Forma project was formed. The project is a multidisciplinary collaboration spanning 42 laboratories and 19 public research organizations. Their goal is to develop and implement a system of in-situ sensors that can collect data from various environments, particularly aquatic ones. By monitoring any changes in the environment due to natural or human causes, it is possible to support socio-economic experiments to work towards creating a sustainable and equitable future. (*Le Project*, 2024)

This research looks primarily at the characteristics of these aquatic sensors. The sensor utilizes a LED emitting at 270 nm that collects absorbance and fluorescence data, as well as a LED emitting at 370 nm that collects only fluorescence data. Aquatic dissolved organic matter studies have not typically utilized a wavelength of 270 nm. Analysis should be conducted using both the prototypes for the in-situ probes and classical spectrophotometers and fluorometers in laboratory settings. The data collected and discussed in this research focuses on adding to the sample database and interpreting the correlations within the data.

Microbial activity can be monitored by looking at the protein-like fluorescence in a water sample. Since microbial activity is closely related to water quality, if fluorescence spectroscopy with an excitation wavelength of 270 nm can be used to determine the level of protein-like fluorescence, a portable probe with these capabilities would allow for quick water quality monitoring in the field. This would be an invaluable tool that would save much time and money, but in order to ensure this is a feasible option, further characterization of samples must be done to confirm the intensity being measured is protein-like fluorescence.

Background

Evolution of Ecosystem Monitoring

The Terra Forma project is aimed at monitoring the changes in the environment so that socio-economic experiments can be proposed to ensure a sustainable and equitable future. One way of monitoring environmental changes is by looking at dissolved organic matter (DOM), tryptophan, and other compounds in water. Various methods have been used to characterize organic matter in both soil and water, such as UV-visible spectroscopy and fluorescence spectroscopy. The UV-visible studies focus on absorbance values at 250, 254, 300, 365, and 400 nm, as well as the slope of the spectra (Zhang et al., 2023). The studies that utilize fluorescence looked at the characteristics that can be observed utilizing samples excited at wavelengths of 254 nm (Zsolnay, 2003), 310 nm (Huguet et al., 2009), and 370 nm (McKnight et al., 2001). These studies suggest using a mixture of both UV-visible and fluorescence spectroscopy is a promising way of quickly checking water composition outside of a lab environment via a portable probe. A probe with a LED emitting at 270 nm for fluorescence and UV-visible spectra and at 370 for fluorescence spectra has been proposed, but before being implemented, more testing should be done with water samples at the 270 nm wavelength to determine how well this wavelength can characterize the samples.

Water Composition and Characterization

In order to determine what contaminants were and were not present in the water samples collected, each water sample needed to be tested and characterized with a variety of methods. These methods looked at the amount of dissolved organic matter (DOM), the acid types, the molecular weight of the DOM, level of humification, and origin of DOM. This data was collected using UV-visible spectroscopy, fluorescence spectroscopy at a plethora of excitation wavelengths, and total organic carbon analysis. With this information, the presence of protein-like fluorescence (PLF) can be confirmed.

Dissolved Organic Matter

Dissolved organic matter can be found in various places throughout the environment, including in aquatic environments where it plays a vital role (Huguet et al., 2009). In order to be considered ‘dissolved’ the matter must be small enough to become incorporated into the water. The most widely accepted designation for DOM is anything smaller than 0.45 μm (Zsolnay, 2003). Anything larger is considered particulate organic matter. There are primarily two different sources that DOM can originate from, the first being microbially through the release of algae or bacteria. The second type of origin is from terrestrial sources such as decomposition of plants,

soil, and other organic matter (McKnight et al., 2001). Regardless of the origin, the majority of DOM is dissolved humic substances (Birdwell & Engel, 2010).

Humus is a type of organic matter that is naturally formed from long-term decomposition of biomass such as plant or animal substances. These humic substances can range in color from black to yellow or white. When in aqueous environments these substances may also cause an odor or taste (*Humus | soil component*, 2019). Humus can be characterized by either being primarily composed of humic acids or of fulvic acids, but fulvic acids are typically more common (Birdwell & Engel, 2010). The fulvic acids are the components that did not decompose in the humification process (Ohno, 2002)

Dissolved organic matter can be analyzed by looking at the dissolved organic carbon (DOC) concentration. This can be done through a total organic carbon analysis, and the LRGP utilizes a Shimadzu TOC-VCSH for this purpose.

UV-Visible Spectroscopy

Ultraviolet-visible spectroscopy (UV-visible spectroscopy) is a type of spectroscopy used to determine how much light a sample absorbs and at what wavelength the absorption occurs. The machine uses a combination of two lights, one that emits electromagnetic radiation in the ultraviolet region and one that is used for the visible portion of the spectrum. These lights excite the molecules from a ground state to an excited state. In order to obtain a range inclusive of the ultraviolet range, a quartz cuvette must be used to hold the samples (Pavia et al., 2001).

At the LRGP, a UV-2600 UV-Vis Spectrophotometer from Shimadzu is used with a quartz cuvette. This single monochromator machine can measure a large wavelength range, but this study focuses on the 200 to 600 nm range. The machine was outfitted with an attachment that can hold cuvettes anywhere from 1 cm by 1 cm to 1 cm by 10 cm. In this study, 1 cm by 1 cm and 1 cm by 2 cm cuvettes were used. To begin testing, ultra-pure water was placed into a cuvette as a ‘blank’ to calibrate the machine for the samples to come.

UV-Visible Spectra Analysis

The UV-visible spectroscopy yields absorbance spectra that are useful for determining the relative molecular weight, aromaticity, and acid composition of DOM. The specific UV absorbance at 254 nm ($SUVA_{254}$) is what is related to aromaticity. By using the absorbance at 254 nm, the DOC concentration, and the optical path length (d) in Equation 1 below, the $SUVA_{254}$ is determined.

$$SUVA_{254} = A_{254}/d/DOC \quad (1)$$

If the $SUVA_{254}$ is greater than 5, the water sample is considered to have a humic character, and if the $SUVA_{254}$ is between 2 and 2.5, the water sample has a non-humic character (Assaad et al., 2015). This is related to aromaticity since humic acids contain aromatic compounds (Capasso et al., 2007). Therefore, greater aromaticity is indicative of humic character. The samples with a non-humic character may still contain some humic substances, but in low enough quantities that it does not represent the overall character of the sample.

To determine if the humic substances in the sample are primarily composed of humic acids or fulvic acids, a ratio of the absorbance at 300 nm to the absorbance at 400 nm can be used. This ratio is referred to as E4/E5. If the ratio yields a value less than 3.5, the water sample is primarily composed of humic acid, and if the value is greater than 3.5, the sample is primarily fulvic acid.

There are two values that can be calculated from the UV-visible spectra data to determine the relative molecular weight of DOM, the spectra slope and a ratio referred to as E2/E3. Both the slope and the ratio will decrease when the molecular weight increases. This means that while it cannot tell the exact molecular weight, it can tell if a sample has a higher or lower molecular weight than the other samples. The E2/E3 ratio is a ratio of the absorbance as 250 nm to the absorbance at 265 nm. The spectral slope equation is shown below.

$$\alpha(\lambda) = \alpha(\lambda)^{[S_{\lambda_0-\lambda}(\lambda_0-\lambda)]} \quad (2)$$

For this to accurately show the relative molecular weight, the slope between 275 and 295 nm ($S_{275\text{-}295}$) should be used. Where $\alpha(\lambda)$ is the Napierian absorption coefficient ($\alpha(\lambda) = 2.303 \cdot A(\lambda)/d$) (Pons et al., 2023).

Fluorescence Spectroscopy

Fluorescence Spectroscopy is an extremely useful analytical technique when looking at water samples, as it can be used to identify a sample's components. This type of spectroscopy works by making the fluorophores within a sample briefly absorb light, exciting it to a higher energy level. After a certain period of time, the fluorophores emit the light as they return to their ground state (Ghisaidoobe & Chung, 2014).

While fluorescence spectroscopy is an invaluable tool, there are limitations such as the inner filter effect (IFE). There are two parts to the inner filter effect, the primary IFE and the secondary IFE. The primary inner filter effect occurs when a sample has a high concentration, causing only the side of the cuvette directly facing the excitation light to fluoresce strongly. In turn, the center of the cuvette experiences a decrease in emission, causing the fluorimeter to measure the emission spectra lower than it actually is. The secondary inner filter effect occurs when the excitation and emission spectra are close to significantly overlap, causing the light

emitted in the center to be absorbed itself. Both types of the IFE can be corrected, either mathematically or by dilution. Since dilution can lead to contamination issues, mathematically applying UV-visible absorbance data to the fluorescence data using Equation 3.

$$F_c = F_o \times 10^{\left(\frac{A_{exc}+A_{em}}{2}\right)} \quad (3)$$

In this equation, F_c is the corrected fluorescence intensity, F_o is the fluorescence intensity observed by the fluorimeter, A_{exc} is the absorbance at the excitation wavelength, and A_{em} is the absorbance value at the emission wavelength (Larsson et al., 2007). With this correction applies, the fluorescence spectra can be more accurately used.

The LRGP utilizes a F-2500 Fluorescence Spectrophotometer from Hitachi Digilab and the samples were placed in a 1 cm x 1 cm quartz cuvette. Each water sample was scanned at six different settings, and each ultra-pure water 'blank' was scanned at five different settings. The six scans used on the samples were a synchronous scan and excitation wavelengths of 254, 270, 275, 310, and 370 nm. The synchronous scan kept the difference between the emission and excitation wavelengths constant at $\Delta\lambda = 50$ nm. For this reason, this fluorescence scan is referred to as SF50. The SF50 spectra was analyzed by decomposition of the spectra into Gauss functions as later described to identify fluorophores. One of the 'blank' scans was called the Raman scan and was used to check the machine's stability prior to each set of samples being tested. The other four settings were SF50 and excitation wavelengths of 270, 275, and 370 nm.

Fluorescence Spectra Analysis

The fluorescence spectra obtained with an excitation wavelength of 254 nm was used to calculate the humification index (HIX). This index is a ratio of the sum of emission intensities between 435 and 480 nm to the sum of intensities between 300 and 345 nm. This can be seen in Equation 4.

$$HIX = \frac{H}{L} = \frac{\Sigma I_{\lambda em(435\ nm-480\ nm)}}{\Sigma I_{\lambda em(300\ nm-345\ nm)}} \quad (4)$$

This ratio can be used since humification and aromaticity are closely associated. Increasing the carbon to hydrogen ratio indicates an increase in aromatic compounds and therefore humic substances (Zsolnay, 2003). Since this ratio is a ratio of sums of intensities, the inner filter effect does not need to be taken into account when calculating HIX, since it affects the whole spectrum the same way (Ohno, 2002).

Another index that can be determined from the fluorescence data is the index of recent autochthonous contributions (BIX). The BIX is a ratio of intensities from a spectrum excited with a wavelength of 310 nm and is used to determine if a β fluorophore, associated with

biological activity, is present in the sample. It is based on a ratio of intensities at 380 and 430 nm as seen in Equation 5.

$$BIX = \frac{I_{\lambda em=380\ nm}}{I_{\lambda em=430\ nm}} \quad (5)$$

The β fluorophore has a maximum intensity at an emission wavelength of 380 nm, and the maximum intensity of the α band is found at 430 nm. Therefore, an increase in BIX indicates an increase in the β fluorophore. Specifically, higher BIX values are primarily of autochthonous origin, meaning the humic substances were created *in situ.* The lower BIX values correspond to an allochthonous origin, meaning the humic substances were created somewhere else and traveled to the area where the sample was collected (Huguet et al., 2009). This can be confirmed from the Gauss parameters calculated from the SF50 spectra.

The fluorescence spectra from an excitation wavelength of 370 nm can be used to calculate the fluorescence index (FI). The 370 nm ultra pure water spectra was subtracted from the 370 nm sample spectra in order to account for the Raman scattering and mitigate the effect of IFE. This uses the intestines at 450 and 500 nm, as seen in Equation 6. The FI is used to identify if the humic substances, specifically the fulvic acids, within a sample were created through microbial or terrestrial means.

$$FI = \frac{I_{\lambda em=450\ nm}}{I_{\lambda em=500\ nm}} \quad (6)$$

If the acids are microbially derived, they will have an index value of ~1.9 and are autochthonous DOM. If the acids are terrestrially derived, they may be autochthonous or allochthonous and will have a FI of ~1.4 (Mc Knight et al., 2001).

Gauss Identification

The fluorescence spectra can most easily be characterized and interpreted by the decomposition of the fluorescence spectra into Gauss functions. This decomposition allows fluorophores within a synchronous spectrum to be isolated and represented by a Gauss shape, as shown in Equation 7.

$$f(\lambda) = a \cdot e^{\frac{(\lambda-b)^2}{2c^2}} \quad (7)$$

The height of a fluorophore peak is represented by “a”, the center of the peak by “b”, and the width of the peak by “c”. These parameters are determined using a software that utilizes a method within the Fortran code that relies on sequential quadratic programming. (Assaad et al., 2015)

In uncontaminated water samples, there are both humic-like and protein-like fluorophores. The humic-like fluorophores are those that resemble both humic and fulvic acids. The protein-like fluorescence (PLF) can be used to monitor water quality, as it is composed of both tryptophan and tyrosine, aromatic amino acids that fluoresce at an excitation wavelength around 270-280 nm. Tryptophan in particular can be used as an indicator of human involvement, as it can originate from sewage or farm waste. While PLF is not necessarily indicative of microbial contamination, it does indicate a level of biological activity that could be caused by DOM being microbially derived (Birdwell & Engel, 2010).

Materials & Methodology

Sample Collection Locations

This study analyzed five separate sets of water samples, FR50, FR51, Elter UK, Vosges, and Cluster Eau, taken from four different general locations. The Vosges sample set was collected from various water sources along the Vosges Mountain range in Eastern France.

The second of these locations was Boucq, France and the forest (Forêt de la Reine) a few kilometers away. Sample sets FR50 and FR51 both came from this area, FR51 in the winter and FR50 in the warmer weather. There are a series of streams and small ponds in the forest that originate from a non-potable source in the town. Both FR50 and FR51 samples were obtained from the rivers, but FR51 does not include samples from the ponds, as they were frozen over at the time of collection. This is noted in Table A1 in the Appendix. It should be noted that while most of these samples were clear in appearance, FR50 Samples 9 and 11 had a yellow color.

The Elter UK sample set came from two separate areas in the United Kingdom. Samples 1 through 10 came from a river (Allt a Mharcaidh) in the Cairngorms National Park in Scotland. These samples were clear in appearance.

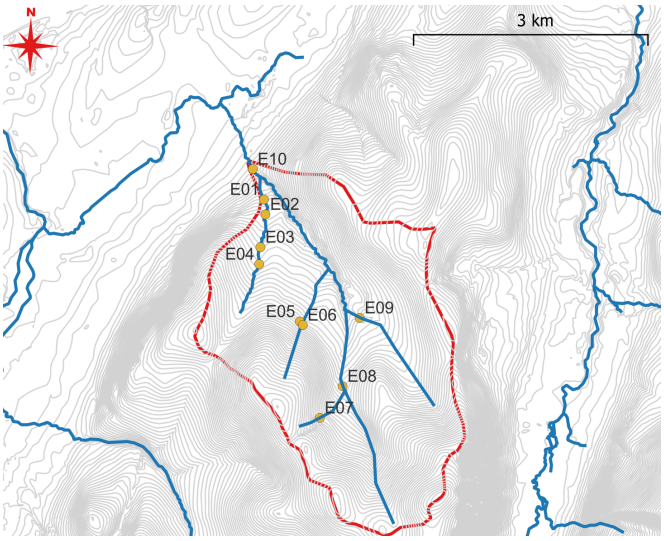

Figure 1. Map indicating where Elter UK Samples 1 through 10 were collected in relation to each other.

Samples 11 through 33 came from rivers (Trout Beck and Moss Burn) within the North Pennines AONB nature preserve in northern England. While most of the samples were collected successively along the rivers, Samples 12, 19, 21, 23, 25, 27, 29, and 31 were each collected from small tributaries feeding the Trout Beck River but not part of the main river. Elter UK Samples 11 through 20 had a yellow-orange appearance, and Samples 21 through 33 had a slightly yellow appearance.

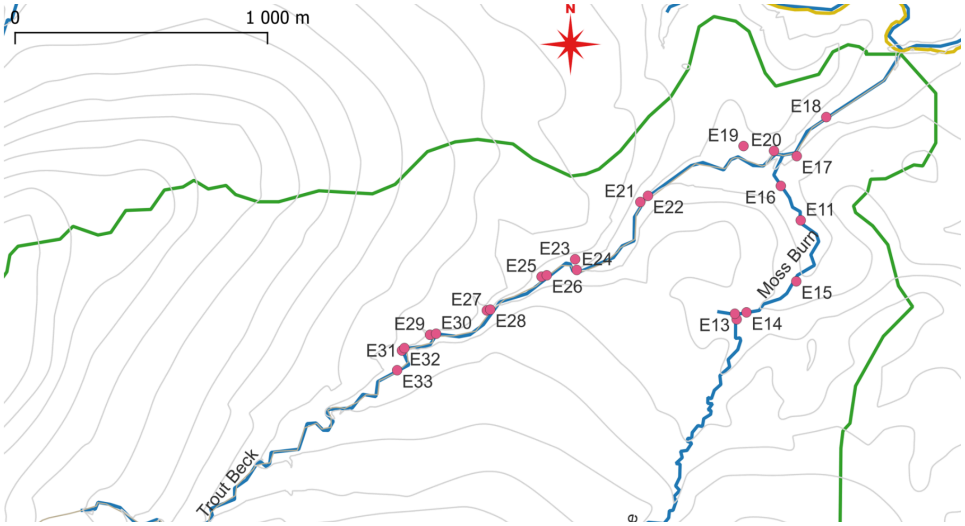

Figure 2. Map indicating where Elter UK Samples 11 through 33 were collected in relation to each other.

The final sample set, Cluster Eau, came from a variety of places in eastern France near the Switzerland border. These locations range from 50 m to 20 km from Lake Geneva. The samples were collected by primary school children involved in a citizen science project.

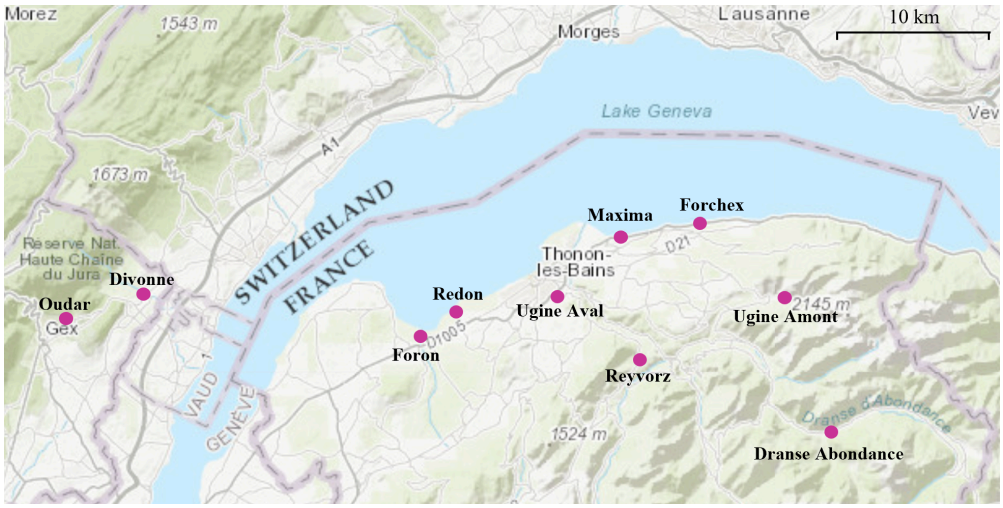

Figure 3. Map indicating where each of the Cluster Eau samples were collected. The image was retrieved from *Lake Geneva topographic map, elevation, terrain*.

Sample Collection

All samples were collected using a plastic cup-like collection tool. The cup was submerged in the water then emptied in order to 'rinse' it. The cup was rinsed one to two times before each sample was collected. The sample water was collected and stored in clean propylene bottles that were rinsed via the same process as the collection cup. These bottles were then stored

in insulated bags to keep cool while in transit and placed in a laboratory cold room for long term storage. The cold room was kept dark and at a constant temperature of 4 °C.

Sample Preparation

Various tests were completed using the water samples, but there were two main filtration methods used for these tests. The first filtration method utilized a 10 mL syringe with a 45 µm Phenex filter. This filtration is applied in the lab for the samples collected near Nancy and on-site for the samples collected in UK and by Cluster Eau. Approximately 2.5 mL was placed into a small glass vial for ion chromatography, and a glass test tube with approximately 10 mL for analysis. The remainder of the unfiltered sample was then filtered and stored in a cold room for future use, including UV-Vis spectroscopy and fluorescence spectroscopy. For this filtration, a 20 mL syringe with a Swinnex filter was used. All samples were stored in 100 mL VWR® bottles made of polypropylene with a screw on top to be stored for future use.

Testing

UV-Visible Light Spectroscopy

The UV-visible spectrum from each water sample was tested using the UV-2600 UV-Vis Spectrophotometer from Shimadzu and quartz cuvettes. This machine was designed to hold 1x1 cm cuvettes, but an additional attachment was designed so that the machine would be capable of holding cuvettes anywhere from 1x1 cm to 1x10 cm. Each scan illuminated the sample with light between 200 and 600 nm, and deionized water was used to calibrate the machine before each set of samples was tested. For each sample, the cuvette was filled with the next sample to be analyzed, emptied, and filled again to rinse between samples. The FR50, FR51, Elter UK, and Cluster Eau samples were each tested with a 1x1 cm cuvette, but the Vosges samples were tested in a 1x2 cm cuvette.

Fluorescence Spectroscopy

The fluorescent spectrum from each water sample was tested using a F-2500 Fluorescence Spectrophotometer from Hitachi Digilab and a 10x10 mm Quartz SUPRASIL℗ cuvette from Hellma Analytics. For each sample, the cuvette was filled with the next sample to be analyzed, emptied, and filled again to rinse between samples. Each water sample was run at an excitation wavelength of 254, 270, 275, 310, and 370 nm in addition to a synchronous spectrum, as shown in Table 1. To standardize the results and account for the Raman effects, a cuvette of deionized water was scanned at 270, 275, and 370 nm in addition to a specific Raman scan for each series of samples.

Table 1. Specific settings used for fluorescence spectroscopy analysis.

Scan Name	Excitation Wavelength (nm)	Start of Emission Wavelength (nm)	Excitation Slit Width (nm)	Emission Slit Width (nm)
raman_eau	350	340	2.5	5
eau_em_254	254	270	2.5	2.5
eau_em_270	270	290	2.5	2.5
eau_em_275	275	295	2.5	2.5
eau_em_310	310	330	2.5	2.5
eau_em_370	370	390	2.5	2.5
eau_sync_50	230	280	2.5	2.5

Gauss Identification

The synchronous fluorescence spectrum from the SF50 scan of each sample was analyzed and corrected using the Raman scan. This allowed for the Gauss decomposition with the assistance of Fortran software as used in (Assaad et al., 2015). This allowed for fluorophores to be identified and analyzed from the spectra peaks.

Results & Discussion

Fluorophore Identification

The software identified a total of five distinct fluorophores. For all of the sample sets, the average peaks of each fluorophore were determined to be 275, 307, 330, 355, and 374 nm. These values deviated at most +/- 10 to 14 nm from the averages, as can be seen in Table 2.

Table 2. Peaks at which fluorophores were found.

Gauss Parameter	Avg. Peak Wavelength (nm)	Highest Peak Wavelength (nm)	Lowest Peak Wavelength (nm)	Deviation from Avg. Peak Wavelength (nm)
B1	275	265	284	10
B2	307	300	320	13
B3	330	320	340	10
B4	355	349	365	10
B5	374	360	380	14

The first fluorophore (B1) is caused by protein-like fluorescence, as this is within the range in which proteins like tryptophan typically tend to fluoresce. The other four fluorophores are indicative of other humic substances. The total fluorescence of the first fluorophore of each sample was calculated using the Fortran code, and labeled F1

DOM Characterization

Various studies have been done that define general ranges for DOM characteristics associated with certain values, such as HIX, BIX, and FI. These values were applicable to the data collected, but some additional specification was also required. The values in Table 3 for HIX and BIX are based on research from Zsolnay (2003) and Huguet et al. (2009) respectively, and the FI values based on research from McKnight et al. (2001). McKnight defined DOM of a terrestrial origin to have a FI of ~1.4 and ~1.9 for microbial origin. Since this data showed a wider range of values, ~1.4 was clarified to mean 1.2-1.6, and ~1.9 was made to specifically refer to anything above 1.6. Looking at the data using this range showed to be reasonable given the other values used to characterize DOM.

Table 3. Values from fluorescence spectra that determine sample characteristics.

HIX values	DOM Characteristics
>16	Strong humic character/important terrigenous contribution
10-16	Strong/Important humic character; use other characteristics to confirm terrigenous or autochthonous contribution
6-10	Important humic character and weak recent autochthonous component
4-6	Weak humic character and important recent autochthonous component
<4	Biological or aquatic bacterial origin
BIX values	**DOM Characteristics**
>0.7	Low autochthonous component
0.7-0.8	Intermediate autochthonous component
0.8-1.0	Strong autochthonous component
>1.0	Biological or aquatic bacterial origin
FI values	**DOM Characteristics**
1.2-1.6	terrestrial origin
>1.6	microbial origin

The only change in HIX value classification from Huguet's ranges was the addition of the 10-16 range being explicitly defined. Samples in this range have a strong to important humic character, but in order to determine if there is important terrigenous or autochthonous contribution, other indexes must be considered. It should be noted that HIX values over 31 showed no indication of protein-like fluorescence. Huguet's proposed BIX values were consistent with the data collected in this study, but it should be noted that BIX values under 0.4 exhibited little to no PLF.

Similarly, to the fluorescence indexes, the indexes determined with UV-visible data were further specified. The $SUVA_{254}$ values listed in Assaad et al. (2015) used values of >5 for samples with a humic character and <2.5 for samples with a non-humic character. The samples analyzed in this study were consistent with these findings, however, samples <3.0 were considered to have a non-humic character. Any samples between 3.0 and 5.0 needed to consider other factors to determine the presence or absence of humic character. No alterations were made to the E4/E5 values that indicate if the acids present are primarily fulvic or humic. All of the 97 samples had a primarily fulvic acid composition, which is to be expected as they are more common than humic acids.

Table 4. Values from UV-visible spectra that determine sample characteristics.

SUVA254 values	DOM Characteristics
>5.0	humic character
3.0-5.0	mixture of humic and non-humic character; use other characteristics to confirm humic or non-humic character
<3.0	non-humic character
E4/E5 values	**DOM Composition**
<3.5	mostly humic acid
>3.5	mostly fulvic acid

Microbially Derived DOM with High PLF

After the DOM of each sample was analyzed, characteristics were noted in the FR51 samples, the Cluster Eau samples, and FR50 Samples 1-8 and 13-17. Each of these samples was determined to have a non-humic character via the $SUVA_{254}$ and microbial origins via the FI. The BIX indicated the majority of the samples displayed strong autochthonous components, meaning that the DOM originated in or very close to the place the sample was collected. This suggests that there is microbial activity at the place the sample was collected, as the FI confirmed. There were a few samples present that showed biological activity or intermediate, rather than strong autochthonous components, but this only had a small impact on the data, as seen in Table 5.

Table 5. Cluster Eau, FR51, and FR50 Samples 1-8 & 13-17 variations in F1.

Humic Character	F1
Important	1651-2423
Weak	1785-2761
Biological origin	1538-6777
Combined Data	1538-6777

These are the highest F1 values among the data, and they do not vary greatly based on humic character. This is because while the humic character can still be considered in samples with non-humic character, it will cause very little variation. These samples containing the highest levels of protein-like fluorescence makes sense given the locations they were obtained. The Cluster Eau sample was taken from the rivers around Lake Geneva and both the FR51 and applicable FR50 samples came from small rivers within the forest that flow from a non-potable source in a local village. Both of these locations are ones that may be subject to contamination from human or other sources, leading to higher microbial activity. This is reflected in the F1 values.

Terrestrially Derived DOM with Low PLF

Elter UK Samples 11 to 33 all display the same DOM characteristics. They all have a humic character, terrestrial origins, and a strong humic character with important terrigenous contribution. These characteristics are consistent despite being obtained via different calculations and spectra. The samples also have a low autochthonous component, meaning that the majority of the DOM did not originate from microbes in the area the samples was collected, rather is displaced terrestrial matter. This is consistent with the location, since the samples were collected in a nature preserve, largely protecting it from major human interactions that could result in increased microbial activity. The lack of biological activity is reflected in the F1 values, as they are significantly lower, running from 0-623. The majority of samples with an F1 of zero corresponded with the samples that were taken from water next to the main river. Since a river has more potential for contamination somewhere upstream, this is consistent with the theory that the decrease in F1 is due to lack of human involvement.

Elter UK Samples 1-10 are similar to Samples 11-33 in the sense that they are both terrestrial and have low autochthonous contribution, but Samples 1-10 have a non-humic character. These samples have F1 values 384-920, which is slightly higher than the rest of the sample set, but still lower than any microbially derived samples.

DOM with Varied Derivation with Intermediate PLF

There was a slight amount of variation in FR50 Samples 9, 10, 11, and 12 varied quite a bit from the rest of the FR50 samples. The samples are of terrestrial origins, weak humic character, and low autochthonous components. The PLF has an intensity of 1600-2613 for these samples. These four samples were taken from ponds within the forest that were not collected in the FR51 sample set since they had frozen over. This explains the mixture of high F1 despite the DOM being primarily terrestrial in origin. Most of the DOM is likely a result of organic matter falling into the ponds and decomposing, but there are also small animals that can contaminate these ponds via urination or methods which would increase the concentration of tryptophan and therefore PLF.

The Vosges samples displayed the widest variation of DOM characteristics out of the five sample sets. The majority of these samples have a humic character, but about ⅓ have microbial origins and the other ⅔ have terrestrial origins. The alternation between the microbial and terrestrial samples does not follow a certain trend. The microbial samples have a strong to intermediate autochthonous component with either a weak humic character or biological origins. These samples had F1 values ranging from 389-1040. The terrestrial samples mainly had a low autochthonous component with intermediate to weak humic components. These samples had slightly lower F1 values ranging from 237-823. None of the samples had extremely high values, as there was either a terrestrial origin or humic character indicating a lower quantity of PLF would be present.

270 nm and 275 nm Fluorescence Spectra

Once the samples were thoroughly characterized, the fluorescence spectra at 270 and 275 nm was analyzed. To ensure that the spectra being analyzed is consistent with the spectra that will be read from the probe, the responsivity curve of the specific photodiode that will be used was applied to the spectra. Since the probe will read the sum of intensities from 270 to 290 nm when excited with a wavelength of 270 nm, the corresponding corrected intensities were summed for each sample. For the 275 nm excitation wavelength, the intensities were summed from 275 to 295 nm. These values were plotted against the F1 fluorophore to see the correlation between the PLF and the spectra the probe will read as seen in Figures 4 and 5.

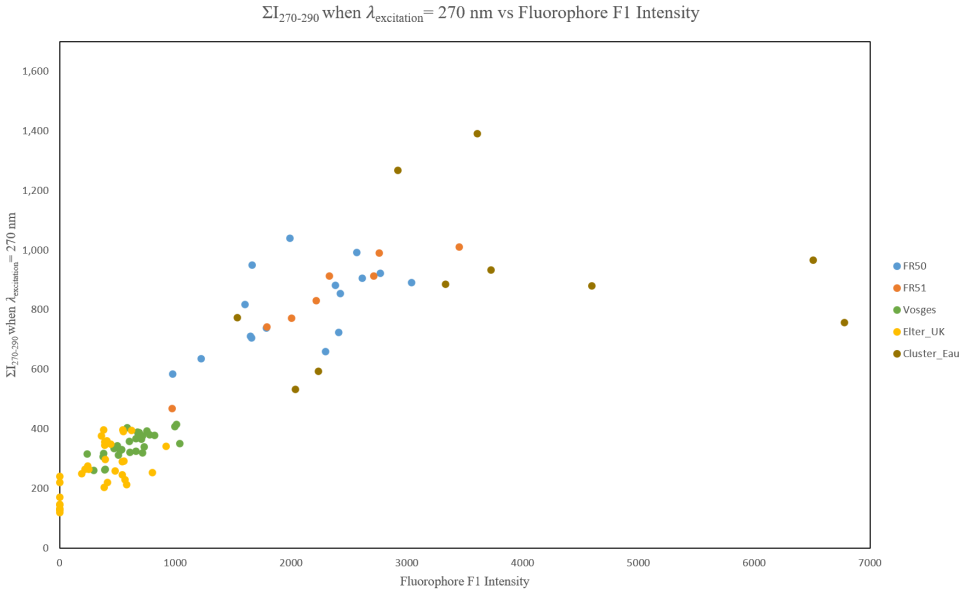

Figure 4. Sum of the 270 nm spectra versus the intensity of the F1 fluorophore.

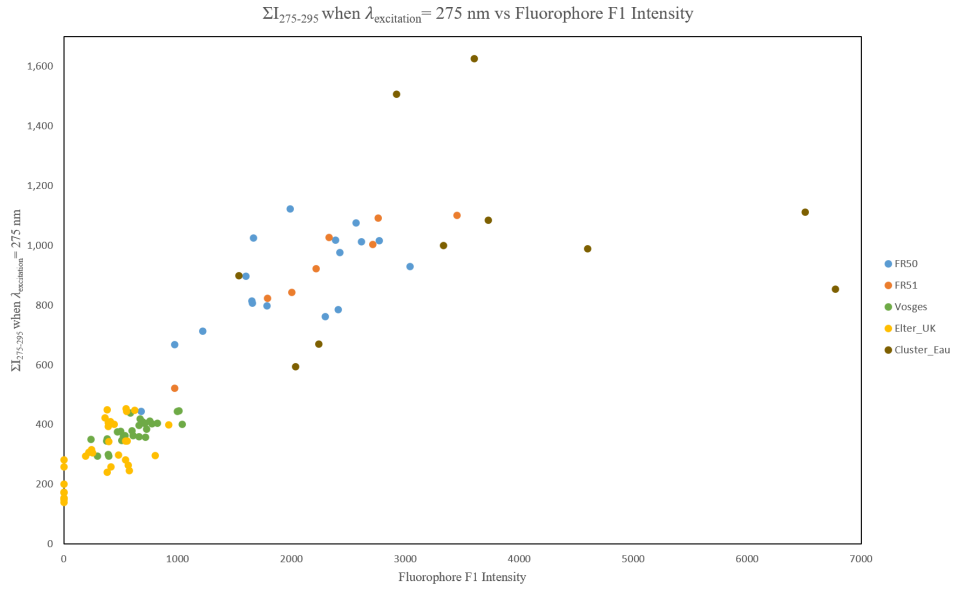

Figure 5. Sum of the 275 nm spectra versus the intensity of the F1 fluorophore.

With the exception of a few samples with F1 values of over 6000, there is a generally linear trend between the sum of spectra and the PLF, signifying a direct correlation. This is true for both the 275 nm spectra, which is the wavelength at which the F1 fluorophore peaks, and for the 270 nm spectra, which is the excitation wavelength the probe will use. Figure 6 highlights the relationship between the sum of intensities at 270 and 275 nm and F1. With a few exceptions, the trends are reflected well.

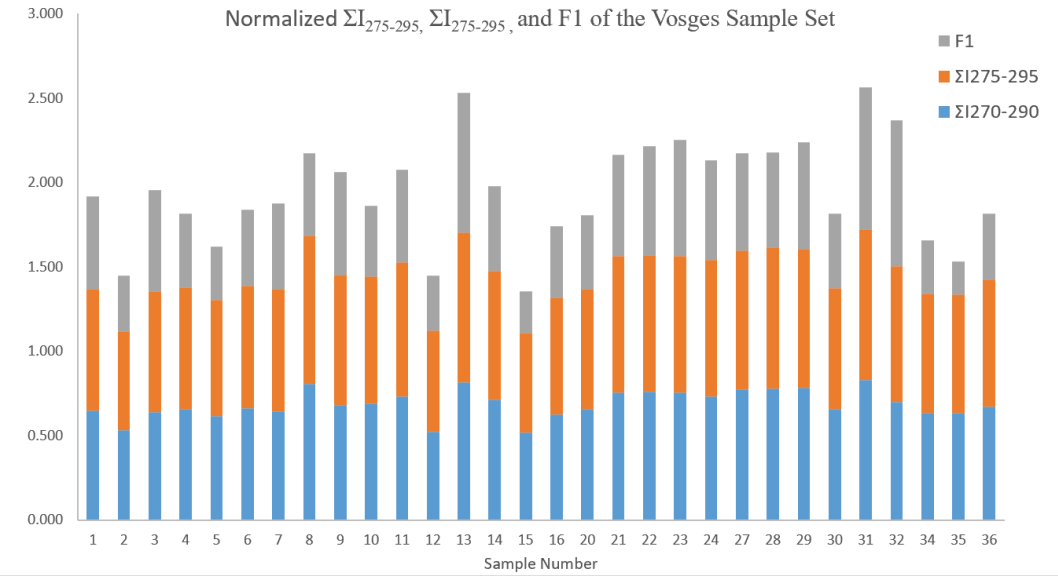

Figure 6. Normalized correlation between the sum of intensities for 270 nm, sum of intensities for 275 nm, and the intensity of theF1 fluorophore.

The F1 fluorophore was already known in this case due to the SF50 spectra, but in order to find this data from the probe, an equation must be made. Using the average scale between the highest sum of intensities and F1 fluoride and the average sum of intensities of samples with a F1 value of zero, Equation 8 was constructed.

$$F_{PLF} = 3.018 \cdot \Sigma(I_{270-290}) - 455.72 \quad (8)$$

In this equation, F_{PLF} is the fluorescence intensity corresponding to protein-like fluorescence and $\Sigma I_{270\text{-}290}$ is the sum of intensities from 270 nm to 290 nm after the responsivity curve has been applied. This yields a value for PLF that deviates on average ~27% from the F1 fluorophore determined by SF50. Table 6 highlights the similarities in the trends between F1 and F_{PLF}. Even if the values are slightly different, since extremely high values are scaled down a bit, the trends exhibited are the same.

Table 6. Trends exhibited by F1 and F_{PLF}.

$SUVA_{254}$	**F**	**BIX**	**HIX**	**HIX**	**F1**	**F_{PLF}**
Character	Origin	Autochthonous Component	Humic Character	Contribution or Component	-	-
non-humic	microbial	Strong/ Intermediate	Strong/ Important	Terrigenous/ Weak Recent autochthonous	678-1222	701-1462
non-humic	microbial	Strong/ Intermediate	Important	Weak recent autochthonous	1651-2423	1691-2411
non-humic	microbial	Strong/ Intermediate	Weak	Important recent autochthonous	1785-2761	1772-2680
non-humic	microbial	Strong/ Intermediate	Biological	Aquatic bacterial origin	1538-6777	1154-3744
humic	terrestrial	Low	Strong	Important terrigenous	0-623	-97-743
humic & non-humic	terrestrial	Low	Weak	Important recent autochthonous	1600-2613	1536-2541
non-humic	terrestrial	Low	Varied	Varied	384-920	187-575
humic	microbial	Strong/ Intermediate	Weak/ Biological	Important recent autochthonous/ Biological origin	389-1040	334-796
humic	terrestrial	Low	Important/ Weak	Weak/ Important recent autochthonous	237-823	329-763

Figure 7 shows the sum of spectra at 270 nm and 275 nm replotted against the new F_{PLF} fluorophore from Equation 8. The data follows the same trends as the F1 fluorophore with a closer correlation to the spectra at 270 and 275 nm.

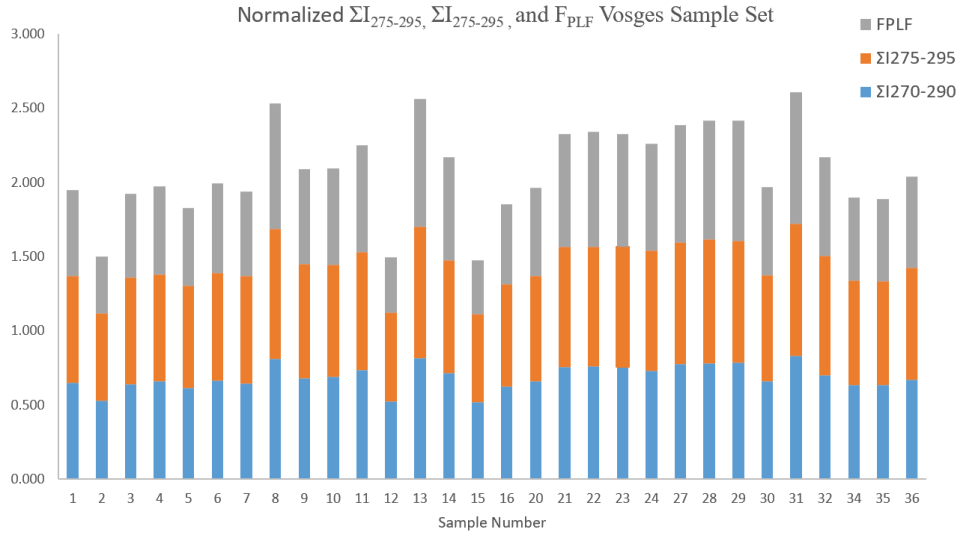

Figure 7. Normalized correlation between the sum of intensities for 270 nm, sum of intensities for 275 nm, and the F_{PLF} fluorophore.

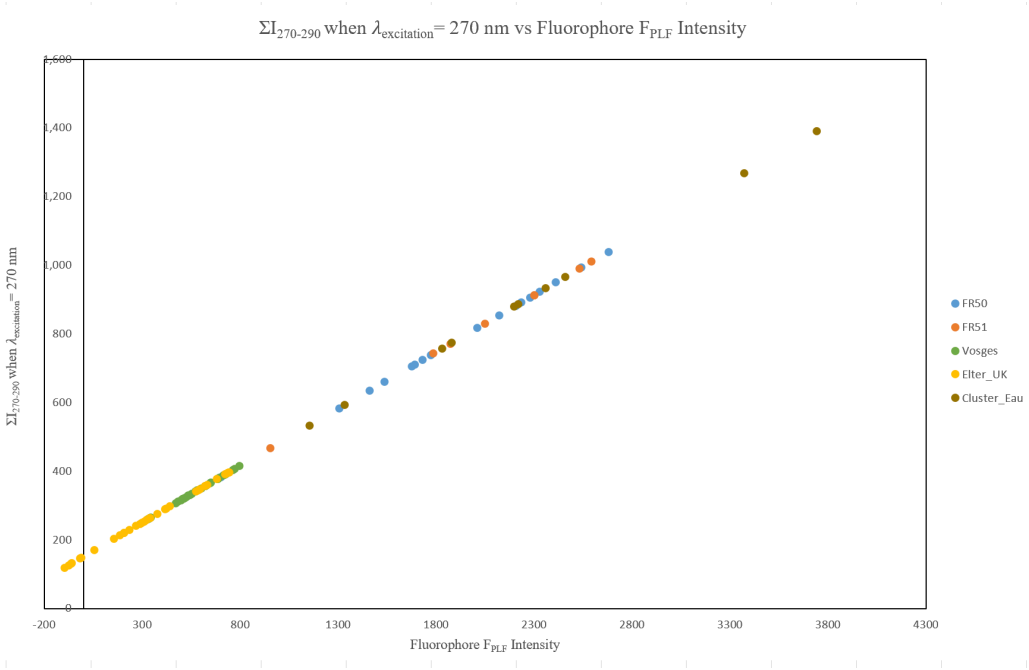

Figure 8. Sum of the 270 nm spectra versus the intensity of the F_{PLF} fluorophore.

The correlation between the protein-like fluorescence may not be as linear as Figure 8 in reality, but within a range of around 27%, this correlation is round to be accurate. By fitting the data to a simple equation, it can be seen that a 270 nm fluorescence probe can determine protein-like fluorescence and therefore be an indicator of microbial activity and water quality.

Conclusion & Recommendations

This study investigated the characteristics of dissolved organic matter that can be observed at certain excitation wavelengths through the use of fluorescence spectroscopy. Characterization of samples was performed via TOC, UV-visible spectroscopy, and fluorescence spectroscopy. By looking at these characteristics, the quality of the water being analyzed could be determined. However, since this required multiple types of tests, the study focused on the possibility of using fluorescence spectroscopy with an excitation wavelength of 270 nm to quickly determine water quality. In this case, water quality was determined by the level of protein-like fluorescence present in a sample.

This protein-like fluorescence was proven to be more present in samples in which the dissolved organic matter was a result of microbial activity rather than terrestrial means. This is what was expected, and once this was checked, the samples were analyzed with excitation wavelengths of 270 and 275 nm. It was clear that there is a direct relationship with the fluorescence spectra at 270 nm and the intensity of the F1 fluorophore. This confirms that it is possible to utilize fluorescence spectroscopy with an excitation wavelength of 270 nm to determine the levels of protein-like fluorescence present in a water sample. This confirmation makes it possible to monitor water quality via a probe with a 270 nm LED light for quicker, easier analysis of water quality in the field over periods of time.

An equation was then developed to apply to data that could be obtained from the probe. Further investigation is recommended to refine the equation used to transform the fluorescence data to intensity of protein-like fluorescence. This study looked at DOM characteristics across 97 water samples from various locations, however to better refine the equation, more data points should be analyzed and considered.

The ability to monitor changes in water quality quickly is essential because regardless if the quality is affected by natural or human causes, knowing sooner makes it possible to make changes and work towards fixing any issues. This would allow for a more sustainable and equitable future. This probe would be an invaluable tool that would save much time and money, which is why this DOM characterization and confirmation of the excitation wavelength what must be used is essential.

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Appendix

Table A1. FR50 and FR51 sample numbers that have the same sampling location.

Sample Set	Sample	Sample Name	Sample Set	Sample	Sample Name
FR50	1	B1_Han	FR51	1	B1_Han
FR50	2	Cure	-	-	-
FR50	3	B1 PG	FR51	2	B1 PG
FR50	4	B1E	FR51	3	B1E
FR50	5	B1_caméra	FR51	4	B1_caméra
FR50	6	B1_buse	FR51	5	B1_buse
FR50	7	B1_chemin	FR51	6	B1_chemin
FR50	8	B1 amont D2	-	-	-
FR50	9	B1_D1	-	-	-
FR50	10	B1_D2	-	-	-
FR50	11	B1_mare amont	-	-	-
FR50	12	B1_mare aval	-	-	-
FR50	13	B2 chem	FR51	7	B2 chem
FR50	14	B2 cam	FR51	8	B2 cam
FR50	15	W forêt	-	-	-
FR50	16	W pieux	-	-	-
FR50	17	Dame Pré	-	-	-

Table A2. Compilation of values from UV-visible and fluorescence analysis that denote DOM characteristics.

Sample	DOC (mg/L)	$SUVA_{254}$	E2/E3	E4/E5	$S_{275-295}$	HIX	BIX	FI	F1	$\Sigma I_{270-290}$ (λ_{ex}=270 nm)	$\Sigma I_{275-295}$ (λ_{ex}=275 nm)
FR50	FR50	FR50				FR50			FR50	FR50	
FR50_1	1.810	2.099	8.400	11.500	0.013	1.546	0.934	1.879	2410	725	786
FR50_2	1.888	1.801	9.500	10.000	0.014	1.228	0.982	1.902	3042	892	930
FR50_3	2.314	2.074	7.571	6.750	0.012	10.534	0.961	1.797	678	383	445
FR50_4	4.256	2.890	5.955	6.364	0.013	8.957	0.858	1.760	1658	706	808
FR50_5	4.566	2.781	6.091	6.455	0.013	12.028	0.829	1.738	976	584	669
FR50_6	4.592	2.809	6.000	6.083	0.013	5.858	0.830	1.738	1785	738	798
FR50_7	5.059	2.925	5.571	5.533	0.014	9.525	0.815	1.718	1651	711	815
FR50_8	5.199	2.962	5.621	5.438	0.013	11.246	0.788	1.723	1222	635	714
FR50_9	17.890	5.931	3.914	3.963	0.012	4.155	0.641	1.558	1600	817	897
FR50_10	14.270	4.478	4.244	4.261	0.013	4.875	0.677	1.586	2566	993	1077

FR50_11	23.850	3.899	4.307	4.382	0.012	8.320	0.546	1.576	2298	660	763
FR50_12	26.640	3.709	4.213	4.300	0.012	5.281	0.595	1.487	2613	906	1012
FR50_13	8.575	2.636	5.558	5.818	0.014	5.527	0.792	1.710	1989	1039	1123
FR50_14	7.632	2.935	5.619	5.682	0.014	6.235	0.768	1.706	1665	950	1025
FR50_15	8.948	2.425	5.381	5.000	0.016	3.921	0.800	1.677	2769	922	1017
FR50_16	10.710	2.437	5.231	4.862	0.016	6.408	0.774	1.597	2384	882	1019
FR50_17	8.824	2.607	5.762	5.435	0.015	10.092	0.798	1.716	2423	854	977
FR51	FR51	FR51				FR51			FR51	FR51	
FR51_1	1.608	2.239	10.000	11.000	0.013	5.216	0.953	1.875	974	468	522
FR51_2	2.451	1.918	9.800	14.000	0.018	2.260	0.918	1.833	3454	1010	1101
FR51_3	2.101	3.046	7.444	7.400	0.016	3.963	0.888	1.833	2004	771	843
FR51_4	2.337	2.867	7.000	7.800	0.016	4.681	0.854	1.775	2217	830	923
FR51_5	2.323	3.142	6.909	7.000	0.015	3.773	0.911	1.772	2715	913	1004
FR51_6	2.437	2.872	7.300	7.800	0.016	5.441	0.862	1.762	1790	743	824
FR51_7	2.956	2.909	7.583	8.000	0.016	5.708	0.873	1.834	2329	913	1027
FR51_8	2.953	2.946	7.077	8.167	0.015	4.634	0.881	1.840	2761	990	1092
Vosges	Vosges	Vosges				Vosges			Vosges	Vosges	
vosges_1	1.763	5.048	5.471	5.667	0.013	3.883	0.707	1.615	662	324	360
vosges_2	2.024	5.435	5.650	5.455	0.015	5.664	0.664	1.556	396	265	294
vosges_3	2.238	4.915	5.136	5.000	0.015	4.668	0.708	1.634	718	320	358
vosges_4	2.330	5.236	5.773	6.091	0.014	5.923	0.691	1.599	523	328	362
vosges_5	4.920	6.402	5.311	5.710	0.014	17.254	0.608	1.507	377	307	346
vosges_6	3.969	6.727	5.189	5.667	0.014	7.247	0.572	1.447	539	331	362
vosges_7	4.692	7.289	4.693	4.976	0.012	13.008	0.554	1.414	608	321	363
vosges_8	4.829	5.612	5.936	6.042	0.015	8.971	0.768	1.583	585	404	439
vosges_9	2.481	3.507	6.846	6.429	0.017	4.894	0.888	1.645	729	340	385
vosges_10	5.740	6.498	5.203	5.526	0.015	11.011	0.589	1.478	498	344	379
vosges_11	3.699	6.218	5.386	5.609	0.014	6.181	0.622	1.472	660	367	397
vosges_12	1.956	4.601	5.813	6.125	0.015	8.923	0.787	1.693	389	262	299
vosges_13	1.706	4.924	5.800	5.625	0.016	2.255	0.781	1.665	997	407	444
vosges_14	2.579	5.545	5.880	5.923	0.015	5.158	0.755	1.633	601	357	380
vosges_15	3.743	5.851	5.256	5.591	0.014	15.455	0.659	1.544	294	260	295
vosges_16	2.016	5.010	5.833	6.000	0.015	5.910	0.820	1.605	508	311	346
vosges_20	3.147	5.847	5.727	5.941	0.015	6.325	0.645	1.577	526	329	355
vosges_21	3.195	5.603	5.286	5.500	0.015	5.197	0.655	1.535	715	377	406
vosges_22	2.540	5.157	6.429	6.700	0.016	3.886	0.672	1.639	776	381	403
vosges_23	2.490	5.663	5.577	5.571	0.015	3.656	0.652	1.505	823	378	405
vosges_24	4.598	7.503	4.733	5.125	0.013	7.779	0.557	1.411	707	366	405
vosges_27	5.051	6.910	5.056	5.472	0.014	7.215	0.666	1.527	690	387	411
vosges_28	3.862	6.370	5.271	5.480	0.014	6.849	0.730	1.596	672	389	419

vosges_29	4.074	6.259	4.943	5.370	0.014	5.160	0.659	1.492	756	392	412
vosges_30	2.038	5.152	6.056	6.222	0.016	4.824	0.849	1.619	529	329	358
vosges_31	1.494	4.752	6.083	5.429	0.015	1.601	0.741	1.616	1011	415	446
vosges_32	0.911	3.185	6.000	3.750	0.019	1.516	0.775	2.482	1040	350	401
vosges_34	4.177	6.225	4.963	5.250	0.014	12.361	0.645	1.501	383	318	352
vosges_35	5.324	6.724	5.333	5.657	0.015	17.151	0.643	1.511	237	316	351
vosges_36	3.559	4.861	6.393	6.357	0.017	11.745	0.711	1.639	470	335	377
Elter_UK	Elter_UK	Elter_UK				Elter_UK			Elter_UK	Elter_UK	
Elter_UK_1	5.616	3.187	4.279	4.826	0.012	13.084	0.464	1.364	480	258	298
Elter_UK_2	5.280	3.352	4.333	5.000	0.012	14.157	0.510	1.334	543	289	346
Elter_UK_3	5.413	3.307	4.279	5.045	0.013	8.358	0.514	1.363	920	342	400
Elter_UK_4	4.599	3.196	4.194	4.600	0.012	8.803	0.513	1.383	555	292	344
Elter_UK_5	2.465	0.811	6.667	5.000	0.025	1.556	0.718	1.667	564	229	263
Elter_UK_6	2.827	1.097	5.333	6.000	0.013	3.184	0.551	1.488	578	213	246
Elter_UK_7	2.202	0.772	6.000	9.000	0.017	1.421	0.758	1.574	801	254	297
Elter_UK_8	3.774	1.643	4.571	5.429	0.013	11.682	0.526	1.378	384	203	240
Elter_UK_9	4.188	2.507	4.154	4.714	0.012	14.316	0.487	1.283	414	220	259
Elter_UK_10	4.508	2.684	4.310	5.000	0.012	10.792	0.487	1.338	543	247	282
Elter_UK_11	18.450	6.016	3.757	4.199	0.011	26.747	0.432	1.315	252	264	305
Elter_UK_12	21.400	6.299	3.739	4.216	0.010	37.928	0.414	1.273	0	220	259
Elter_UK_13	17.930	5.884	3.814	4.304	0.011	27.186	0.450	1.325	221	263	307
Elter_UK_14	19.620	6.009	3.782	4.239	0.010	30.639	0.432	1.297	193	250	294
Elter_UK_15	21.090	5.936	3.777	4.268	0.010	31.880	0.419	1.281	0	240	282
Elter_UK_16	18.990	5.803	3.746	4.170	0.010	28.071	0.449	1.307	243	276	317
Elter_UK_17	13.750	5.622	3.813	4.248	0.011	20.510	0.479	1.325	442	348	401
Elter_UK_18	14.420	5.465	3.830	4.261	0.011	19.028	0.478	1.309	391	345	394
Elter_UK_19	31.570	5.553	3.993	4.774	0.011	40.396	0.365	1.231	0	145	174
Elter_UK_20	15.660	5.658	3.788	4.206	0.011	23.684	0.464	1.291	397	298	343
Elter_UK_21	31.990	5.942	3.881	4.546	0.011	50.292	0.368	1.238	0	126	149
Elter_UK_22	11.780	5.628	3.816	4.250	0.011	17.506	0.487	1.334	623	395	449
Elter_UK_23	31.860	5.832	3.904	4.595	0.011	37.082	0.357	1.211	0	147	171
Elter_UK_24	11.540	5.685	3.847	4.253	0.011	16.955	0.501	1.335	553	391	444
Elter_UK_25	32.640	5.781	3.925	4.668	0.011	44.302	0.367	1.220	0	119	140
Elter_UK_26	12.520	5.735	3.756	4.171	0.011	17.623	0.481	1.341	390	356	406
Elter_UK_27	33.840	5.892	3.935	4.636	0.011	40.135	0.379	1.271	0	130	153
Elter_UK_28	11.590	5.729	3.806	4.228	0.011	14.151	0.483	1.339	547	397	453
Elter_UK_29	33.310	5.995	3.883	4.539	0.011	41.094	0.382	1.261	0	132	154
Elter_UK_30	12.230	5.429	3.914	4.463	0.011	20.633	0.477	1.332	410	361	411
Elter_UK_31	29.260	5.995	3.875	4.490	0.011	40.325	0.387	1.271	0	170	201

Elter_UK_32	11.130	5.597	3.844	4.255	0.011	19.400	0.496	1.364	363	377	423
Elter_UK_33	10.600	5.415	3.876	4.306	0.011	18.654	0.499	1.351	380	397	449
Cluster_Eau	Cluster_Eau	Cluster_Eau				Cluster_Eau			Cluster_Eau	Cluster_Eau	
DRANSE_ABAONDANCE	1.400	1.000	5.333	3.500	0.011	0.518	1.000	2.023	3727	933	1085
UGINE_AMONT	1.650	1.877	5.500	5.333	0.015	1.288	0.880	1.759	3333	886	1000
REDON	1.800	1.886	5.286	4.750	0.015	2.380	1.016	1.907	2238	593	669
FORCHEX	1.900	1.787	6.000	4.250	0.014	3.177	0.924	1.774	2035	533	594
UGINE_AVAL	2.230	2.061	5.444	4.167	0.014	2.073	1.228	1.835	6507	966	1113
REYVORZ	2.650	2.602	5.538	5.143	0.014	3.988	0.884	1.737	6777	757	854
OUDAR	1.830	1.312	6.500	4.000	0.016	0.676	0.841	1.808	2922	1269	1508
FORON	2.370	2.234	5.600	4.833	0.015	2.790	0.977	1.801	1538	774	899
DIVONNE	3.010	1.264	5.714	4.750	0.019	1.626	0.825	1.694	4599	880	990
MAXIMA	2.190	1.916	6.429	5.500	0.017	1.227	0.976	1.864	3606	1392	1626