



# Master's Thesis

Master's Programme in Applied Environmental Science – Ecosystem Services and Nature Resource Management, 60 credits



## The Specific Influence of Iron and Carbon on Brownification of Southern Swedish Freshwater

Environmental Science, 22.5 credits

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ThankGod Akusiobi

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## **Abstract**

As the soil in southern Sweden recovers from acidification during the 20<sup>th</sup> Century, iron (Fe) and dissolved organic carbon (DOC) increasingly enter freshwater systems, resulting in "brownification." This process, characterised by light absorption and scattering of waterborne particles and dissolved molecules, causes a brown-yellow colouration that disrupts freshwater ecosystems. Although extensive research has shown that DOC and Fe strongly associate and transport together, their combined effect on light absorption remains poorly understood. This study assessed water quality from Lake Bolmen to investigate how DOC influences Fe light-absorbing properties. Using the data obtained from field samples, a laboratory study was designed and conducted where known concentrations of DOC and Fe were mixed in artificial river water and analysed using UV-Vis spectroscopy. The results revealed that Fe light absorption is markedly enhanced at very low C ratios, beyond which the impact of this enhancement on overall brownification is minimal. These findings provide new insights into the light-absorbing behaviours of Fe and DOC and suggest priorities for future brownification mitigation strategies.

Keywords: Brownification, Lake Bolmen, Freshwater, DOC, Iron, Land use

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# 1. Introduction

## 1.1 Background

Changes in land use, climate change, and other human activities have all contributed to the degradation of freshwater ecosystems (Bayramoglu et al., 2020). A significant threat to these ecosystems can disrupt their services, leading to water and food shortages, poor living conditions, and further climate change (Cowx and Portocarrero Aya, 2011). Preserving freshwater ecosystems is essential for maintaining a healthy lifestyle (Darwall et al., 2018). The movement of nutrients, organic materials, trace metals, and other harmful substances during urban runoff can degrade water quality (Faulkner et al., 2000). Dissolved organic carbon (DOC) entering freshwater from humic materials leads to water browning compared to its normal colour or brownification. The level of DOC in freshwater is largely influenced by changes in land use, atmospheric sulfur deposition, and the predominant soil type in the catchment area (Yang et al., 2012; Porcal et al., 2009).

Brownification can affect the ecological balance of freshwater systems (Van Dorst et al., 2018). This effect includes less light reaching underwater plants, which decreases fish populations and reduces photosynthesis in the water (Kritzberg et al., 2020). High DOC concentrations also complicate water treatment processes, affecting the supply of clean drinking water (Forsius et al., 2017; Keeler et al., 2015). The water quality of Lake Bolmen has deteriorated due to brownification, with a 50% increase in color over the past 40 years (Klante et al., 2021).

Brownification is not only caused by elevated concentrations of DOC but also iron (Fe), as demonstrated extensively in a large range of studies (Härkönen et al., 2023; Kankaala et al., 2019; Nydahl et al., 2019; Sepp et al., 2018). Although DOC in freshwater comes mainly from the soil (Graeber et al., 2012), the binding of DOC and Fe means that DOC and Fe are often found together and contribute cooperatively to brownification (Weyhenmeyer et al., 2014). An increase in DOC concentration leads to an increase in Fe concentration, providing a mechanism for how DOC assists with Fe transport in freshwater systems. The relationship breaks down when DOC becomes saturated with Fe, as DOC no longer has free functional groups to stabilise complexes and transport Fe (Xiao et al., 2013).

While it is well evidenced that there is a relationship between Fe and DOC concentrations regarding their transport and stability, there is limited understanding of their combined effect on light-absorbing properties, that is, their combined contribution to brownification. Complexing Fe with DOC has been suggested as a mechanism for enhancing the light absorption ability of Fe (Weyhenmeyer et al., 2014). The precise significance and extent of this interaction on brownification remain unexplored. Understanding how Fe enhances light absorption through complexation with DOC is essential for assessing its impact on brownification and developing mitigation strategies.

This research aims to study the effect of complexation between DOC and Fe on the light-absorbing properties of artificial freshwater. Firstly, artificial freshwater was designed to replicate the water quality of natural freshwater found in the Lagan river system, including Lake Bolmen, in southern Sweden. Secondly, the artificial freshwater was then used in a laboratory-based study where known concentrations of DOC and Fe were mixed and analysed

by photospectrometry. Through this experimental design, I examined how DOC and Fe interact through complexation, influencing the absorbance of light and thereby contributing to brownification. By carefully controlling the concentrations of DOC and Fe, I was able to identify that Fe-DOC complexes had a higher absorbance than the values of individual components, suggesting a synergistic, enhancing effect.

## **1.2 Research Question**

1. Does DOC affect the absorbing properties of iron?

## 2. Methods

### 2.1. Sampling

The water samples used for the experimental study were collected from Lake Bolmen and its tributaries. The samples were taken to the laboratory for qualitative analysis to understand the chemistry behind the browning colour of southern Sweden's freshwater. The samples from Lake Bolmen and its tributaries were collected in the winter.

### 2.2. Quantification of Iron

#### 2.2.1. Stock solution of iron

A 1 mg/L Fe stock solution was produced by serial dilution of a 10 mg-Fe/L Fe stock solution with deionised water. Firstly, 1 L stock of a 10 mg-Fe/L Fe solution was produced by dissolving 0.0484 g  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in 1 L deionised water in a 1 L volumetric flask. Secondly, 100 mL of 10 mg-Fe/L Fe solution was then measured in a 100 ml volumetric flask, to which 900 mL of water was added to produce 1 L of 1 mg-Fe/L Fe stock solution. The flask was shaken multiple times to ensure thorough mixing and stored in a glass bottle.

#### 2.2.2 Preparation of solutions for calibration

A series of solutions with known Fe concentrations were prepared by diluting a 1 mg-Fe/L stock solution into 20 mL total volumes (Table 2).

*Table 1: Composition of solutions of known Fe concentrations for calibration.*

Solution	V(1 mg-Fe/L) (mL)	V (water) (mL)	Final V (mL)	Final concentration (mg-Fe/L)
1	0.0	20.0	20	0.00
2	0.8	19.2	20	0.04
3	2.0	18.0	20	0.10
4	4.0	16.0	20	0.20

The solutions were then treated with 5 drops of thioglycolic acid/ammonium thioglycolate solution (Merck Millipore MQuant Iron Test), sealed with a screw cap, shaken by hand for 1 min, and then allowed to stand for 5 min. The presence of iron caused the solution to turn purple/blue due to forming a Fe-thioglycolate reporter complex (Rahman et al., 2020).

#### 2.2.3 Photometric quantification of iron concentration – calibration

All spectroscopic and photometric analysis was conducted using a UV- 1900i SHIMADZU spectrophotometer in 1 cm quartz cuvettes. Initially, the absorbance maximum for the purple/blue reporter complex was found at 561 nm compared to a deionised water background. Since the prolonged exposure to visible light during analysis caused the colour of the reporter complex to intensify, the solution was then replaced by a second aliquot. Photometric data was then obtained at a single wavelength ( $\lambda_{\text{abs}} = 561 \text{ nm}$ ) within 1 s of each sample being present in the spectrophotometer to minimise unwanted intensification of colour. The absorbance values obtained at each concentration were then used to construct a calibration curve. The

quality of the calibration curve was assessed by the coefficient of determination ( $R^2$ ), which indicated the linearity of the line that best fit the obtained data points.

#### **2.2.4 Sample preparation for photometric analysis**

The six water samples were filtered using a syringe-driven filter (0.45  $\mu\text{m}$ ). The suitability of the water samples for iron tests was assessed colourimetrically to ensure the Fe concentrations were within the approximate range of the calibration curve. This was achieved by taking 20 mL of each sample into a glass tube, adding 5 drops of thioglycolic acid/ammonium thioglycolate solution, sealing, shaking for 1 min and then allowing it to react for 5 min. The colour was then compared to the colour scale accompanying the Fe test kit (Merck Millipore MQuant Iron Test). Samples judged to have a higher Fe concentration than those in the calibration range (i.e. 0.2 mg-Fe/L) were then diluted 10 times. The diluted samples were then treated with thioglycolic acid/ammonium thioglycolate solution using the same method for photometric analysis.

#### **2.2.5. Photometric quantification of iron concentration – samples**

Absorbance values at  $\lambda_{\text{abs}} = 561 \text{ nm}$  were obtained for the six samples collected from Lake Bolmen (diluted as required) treated with and without thioglycolic acid/ammonium thioglycolate solution. Absorbance values of untreated (that is, without thioglycolic acid) but diluted samples were then subtracted from the treated, diluted sample so that the absorbance of light at that given wavelength can only be attributed to the presence of the Fe-thioglycolate reporter complex. “Background-removed” absorbance were then converted to Fe concentrations using the calibration curve and multiplied by 10 to account for the 10x dilution.

#### **2.2.6. Water colour (Brownness)**

The colour (brownness) of the filtered water sample was analysed as a function of the absorbance of light using a UV-1900i SHIMADZU spectrophotometer. Measurements were taken in 1 cm quartz cuvettes at a wavelength of 420 nm

### **2.3 Analysis of other water quality parameters**

#### **2.3.1 Conductivity and pH Measurements**

The conductivity of the water samples was measured using a HORIBA Compact Conductivity Meter (LAQUAtwin EC11), which indicates the total dissolved ionic content in the water. The pH values of the water samples were determined using a ThermoScientific Eutech Elite pH Spear.

#### **2.3.2 DOC measurements**

The six water samples were filtered using a syringe-driven filter (0.45  $\mu\text{m}$ ) and analysed using the SHIMADZU Total Organic Carbon Analyzer (TOC-L). Because filtered samples were analysed, it was assumed that the final *total* organic carbon (TOC) value corresponded to the *dissolved* organic carbon (DOC) value.

#### **2.3.3 Data analysis**

The experimental data were collected and analysed using Microsoft Excel. Linear regression analysis was performed in Excel by modelling a linear trendline, and the degree of correlation was interpreted from the calculated coefficient of determination ( $R^2$ ) values.

## **2.4 Laboratory study to explore Fe-DOC interactions**

### **2.4.1. Preparation of artificial freshwater**

Artificial freshwater was prepared in the laboratory to control its conductivity, alkalinity, and pH. Data from collected natural water samples informed the formulation of the artificial freshwater, ensuring that all parameters were within the target ranges. Into 1 L of deionised water, 0.126 g of  $\text{NaHCO}_3$  (equal to an alkalinity of 1.5 mM) was dissolved, and the conductivity and pH were adjusted to 650  $\mu\text{S}/\text{cm}$  (using a total dissolved solids standard) and 6.8 (using  $\text{NaOH}$  or  $\text{HCl}$ ), respectively. This produced a 10x artificial freshwater solution. To produce 1x artificial freshwater (alkalinity at 0.15 mM, conductivity at 65  $\mu\text{S}/\text{cm}$  and pH 6.8), the 10x solution was diluted 10x with deionised water.

### **2.4.2. Preparation of DOC and Fe stock solutions**

The laboratory experimental design also enabled me to achieve specific DOC and Fe concentrations for the experiment. For a model DOC specie, the sodium salt of lignic acid was used due to its high solubility, processibility, and suitable chemical functionality. A 1 g-C/L model DOC solution was prepared by dissolving 0.0778 g sodium salt of lignic acid in 50 ml of deionised water.  $\text{FeCl}_3$  was used as the model Fe specie (in the form of  $\text{Fe}^{3+}$  free ions). For Fe solution, 1g-Fe/L stock solution was prepared using 0.2427g of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  dissolved in 50ml of deionised water.

To run the laboratory study, the 1 g/L stock solutions of DOC and Fe were diluted to 20 mg/L into a mixture of deionised water and 10x artificial freshwater stock. To produce a 1x artificial freshwater containing 20 mg/l DOC or Fe, 5 ml of the 10x artificial freshwater was mixed with 1 ml of the 1g/L DOC stock or 1g/L Fe stock and diluted with 44 ml deionised water to yield 50 mL of stock solution.

### 2.4.3. Experimental design

Different mixtures of Fe and DOC with specific, known concentrations were produced according to a 6 x 6 experimental matrix (Fig. 1). By varying the concentration of one additive compared to a fixed concentration of the other, it is possible to see how the light absorption is affected by each additive independently. In each row, from left to right on the experimental matrix (Fig. 1), the Fe concentration increases from 0 to 10 mg/L at 2 mg/L intervals. In each column, from bottom to top, the DOC (specifically C) concentrations increase from 0 to 10 mg/L at 2 mg/L intervals. This design examined a large range of different Fe/DOC mixtures.

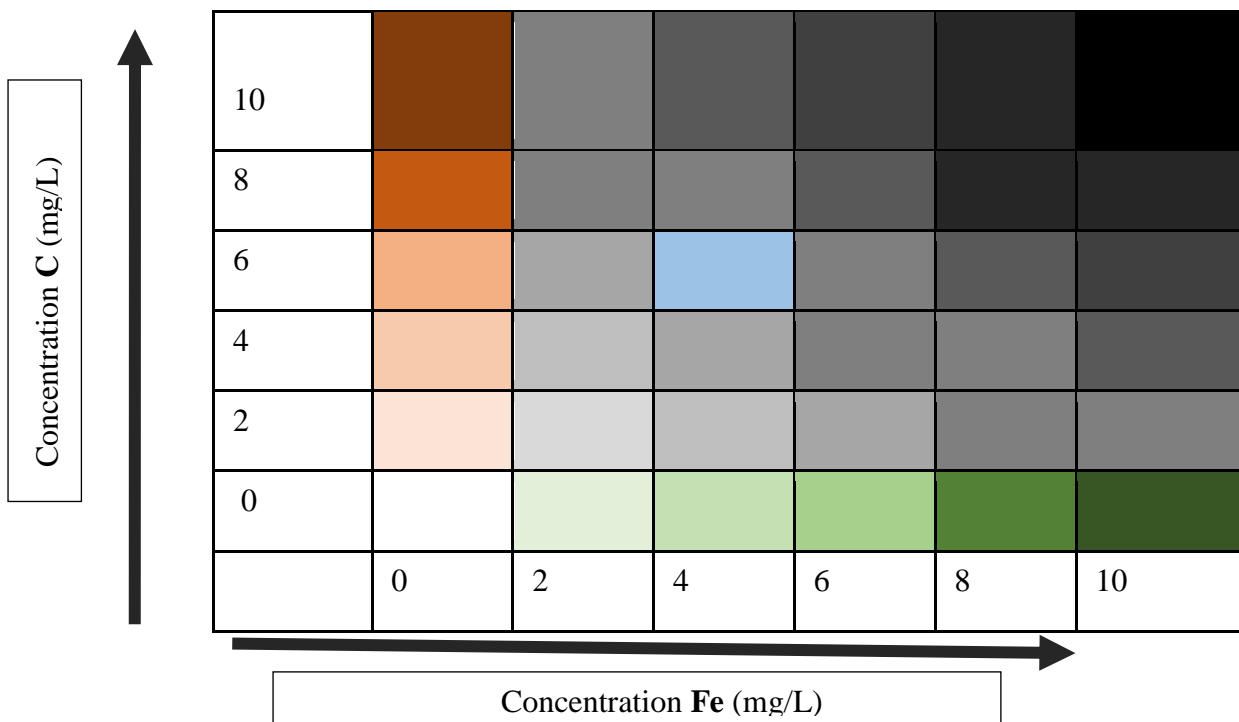


Figure 1: Experimental matrix showing the different concentrations of DOC (according to the specific concentration of carbon, C) and iron (Fe) in mg/L in artificial freshwater solutions. The preparation for the sample in the cell highlighted in blue is shown in Section 2.4.4. The cells highlighted in green (increasing Fe concentration at 0 mg-C/L DOC) and red (increasing C (DOC) concentration at 0 mg-Fe/L Fe) represent experiments using calculated additive concentrations as described in section 3.2.2.

### 2.4.4. Experimental procedure

Mixtures of 20 mg/L DOC and Fe solutions in 1x artificial freshwater at specific, known concentrations were mixed with 1x artificial freshwater in different wells of 2 3x4 well plates (volume per well approximately 3 mL) according to the experimental matrix (Fig. 1).

An example procedure for preparing samples is presented here and refers to the cell highlighted in blue in Figure 1, where Fe concentration is 4 mg/L and C is 6 mg/L. 0.6 ml of 20 mg/L DOC (or C) stock solution in 1x artificial freshwater and 0.4 ml 20 mg/L Fe stock solution in 1x

artificial freshwater were transferred to the corresponding cell using micropipettes. The mixture was then diluted to 2 mL with 1x artificial freshwater, yielding the correct concentrations of Fe and C.

The samples were then left for 1 h. Then, the water colour was examined by photospectroscopy in the same procedure as per section 2.2.6., however, the absorbances at 400, 420 and 440 nm were obtained. Then, the average value was calculated and used. Through this, the signal-to-noise ratio was optimised.

### 3. RESULTS

#### 3.1 PART 1: Water quality assessment of natural freshwater samples

##### 3.1.1. The relationship between Fe and DOC

The water quality of the six freshwater samples was assessed and compiled (Table 2). Linear regression analysis showed that Fe and DOC concentrations are linearly correlated ( $R^2 = 0.97$ ; Fig. 2). This indicates that an increase in DOC concentration leads to a similar increase in Fe concentration due to the synergic effect of DOC and Fe through complexation. A similar analysis also showed that water colour correlated linearly to the DOC concentration (Fig. 3;  $R^2 = 0.99$ ) and Fe concentration (Fig. 4;  $R^2 = 0.97$ ). It was inferred that both DOC and Fe can contribute to brownification; however, the positive correlation was an artefact of the correlation between the concentrations themselves (Fig. 2). It was also impossible to deconvolute the relative contribution to water colour (or brownification).

Table 2: Water quality assessment for six water samples taken from the Lagan water system (e.g. Lake Bolmen) in southern Sweden, including pH, conductivity, Fe concentration (mg-Fe/L), DOC expressed as a concentration of carbon, C (mg-C/L), water colour (determined as a function of the absorbance of light at 420 nm); and the carbon-to-iron ratio

Location	pH	Conductivity ( $\mu\text{S/cm}$ )	[Fe] (mg-Fe/L)	DOC (mg-C/L)	Water colour (absorbance at 420 nm)	C:Fe ratio
Tiraholm	6.72	65	0.37	13.88	0.08	37.5
Lillån	6.27	67	1.17	16.91	0.10	14.5
Storån	6.29	48	0.80	15.96	0.10	20.0
Norra hamnen	6.00	52	0.94	16.95	0.10	18.0
Piksborg	6.60	100	0.54	13.53	0.07	25.1
Muråns dike	4.23	59	2.16	24.97	0.15	11.6

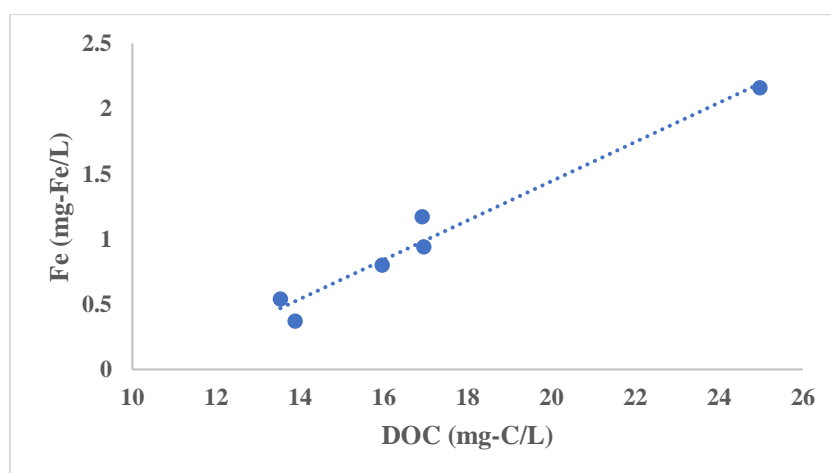


Figure 2: Correlation between DOC (mg-C/L) and concentration of Fe (mg-Fe/L)

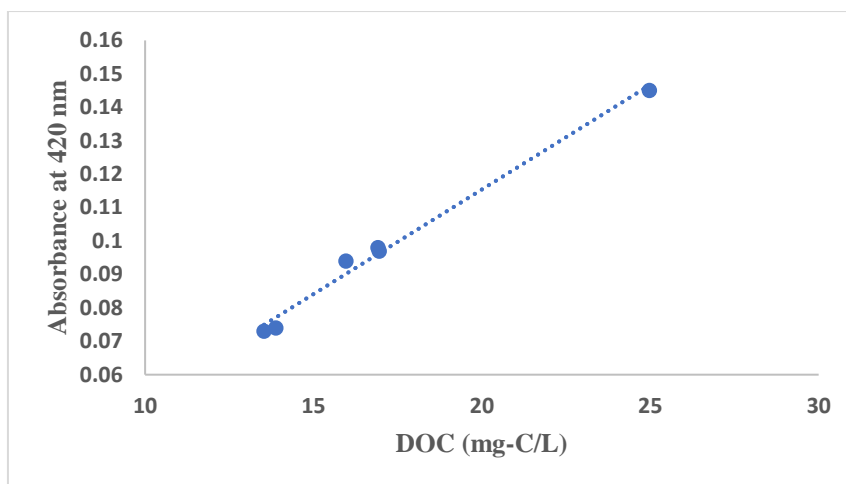


Figure 3: Absorbance at 420nm against the concentration of DOC (mg-C/L)

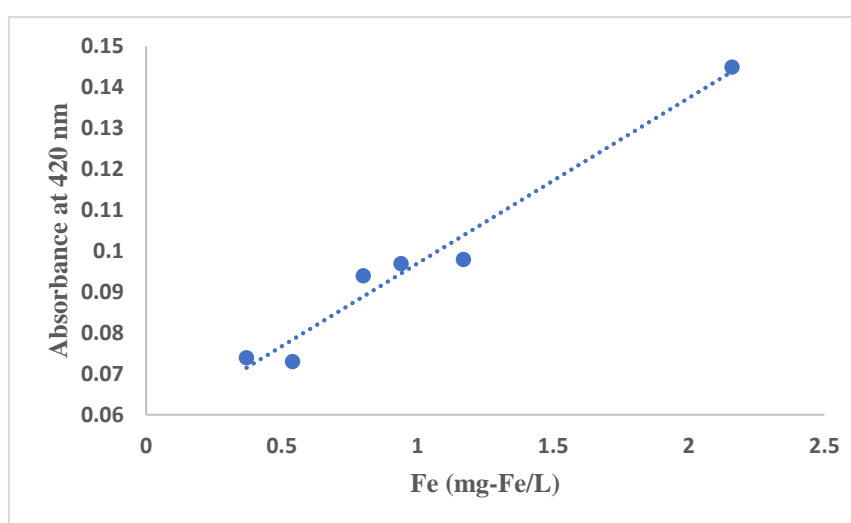


Figure 4: Absorbance at 420 nm against the concentration of Fe (mg-Fe/L)

### 3.1.2. Design of artificial freshwater and experimental parametrisation

The data presented in Table 2 established the boundary values for preparing an artificial freshwater that simulates the freshwater found in the Lagan river system – Lake Bolmen. The artificial freshwater was then used in laboratory-based studies of DOC and Fe complexation (see Section 3.2).

The water from the samples had a pH range of 4.23 - 6.72. However, 6.8 was selected as the pH for artificial freshwater because of concerns with the stability of lignic acid at lower pH values. pH 6.8 is still considered a reasonable pH value for this water system (Borgström, 2020). Conductivity ranged from 48-100  $\mu\text{S}/\text{cm}$ , representing freshwater's low salinity. The conductivity of the artificial freshwater was set to 65  $\mu\text{S}/\text{cm}$ , which is well within this range. Alkalinity was not measured in this study. However, a typical alkalinity value obtained from previous Lake Bolmen studies was used, represented as 0.15 mM  $\text{HCO}_3^-$  (Borgström, 2020).

The DOC concentrations ranged from 13.53-24.97 mg-C/L, while Fe concentrations ranged from 0.37-2.16 mg-Fe/L. DOC was, therefore, in much higher concentrations than Fe, leading

to high carbon-to-iron (C:Fe; calculated at the DOC concentration divided by the Fe concentration) ratios between 11.6-37.5 (Table 2). In the current study, much lower C: Fe ratios (up to 5) were examined to investigate the effect of DOC on the absorbing properties of Fe and conditions where the Fe ions may be either partially or fully complex to capacity with DOC molecules.

## 3.2 PART 2: Laboratory study into the effect of DOC on the absorbing properties of Fe

### 3.2.1. The comparative light-absorbing ability of Fe and DOC

A laboratory experiment was designed to examine how different concentrations of iron (Fe) and carbon (C) affect light absorbance and to study the combined effects of dissolved organic carbon (DOC) and iron on their light-absorbing properties. Absorbance values at 400, 420 and 440 nm were obtained from samples with specific, known concentrations of DOC and Fe in artificial freshwater (see Section 3.1). The average of the absorbance values was then calculated.

Average absorbance (400-440 nm) was linearly correlated to both DOC ( $R^2 = 0.99$ ) and Fe concentration ( $R^2 = 0.99$ ; Fig. 5). The linearity of both DOC and Fe shows that they both follow the Beer-Lambert law (direct proportionality between absorbance and concentration). The different gradients suggest a different apparent total attenuation coefficient, which reflects how effective the absorbing species is at absorbing light of a specific wavelength. The higher the gradient, the higher the effectiveness. Comparing the gradients of the linear trends in Fig. 5 shows that Fe is 1.95 times more effective at absorbing light between 400-440 nm per unit mass concentration.

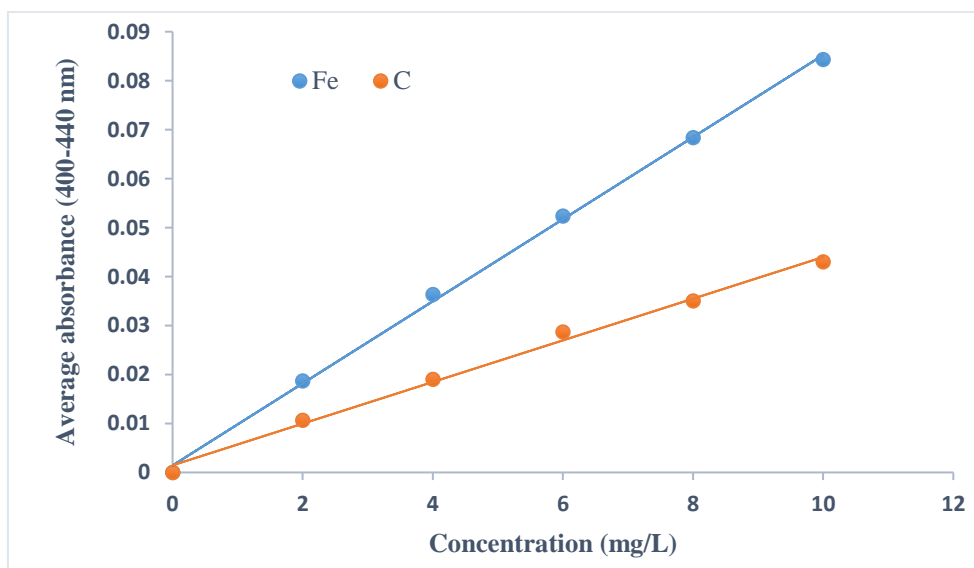


Figure 5: Average absorbance at (400-440nm) against the DOC (orange) and Fe (blue) concentration in units mg/L.

The average absorbance values obtained between 400-440nm of all samples were linearly correlated to the total concentration in mg/L (that is, concentration DOC + concentration Fe; Fig. 6). However, due to the differences in absorbing properties of DOC and Fe, the  $R^2$  value ( $R^2= 0.91$ ) is comparatively low, since similar total concentrations may have different proportions of Fe and DOC. This is shown by the spread of absorbance values for a given total concentration, an example highlighted with a red circle in Fig. 6. As shown for real water samples; it is, therefore, impossible to convert absorbance values into concentrations of DOC and Fe directly.

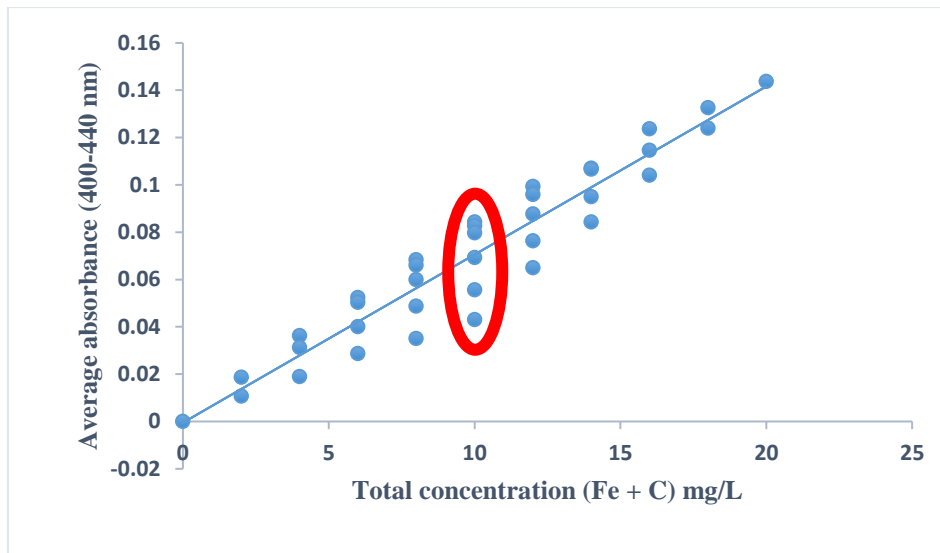


Figure 6: Average absorbance at 400-440 nm against total concentration (Fe + DOC) in mg/L. An example of different absorbance values at similar total concentrations is shown with a red circle.

In order to account for the difference in absorbing properties, total concentrations were recalculated into *effective* total concentrations, where the concentrations of Fe and DOC were recalculated according to their relative absorbing ability and then added together:

$$((\text{concentration Fe}) \times 1.95) + ((\text{concentration C}) \times 1) = \text{effective total concentration}$$

Replotting the average absorbance at 400-440 nm against the effective total concentration yields a stronger linear correlation ( $R^2 = 0.99$ ) than seen in Fig. 6 (Fig. 7). This confirms the validity of the observed differences in the absorbing ability of Fe and DOC per unit of mass concentration.

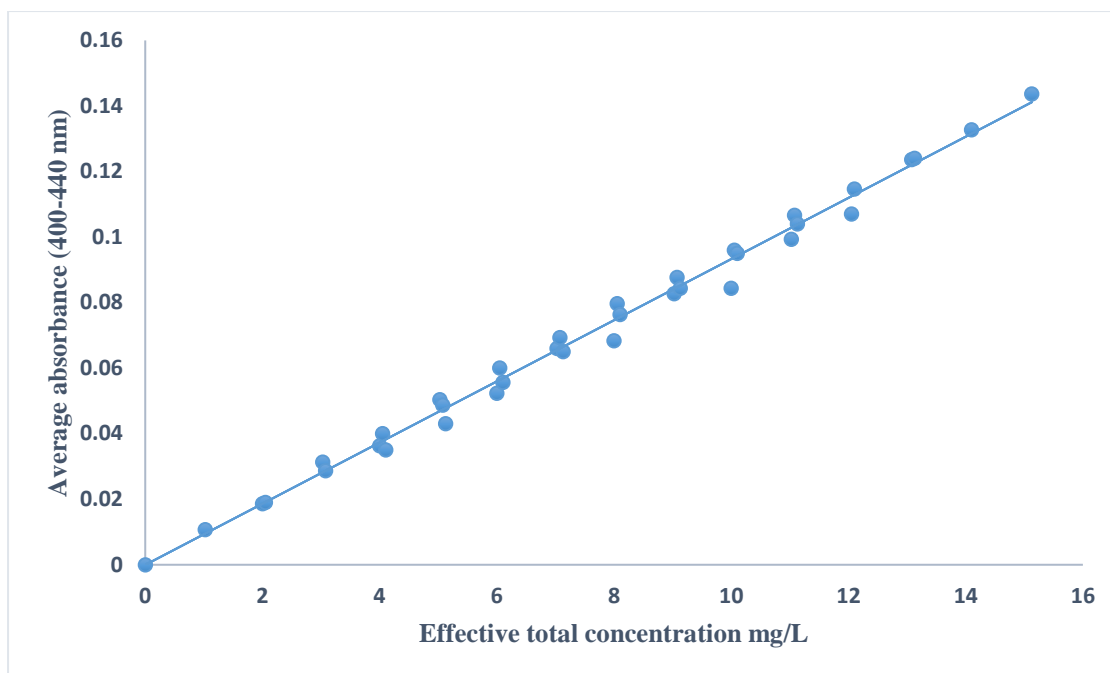


Figure 7. Average absorbance at 400-440 nm against effective total concentration (mg/L).

### 3.2.2. The enhancement of light absorption of Fe through complexation to DOC

To investigate the possible enhancement of light-absorbing properties of Fe through complexation to DOC, the absorbance values obtained for each experiment in each cell were compared to calculated *additive* absorbance. Additive concentrations were calculated by adding the absorbance values for the corresponding Fe and DOC concentrations where they were separated from each other (i.e. from samples obtained from the cells highlighted in green and red in Fig. 1).

$$\text{Absorbance (Fe in 0 mg-C/L DOC) + absorbance (DOC in 0 mg-Fe/L Fe)} \\ = \textit{additive absorbance}$$

These values represent an absorption value which may be obtained if, when combined, Fe and DOC produce no net loss or gain in absorbing ability when complexed in solution. The degree of change in absorbing properties was therefore calculated as a percentage difference between the *real* absorbance of a Fe/DOC mixture and the corresponding *additive* absorbance:

$$(\textit{real absorbance} - \textit{additive absorbance}) / \textit{additive absorbance} \times 100 = \\ \text{\% difference in absorbance compared to additive absorbance.}$$

The values for % difference in absorbance with increasing Fe concentrations under constant DOC concentrations showed that DOC/Fe mixtures had an enhanced absorption compared to when they were separate (Fig. 8). At lower DOC concentrations (C = 2 mg-C/L and 4 mg-C/L), the % difference increased up to a certain point and then decreased. However, the “peak” moved to a higher Fe concentration. Within the experimental design, similar peaks were not observed when the DOC concentration was higher (6, 8 and 10 mg-C/L).

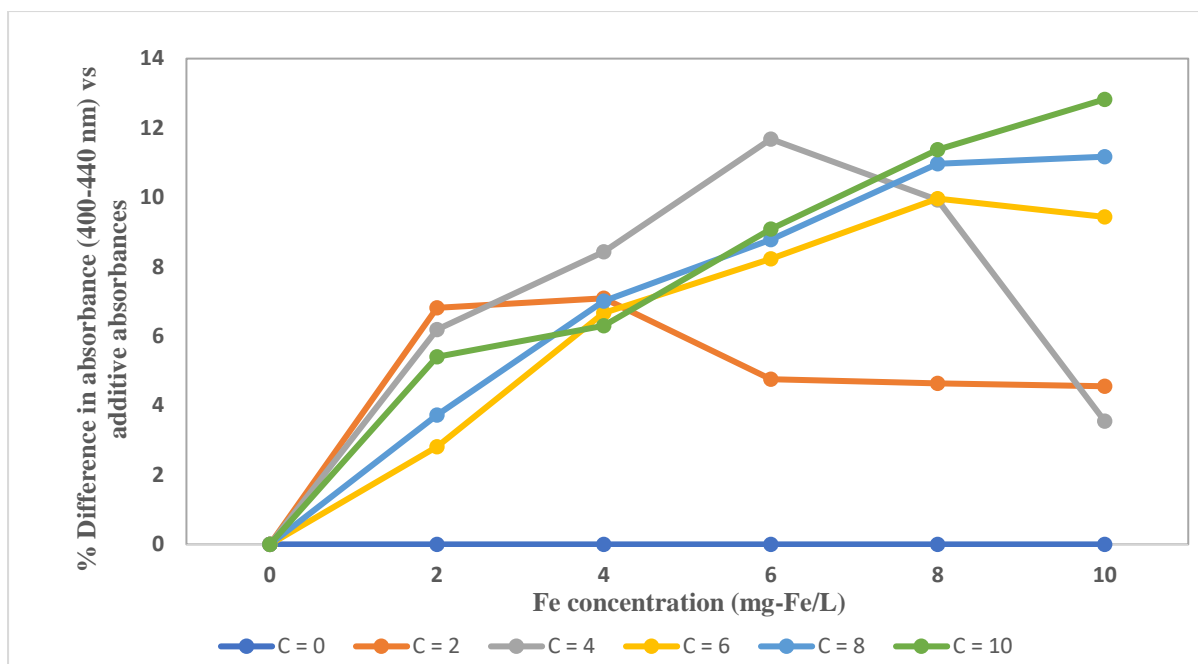


Figure 8: Fe concentrations (under different DOC concentrations; 0 (dark blue), 2 (orange), 4 (grey), 6 (yellow), 8 (light blue) and 10 (green) mg-C/L) against % difference in absorbance compared to additive absorbance.

Under the assumption that a peak would be observed for each DOC concentration, a polynomial trendline was modelled (second order for all except C = 2 mg-C/L, where a third order trendline was modelled) for each DOC concentration and forecasted to see at which Fe concentration a peak would be found (Fig. 9). The Fe concentration at each peak in experimental group where the DOC concentration was fixed were linearly correlated to the DOC concentration ( $R^2 = 0.99$ ; Fig. 10). This value represents the C: Fe ratio at which the enhancement of light absorption was greatest. The average C: Fe ratio of all experimental groups was  $0.67 \pm 0.064$ .

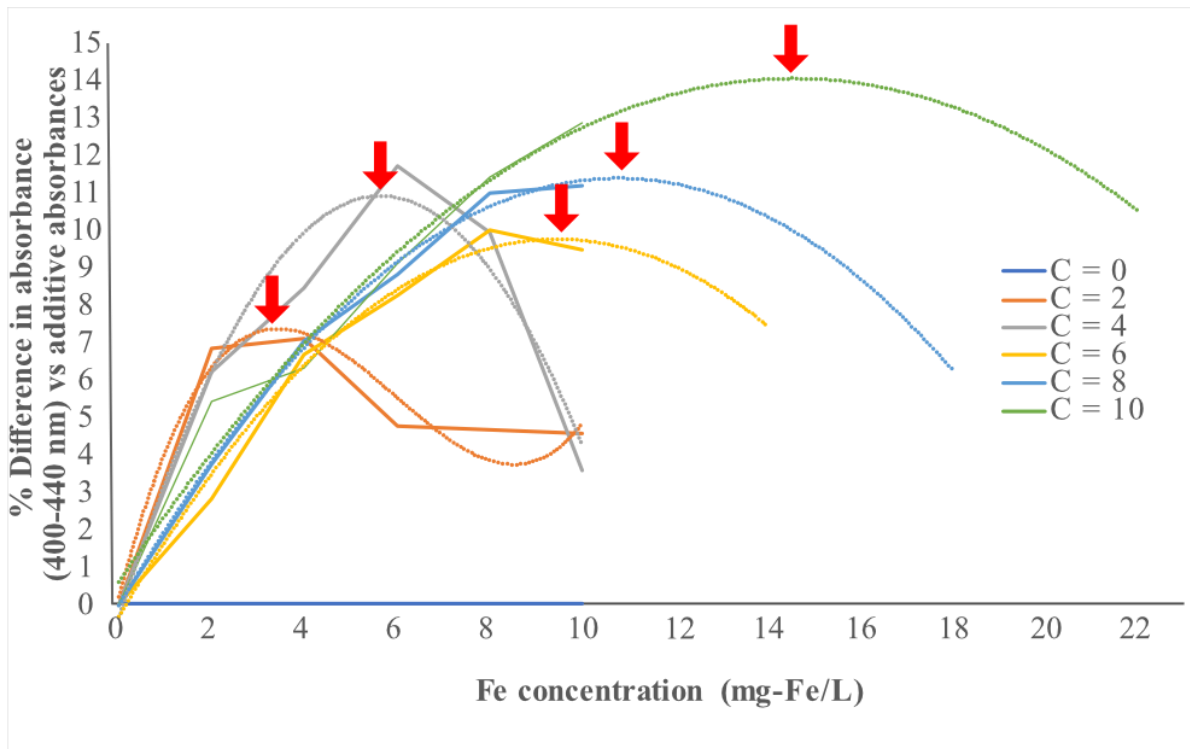


Figure 9: *Fe concentrations (under different DOC concentrations; 0 (dark blue), 2 (orange), 4 (grey), 6 (yellow), 8 (light blue) and 10 (green) mg-C/L) against % difference in absorbance compared to additive absorbance with forecasted polynomial trendlines. The peak of each trendline is shown with red arrows, representing Fe concentrations at which the absorption enhancement was highest.*

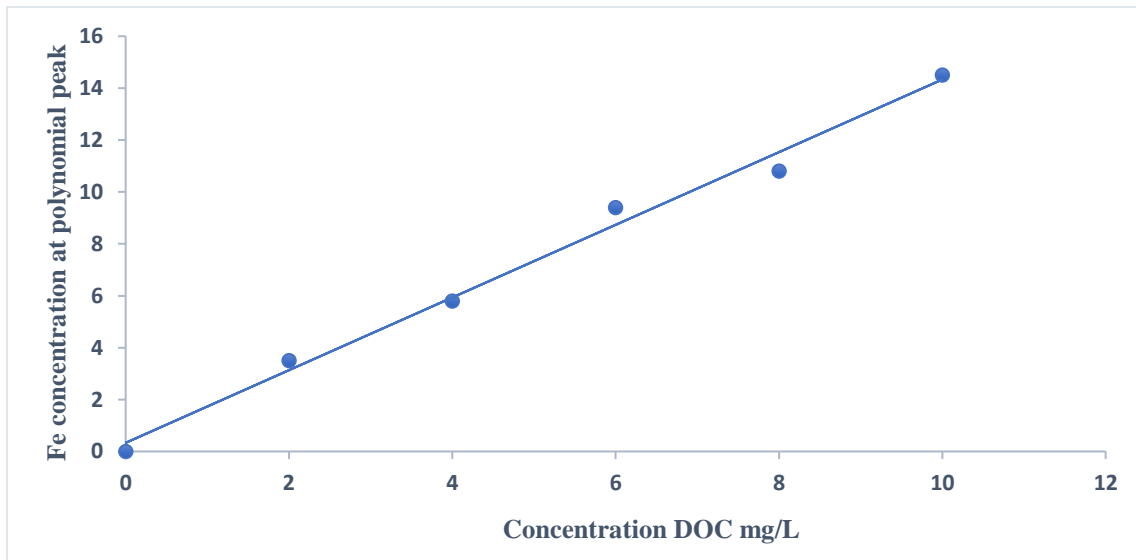


Figure 10: *DOC concentration (mg-C/L) against the Fe concentration (mg-Fe/L) at the peak of the (forecasted) polynomial trendlines obtained from experimental groups with different fixed DOC concentrations from Figure 9.*

Polynomial trendlines also revealed the maximum possible enhancement in absorption through the formation of Fe-DOC complex according to the Fe concentration when the C:Fe ratio is 0.67 (Fig. 11). These values were taken as the % difference in absorbance at each trendline peak (as shown in Fig. 9). Generally, the lower the Fe concentration, the lower the possible enhancement observed. A trend was plotted as an exponential decay, not linear, since a zero Fe concentration would logically lead to a zero enhancement in absorbance (i.e., intercept at the origin). The trendline represents an interpolation from experimental Fe concentrations (2-10 mg-Fe/L) down to realistic Fe concentrations found in the freshwater samples (0.37-2.16 mg-Fe/L; Table 2), not taking into account DOC concentrations. It is, therefore, possible to estimate that the enhancement of light absorption due to Fe-DOC complex formation in natural freshwater Fe levels is 1-7% if the C:Fe ratio is 0.67.

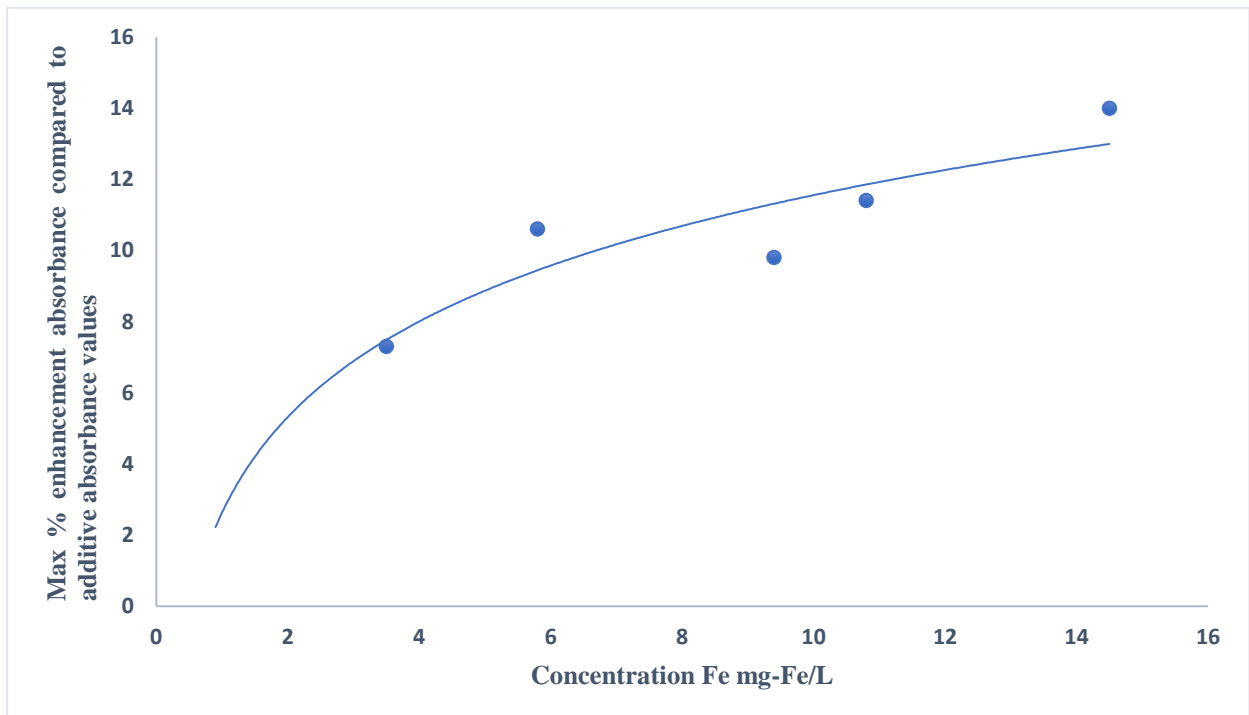


Figure 11: A curve plot showing the max percentage enhancement absorbance compared to additive absorbance value against the concentration of Fe in mg/L.

#### 4. DISCUSSION

The purpose of using natural water samples collected from Lake Bolmen is to analyse the data and use the data to formulate values, such as pH and conductivity, for producing artificial freshwater and to aid in the parametrisation of C and Fe concentration (see section 3.1.2). It was also beneficial to obtain a new analysis of water samples to check that the ecological status of Lake Bolmen has not changed significantly since last year (Borgert and Liess, 2023). The C: Fe ratio indicates the amount of carbon relative to Fe in the sample locations. For example, Tiraholm has a high C:Fe ratio of (37.5), which shows high carbon prevalence to Fe (Table 2), influencing the solubility and mobility of Fe in the water. Muråns dike shows a low C:Fe ratio of (11.6) (Table 2), which shows a relatively high Fe prevalence compared to C and low pH, which is suggested to affect the solubility and oxidation of Fe and the interaction with DOC. The conductivity values indicating the presence of dissolved ions in the water show that Piksberg has the highest conductivity value (100  $\mu\text{S}/\text{cm}$ ), which can be suggested to affect the complexity and interaction of Fe and DOC in the water (Lønborg et al., 2020). Because these may have a meaningful effect on iron solubility and the formation of Fe-DOC complexes, then they should be explored in further studies.

When comparing the relative contribution of Fe and DOC (expressed as C) as obtained from the laboratory studies, Fe is 1.95 times more efficient at absorbing (400-440 nm) than DOC per mass unit concentration (Fig. 4). This is in agreement with previous research which suggested that Fe has a more significant impact on brownification per unit of mass concentration than DOC (Kritzberg and Ekström, 2012). However, although Fe is shown to be better at absorbing light, in the natural samples, DOC concentrations are markedly higher than Fe (cf. C: Fe ratios are higher than 10). DOC, therefore, has the most significant contribution to brownification. I observed an enhancement of Fe due to the complexation with DOC (as seen in Fig. 11).

This enhancement reached a maximum at a very low C: Fe ratio ( $0.67 \pm 0.064$ ). This maximum represents the point at which Fe is saturated with DOC, that is, no more space or charge density available to accommodate more DOC molecules (See section 3.2.2). The maximum enhancement observed due to complexation between Fe and DOC in the Fe concentrations range in natural freshwater (0.4 – 2.4 mg-Fe/L) and at a C:Fe ratio of 0.67 was 1-7% (Fig. 11). However, in natural freshwater samples where the C: Fe ratio exceeds 10, any enhanced absorbance of light by the formation of Fe-DOC complexes will have a negligible contribution to brownification. That is, the absorbing properties of natural freshwater leading to a brown colour is dominated by DOC.

I used sodium salt of lignic acid as the modelled DOC compound due to its high solubility and the presence of complex-forming carboxylic acid groups. Due to differences in the chemistry of molecules, it would be suggested that there could be a different result therefore, it is interesting to repeat. However, DOC is more complex than just a content of lignic acid, where humic acid is a major contributor. Humic acids are highly complex and with a wide range of complex-forming functional groups such as carboxyl acid, alcohol and amine (Deng et al., 2021) (Wu et al., 2017); humic acids are, therefore, also suitable to model DOC molecules

(Klučáková, 2022). It would be interesting to repeat this experiment in the future using either humic acids themselves or a mixture of lignic acid and humic acids to understand better the Fe-DOC interactions which contribute to brownification.

The water samples collected for this experimental research were across a wide range of pH values. The wide range of pH values indicates that there will be large variability across a single water system regarding the accumulation and positioning of DOC and the extent of photochemical reactions occurring in the freshwater system. The pH will reflect the variability of the inhomogeneity, which shows different parts and different types of chemistry taking place at any given time (Porcel et al., 2009). pH influences the speciation of Fe and the ionisation of functional groups on the DOC (Shirokova, 2022). During the ionisation, the functional group of DOC have fewer negative charge ions, which influences the binding of  $\text{Fe}^{2+}$  (Bhattacharyya et al., 2019). Ferric ion ( $\text{Fe}^{3+}$ ) is more soluble at lower pH levels and can form more stable complexes with DOC, whereas at higher pH levels,  $\text{Fe}^{3+}$  tends to precipitate unless it complexes with DOC (Pierson-Wickmann et al., 2021). Because of stability concerns of lignic acid at lower pH values, 6.8 was selected as the pH for artificial freshwater during my study (see section 3.1.2). There is a possibility of using a wide range of pH to replicate this study and investigate the effect of pH on the interaction of Fe-DOC.

Since the interaction of DOC and Fe is the major factor promoting freshwater brownification, more studies should be conducted to develop ways to mitigate the inflow of these molecules in the freshwater or suggest an industrial means of treating freshwater brownification. Water browning affects the social lifestyle humans derive from freshwater, for example, making the water unhealthy for swimming and fishing and increasing the cost of drinking water (Williamson et al., 2015). Water browning alters the ecological interaction of aquatic inhabitants by interrupting the behaviour of zooplankton and phytoplankton in freshwater (Williamson et al., 2015). An innovative and sustainable technique for treating water browning should be considered to obtain good water quality and reduce drinking water costs. This research may be used for future reference studies when investigating the relationship between Fe and carbon in water and analysing the effect of Fe and DOC on brownification, not only to get insight from a concentration perspective but to understand the absorbance perspective of both Fe and DOC.

## 5. CONCLUSION

The presence of DOC and Fe in the water is the major cause of water browning in southern Sweden. This brown water has altered the ecological and social essence of freshwater and has increased the cost of drinking water. Climate change, poor land use management, atmospheric acid deposition and hydrological alteration are responsible for the mobility of DOC and Fe in freshwater. The interaction of DOC and Fe to form a DOC-Fe complex enhances light absorption and changes the water colour to brown. The increase in carbon concentration will enhance Fe to absorb light but to a very low point. DOC will keep enhancing Fe until DOC is saturated; any addition of Fe will be masked off or precipitated out. Undoubtedly, DOC assists with the transport of Fe through complexation. However, any contribution to brownification by the complexation on the enhancement of Fe's light-absorbing properties can be disregarded. Minimising these human activities or providing a sustainable and innovative solution that will help mitigate some of the ongoing environmental issues like brownification should be encouraged and supported by the government. Sustainable freshwater management practices should be encouraged and implemented to prevent the influx of DOC or Fe in the water bodies. Understanding the carbon-to-iron (C:Fe) ratio will help the environmental specialist and chemists detect the relative amount of Carbon to Fe and employ means for optimising water treatment processes.

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