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ABSTRACT

FLUORESCENCE SPECTROSCOPY FOR THE CHARACTERIZATION OF HUMIC AND FULVIC ACIDS AND THEIR DISINFECTION BY-PRODUCTS FORMATION POTENTIAL

by
Ishvinder H Kochar

Natural Organic Matter (NOM) plays a major role in the formation of undesirable organic by-products following disinfection/oxidation of drinking water. It is suspected that most precursors to disinfection by-products (DBPs) are humic although non-humic substances have not been studied and are suspected of also contributing to DBPs. NOM reacts with many of the disinfectants used to treat drinking water, such as chlorine, chloramine, and ozone, to form a variety of DBPs. Many of these DBPs have adverse health effects in humans (i.e. carcinogenic or mutagenic effects). The primary DBPs of concern include the trihalomethanes (THMs), haloacetic acids (HAAs), and haloacetonitrile (HANs). The Spectral Fluorescent Signatures (SFS) technique was developed for the identification of the humic acids (HA), fulvic acids (FA) and non-humic substances by fluorescence. The SFS is the total sum of emission spectra of a sample at different excitation wavelengths, recorded as a matrix of fluorescent intensity in coordinates of excitation and emission wavelengths, in a definite spectral window. For the characterization of NOM in raw water, and determination of DBP formation reactivities, samples were prepared using river HA and FA, soil HA and FA and peat HA and FA in varying concentrations from 0.5 to 10 ppm. UV-254, TOC, DBP formation potential, SFS tests were conducted on each matrix of samples.

FLUORESCENCE SPECTROSCOPY FOR THE CHARACTERIZATION OF HUMIC AND FULVIC ACIDS AND THEIR DISINFECTION BY-PRODUCTS FORMATION POTENTIAL

by
Ishvinder H Kochar

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APPROVAL PAGE

FLUORESCENCE SPECTROSCOPY FOR THE CHARACTERIZATION OF HUMIC AND FULVIC ACIDS AND THEIR DISINFECTION BY-PRODUCTS FORMATION POTENTIAL

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To
GOD "THE ALMIGHTY"
And
my beloved family

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TABLE OF CONTENTS

TABLE OF CONTENTS
(Continued)

TABLE OF CONTENTS
(Continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES
(Continued)

Figure **Page**

LIST OF FIGURES
(Continued)

Figure **Page**

LIST OF FIGURES
(Continued)

Figure **Page**

LIST OF FIGURES
(Continued)

Figure **Page**

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Figure **Page**

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(Continued)

Figure **Page**

CHAPTER 1

INTRODUCTION

1.1 Objective

The objective of this thesis was the development of a rapid analytical technique for the determination of natural organic matter fractions (NOM) in natural water, and to be used as a predictive tool for disinfection by-product (DBP) formation due to the presence of humic substances in drinking water sources. The thesis discusses ongoing research in the development of a rapid analytical technique for the determination of three NOM fractions in natural water (i.e. humic acid, fulvic acid and non-humic substances). The technique currently being developed called "Spectral Fluorescent Signatures" (SFS) has the potential to differentiate humic from non-humic and humic acid from fulvic acid originating from point sources or non-point sources inputs. The research conducted to meet these objectives was part of an ongoing study intended to correlate natural organic matter fractions (humic substances and non-humic substances) in natural water to the total organic carbon (TOC), ultra-violet absorbance at 254 nm (UV-254), and DBPs using SFS. Funding for the project was provided by the New Jersey Department of Environmental Protection (NJDEP) (Taha F. Marhaba, Principal Investigator). This technique would be of interest to the regulatory agencies, water treatment purveyors, wastewater treatment authorities, and consultants.

The aquatic samples consisted of standard Fulvic Acid (FA) and Humic Acid (HA) from the Suwannee river, sampled near Fargo, Georgia, U.S.A. both isolated by XAD-8 resin technique and obtained from International Humic Substances Society

(IHSS) (St. Paul, MN). Standards for peat HA and FA, soil HA and FA were also obtained from IHSS. Working solutions were prepared from the river HA and FA, soil HA and FA, peat HA and FA standards for varying concentrations up to 10 ppm. Mixtures of various proportions of river HA and FA were also prepared.

In an attempt to characterize NOM fractions in natural water into HA, FA and non-humic substances, a sample matrix containing HA and FA at various concentrations were analyzed. Four parameters were utilized namely: a) major and minor peak in terms of the excitation and emission coordinates, (b) the fluorescent intensity at these locations, (c) the approaching gradient at these peaks or slope, and (d) the integration of the peak spectra or area under the curve where the peak occurs. To examine the reactive components of organic matter in relation to DBP formation (using TOC, UV, SFS as parameters), the HA and FA standard solutions were chlorinated with 100 ppm Cl_2 using calcium hypochlorite. These chlorinated samples were incubated for seven days at 25°C. After seven days, the samples were dechlorinated with ammonium chloride and analyzed for trihalomethane (THM), haloacetonitrile (HAN) and haloacetic acid (HAA) formation by liquid-liquid extraction followed by gas chromatographic analysis. Fluorescence measurements were taken for HA and FA samples before and after chlorination to provide a visual means of determining which organic fractions react to form DBPs. Data from both the analysis were analyzed to determine the relationship between fluorescence and DBP formation.

CHAPTER 2

BACKGROUND

2.1 Drinking Water Concerns

Natural organic matter (NOM) is a term used to describe the organic material typically present in natural water. The role of humic substances in water chemistry is receiving increasing attention because they are known to complex trace metals. They are a source of methyl groups for the production of chlorinated methane's in water treatment and are implicated in the complexation or solubilization of pesticides and hydrocarbons in the aqueous environment (Reuter *et al.*, 1977).

NOM plays a major role in the formation of undesirable organic by-products following disinfection/oxidation of drinking water. NOM can be divided into humic and nonhumic substances. Humic substances are among the major constituents of NOM in natural water and are more hydrophobic in character compared to non-humic substances, comprising of HA and FA (Collins *et al.*, 1986). It is suspected that most precursors to DBPs are humic although non-humic substances have not been studied well and are suspected to also contribute to DBP formation. NOM reacts with many of the disinfectants used to treat drinking water, such as chlorine, chloramine, and ozone, to form a variety of DBPs (Marhaba *et al.*, 1998). Many of these DBPs have adverse health effects in humans (i.e. carcinogenic or mutagenic effects). The primary DBPs of concern include the THMs, HAAs, and HANs (Marhaba *et al.*, 1998).

2.2 Humic and Non-Humic Substances

NOM can be divided into humic and nonhumic fractions; the humic fraction is more hydrophobic in character. The nonhumic fraction is less hydrophobic in character and comprises hydrophilic acids, proteins, amino acids, and carbohydrates. Adsorption chromatography using a XAD-8 resin is utilized to fractionate the dissolved organic matter into hydrophilic and hydrophobic fractions (Thurman *et al.*, 1984; Aiken *et al.*, 1979). The hydrophobic fraction of the aquatic organic matter isolated by adsorption chromatography represents an "operational" definition of aquatic humic substances (Davis *et al.*, 1980; Thurman 1984; Weber 1975; Thurman *et al.*, 1984; Aiken *et al.*, 1979). The hydrophilic fraction of the aquatic organic matter, represented by the material not adsorbed onto the XAD-8 resin, is "operationally" defined as non-humic substances, which comprise the remainder of the dissolved organic matter. Simple carbohydrates, uronic acids, and hydroxy acids represent examples of typical hydrophilic molecules, which will not adsorb to the XAD-8 resin. (Collins *et al.*, 1986).

2.3 The Nature of Humic Substances

Humic substances are significant in aquatic systems for several reasons. They impart a brown/yellow color to the water (Black and Christman, 1963; Packham, 1964; Bennett and Drikas, 1993), they can complex with metals (Reuter and Perdue, 1977) and organic pollutants such as pesticides (McCarthy, 1989), and most significantly, they are the precursors of mutagenic halogenated compounds in water formed after chlorination (Bellar *et al.*, 1974; Rook, 1974, 1977).

Humic substances are among the major constituents of NOM in natural water and are more hydrophobic in character, comprising of humic acids (HA) and fulvic acids (FA) (Collins *et al.*, 1986).

The humic substances are a complex group of organic materials whose structure is not well defined. Fujita *et al.* (1998) defined HA, as the fraction of the concentrated DOM that is insoluble at pH < 2, and the FA as the fraction that remains soluble at pH<2 and is sorbed onto Amberlite XAD-8 resin. The H:C ratio indicates the degree of unsaturation in the carbon skeleton; a low H:C ratio reflects greater unsaturated and aromatic character (Thurman, 1985). Steelink (1985) reported that H:C ratios are clustered around 1.0 for most soil and aquatic humates and fulvates. Ratios above 1.3 indicated that the material may be a nonhumic substance. Another definition of HA and FA as defined by Thurman *et al.* (1981) is humic substances, which are soluble in base and acid (i.e. FA) or soluble in base and insoluble in acid (i.e. HA). In general, FA are low molecular weights, less hydrophobic compared to HA and highly soluble, HA are of high molecular weight, more hydrophobic than and not as soluble as FA (Rebhun *et al.*, 1996).

2.4 Disinfection By-Products

Water intended for potable purposes invariably contain microorganisms that may be harmful to human health. For this reason, it is necessary to treat water with a disinfectant. Various available disinfectants are chlorine, ozone, chlorine dioxide, potassium permanganate and chloramines. Chlorine has been the disinfectant of choice for nearly 100 years and is used by majority of water treatment systems for the protection

against waterborne diseases. Addition of chlorine to water in the presence of naturally-occurring organic matter results in the formation of numerous disinfection by-products, which have the potential of causing adverse health effects (Marhaba *et al.*, 1998). Most of the organic halides are generated with chlorination, some of which are carcinogenic, mutagenic or possibly teratogenic (Simmon and Tardiff, 1978; Cantor *et al.*, 1978). Chlorine disinfection by-products are considered to be more of a concern than the by-products resulting from the use of other oxidants, such as ozone or chloramine. Chlorinated DBPs form when free chlorine (HOCl) is added to water. (Marhaba *et al.*, 1998). Chlorine acts as an oxidant and reacts with the natural organic matter (NOM) present. The generalized equation describing the formation of the halogenated DBPs is:

$$HOCl + Br^- + NOM \Rightarrow \text{THMs and Other Halogenated DBPs}$$

The major halogenated DBPs that are commonly identified from chlorine treatment are THMs, HAAs, HANs, cyanogen halides and halopicrins (Krasner *et al.*, 1989; Stevens *et al.*, 1989). Some of the major types of these DBPs are listed in table 1.

Table 1. Chlorinated DBPs
Source [Marhaba *et al.*, 1998]

Chemical Class	Chemical Compound
Trihalomethanes (THMs)	Chloroform
	Bromodichloromethane
	Dibromochloromethane
	Bromoform

Table 1. (Continued)

Chemical Class	Chemical Compound
Haloacetic Acid (HAAs)	Monochloroacetic Acid (MCAA)
	Dichloroacetic Acid (DCAA)
	Trichloroacetic Acid (TCAA)
	Monobromoacetic Acid (MBAA)
	Dibromoacetic Acid (DBAA)
Haloacetonitrile (HANs)	Dichloroacetonitrile
	Trichloroacetonitrile
	Dibromoacetonitrile
	Bromochloroacetonitrile
Cyanogen Halides	Cyanogen Chloride
	Cyanogen Bromide

In the absence of bromide ion (Br^-), only the chlorinated by-products are formed. In the presence of bromide ion, free chlorine (HOCl) rapidly oxidizes bromide ion to hypobromous acid (HOBr), which then reacts, along with the remaining HOCl, with NOM to produce the mixed chloro-bromo DBPs (Dore *et al.*, 1988; Marhaba, 1993).

It has been found that THMs and HAAs are the most common DBPs found in the treatment process (Krasner *et al.*, 1989; Grenier *et al.*, 1992). Early studies have mainly focused on formation of THMs (Kavanaugh *et al.*, 1980; Engerholm and Amy, 1983; Urano *et al.*, 1983; Adin *et al.*, 1991). The US Environmental Protection Agency (USEPA) has set a maximum contaminant level (MCL) of 100 μg/L for Total Trihalomethanes (TTHMs) and has proposed a new MCL of 80 μg/L in Stage 1 of the Disinfection/Disinfection By-Product Rule (D/DBP Rule) (USEPA, 1994). In addition to these standards, a MCL for HAA5 of 60 μg/L is proposed (USEPA, 1994). TTHMs is

defined as the sum of four individual THMs: chloroform, bromoform, dibromochloromethane and bromodichloromethane. HAA5 is defined as the sum of five HAAs listed in Table 1: MCAA, DCAA, TCAA, MBAA, and DBAA. Stage 2 of the D/DBP Rule may lower the MCLs for TTHMs and HAA5 to 40 μg/L and 30 μg/L, respectively (USEPA, 1994b). Hence, a determination of the organic substances responsible for forming the DBPs is important for the minimization of DBP formation in water treatment systems. But THMs only represent 5-20% of the total organic halide (TOX) formed during the chlorination process (Christan *et al.*, 1983). With recent advances in the identification of DBPs and their health effects, a greater appreciation for the non-THM organic halides exits (Reckhow and Singer, 1984; Christan *et al.*, 1983; Miller and Uden, 1983; Suffet *et al.*, 1976). The levels of THMs and HAAs formed upon chlorination of natural water depend on several operational conditions, such as chlorine dosage and free chlorine contact time, as well as water quality conditions, such as organic content, bromide, temperature and pH.

2.5 Fluorescence Spectroscopy

One potentially useful tool in the analysis of humic substances is fluorescence spectroscopy. Fluorescence occurs when radiation is absorbed, and the excited species formed loses part of its excess energy by a non-radiative means. Then the remaining energy is emitted as radiation. This radiation is of a longer wavelength and lower energy than that which caused the excitation. At the ground state, the molecule absorbs light and transits to the excited state. The molecule loses a portion of the exciting energy as vibrational energy, transits to a lower vibration level with no radiation emitted, and then

returns to the ground state while emitting a kind of optical energy. This is called "fluorescence". Figure 1 illustrates this phenomenon.

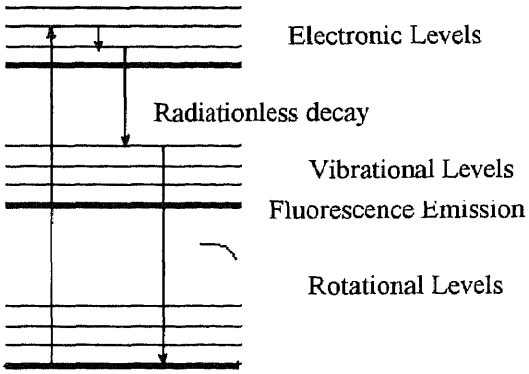


Figure 1. Fluorescence Phenomenon.
Source: [Kebbekus *et al.*, 1998] [Hitachi manual, 1988]

The typical method used to investigate the organic properties of water is UV absorbance (often at wavelength 254 nm). Fluorescence is, in essence, the reverse of absorption, as it measures the light that is absorbed and then released (Senesi, 1990). Conventional fluorescence spectra are obtained by using one of two spectroscopic modes. The emission spectrum is recorded by measuring the relative intensity of radiation emitted as a function of wavelength for a fixed excitation wavelength. Or, the excitation spectrum is recorded by measuring the emission intensity at a fixed wavelength while varying the excitation wavelength (Senesi, 1990).

Optical emission different from fluorescence may be observed during the analysis. This will often be one of three types of scattering effects: (1) Rayleigh scattering, (2) Raman scattering, and (3) Second-order Ray scattering. Rayleigh scattering will appear where the excitation wavelength is equal to the emission

wavelength (Senesi, 1990). Raman scattering will appear where the emission wavelength is slightly longer than the excitation wavelength. Second-order Ray scattering will occur where the emission wavelength is equal to twice the excitation wavelength. Scattering peaks are not caused by any organic materials that might be present in the solution [Hitachi Instruction manual, 1988]. Instead, scattering peaks are due to interaction between the light being used to analyze the sample and the solvent that the sample has been prepared in.

Table 2. Location of Raman Peaks for Typical Solvents
Source: [Hitachi Instruction manual, 1988]

Excitation	**Emission Wavelength**		
Wavelength	**Water**	**Ethanol**	**Chloroform**
248	271	267	------
313	350	344	346
365	416	405	410
405	469	459	461
436	511	500	502

Table 2 lists the location of Raman peaks for some typical solvents. Peaks due to scatter can be differentiated from a peak due to fluorescence by the fact that emission wavelength of a fluorescence peak remains constant for varying excitations, while a scattering peak will have a different emission wavelength for every excitation wavelength.

Fluorescence measurements involves the following components, shown schematically in figure 2: a source of excitation radiation (commonly a xenon discharge lamp), a device for selecting the desired excitation wavelength (generally

monochromators in spectrofluorimeters), a sample cell (preferentially a quartz cell), a device for selecting the desired emission wavelength (again a monochromator), a detector (almost universally photomultiplier tubes) and a readout device (generally a recorder output or, preferentially, a microprocessor for data analysis).

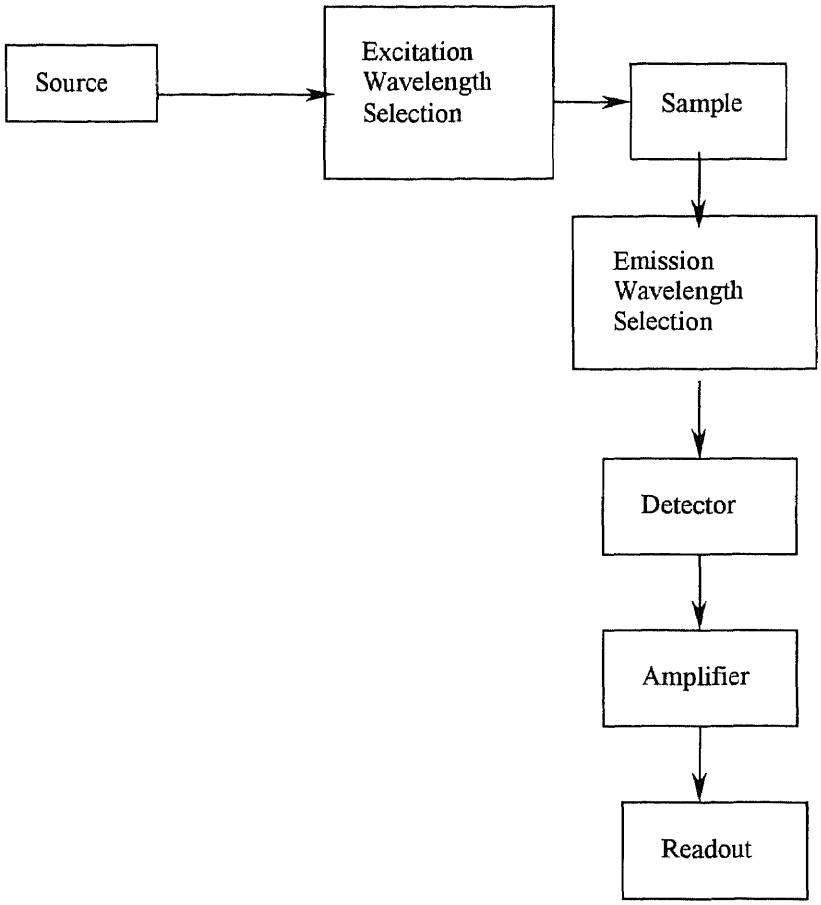


Figure 2. Typical Luminescence Instrument
Source [Senesi, 1990]

2.6 Spectral Fluorescence Signatures

The Spectral Fluorescence Signatures (SFS) is the total sum of emission spectra of a sample at different excitation wavelengths, recorded as a matrix of fluorescent intensity in coordinates of excitation and emission wavelengths, in a definite spectral window. Analysis of natural water samples using excitation-emission matrix spectroscopy (3-D fluorescence contouring) shows this technique to have the potential in characterizing the nature and source of NOM in natural water. The method of fluorescent diagnostics is an effective tool for the analysis of organics in water media. It has high sensitivity and allows analysis to be performed without pretreatment of water samples (Babichenko *et al.*, 1995). The analysis tool makes it possible to build an on-line diagnostic system, which measures the emission of (luminescence) the substance upon its return to the normal state. More importantly, because this quantified amount of energy is dependent on the structure of the compound molecules, it is considered a signature, which is unique to the nature of the compounds present.

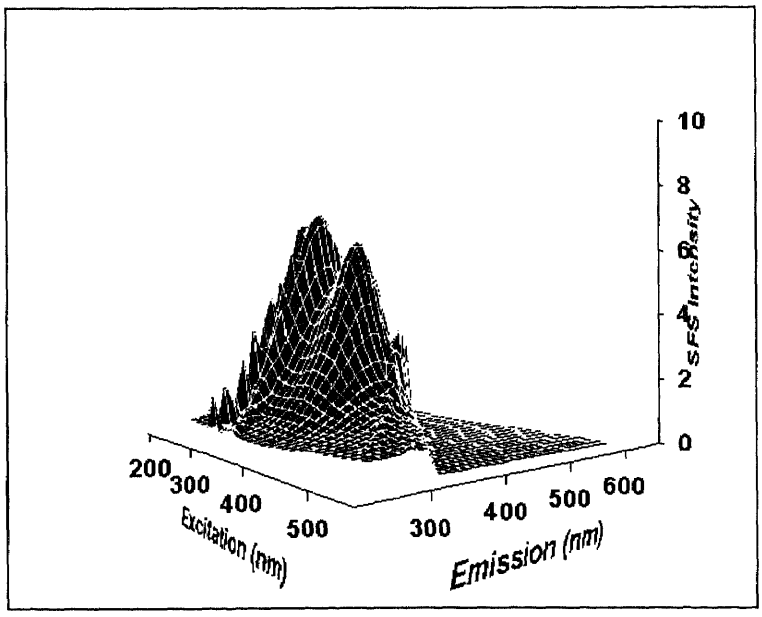


Figure 3. Spectral Fluorescence Signature

Figure 3 is an example of what the signature for a sample might look like. Correlations have been established between fluorescence and dissolved organic carbon (DOC), and fluorescence has been found to be a function of NOM source, pH and molecular weight (Owen *et al.*, 1993). The ultimate goal of this project was to establish correlations between fluorescence and TOC, UV-254 for acid standards in order to use them to predict concentrations, and ultimately reduce the formation of DBPs in water treatment facilities.

CHAPTER 3

METHODOLOGY

3.1 Experimental Objectives

To meet the research objectives discussed in Chapter 1, TOC, UV-254, SFS, DBP formation potential tests were conducted on a series of river HA and FA, soil HA and FA and peat HA and FA for concentrations varying from 0.5 to 10 ppm. A model of SFS database was created using the working samples from the river HA and river FA standards.

3.2 Materials

All the chemicals used in this investigation were of the highest quality available. The HA and FA standards were purchased from IHSS (St. Paul, MN), and were used in the condition in which they were received. Standards used for the calibration of instruments were purchased from Ultra Scientific Corp. (N. Kingstown, RI), Aldrich Chemical Corp. (Milwaukee, WI), Supelco Corp. (Bellefona, PA), or Sigma Corp (St. Louis, MO). Pure forms of the DBPs were purchased from Aldrich Chemical Corp. (Milwaukee, WI), and reagents were purchased from Fisher Scientific Corp. (Pittsburg, PA). HA and FA standards were prepared in the laboratory and stored at 4 °C.

3.3 Total Organic Carbon (TOC) Analysis

TOC of the samples were conducted in accordance with the persulfate/100°C method (Standard Methods No. 5310-D, 1995). TOC-700 analyzer (O.I. Corp., College Station, TX.) was used. 5% phosphoric acid was used to first acidify the sample, which was then purged of total inorganic carbon (TIC) by nitrogen. Sodium persulfate was subsequently introduced as an oxidant in the process for the oxidation of the organic compounds at 100 °C. As CO_2 is purged and trapped at the end of the oxidation process, an infrared photometric beam was used for the analysis of carbon mass. The analyzer was regularly calibrated with 1000-ppm potassium hydrogen phthalate (KHP) standard in either the TIC or TOC calibration mode, as recommended by the manufacturer. The instrument was equipped with an auto-sampler to ensure an identical injection for all samples. The calibration parameters were stored in the instrument's memory, and data was logged using the attached printer. The data from the printout was logged in a spreadsheet for analysis.

3.4 Fluorescence Spectroscopy

Fluorescence measurements were accomplished by the use of the Hitachi F-3010 spectrophotometer (Tokyo, Japan) equipped with 150 W ozone free xenon lamp. This instrument had single monochromators on both excitation and emission spectrometers. The blaze wavelength was 300 nm for excitation grating and 400 nm for the emission grating. Both grating had a blaze density of 900-groove mm^{-1}. The sample was recorded in a standard 1-cm quartz cuvette. Each spectra signature was formed out of 26 emission spectra which were subsequently measured at excitation every other 12 nm in the 225-

525 nm range. The start wavelength of the emission scans (λ_{em} = 249 nm to 635 nm) at each excitation wavelength was offset by 24 nm ($\Delta\lambda = \lambda_{em} - \lambda_{exc} = 24$ nm).

Raw data was recorded by a computer in the form of *.prn files. The program was set for an optimal stepwise increment of 12 nm both with respect to the excitation as well as the emission. Raw data was then fed into Grams32™ version 5 (Graphic Relational Array Management System) (Galactic Inc, Mass.), which converted each *.prn file into *.spc file. Each spectra *.spc file was linked together via a multi-file utility from which a full 3-D spectra (i.e. SFS) could be viewed at any desired angle. The multi-file spectra could be integrated for the total volume under the curve (using a spreadsheet). Spectra subtraction could also be done to screen out the known undesirable components of the total spectra. These were among the math functions that were employed in this research. The program was also designed to scan the test specimen in either of the 2 modes-forward mode from low excitation wavelength (high-energy state) to high excitation wavelength (low energy state) or reverse mode. This research was done on reverse-mode scanning to minimize the exposure of the sample to low-wavelength radiation and thereby minimize photodegradation. Our data showed that, for the equipment being used, either mode of scanning operation was equally acceptable.

Blank samples consisting of deionized and organic free water were used to identify scattering peaks and serve as a baseline reading. All spectra data were corrected for scattering.

3.5 UV-254 Analysis

UV-254 analysis was conducted using the Varian DMS 300 UV-Visible Spectrophotometer (Mulgrave, Victoria, Australia) using 1-cm path length. The absorbance mode was used for the analysis at the fixed wavelength of 254 nm. UV light source was used for this purpose (monochromator between 310 and 190 nm). The instrument was calibrated using the dichromate solution (Varian Operating Manual, 1987). The instrument was referenced against organic water blanks for the analysis of the actual samples.

3.6 Disinfection By-Products Formation Potential

The DBP formation potential (DBPFP) was obtained when high chlorine doses were applied for 1 week, thus enabling precursors to react. It is a measure of the precursor concentration. The ultimate goal of this investigation was to predict the formation of disinfection by-products using the SFS of a sample. To investigate this, a sample matrix of HA and FA was prepared. SFS of the samples was done using the procedure discussed. The samples were buffered to a pH between 6-7 and chlorinated by a concentration of 100-ppm Cl_2 using calcium hypochlorite. These chlorinated samples were incubated for 7 days at 25 °C. After the incubation period, the samples were dechlorinated with ammonium chloride and analyzed for THMs, HANs and HAAs using liquid-liquid extraction gas chromatography (GC), as described in EPA Method 551.1 and 552.2. In addition to this analysis, a post-chlorination SFS was developed for comparison with the pre-chlorination SFS.

The liquid-liquid extraction GC analysis was performed using a Varian 3400 Gas Chromatograph (Walnut Creek, CA) equipped with two electron capture detectors (ECDs) and an autosampler to ensure that the injection procedure is identical for all samples. The primary column was a DB-1 Column and the confirmation column was a DB-1301 Column from J&W Scientific (Folsom, CA). Data was collected using a computer equipped with PC Minichrom™ software (Cheshire, England). The Minichrom software was used to store the calibration and analytical parameters required for this method.

3.7 Quality Assurance and Quality Control

To ensure the validity of the results of this investigation and to identify the source of any errors, several quality assurance and quality control (QA/QC) measures were utilized. In addition to the fluorescence QA/QC, the TOC analysis and the THM/HAN/HAA analysis both require additional QA/QC protocols.

For all analysis, reagent blanks were tested to ensure there were no impurities or interference's that would alter the results in some unexpected way. Duplicates were run for approximately 10 percent of all samples to ensure that the analysis is repeatable and to determine if any errors were undetected in the experiment. The calibration of all instruments was checked on a regular basis by running samples of known concentrations to determine if recalibration was required.

The THM/HAN analysis required two additional QA/QC measures. Decafluorobiphenyl was added to each sample prior to the liquid-liquid extraction for use as a surrogate standard. In addition to this standard, 4-Bromofluorobenzene (4-BFB) was

added to each sample after the extraction for use as an internal standard. The surrogate standard was used to determine the accuracy of the extraction procedure by comparing its known concentrations with the concentration determined by the GC. The internal standard was added in identical amounts to the extract for each sample. The response recorded from the ECD for the internal standard was used to quantify the amount of other materials present in the sample. For the HAA analysis 2,3-Dibromopropionic Acid was used as a surrogate standard and 1,2,3-Trichloropropane was used as an internal standard.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Scope of Experiments

The results of the experiments discussed in Chapter 3 are presented here in the following six sections. Section 4.2 contains a discussion on the development of a technique to characterize the humic acid, fulvic acid and the non-humic substances in a natural water sample making use of the acid standards. Section 4.3 consists of the relationship between river HA and FA in the analysis of its mixtures. Section 4.4 describes a way to characterize the NOM fractions regions in natural water sample using the results of section 4.2. The relationship between TOC and fluorescence is shown in section 4.5 and the relationship between UV-254 and TOC is presented in section 4.6. Section 4.7 contains a discussion on the relationship between fluorescence and DBP formation in HA and FA along with the relationships between DBP formation and UV-254, DBP formation and TOC and DBP and specific absorbance (ratio of UV-254 and TOC).

4.2 Identification of NOM Fractions Using SFS

Using the working samples from the river HA and river FA standards, a model of SFS database was created. Ten concentrations were established from each standard ranging from 0.5 ppm to 10 ppm. Each concentration was then subjected to a fluorescent spectrophotometric analysis. By developing the SFSs for these standards, it was possible to visually identify the types of organics present in a natural water sample. Data from

each SFS analysis was fed in as part of a 20-file SFS database (ref. Table 2) whose use and results will be discussed next. Additional SFS figures for River HA and River FA can be viewed in Appendix A. SFS figures for Peat HA and FA and Soil HA and FA are shown in Appendix B.

Qualitatively, the two fractions can be identified by examining the following spectral fluorescent signatures shown in figures 4-9.

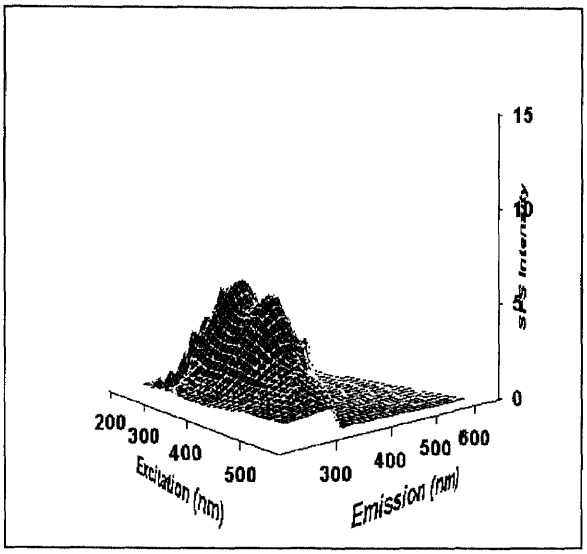


Figure 4. River Humic Acid 10 ppm

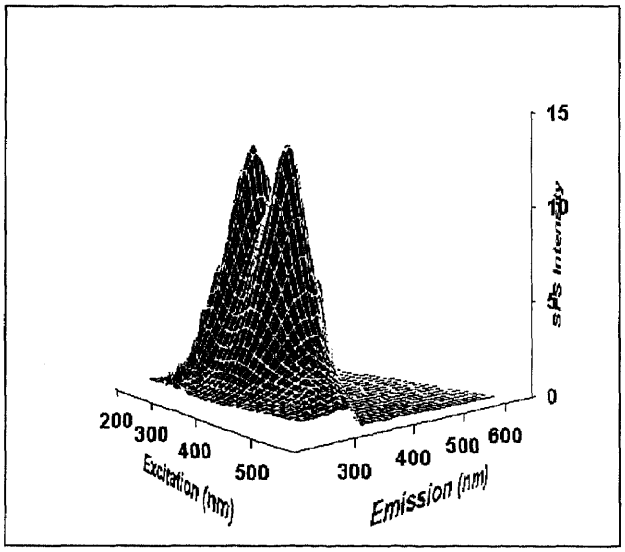


Figure 5. River Fulvic Acid 10 ppm

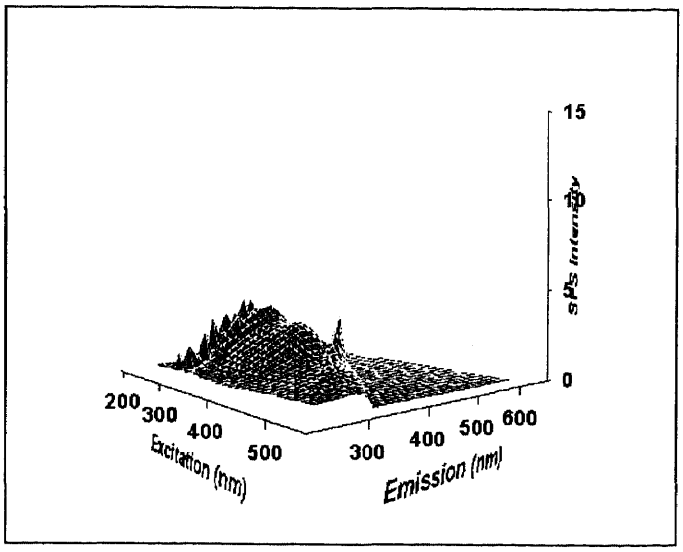


Figure 6. River Humic Acid 4 ppm

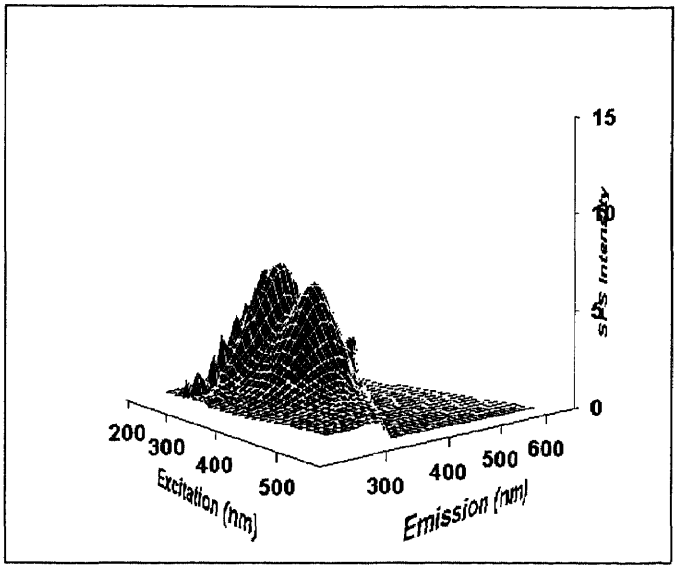


Figure 7. River Fulvic Acid 4 ppm

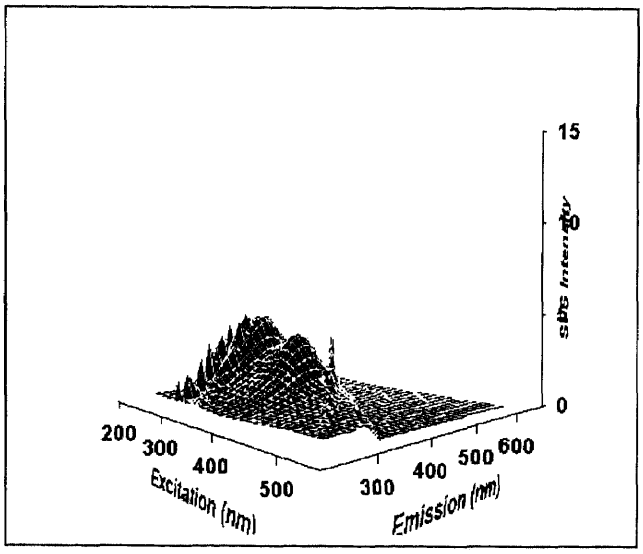


Figure 8. River Humic Acid 2 ppm

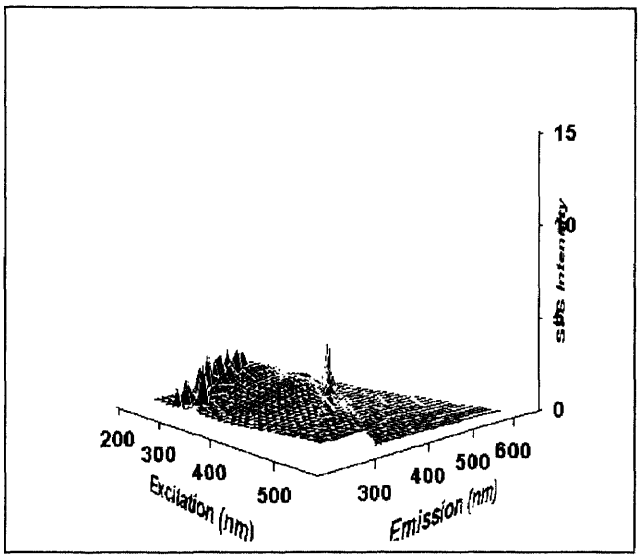


Figure 9. River Fulvic Acid 2 ppm

The two fractions of humic substances, HA and FA, were characterized by double-peak phenomena in an overlapping fluorescing region. They could be well distinguished based on the factors such as fluorescent intensity, slopes and area under a specific spectrum. Although they had similar fluorescence regions, an examination of the spectra did show that slopes pertaining to the peaks of FA fractions were, on average, 70% larger than those of the HA fractions (ref. Table 2). In addition, the integration of the peak spectra was about 44% larger in case of river fulvic acid as compared to that of river humic acid. The peak that was associated with the higher level of excitation energy was defined as the major peak (Ex. 250, Em. 450 wavelength pair) and that associated with the lower excitation energy was defined as the minor peak (Ex. 350, Em. 450 wavelength pair).

Table 3 reflects the specifics related to each of these fractions in the range of 0.5 ppm to 10 ppm. The reason for these differences could be explained to the presence of simpler structural components in FA compared to HA, i.e., a low degree of aromatic substitution and polycondensation and low levels of conjugated chromophores (Senesi *et al.*, 1989).

Table 3. Spectral Fluorescent Signature Database

		Major Spectra				Minor Spectra				
Fraction	**Conc. ppm**	**Peak Intensity**	**Area**	**Slope**	**Slope x Area**	**Peak Intensity**	**Area**	**Slope**	**Slope x Area**	**Total Spectra Area**
Humic Acid	0.5	0.84	302	0.004	2.00	0.67	164	0.004	1.00	3710
	1	1.31	372	0.007	3.00	1.00	209	0.018	4.00	4517
	1.5	1.70	420	0.014	6.00	1.30	243	0.012	3.00	5006
	2	2.02	474	0.012	6.00	1.59	285	0.012	4.00	5713
	2.5	2.34	522	0.018	10.00	1.76	311	0.014	5.00	6240
	3	2.86	582	0.020	12.00	2.15	360	0.020	8.00	6830
	4	3.75	678	0.031	21.00	2.94	466	0.030	14.00	8272
	6	4.60	776	0.045	35.00	3.71	560	0.040	23.00	9838
	8	5.60	907	0.044	40.00	4.65	678	0.040	28.00	11259
	10	6.25	986	0.056	56.00	5.42	774	0.053	42.00	12567
Fulvic Acid	0.5	1.56	405	0.012	5.00	1.18	221	0.009	2.00	4670
	1	2.56	562	0.028	16.00	2.00	320	0.020	7.00	6160
	1.5	3.54	634	0.040	26.00	2.82	411	0.030	13.00	7078
	2	4.56	780	0.055	43.00	3.70	520	0.050	26.00	8627
	2.5	5.24	867	0.064	56.00	4.32	586	0.053	32.00	9636
	3	6.05	975	0.073	72.00	4.90	664	0.072	48.00	10818
	4	7.71	1170	0.094	110.00	6.52	847	0.090	77.00	13032
	6	10.37	1500	0.125	188.00	9.20	1162	0.112	130.00	17034
	8	12.95	1828	0.150	275.00	11.98	1484	0.160	238.00	21084
	10	14.55	2014	0.190	384.00	13.90	1706	0.195	334.00	23760
Mixtures **										
RH0.5 RF3.5	2	10.94	1623	0.130	211.00	9.40	1208	0.120	145.00	18374
RH1 RF3	2	10.30	1489	0.126	188.00	9.00	1153	0.123	142.00	17115
RH1.5 RF2.5	2	9.30	1367	0.114	156.00	8.20	1052	0.110	115.00	15796
RH2 RF2	2	8.27	1281	0.098	125.00	7.06	930	0.089	83.00	14626
RH2.5 RF1.5	2	7.20	1106	0.087	96.00	6.10	827	0.080	67.00	13020
RH3 RF1	2	6.19	1011	0.076	77.00	5.00	629	0.063	40.00	11834
RH3.5 RF0.5	2	5.30	873	0.062	54.00	4.40	629	0.058	37.00	10410

** RH0.5 RF3.5 is defined as 0.5 parts of River Humic Acid + 3.5 parts of River Fulvic Acid

Table 4. Characteristics of the Two Humic Substances Fractions

Fraction	Major Peak Location	Major Peak Slope	Major Peak Area	Minor Peak Location	Minor Peak Slope	Minor Peak Area
Humic Acid	(249-261 Ex, 453 Em)	0.025	602	(321 Ex, 441-453 Em)	0.024	405
Fulvic Acid	(237-249 Ex, 441 Em)	0.083	1073	(321 Ex, 441 Em)	0.08	792
Mixtures of FA & HA	(249 Ex, 441 Em)	0.07	875	(321 Ex, 441 Em)	0.064	642
**Non-Humic Substances	(225-237 Ex, 345-357 Em)	0.02	420	(273 Ex, 357-369 Em)	0.03	370
**Non-Humic Substances	(225 Ex, 609-621 Em)	0.064	327	-	-	-

** These regions have the presence of hydrophilic acid, hydrophilic neutral and hydrophilic base and have been designated as non-humic substances based on the research results of Marhaba *et al.*, 1998 "NOM Fractionation Research".

It is commonly accepted that the FA is responsible for the largest part of the DOM fluorescence observed in natural water (Ewald *et al.*, 1984, 1989; Plechanov *et al.*, 1983). Humic substances account for less than one half of the DOC (Thurman *et al.*, 1981) and that very little is known about the part which contains the so-called hydrophilic substances (Leenheer *et al.*, 1978). This latter part is supposed to contain compounds such as sugar acids, amino acids, fatty acids and carboxylic acids (Leenheer *et al.*, 1981) i.e., compounds with lower molecular weight than humic substances but which may also contribute to the total fluorescence of the natural water.

4.3 Analysis of Mixtures of Acid Standards

Analysis was done on the mixtures (total of seven mixtures) of river HA and river FA in different proportions ranging from 0.5 parts RH + 3.5 parts RF to 2 parts RH + 2 parts RF (total concentration = 2 ppm). From tables 3 and 4, we saw the dominance of river FA over river HA in humic substances. Reducing the proportions of river HA and increasing the proportions of river FA, resulted in the increase of intensity of major and minor peak, slope, product of slope and area, area of the major and minor spectrum which tallied with the work done by (Krasner et. al., 1996), which reported that humic substances (part of hydrophobic fraction) accounted for 46% of the DOC, with a predominance of fulvic over humic acids (43% and 3% respectively). The results are depicted in figures 10-13.

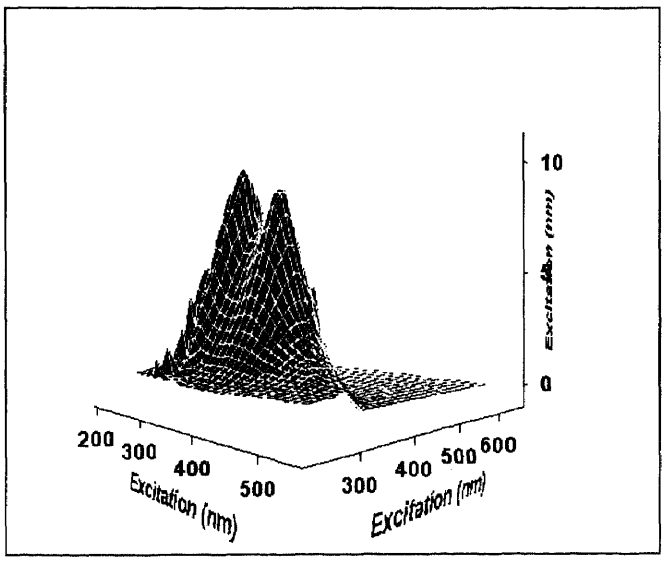


Figure 10. Mixture of Humic Acid (1 part) + Fulvic Acid (3 parts)
(2-ppm mixture concentration)

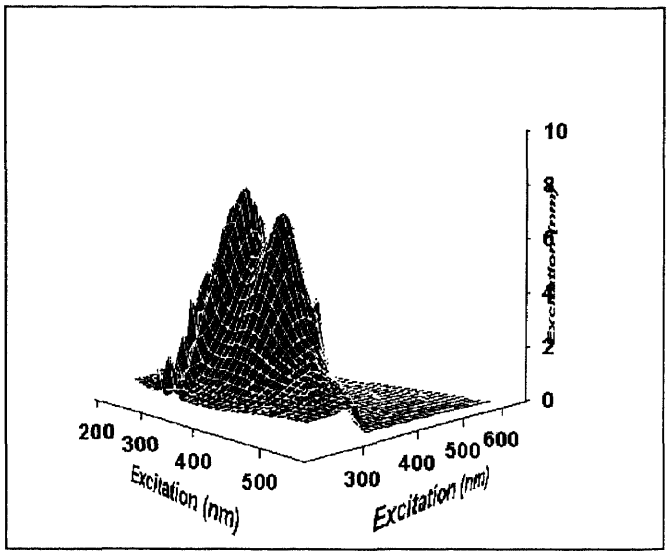


Figure 11. Mixture of Humic Acid (2 parts) + Fulvic Acid (2 parts) (2-ppm mixture concentration)

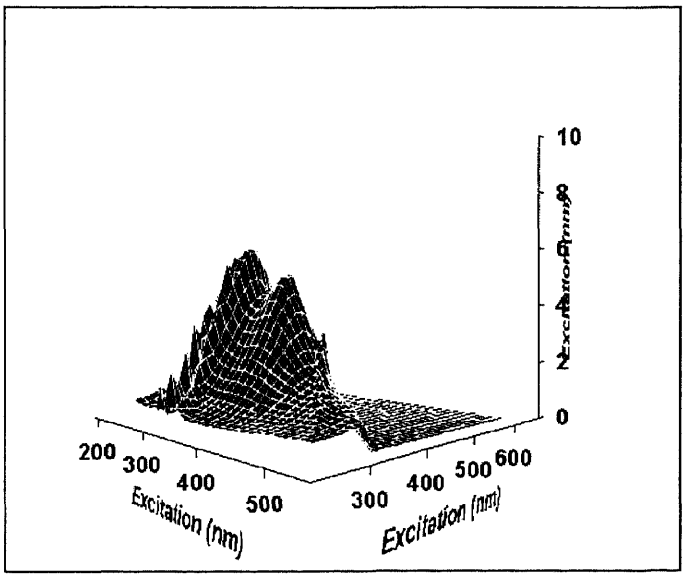


Figure 12. Mixture of Humic Acid (3 parts) + Fulvic Acid (1 part) (2-ppm mixture concentration)

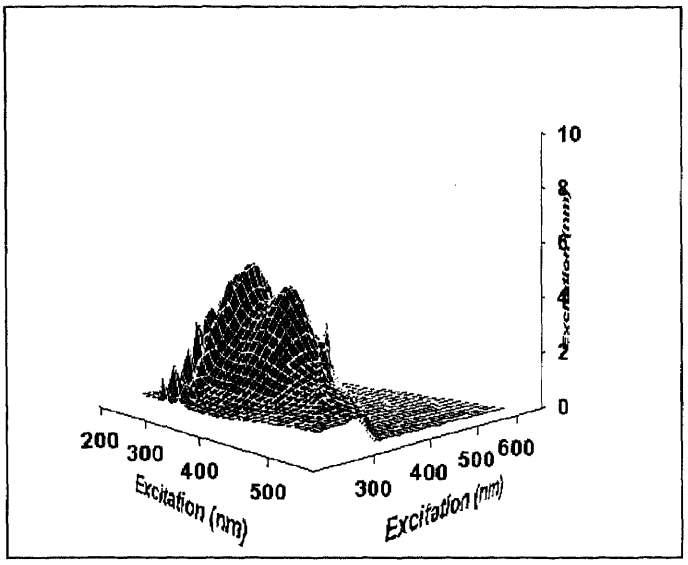


Figure 13. Mixture of Humic Acid (3.5 parts)+Fulvic Acid (0.5 parts) (2-ppm mixture concentration)

Additional SFS figures of the mixtures of River HA and River FA can be viewed in Appendix C.

4.4 NOM Regions in Natural Water Sample

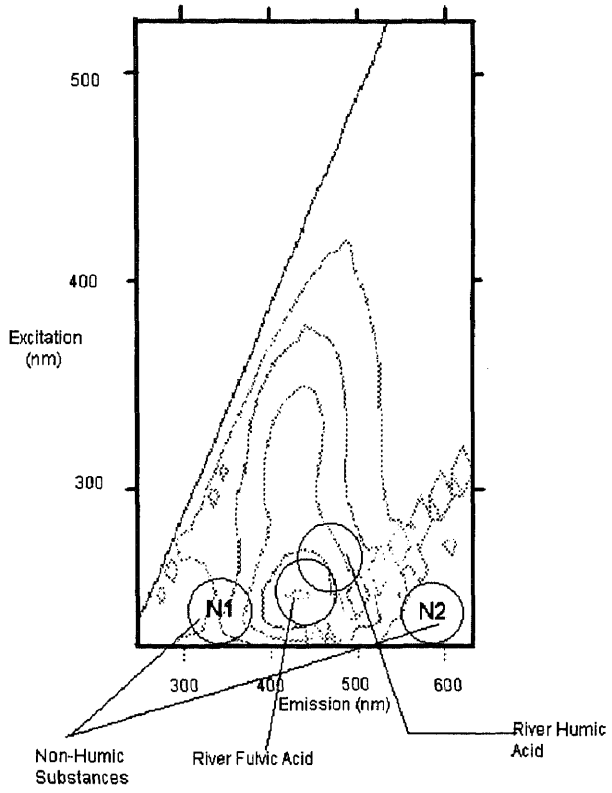


Figure 14. SFS contour of the Passaic River at Little Falls, NJ

During the course of this project, samples from the Passaic river basin, Raritan river, Millstone river were analyzed. Passaic river was selected for this analysis, based on the fact that the SFS signatures obtained from Passaic samples differed slightly from the other samples analyzed.

Figure 14 shows a SFS contour of the Passaic River at Little Falls, NJ. It shows the main fluorescence regions for FA and HA, which correspond to the major peaks' fluorescent SFS regions of all concentrations studied. A separate research study being

performed by Marhaba *et al.* designated regions for major peaks of hydrophilic substances, which were the main components of non-humic substances. Table 4 shows the major and minor peak locations of these regions. Figure 14 shows the location of these non-humic regions on the SFS contour. As shown in figure 14, the natural water SFS contour showed non-humic substances as designated by regions N1 and N2. This was verified by overlaying the HA and FA contours over the natural water contour.

Being a signature, each was expected to be unique in some aspects despite the fact that they may appear overlapped with each other in some cases. As seen from table 3, there are at least 4 criteria for identification that can be used, either singly or jointly, for the identification of humic substance fractions. These are (a) location of major and minor peak in terms of the excitation and emission coordinates, (b) the fluorescent intensity at these locations, (c) the approaching gradient at these peaks or slope, and (d) the integration of the peak spectra or area under the curve where the peak occurs. Figure 14 shows the fluorescent regions, which were found unique in natural water sample collected at the Passaic Valley Water Commission water treatment plant.

4.5 TOC Analysis

It has been known for some time that there is a strong correlation between UV absorbance and total organic carbon (Owen *et al.*, 1993). It was also known that this relationship will vary with the location of the samples being analyzed, and will exhibit seasonal variations as well (Owen *et al.*, 1993). It should be expected that the relationship between TOC and fluorescence would also change with location and with the time of year the samples were collected.

For the purpose of this experiment, each sample that was analyzed for TOC also had its SFS developed. From the signatures, two fluorescence intensity values were tabulated. These points included the two peaks (Major Peak: 250, 450 wavelength pair and Minor Peak: 350, 450 wavelength pair)

These relationships were developed by plotting the fluorescence intensity from one of the two wavelength pairs noted in Section 4.2 along with the TOC values measured for the sample. A linear regression was performed for the plot obtained and the equations generated. These plots can be found in Appendix D. Fluorescence was represented in Table 5 as F, and TOC as C.

Table 5. Relationship between TOC and Fluorescence

Acid Standard	Wavelength Pair (nm) (Excitation, Emission)	Relationship (F=Fluorescence Intensity, C=TOC)	Number of Samples, n	R^2
River HA	Major Peak (250, 450)	F = 2.056C - 1.893	10	0.9773
River HA	Minor Peak (350, 450)	F = 2.3829C - 1.4665	10	0.9912
River FA	Major Peak (250, 450)	F = 0.8314C - 0.9523	10	0.9810
River FA	Minor Peak (350, 450)	F = 0.856C - 0.3884	10	0.9919
Peat HA	Major Peak (250, 450)	F = 0.0977C + 0.0204	10	0.9891
Soil HA	Major Peak (250, 450)	F = 0.0705C + 0.1968	10	0.9852
Soil FA	Major Peak (250, 450)	F = 1.5161C - 0.7487	10	0.9824
Soil FA	Minor Peak (350, 450)	F = 2.2414 C - 0.7974	10	0.9922
Peat FA	Major Peak (250, 450)	F = 0.5575C - 0.9143	10	0.9649
Peat FA	Minor Peak (350, 450)	F = 0.6751C - 0.4518	10	0.9788

The relationships between fluorescence and TOC were stronger using the (350, 450) wavelength pair. In every case where a relation was found for a location using both wavelength pairs, the R^2 value was higher for the (350, 450) wavelength pair.

4.6 UV-254 and TOC

It has been known for some time that there is a strong correlation between UV absorbance and total organic carbon (Owen *et al.*, 1993). For the purpose of this experiment, each sample was analyzed for TOC and UV-254.

These relationships were developed by plotting the TOC with the UV-254 values measured for the sample. A linear regression was performed for the plot obtained and the equations generated. These plots can be found in Appendix E. TOC was represented in Table 6 as C, and UV-254 as A.

Table 6. Relationship between TOC and UV-254

Acid Standard	Relationship (C=TOC, A=UV-254)	Number of Samples, n	R^2
River HA	C = 35.349A - 0.3598	10	0.9611
River FA	C = 43.761A - 0.2186	10	0.9966
Soil HA	C = 5.5856A + 0.1948	10	0.9395
Soil FA	C = 15.837A - 0.469	10	0.9982
Peat HA	C = 7.5679A	10	0.9148
Peat FA	C = 14.389A - 0.7094	10	0.9949

As seen from Table 6, very good correlations exist between UV-254 and TOC for river, soil and peat HA and FA.

4.7 DBP Formation Potential

Since one of the goals of this research was to use fluorescence as a predictive tool for DBP formation, each sample had its SFS developed prior to and after chlorination. The concentrations of DBPs formed during chlorination have been correlated with various nonspecific parameters such as natural-water TOC, UV absorbance, and chlorine demand. These parameters, which reflect the concentration of organics, do not precisely take into account the composition of the organic matrix and the possible changes in the proportions of the macromolecules present. The same two fluorescence points (Major and Minor Peak) were tabulated along with the TTHMs, THANs and HAA6 (total of monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, dibromoacetic acid and tribromoacetic acid) for each sample. A plot of fluorescence versus TTHMs, THANs and HAA6 for river HA and FA was done which can be found in Appendix F. For each plot, linear regression was performed for the plot obtained and the equations generated. The relationships derived from these plots were summarized below in Table 7.

Table 7. Relationship between DBP Formation and Fluorescence

Acid Standard	DBP	Wavelength Pair (nm) (Excitation, Emission)	Relationship (F=Fluorescence, C=DBPFP)	Number of Samples, n	R^2
River HA	TTHM	Major Peak (250, 450)	F = 5.6947 C + 15.26	6	0.8467
River HA	TTHM	Minor Peak (350, 450)	F = 7.8057 C + 14.748	6	0.8673
River HA	THAN	Major Peak (250, 450)	F = 0.6273 C + 3.026	6	0.9662
River HA	THAN	Minor Peak (350, 450)	F = 0.8503 C + 2.983	6	0.968
River HA	HAA6	Major Peak (250, 450)	F = 0.2916 C - 0.4758	5	0.9515
River HA	HAA6	Minor Peak (350, 450)	F = 0.2144 C - 0.305	5	0.9561
River FA	TTHM	Major Peak (250, 450)	F = 1.632 C + 19.072	6	0.8676
River FA	TTHM	Minor Peak (350, 450)	F = 1.9284 C + 19.386	6	0.8538
River FA	THAN	Major Peak (250, 450)	F = 0.2756 C + 2.8866	6	0.7555
River FA	THAN	Minor Peak (350, 450)	F = 0.3291 C – 2.9288	6	0.7593
River FA	HAA6	Major Peak (250, 450)	F = 0.6696 C - 1.4593	6	0.9434
River FA	HAA6	Minor Peak (350, 450)	F = 0.5601 C - 1.347	6	0.9375

From Table 7, it was seen that when the fluorescence was measured at the 350, 450 wavelength pair there was a strong relationship between fluorescence and the formation of disinfection by-products. The DBP results presented here were for river HA and FA.

There exist good correlations between the THMFP and TOC of natural drinking water (Singer *et al.*, 1981; Edzwald *et al.*, 1985). Studies have also shown UV-254 absorbance to be a better surrogate (indicator) than TOC concentrations for chlorinated by-product formation (Najm *et al.*, 1994). The relationship between DBP and UV-254 and DBP and TOC for River HA and FA are shown in Appendix G. A linear regression was performed for the plot obtained and the equations generated. The relationships for river HA and FA derived from these plots are summarized below in Table 8.

Table 8. Relationship between DBP Formation, TOC and UV-254

Acid Standard	DBP	Number of Samples, n	Relationship (U=UV, C=DBPFP)	R^2 (UV)	Relationship (T=TOC, C=DBPFP)	R^2 (TOC)
River HA	TTHM	6	C = 135.47 U + 16.893	0.8566	C = 3.6785 T + 18.477	0.8706
River HA	THAN	6	C = 14.98 U + 3.2021	0.9851	C = 0.3997 T + 3.3912	0.9669
River FA	TTHM	6	C = 101.07 U + 19.487	0.8864	C = 2.5178 T + 19.529	0.8688
River FA	THAN	6	C = 17.602 U + 2.9252	0.8208	C = 0.432 T + 2.9478	0.7809

Table 8 reflects that the TTHM and THAN levels correlate better with UV-254 compared to TOC.

Aromatic character of HA and FA imparts an ability to absorb light in UV range. TOC in natural water can be divided into 2 fractions, a UV-sensitive and a UV-insensitive fraction. Ratio of UV absorbance to TOC which is called specific absorbance provides a relative index of humic contents of DOC (AWWARF, 1993).

The relationship between specific absorbance and DBPs can be seen in Appendix G.

A linear regression was performed for the plot obtained and the equations generated. The relationships for river HA and FA derived from these plots are summarized below in Table 9.

Table 9. Relationship between DBPs and Specific Absorbance (UV-254/TOC ratio)

Acid Standard	DBP	Relationship (F = DBPFP, A = Sp. Absorbance)	Number of Samples, n	R^2
River HA	TTHMs	F = -371.55 A + 39.295	6	0.5512
River HA	THANs	F = -36.07A + 5.4966	6	0.4885
Peat HA	TTHMs	F = 1.7699 A + 5.791	6	0.2240
Peat FA	TTHMs	F = -0.1132 A + 6.8453	6	0.2175
Peat FA	THANs	F = -0.127 A + 1.0942	6	0.594
Soil HA	TTHM	F = 5.377 A + 5.5151	6	0.8721
Soil HA	THAN	F = 8.4094 A + 2.6964	6	0.614
Soil FA	TTHMs	F = -12.638 A + 6.8964	6	0.8505

From table 9, it was seen that the TTHM and THAN levels correlate better with Specific Absorbance (UV-254/TOC ratio) for Soil HA.

A plot of fluorescence versus TTHMs, THANs for Peat and Soil HA and FA was also done which can be found in Appendix H. A linear regression was performed for the plot obtained and the equations generated. The relationships derived from these plots are summarized below in Table 10.

Table 10. Relationship between DBP Formation and Fluorescence

Acid Standard	DBP	Intensity Peak	Relationship (F=Fluorescence, C=DBPFP)	Number of Samples, n	R^2
Soil HA	TTHM	Major Peak	F = 5.1273 C - 28.058	7	0.7325
Soil HA	THAN	Major Peak	F = 2.2171 C - 5.271	7	0.8586
Soil FA	TTHM	Major Peak	F = 1.4 C - 6.32	7	0.9059
Soil FA	TTHM	Minor Peak	F = 1.0727 C - 4.9932	7	0.9079
Soil FA	THAN	Major Peak	F = 3.5 C - 2.05	7	0.3088
Soil FA	THAN	Minor Peak	F = 2.6667 C - 1.7048	7	0.306
Peat HA	TTHM	Major Peak	F = 6.5976 C - 38.099	6	0.9359
Peat HA	THAN	Major Peak	F = 18.042 C - 16.515	6	0.5282
Peat FA	THAN	Major Peak	F = 74.512 C - 76.16	6	0.8628
Peat FA	THAN	Minor Peak	F = 65.61C - 67.888	6	0.8567

Table 10 indicates that the R^2 values for Soil FA were 0.3088 for the major peak and 0.306 for the minor peak and for peat HA were 0.5282. The reason for this seemed to be the high density (insolubility) of soil FA and peat HA compared to water making the acid standard settle to the bottom when a particular test was performed.

Tables 7 and 10 shows that with respect to relation between fluorescence and reactivities peat HA > river HA > soil HA in the formation of THM and river HA > soil HA > peat HA in the formation of HAN. For FA: soil FA > river FA > peat FA in the formation of THM and peat FA > river FA > soil FA in the formation of HAN. Figures 15-17 show the relationship between concentration and TTHMs, THANs, HAA6 for river, soil, peat HA and FA.

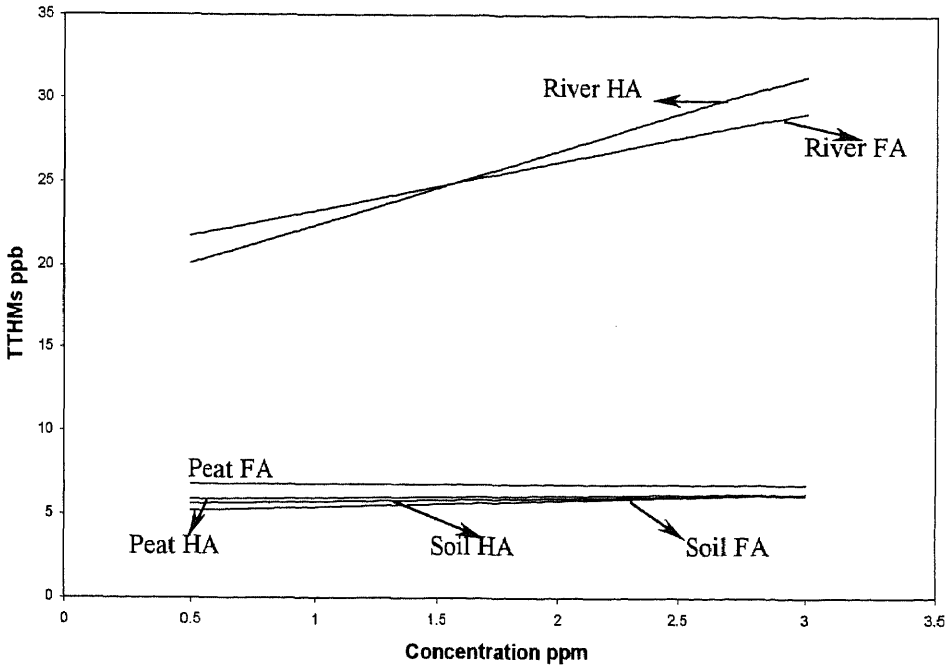


Figure 15. Concentration vs. TTHMs: River, Soil, Peat HA and FA

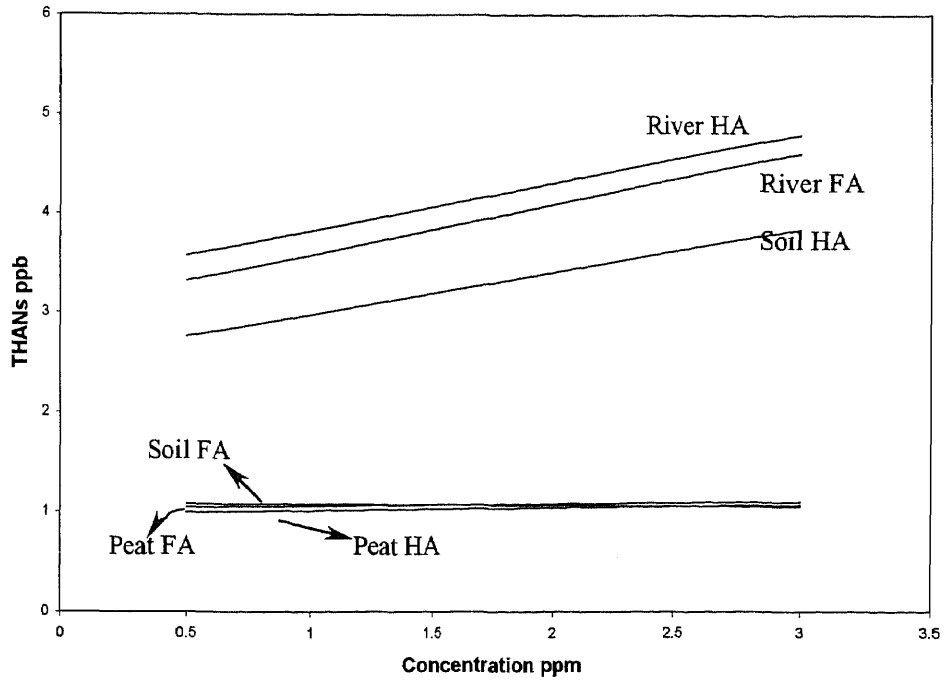


Figure 16. Concentration vs. THANs: River, Soil, Peat Ha and FA

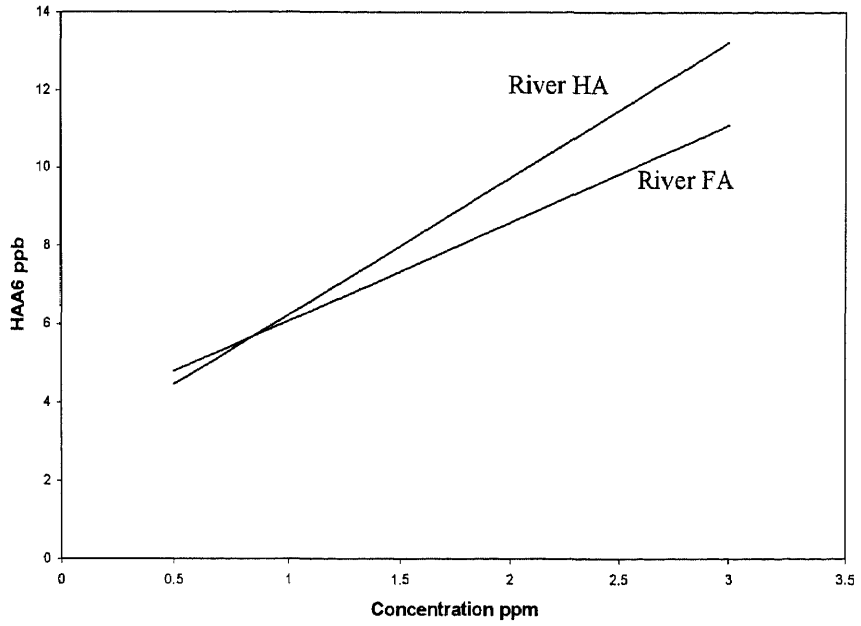


Figure 17. Concentration vs. HAA6: River HA and FA

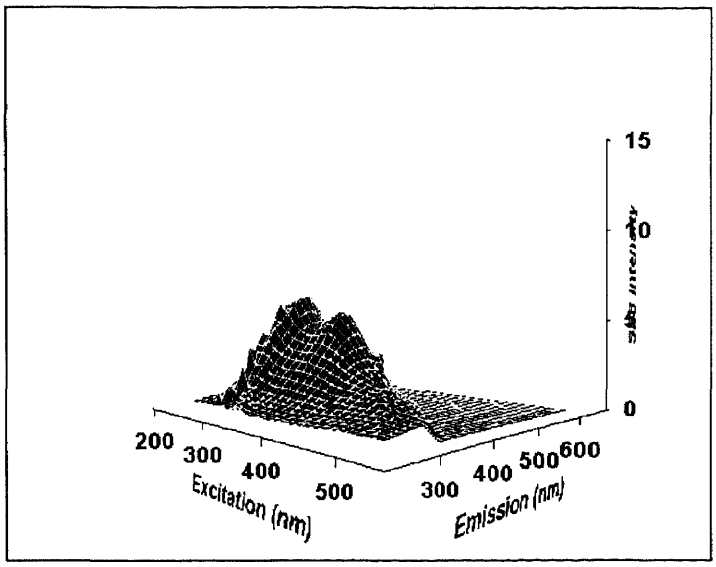
Results from figure 15-17 could be summarized in table 11 in descending order of reactivities to DBP formation.

Table 11. Reactivity of Humics and Fulvics to DBP Formation

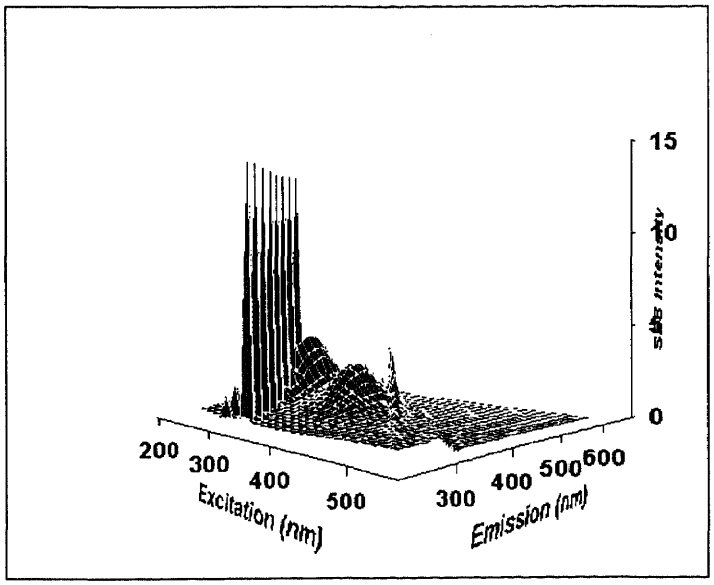
THMs	HANs
River Humic Acid	River Humic Acid
River Fulvic Acid	River Fulvic Acid
Peat Fulvic Acid	Soil Humic Acid
Peat Humic Acid	Soil Fulvic Acid
Soil Humic Acid	Peat Fulvic Acid
Soil Fulvic Acid	Peat Humic Acid

Appendix F also contains the individual break up for the concentrations of four THM and four HAN for river HA and FA at 10 ppm concentration. From the individual breakup it was seen that chloroform was the most predominantly formed DBP.

A post-chlorination SFS was developed for each sample in this experiment. It was interesting to note some of the differences that exist between the pre- and post-chlorination signatures. An example of this is presented in Figure 18.



a) Pre-Chlorination



b) Post-Chlorination

Figure 18. Pre- and Post-chlorination SFS for River HA (10 ppm)

The chlorine dosage of 100 ppm was chosen as it was thought that this would be sufficient to oxidize enough of the organic matter present in the sample to form the maximum DBPs. After chlorination, the peak located at the (350, 450) wavelength pair was greatly diminished, compared to the peak located at the (250, 450) pair. This might begin to explain the stronger relationships between fluorescence at the (350, 450) pair and the other parameters of interest in this research. It might be that the functional groups within a humic substance will fluoresce with greater intensity at the (250, 450) wavelength pair, but the fractions of a humic substance that register as total organic carbon and react to form by-products will fluoresce at the (350, 450) pair. Additional pre- and post-chlorination figures can be viewed in Appendix I.

CHAPTER 5

CONCLUSIONS

5.1 Overview

Fluorescence spectroscopy was used for the characterization of NOM into its various fractions like HA, FA and non-humic substances. Formation potential tests were performed on the river, soil and peat HA and FA to determine reactivities. For the purpose of characterizing NOM, the results for the acid standards were applied to natural water samples. These examinations allowed preliminary quantitative relationships to be established between fluorescence and TOC, UV-254 and TOC, DBP Formation and Fluorescence for river, soil, and peat HA and FA, DBP Formation and TOC, DBP Formation and UV-254. This preliminary investigation has been intended to serve as a basis for future research in this area.

5.2 Humic and Fulvic Acid Standards

The following conclusions could be drawn from the examination of the humic and fulvic acid standards:

1. HA and FA had overlapping spectral fluorescence signatures. These signatures had two peak at an emission wavelength of 450 nm. The first peak was located at an excitation wavelength of 250 nm, and the second was located at an excitation wavelength of 350 nm. The peak that was associated with the higher level of excitation energy was defined as the major peak (Ex. 250, Em. 450 wavelength pair)

and that associated with the lower excitation energy was defined at the minor peak (Ex. 350, Em. 450 wavelength pair).

2. They could be well distinguished based on the factors such as fluorescent intensity, slopes and area under a specific spectrum.
3. Although they had similar fluorescence region, an examination of the spectra does show that slopes pertaining to the peaks of FA fractions were, on average, 70% larger than those of the HA fractions. In addition, the integration of the peak spectra was about 44% larger in case of river fulvic acid as compared to that of river humic acid.

5.3 Analysis of Mixtures of Acid Standards

The following conclusions could be drawn from the examination of the humic and fulvic acid standards by SFS:

1. From the analysis done on the mixtures of river HA and river FA in different proportions ranging, there was a fluorescence predominance of river FA over river HA in humic substances.
2. Reducing the proportions of river HA and increasing the proportions of river FA, resulted in the increase of intensity of major and minor peak, slope, product of slope and area, area of the major and minor spectrum.

5.4 Analysis of NOM Fractions in Natural Water Sample

The following conclusion could be drawn from the examination of the natural water sample using the database created from analysis conducted on river HA and FA samples.

1. Parameters used for characterizing NOM included: (a) location of major and minor peak in terms of the excitation and emission coordinates, (b) the fluorescent intensity at these locations, (c) the approaching gradient at these peaks or slope, and (d) the integration of the peak spectra or area under the curve where the peak occurs.
2. The Spectral Fluorescence Signature (SFS) technique serves as an analytical tool that has the potential to:
    - Rapidly identify, qualitatively and quantitatively, the existence of certain NOM fractions (humic acids, fulvic acids and nonhumic substances).
    - Provide a cost-effective tool for the study of DBP formation potential of NOM fractions.
    - Be used as a tool to track point and non-point source contributions of NOM fractions in source waters.

5.5 Total Organic Carbon

The following conclusions could be drawn from the TOC analysis conducted on the river, soil, and peat HA and FA acid standards.

1. There was a strong correlation between fluorescence and total organic carbon. The correlation would be stronger if the (350, 450) wavelength pair was used for the fluorescence measurements than the (250, 450) pair.
2. The correlation between fluorescence and TOC was strongest for river, soil, and peat HA and FA in descending order.

5.6 UV-254 and TOC

The following conclusions could be drawn from the UV-254 analysis conducted on the river, soil, and peat HA and FA acid standards.

1. There was a stronger correlation between UV-254 and TOC for FA compared to HA.

5.7 Disinfection By-Production Formation

The following conclusions could be drawn from the results of the DBP Formation Potential test conducted on river, soil and peat humic and fulvic acid.

1. There was a strong correlation between fluorescence and the formation of THMs. The correlation was strong irrespective of the wavelength pair, which was used to measure fluorescence. There was not much difference between the R^2 values for major peak (250, 450) or minor peak (350, 450) wavelength pair.
2. There was a strong correlation between fluorescence and the formation of HANs. The correlation was strong irrespective of the wavelength pair, which was used to measure fluorescence. There was not much difference between the R^2 values for major peak (250, 450) or minor peak (350, 450) wavelength pair.
3. There was a strong correlation between fluorescence and the formation of HAAs. The correlation was strong irrespective of the wavelength pair, which was used to measure fluorescence. There was not much difference between the R^2 values for major peak (250, 450) or minor peak (350, 450) wavelength pair.
4. There was a strong correlation between TOC and the formation of DBPs and UV-254 and the formation of DBPs. THMs and HAN correlate better with UV-254 compared

to TOC for river, soil and peat HA and FA. So, UV-254 absorbance was a better indicator than TOC concentrations for chlorinated by-product formation

5. Prior to chlorination, the HA and FA samples showed two peaks in their SFS. The first of these was located at the (250, 450) wavelength pair, and the other was located at the (350, 450) wavelength pair. After chlorination, the peak located at the (350, 450) wavelength pair was greatly diminished, compared to the peak located at the (250, 450) pair. This might be caused by the structure of the acid standards itself.
6. The correlation between the DBPs (TTHMs and THANs) and specific absorbance for Soil HA were the best.
7. With respect to relation between fluorescence and reactivities peat HA > river HA > soil HA in the formation of THM and river HA > soil HA > peat HA in the formation of HAN. For FA: soil FA > river FA > peat FA in the formation of THM and peat FA > river FA > soil FA in the formation of HAN.
8. For the reactivities of humics and fulvics to DBPs river HA, river FA, peat FA, peat HA, soil HA, soil FA in descending order for THMs and river HA, river FA, soil HA, soil FA, peat FA, peat HA in descending order of HANs.
9. From the individual breakup of the THMs and HANs for river HA and FA, it was seen that chloroform was the most predominantly formed DBP.

CHAPTER 6

RECOMMENDATIONS FOR FUTURE RESEARCH

6.1 General

The preliminary research presented here was part of an ongoing investigation into the nature of humic substances in drinking water and their potential for forming harmful by-products. This preliminary work should be considered as a basis for future research. The observations and conclusions reached in the analysis of data need to be confirmed either through additional tests, or by applying the methods used to reach these conclusions to additional materials to determine if the relationships found between fluorescence and traditional testing parameters would hold true for additional substances.

6.2 Mixtures of HA and FA in Different Proportions

One challenge that has to be tackled would be how to read SFS of mixtures, which have different concentrations of different compounds within different fractions. This would require a database consisting of a matrix of different concentrations of HA and FA. Determination of fluorescence properties of different concentration of the two standards when mixed together would be required for this.

6.3 DBP Formation for Additional Humic Substances

The work presented here deals with THM, HAN and HAA acid formation potential test for the Suwannee river HA and FA. This work also includes some tests on peat and soil HA and FA, basically THM and HAN formation potential tests. It would be beneficial to

carry out tests for HAAs on HA and FA derived from other sources. It should be expected that there would be a statistically significant relationship between fluorescence and DBP formation in HA and FA from other sources, which will also help confirm the results of the work done here.

6.4 DBP Formation for Varying Chlorine Dosage

The investigation conducted as part of this project considered only a single chlorine dosage of 100 ppm when looking at the formation of DBPs. The goal for using this dosage was to determine a maximum value of by-product that might form from disinfection by oxidizing most of the organic substances that form the DBPs of concern.

Another study could be to investigate the DBPs that form due to humic materials for a series of chlorine dosages and contact times. By preparing several identical samples matrices of acid standards and chlorinating each matrix with a different dose of chlorine, a series of relationships may be generated by plotting fluorescence vs. DBP formation.

For the purpose of this research formation potential tests were used which determined the levels of DBP precursors present in water. In addition a study can be done using SDS (Simulated Distribution System) test on these samples. SDS tests determine the DBP levels formed under normal chlorination conditions. The differences between FPT and SDS test are the chlorine dosage used and the incubation time. The chlorine dosage used in an SDA test is one that results in a chlorine residual at the end of the incubation period that is comparable to chlorine residuals measured in operating full-scale distribution systems (commonly ranging from 0.5 to 2 mg/L). A significantly higher chlorine dosage is used in a FPT. Similarly, the incubation period of the SDS test is set at a value comparable to the average hydraulic residence time in a distribution

system (commonly ranging from 12 to 48 hours). FPT are commonly incubated for longer periods of time, ranging from 72 to 168 hours.

6.5 DBP Formation for Varying pH

The investigations conducted as part of this project considered pH values between 6-8. An investigation of DBPs formed at different pH would be beneficial.

APPENDIX A

SFS FOR RIVER HA AND FA

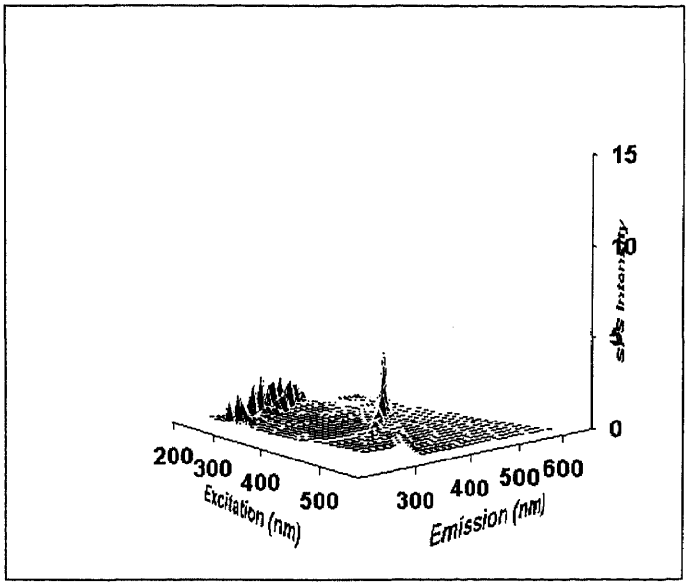


Figure A.1 River Humic Acid 0.5 ppm

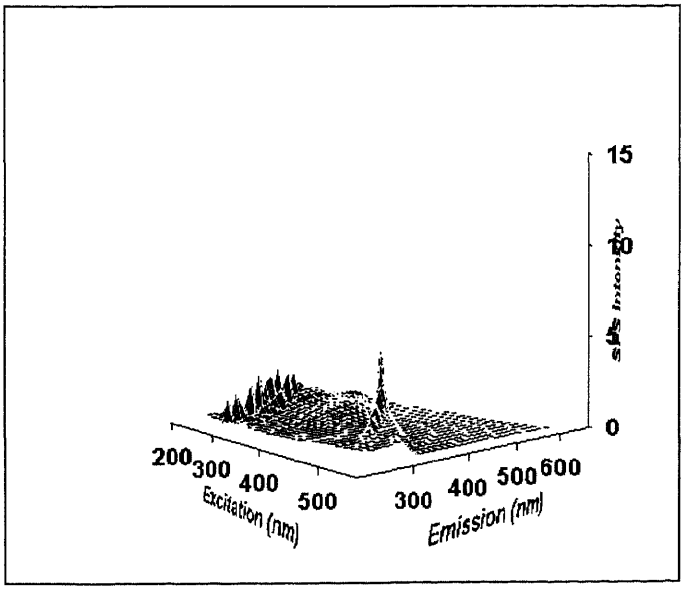


Figure A.2 River Fulvic Acid 0.5 ppm

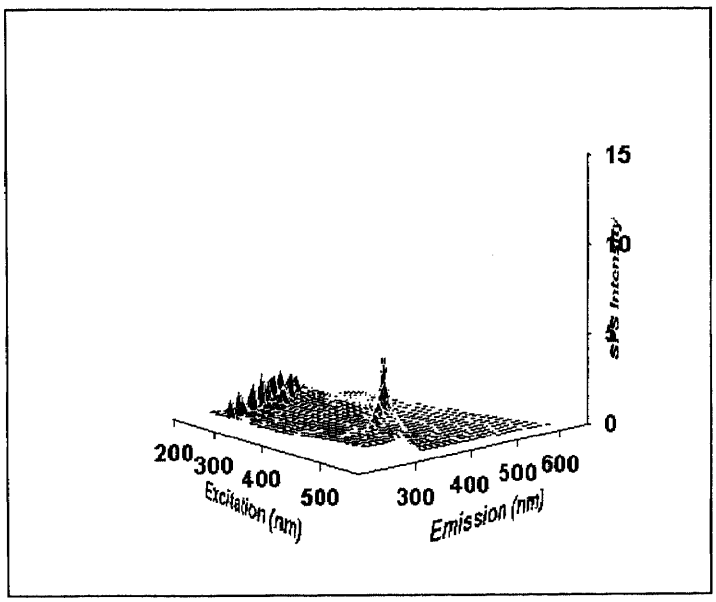


Figure A.3 River Humic Acid 1 ppm

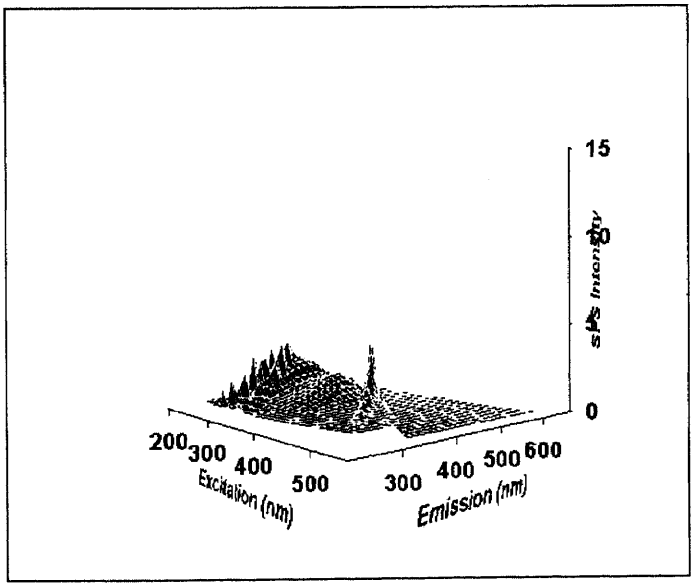


Figure A.4 River Fulvic Acid 1 ppm

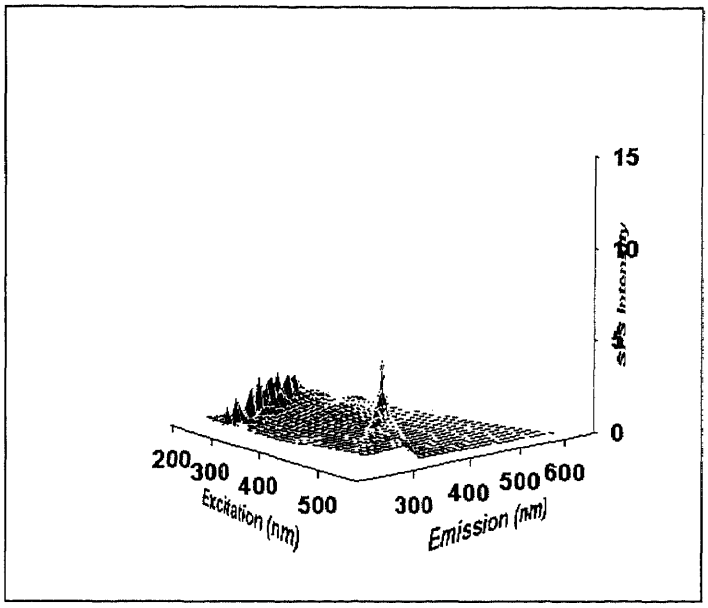


Figure A.5 River Humic Acid 1.5 ppm

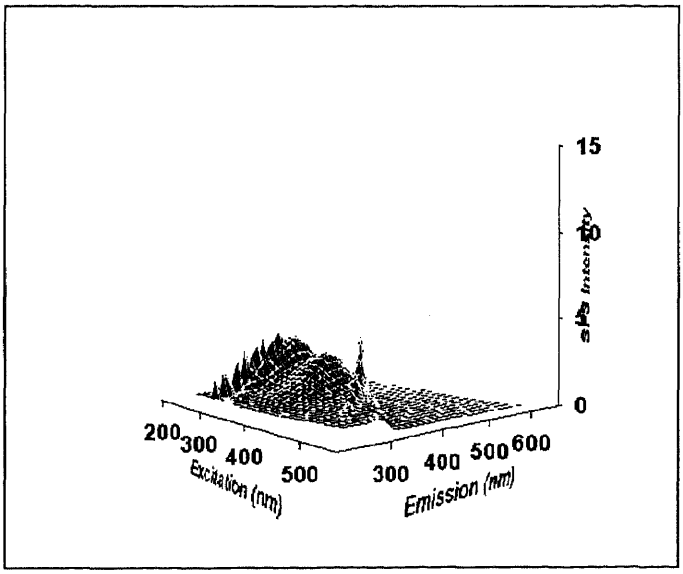


Figure A.6 River Fulvic Acid 1.5 ppm

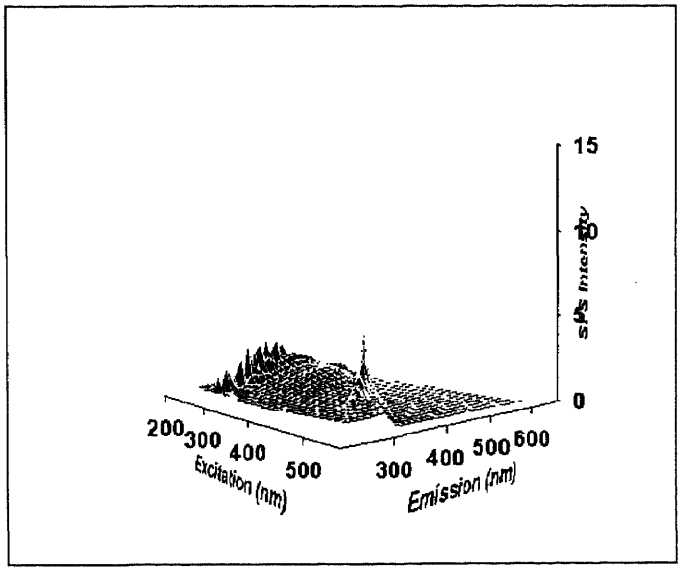


Figure A.7 River Humic Acid 2.5 ppm

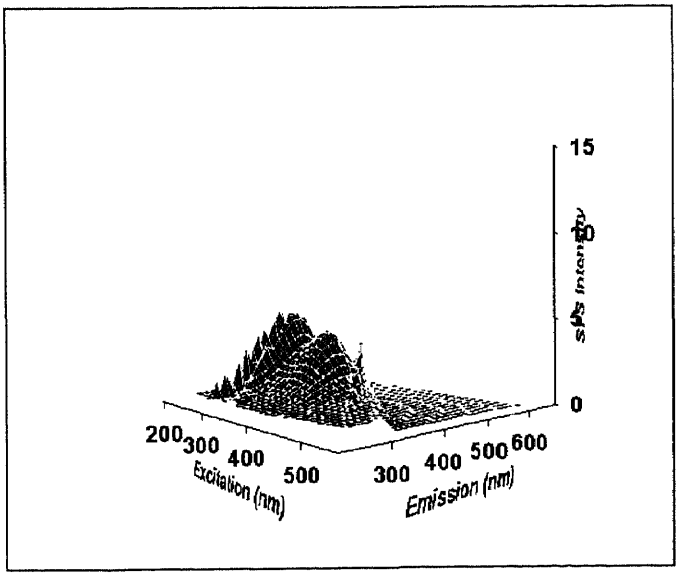


Figure A.8 River Fulvic Acid 2.5 ppm

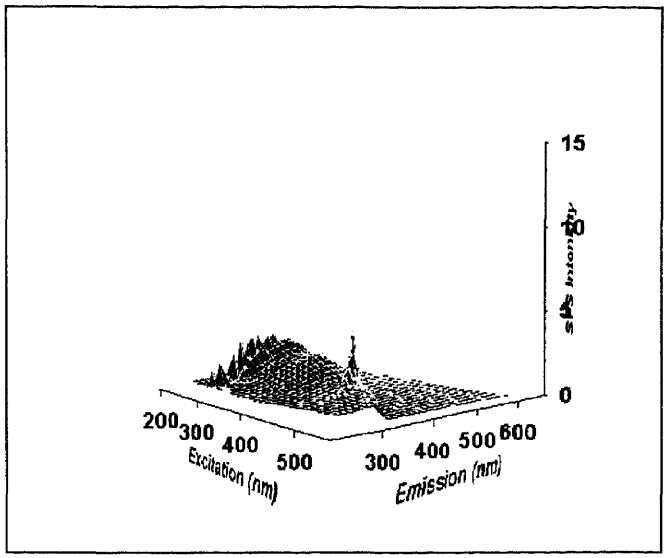


Figure A.9 River Humic Acid 3 ppm

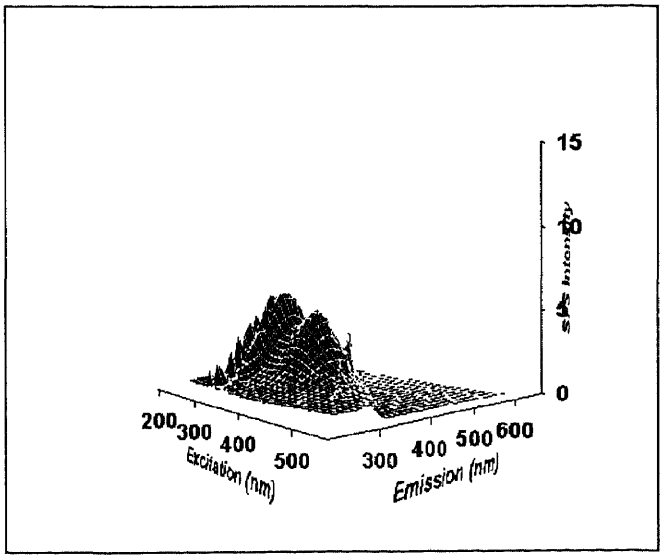


Figure A.10 River Fulvic Acid 3 ppm

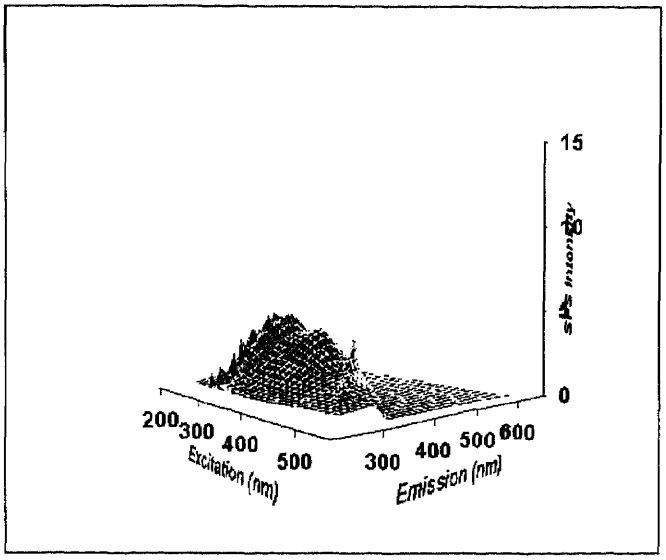


Figure A.11 River Humic Acid 6 ppm

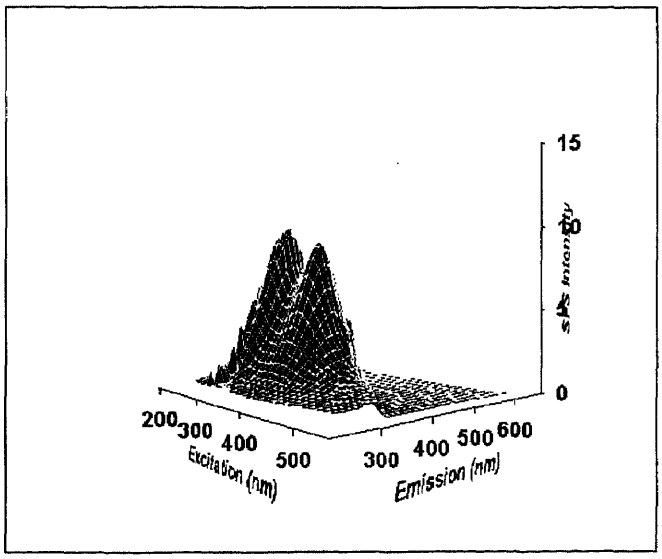


Figure A.12 River Fulvic Acid 6 ppm

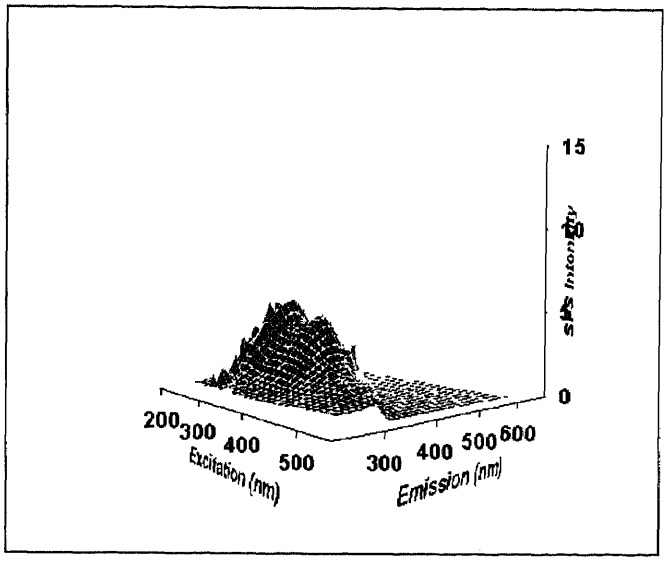


Figure A.13 River Humic Acid 8 ppm

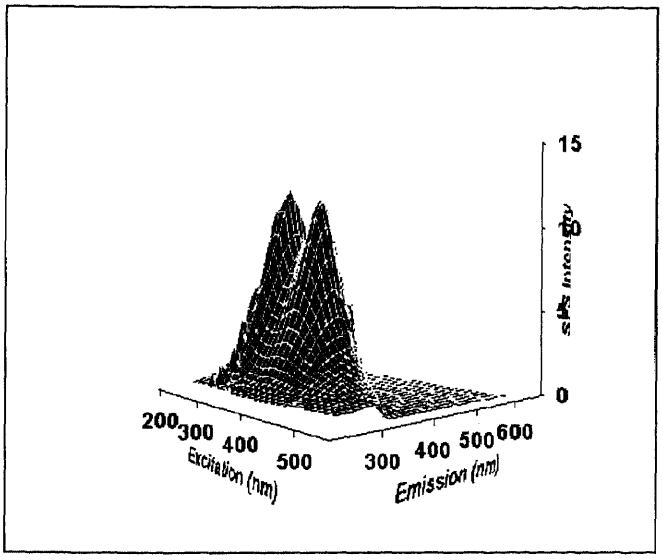


Figure A.14 River Humic Acid 8 ppm

APPENDIX B

SFS FOR PEAT AND SOIL HA AND FA

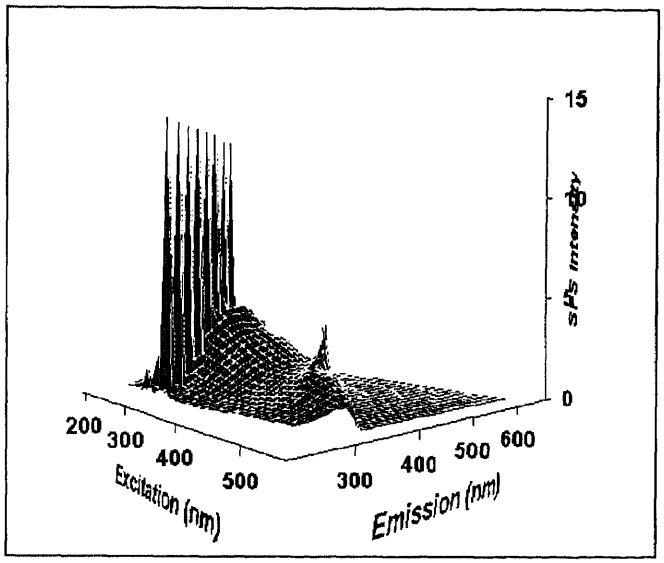


Figure B.1 Peat Humic Acid 4 ppm

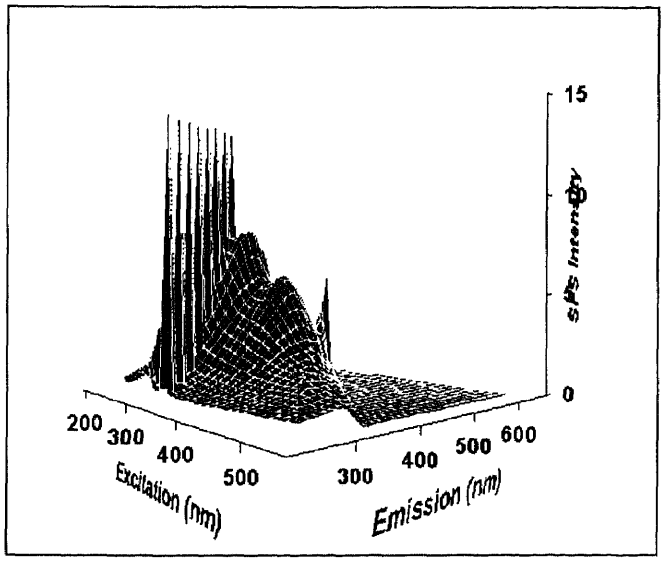


Figure B.2 Peat Fulvic Acid 4 ppm

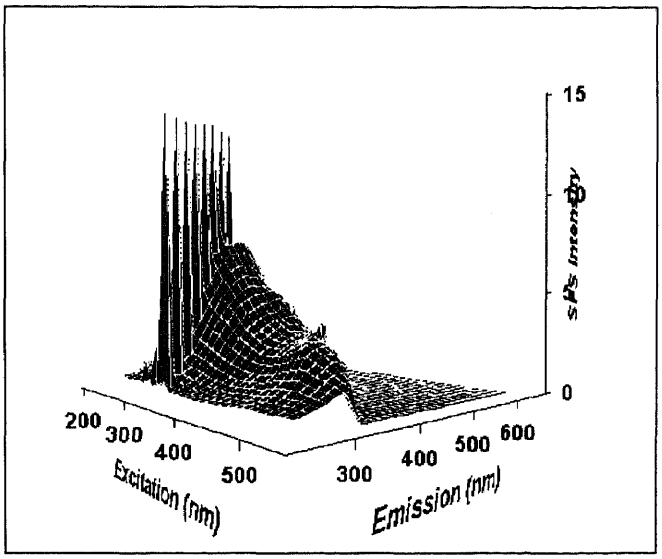


Figure B.3 Peat Humic Acid 8 ppm

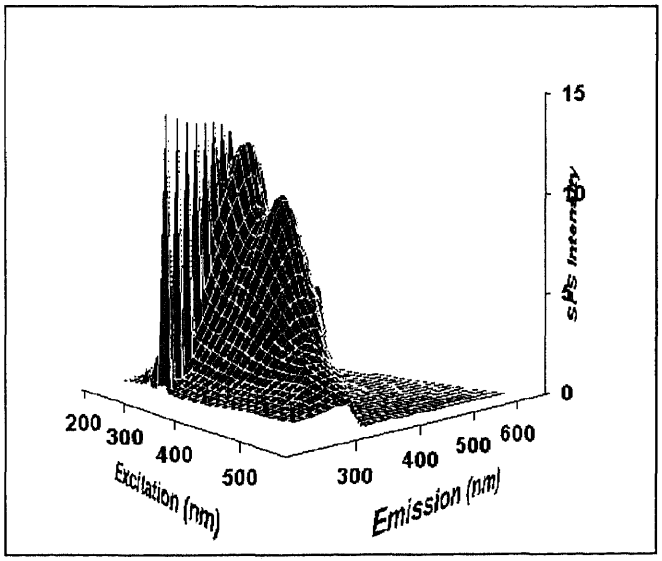


Figure B.4 Peat Fulvic Acid 8 ppm

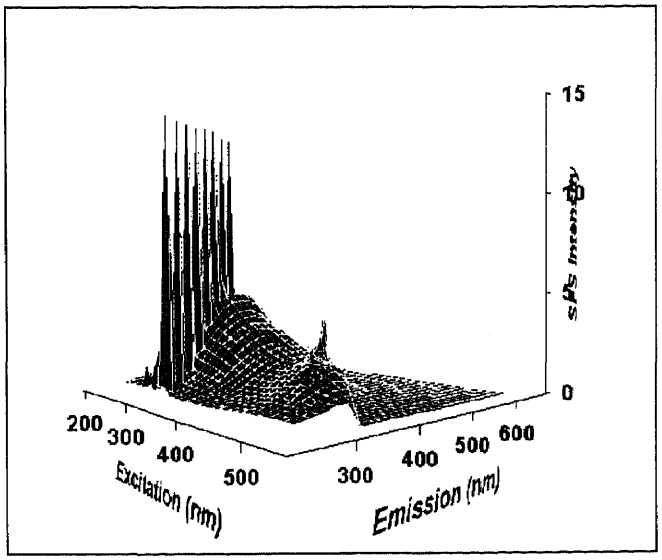


Figure B.5 Soil Humic Acid 4 ppm

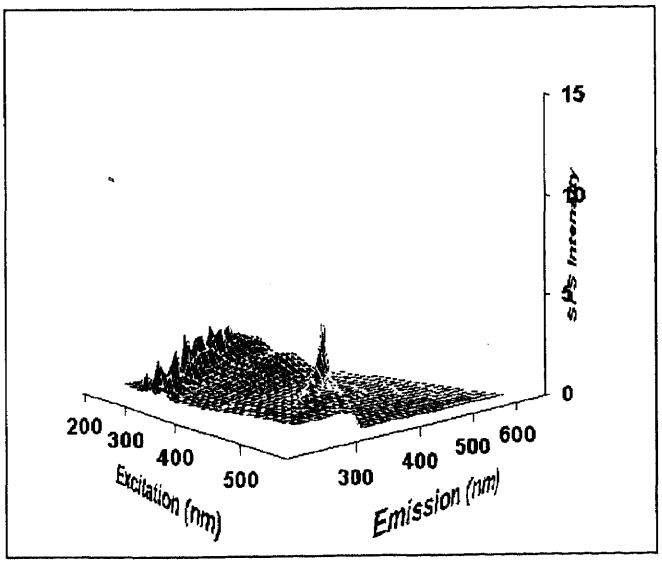


Figure B.6 Soil Fulvic Acid 4 ppm

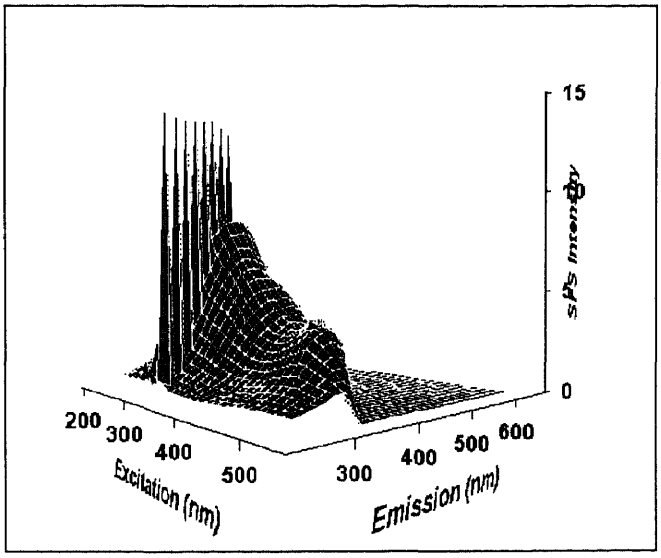


Figure B.7 Soil Humic Acid 8 ppm

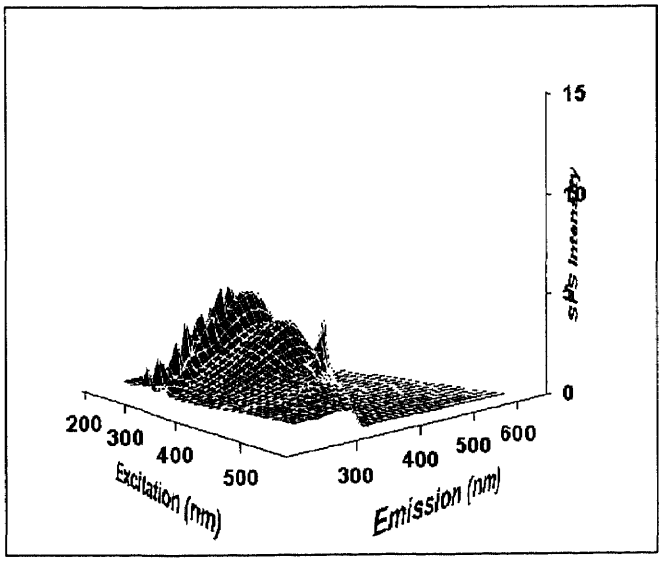


Figure B.8 Soil Fulvic Acid 8 ppm

APPENDIX C

SFS FOR MIXTURES OF RIVER HA AND FA

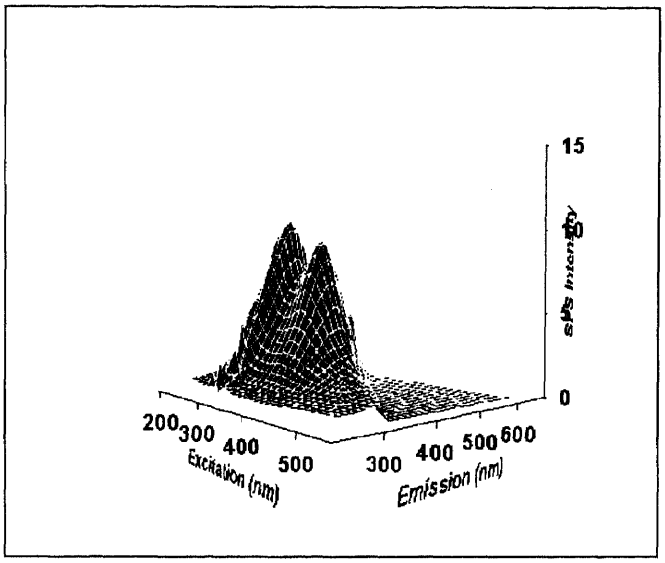


Figure C.1: Mixture of Humic Acid (0.5 part) + Fulvic Acid (3.5 parts)
(2-ppm mixture concentration)

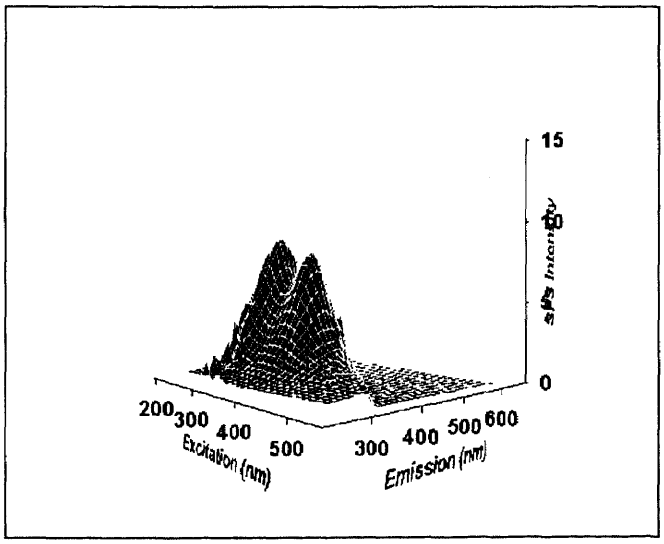


Figure C.2: Mixture of Humic Acid (1.5 part) + Fulvic Acid (2.5 parts)
(2-ppm mixture concentration)

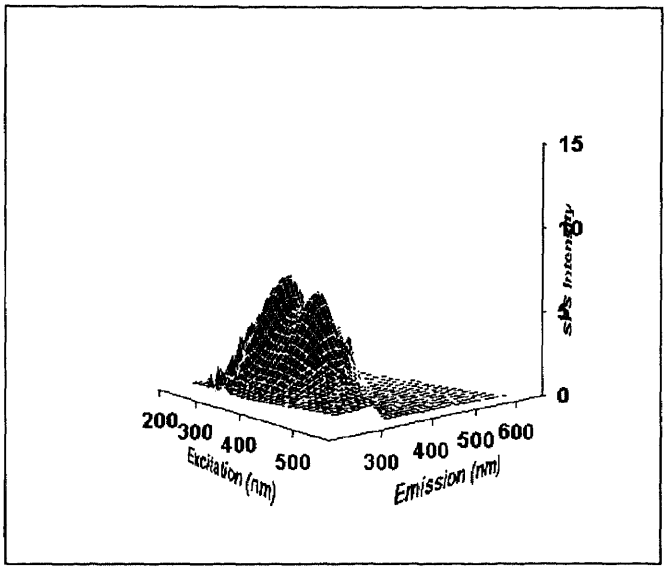


Figure C.3: Mixture of Humic Acid (2.5 part) + Fulvic Acid (1.5 parts)
(2-ppm mixture concentration)

APPENDIX D

TOC VS. FLUORESCENCE GRAPHS

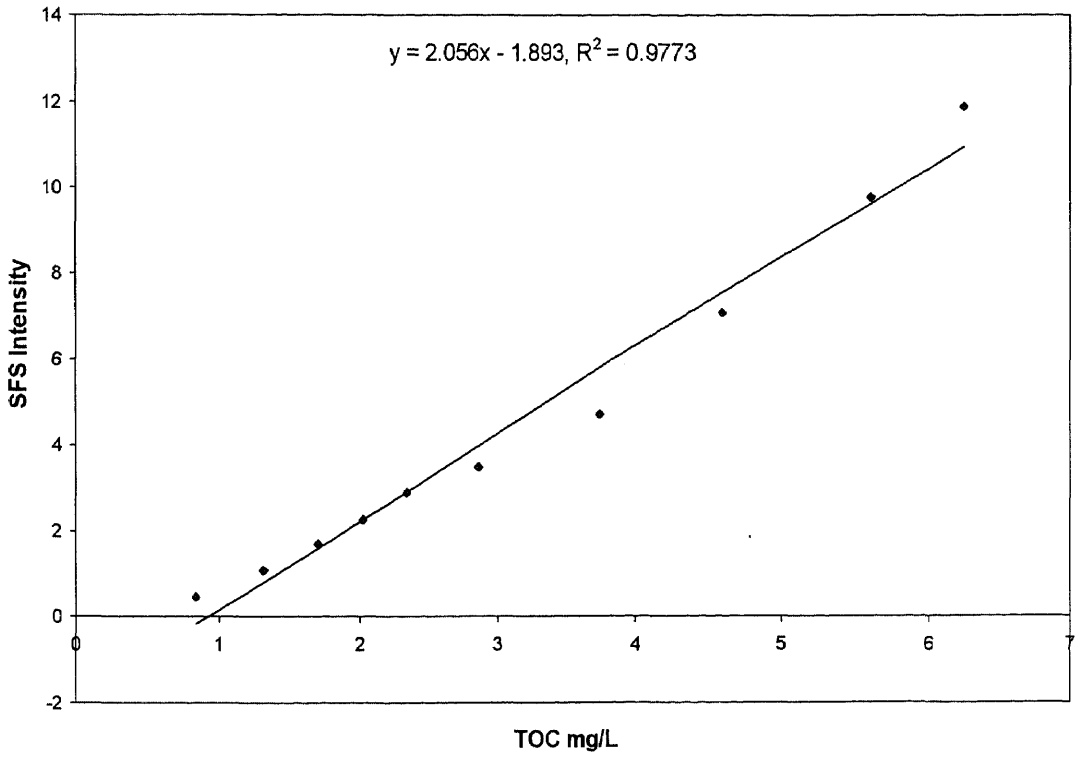


Figure D.1. SFS vs. TOC, River HA measured at Excitation Wavelength 250 nm (Major Peak)

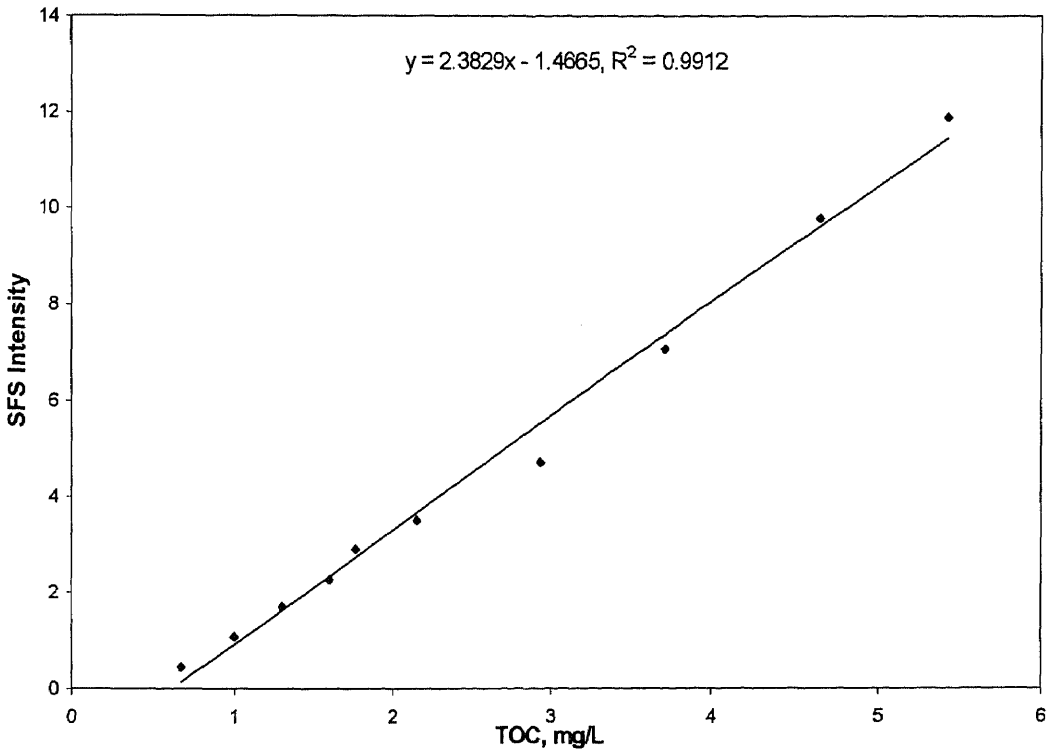


Figure D.2. SFS vs. TOC, River HA measured at Excitation Wavelength 350 nm (Minor Peak)

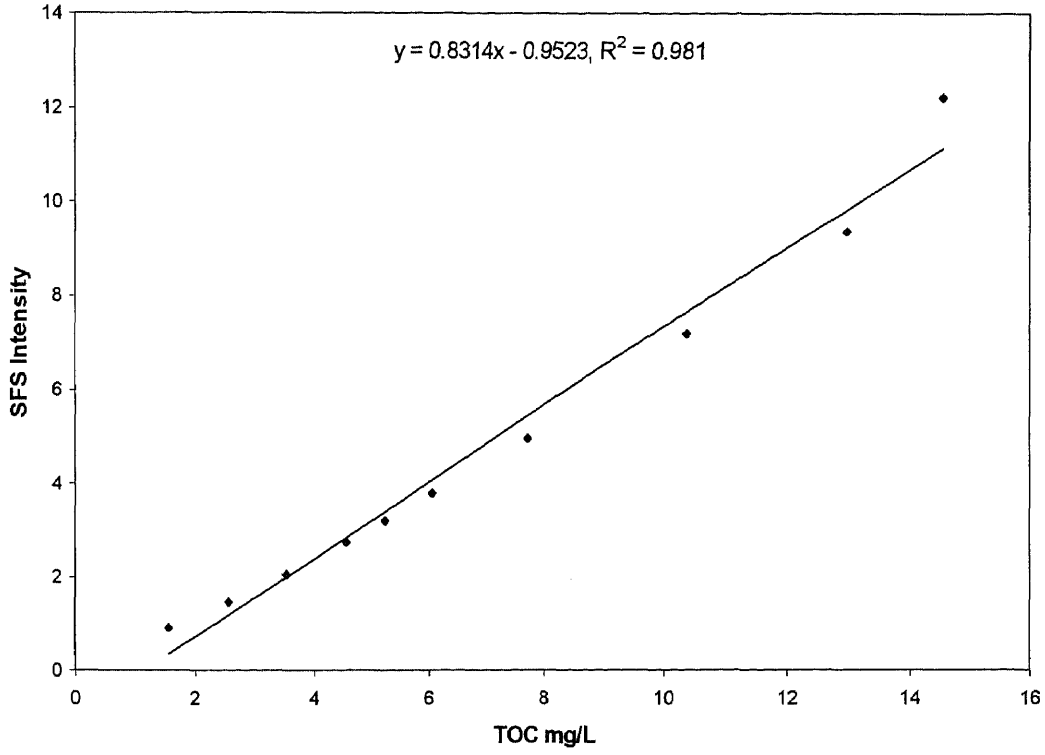


Figure D.3. SFS vs. TOC, River FA measured at Excitation Wavelength 250 nm (Major Peak)

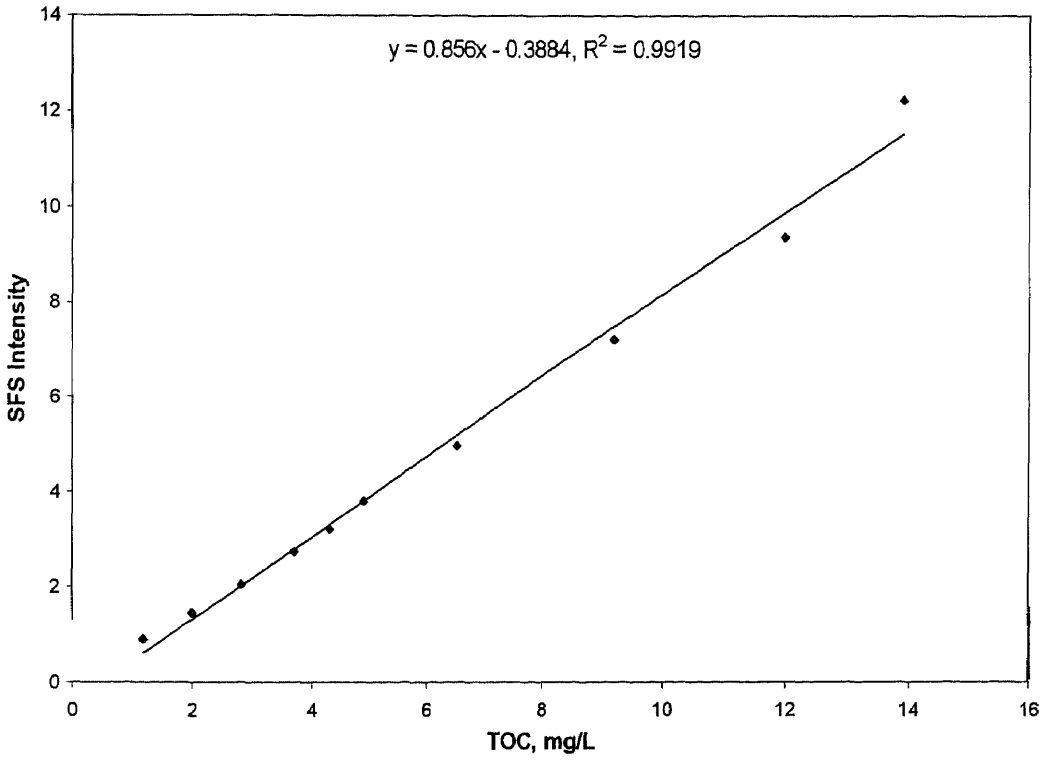


Figure D.4. SFS vs. TOC, River FA measured at Excitation Wavelength 350 nm (Minor Peak)

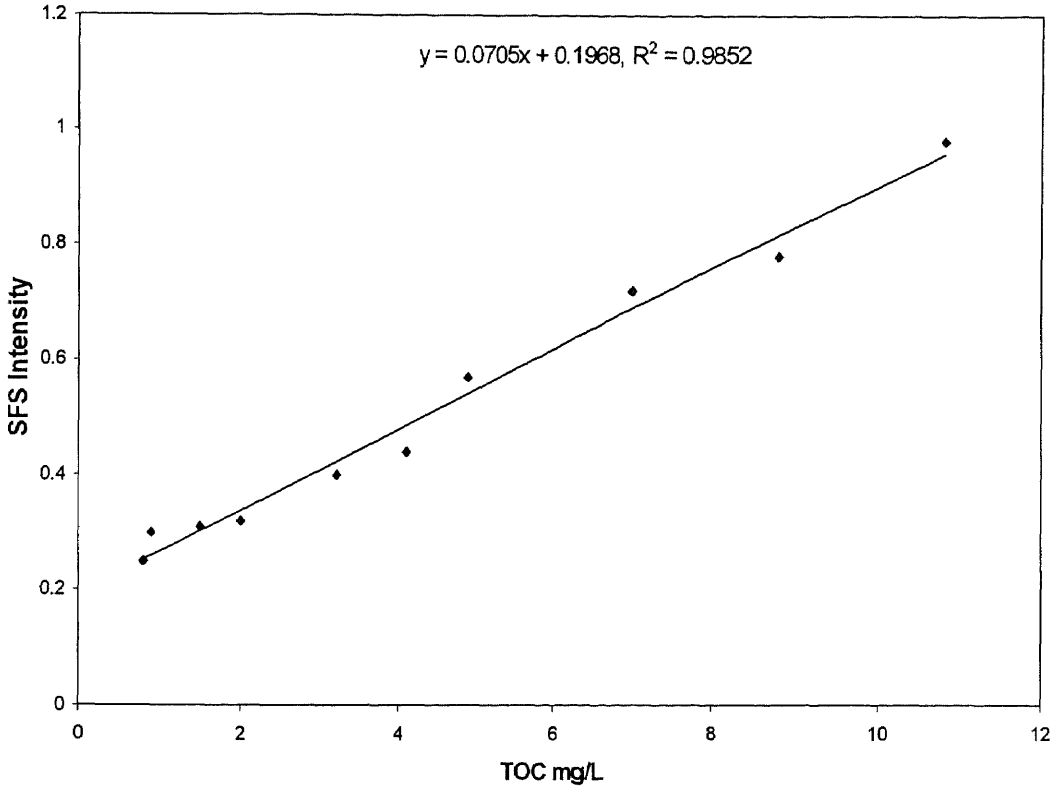


Figure D.5. SFS vs. TOC, Soil HA measured at Excitation Wavelength 250 nm (Major Peak)

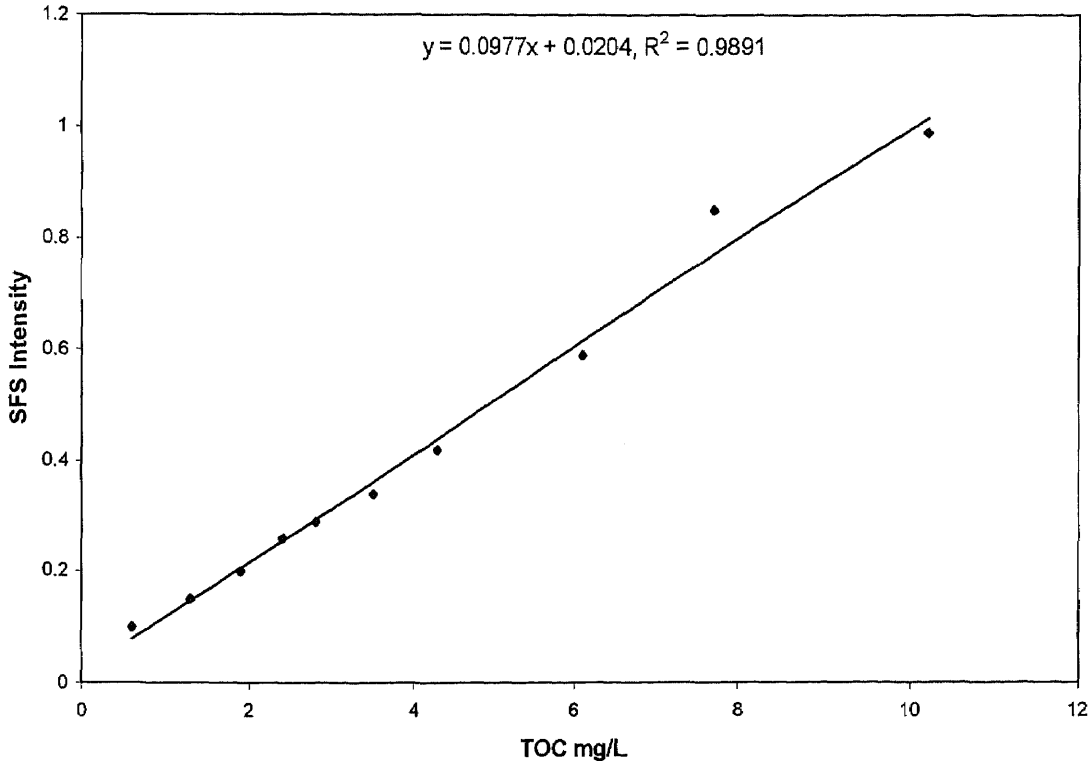


Figure D.6. SFS vs. TOC, Peat HA measured at Excitation Wavelength 250 nm (Major Peak)

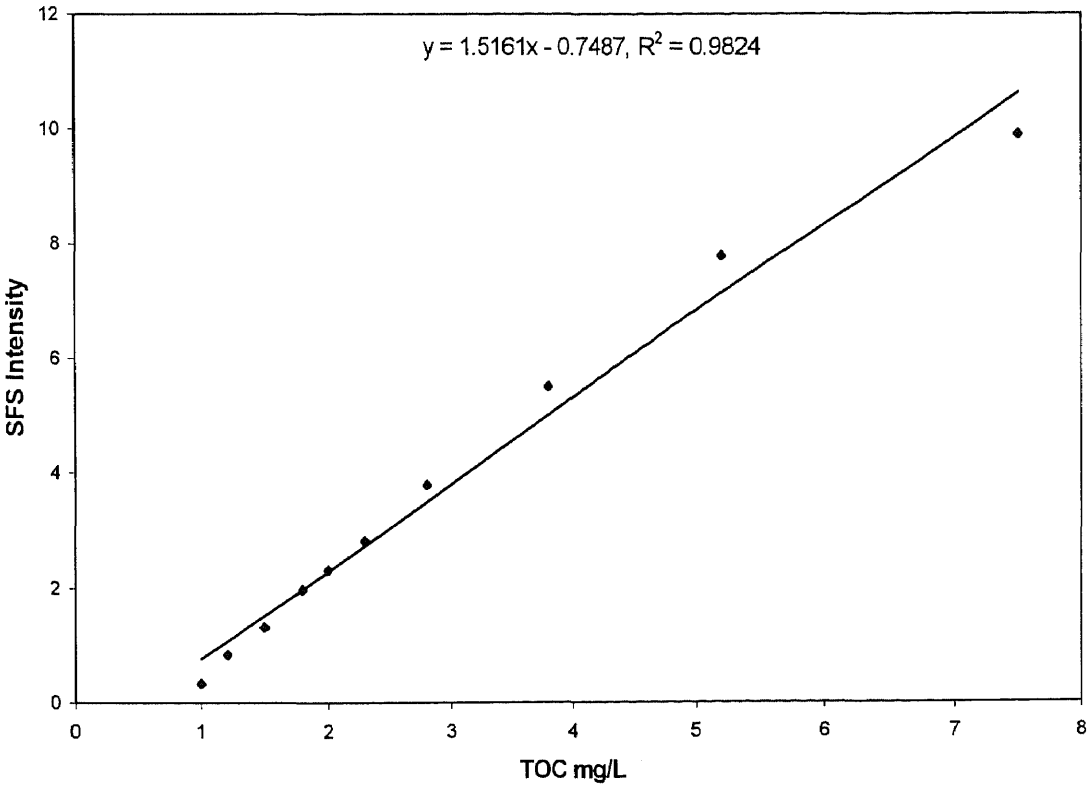


Figure D.7. SFS vs. TOC, Soil FA measured at Excitation Wavelength 250 nm (Major Peak)

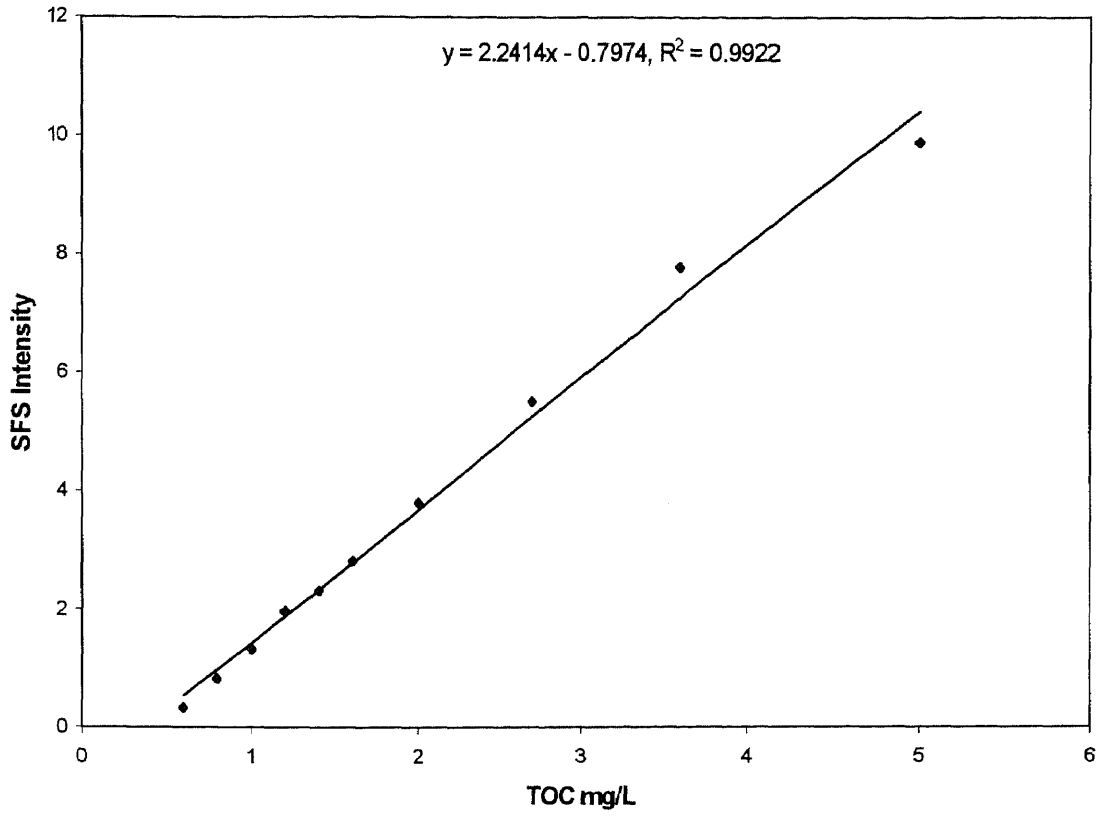


Figure D.8. SFS vs. TOC, Soil FA measured at Excitation Wavelength 350 nm (Minor Peak)

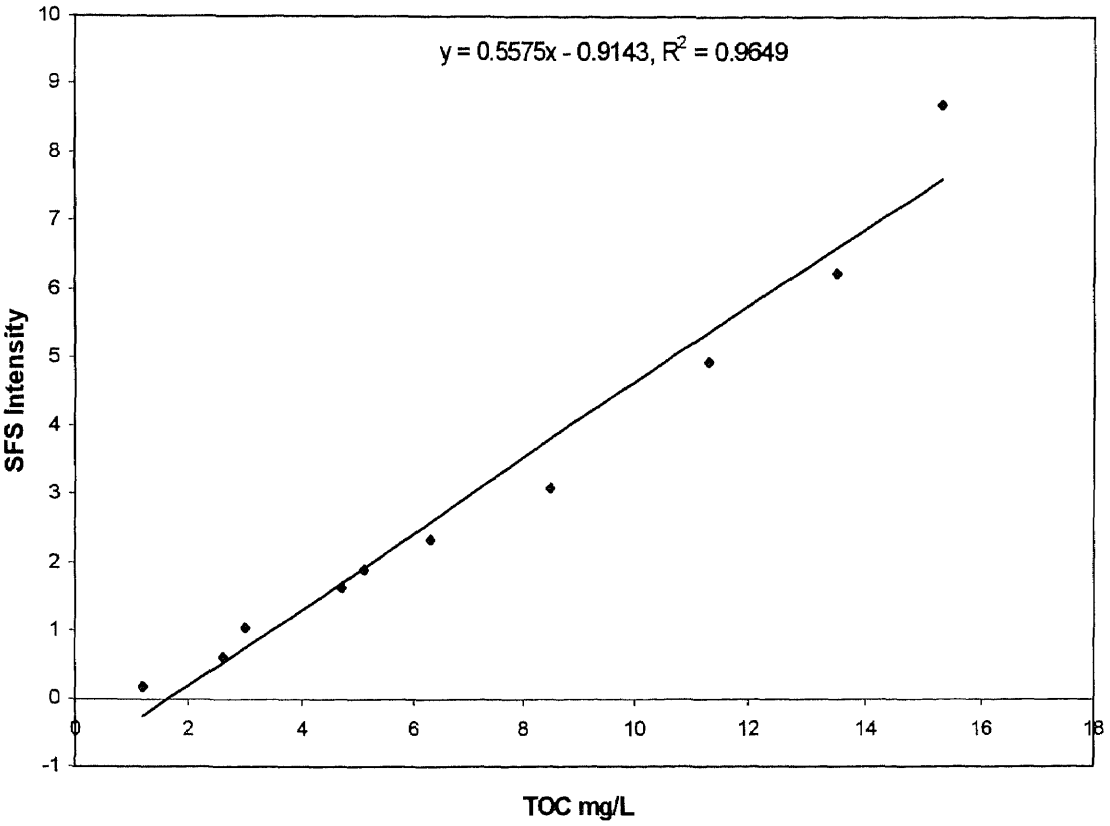


Figure D.9. SFS vs. TOC, Peat FA measured at Excitation Wavelength 250 nm (Major Peak)

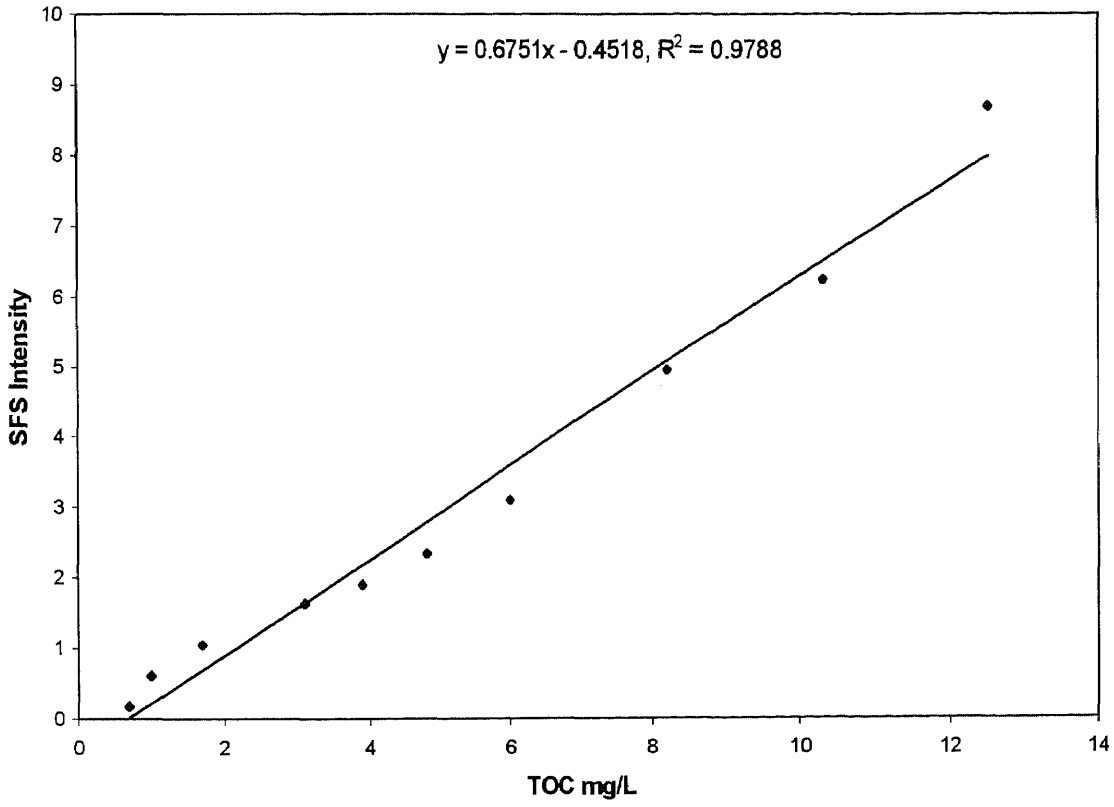


Figure D.10. SFS vs. TOC, Peat FA measured at Excitation Wavelength 350 nm (Minor Peak)

APPENDIX E

TOC VS. UV-254 GRAPHS

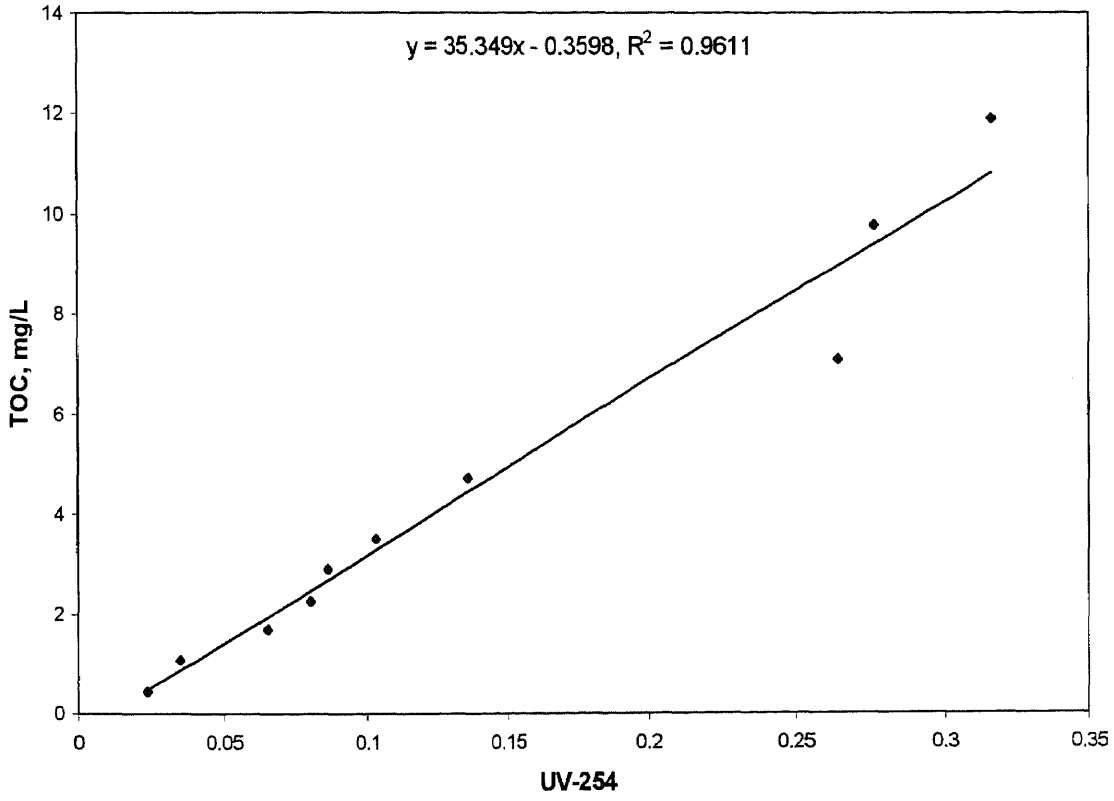


Figure E.1. TOC vs. UV-254, River Humic Acid

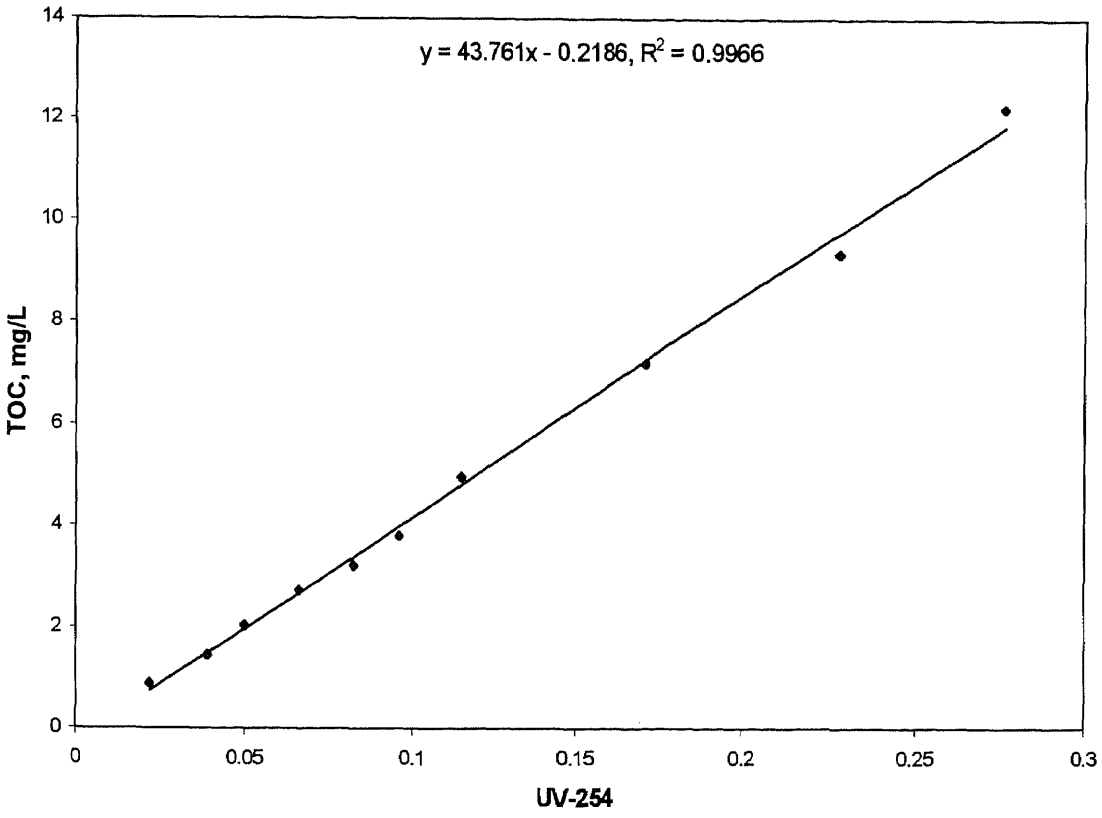


Figure E.2. TOC vs. UV-254, River Fulvic Acid

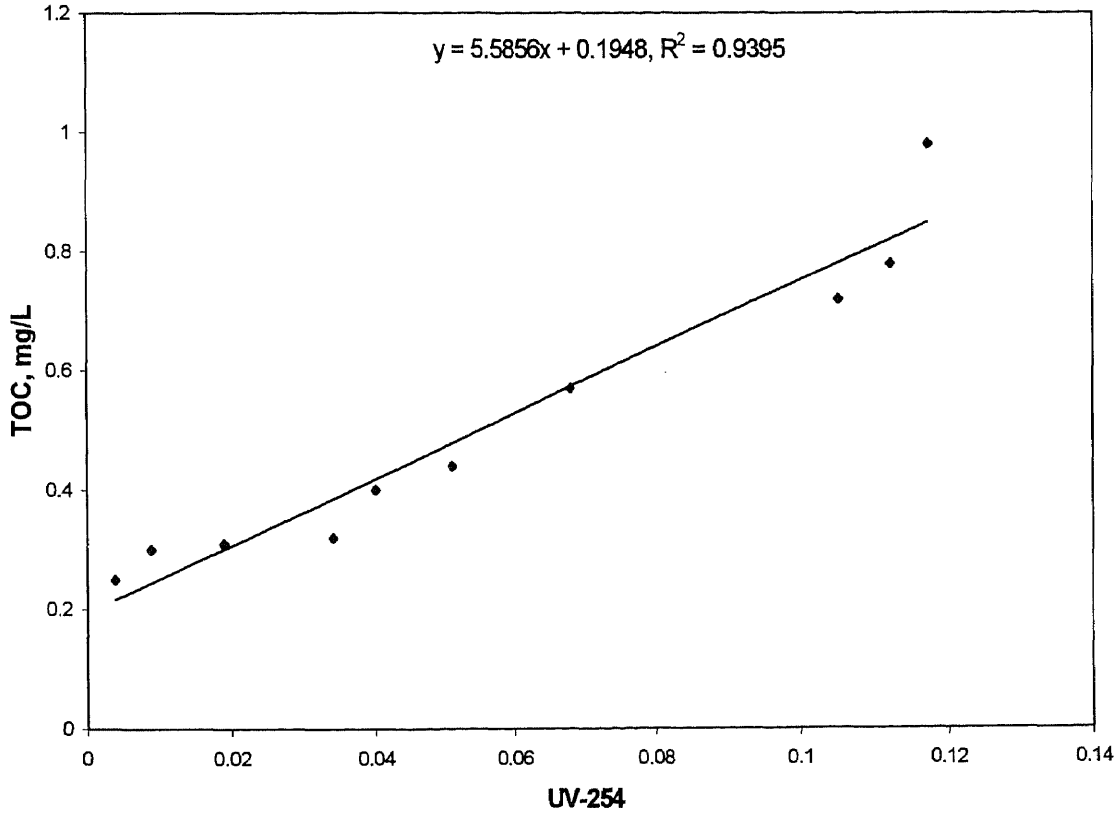


Figure E.3. TOC vs. UV-254, Soil Humic Acid

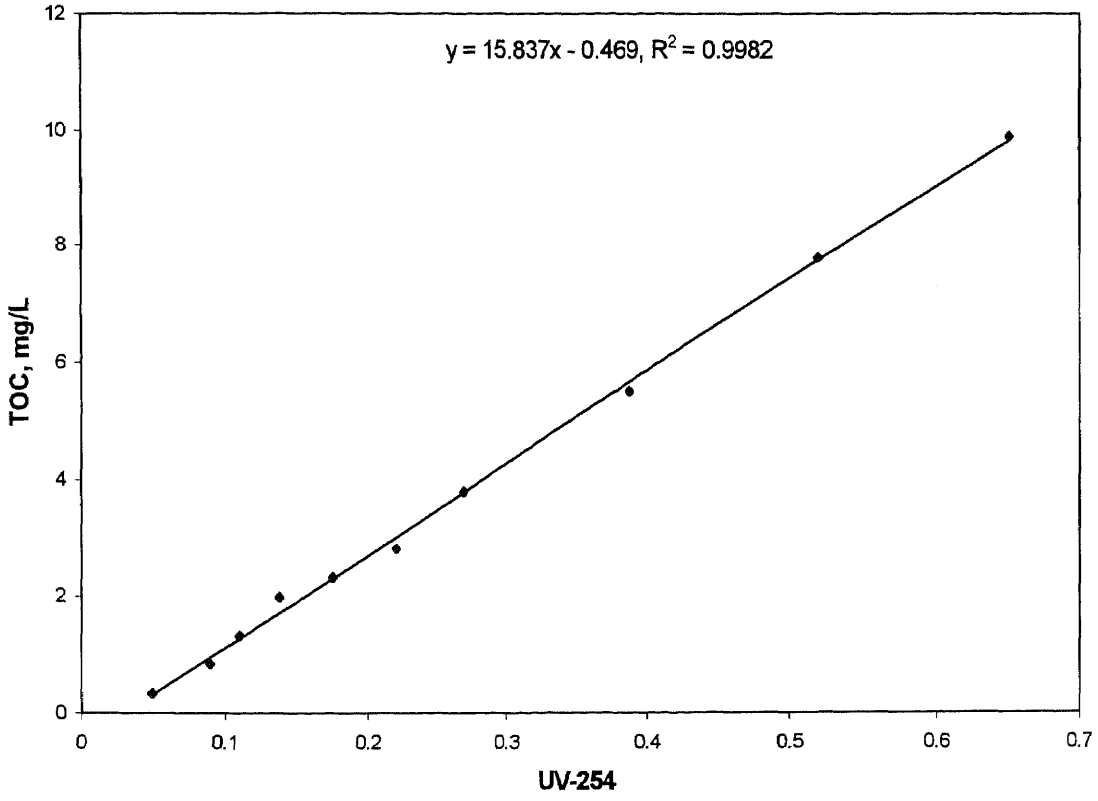


Figure E.4. TOC vs. UV-254, Soil Fulvic Acid

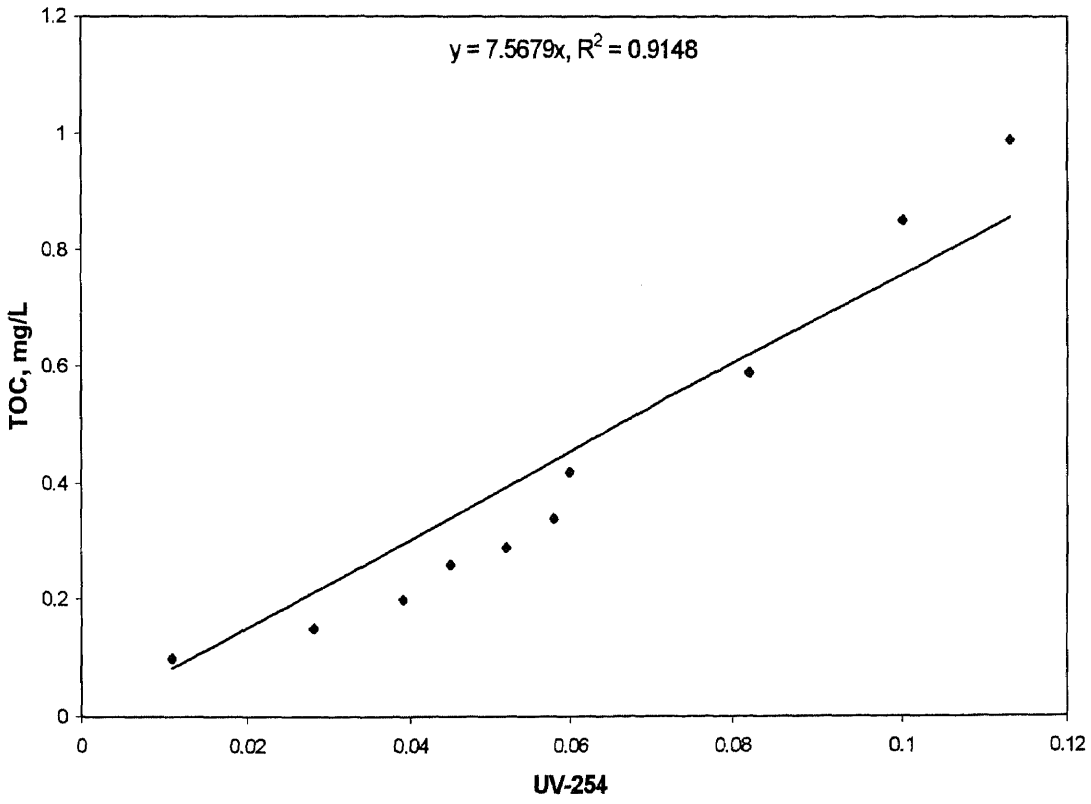


Figure E.5. TOC vs. UV-254, Peat Humic Acid

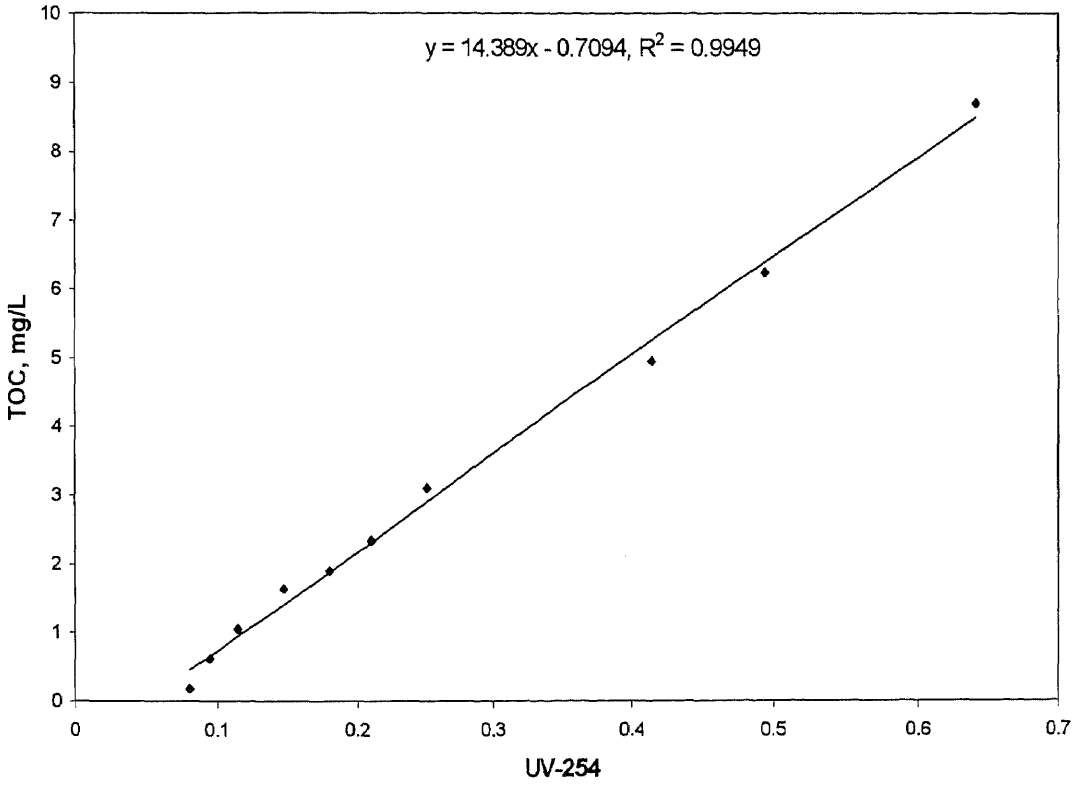


Figure E.6. TOC vs. UV-254, Peat Fulvic Acid

APPENDIX F

DBPFP VS. FLUORESCENCE GRAPHS

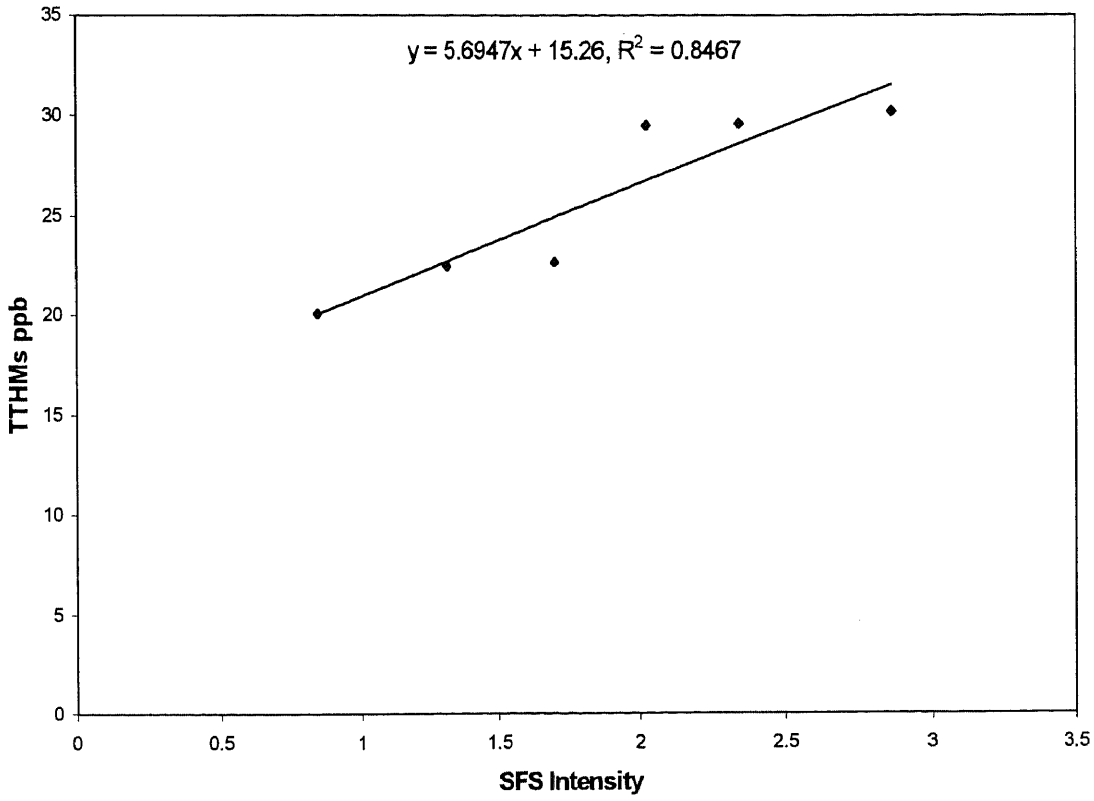


Figure F.1. TTHMs vs. SFS, River HA measured at Excitation Wavelength 250 (Major Peak)

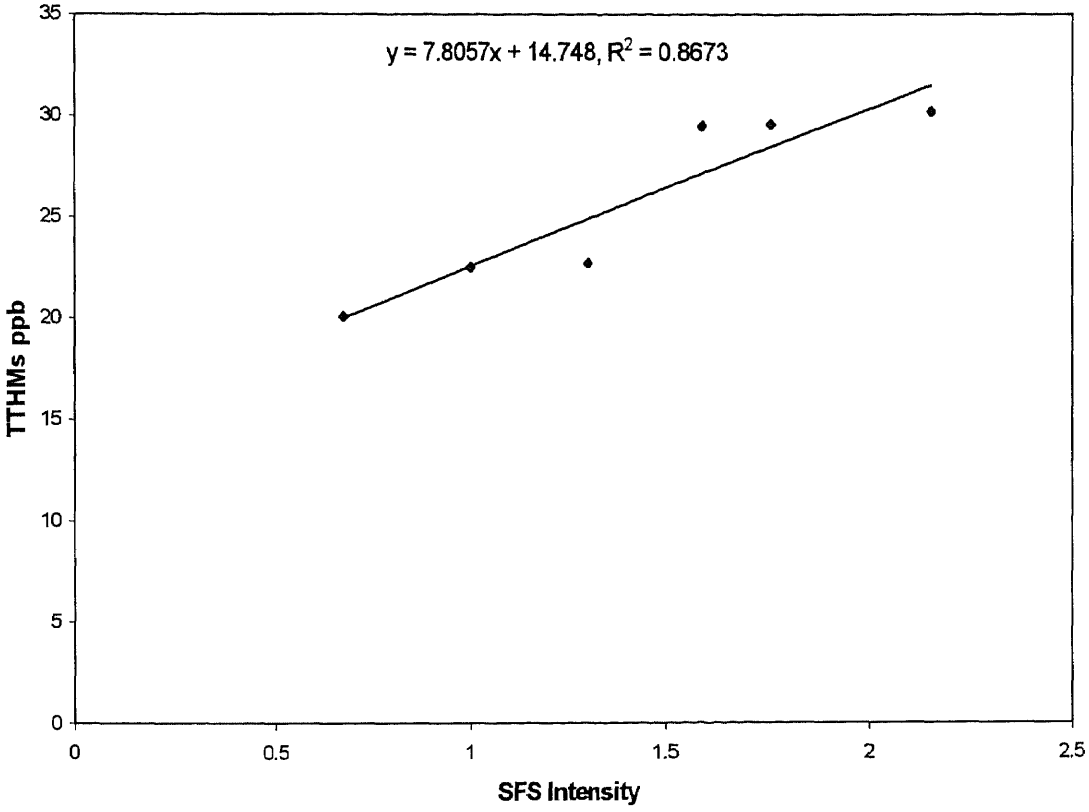


Figure F.2. TTHMs vs. SFS, River HA measured at Excitation Wavelength 350 (Minor Peak)

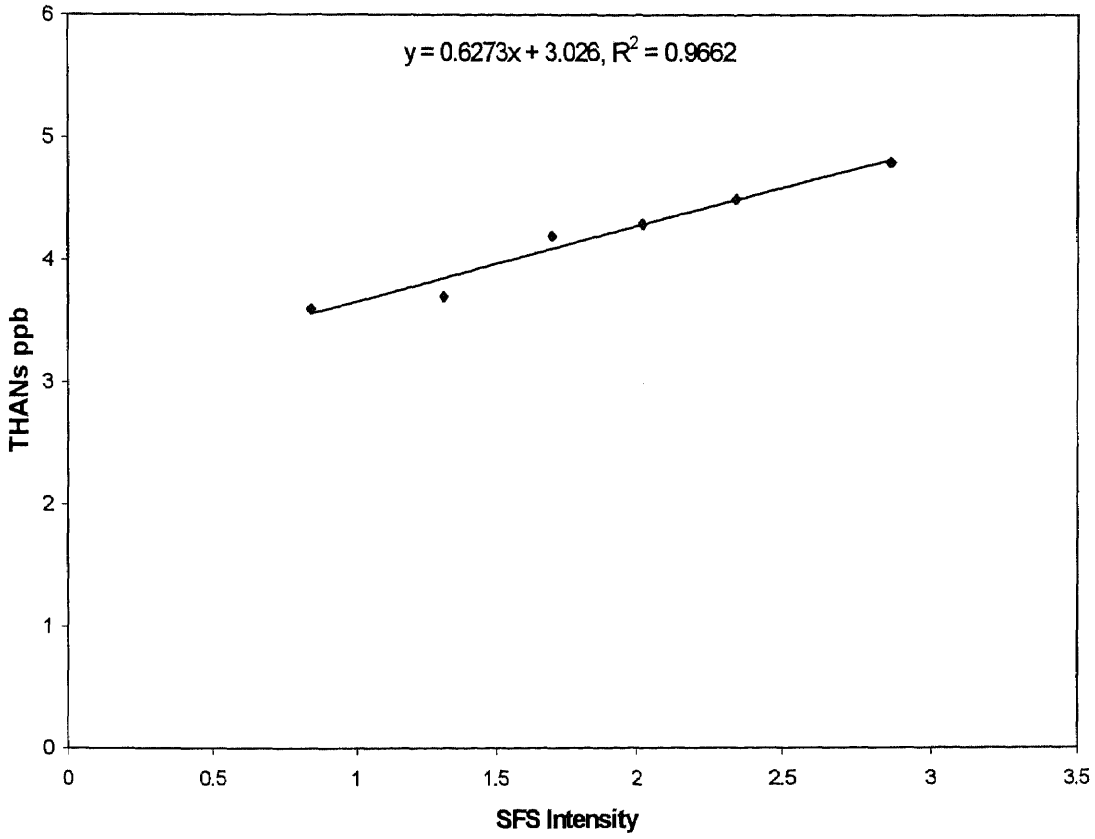


Figure F.3. THANs vs. SFS, River HA measured at Excitation Wavelength 250 (Major Peak)

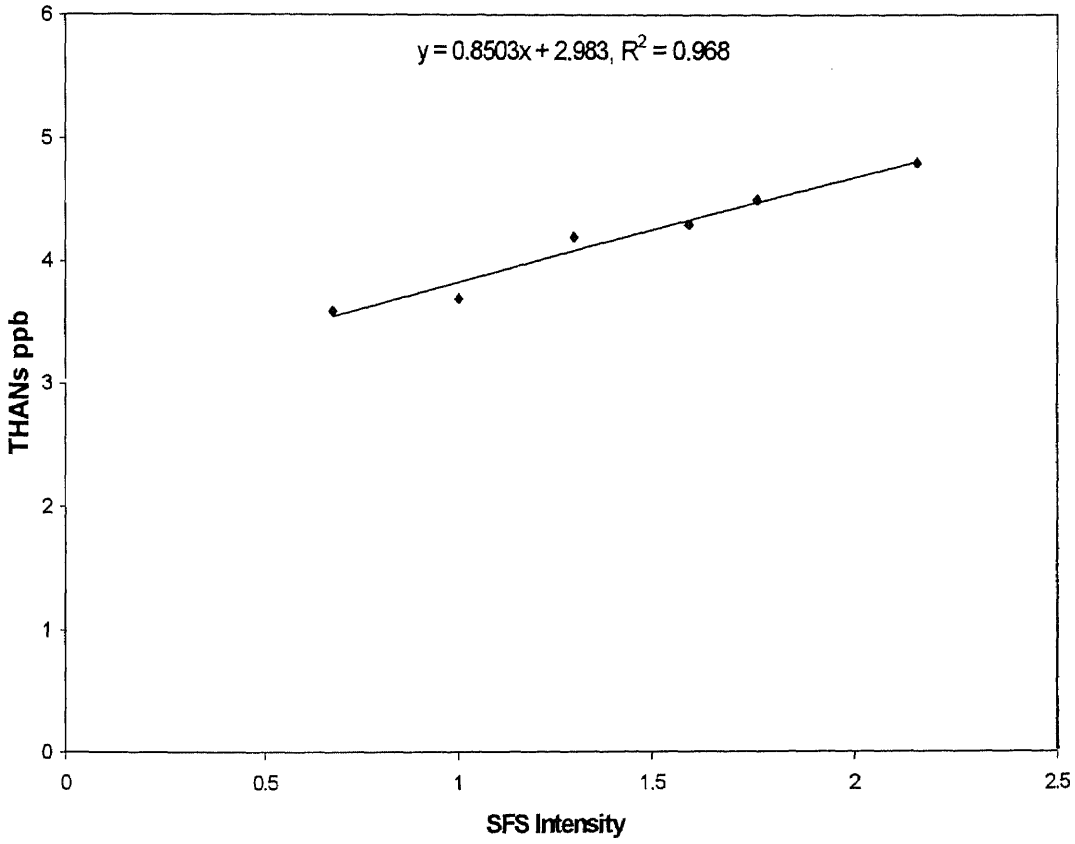


Figure F.4. THANs vs. SFS, River HA measured at Excitation Wavelength 350 (Minor Peak)

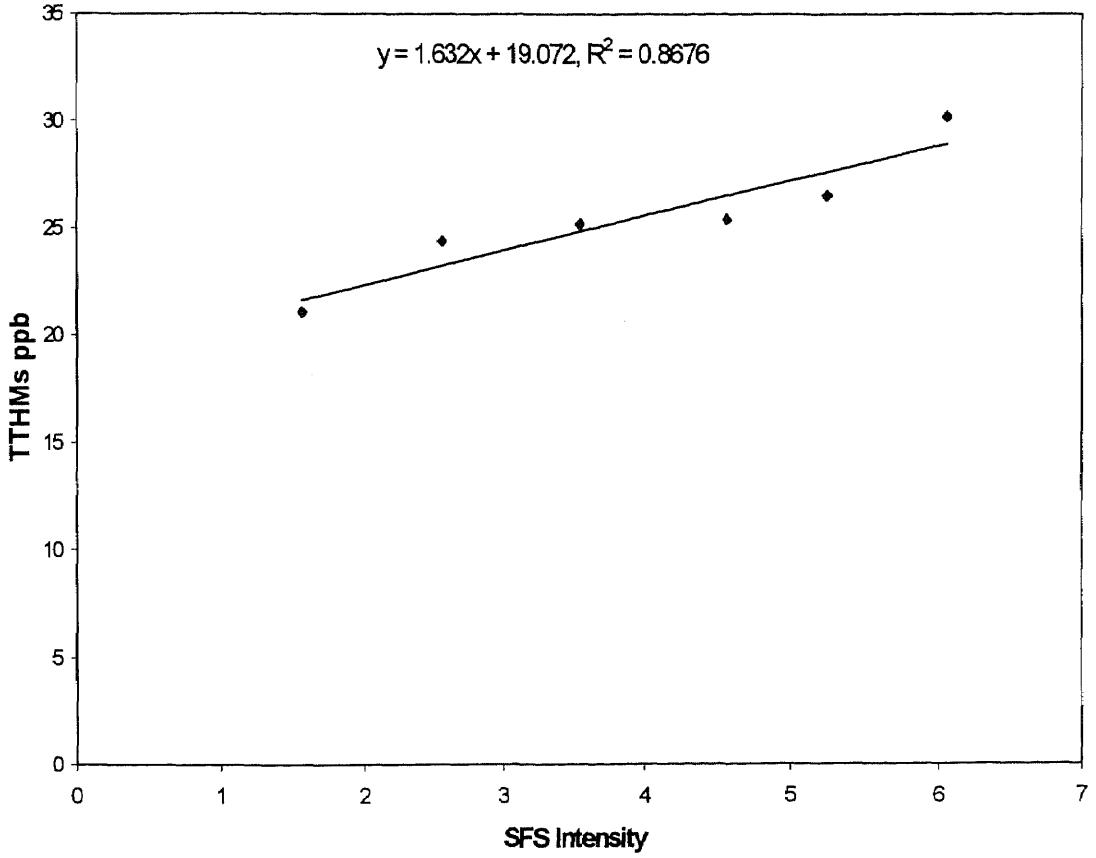


Figure F.5. TTHMs vs. SFS, River FA measured at Excitation Wavelength 250 (Major Peak)

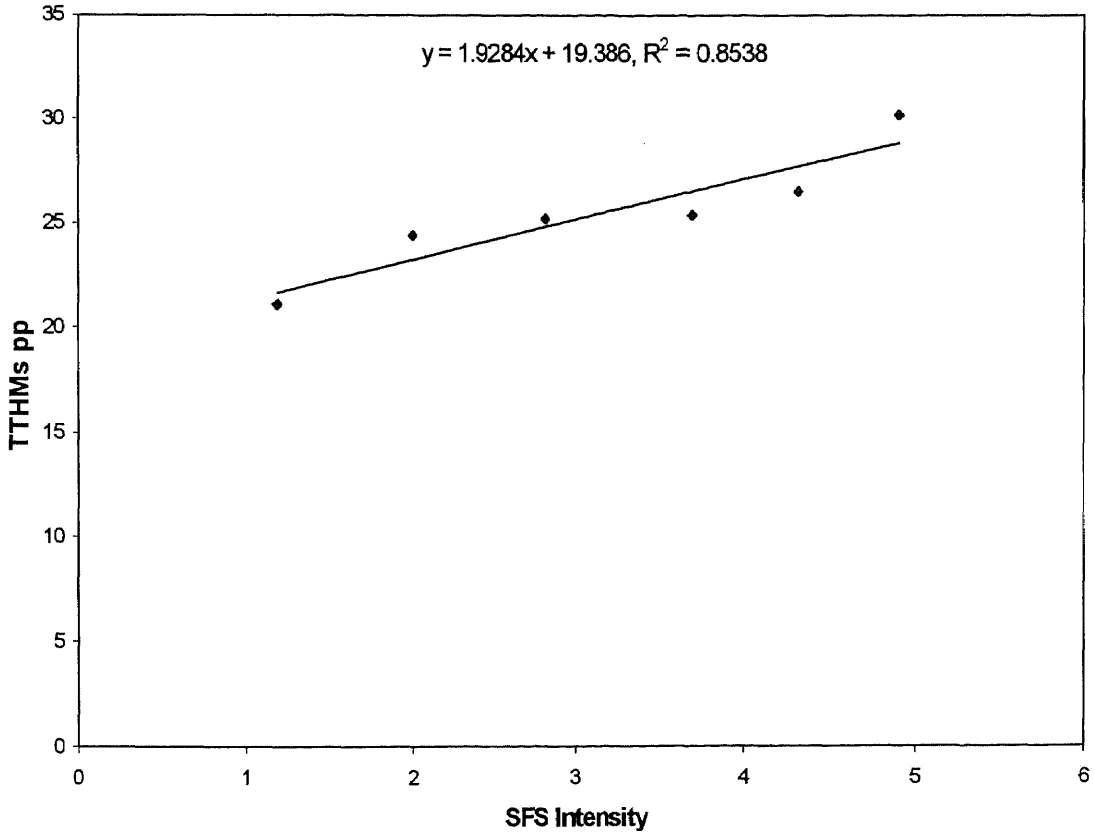


Figure F.6. TTHMs vs. SFS, River FA measured at Excitation Wavelength 350 (Minor Peak)

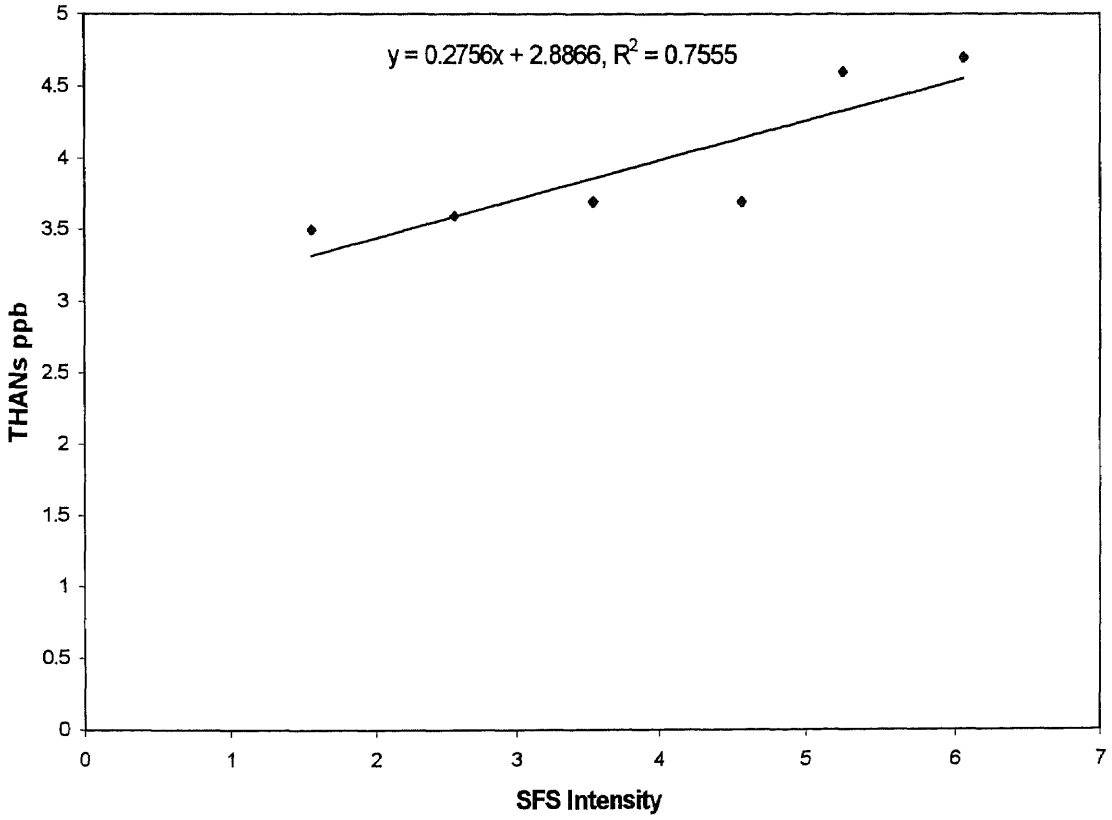


Figure F.7. THANs vs. SFS, River FA measured at Excitation Wavelength 250 (Major Peak)

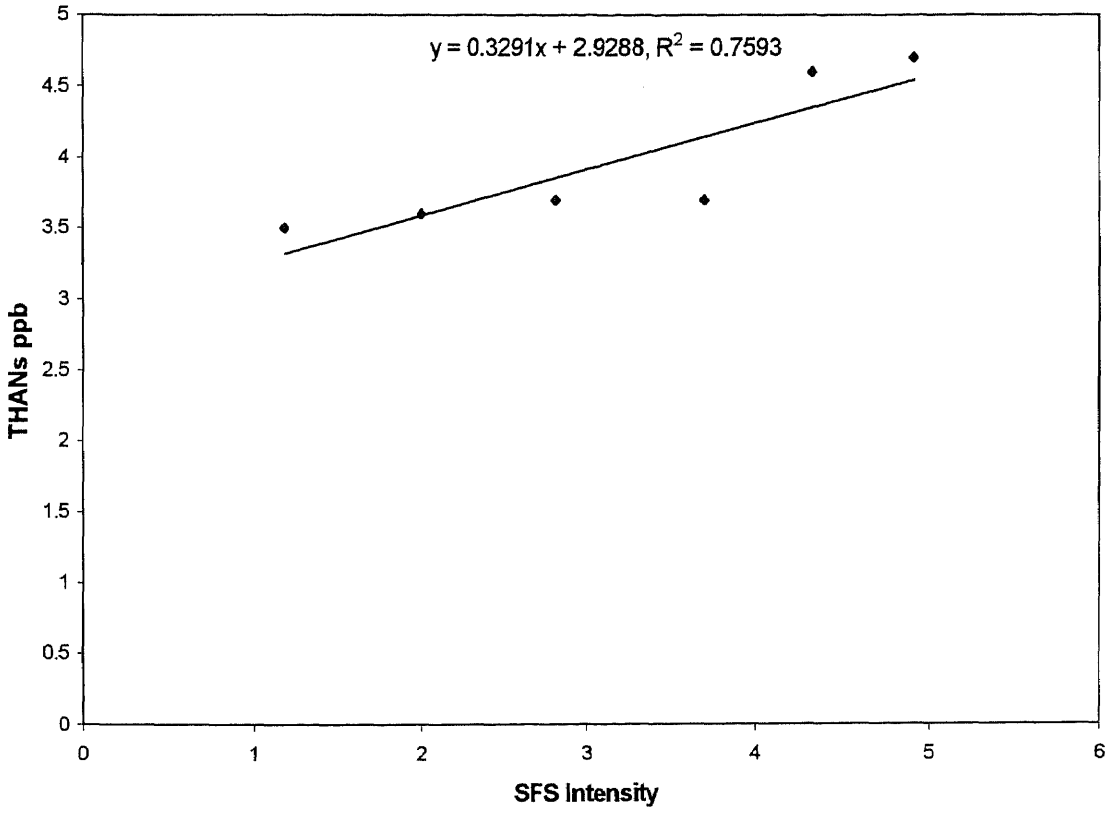


Figure F.8. THANs vs. SFS, River FA measured at Excitation Wavelength 350 (Minor Peak)

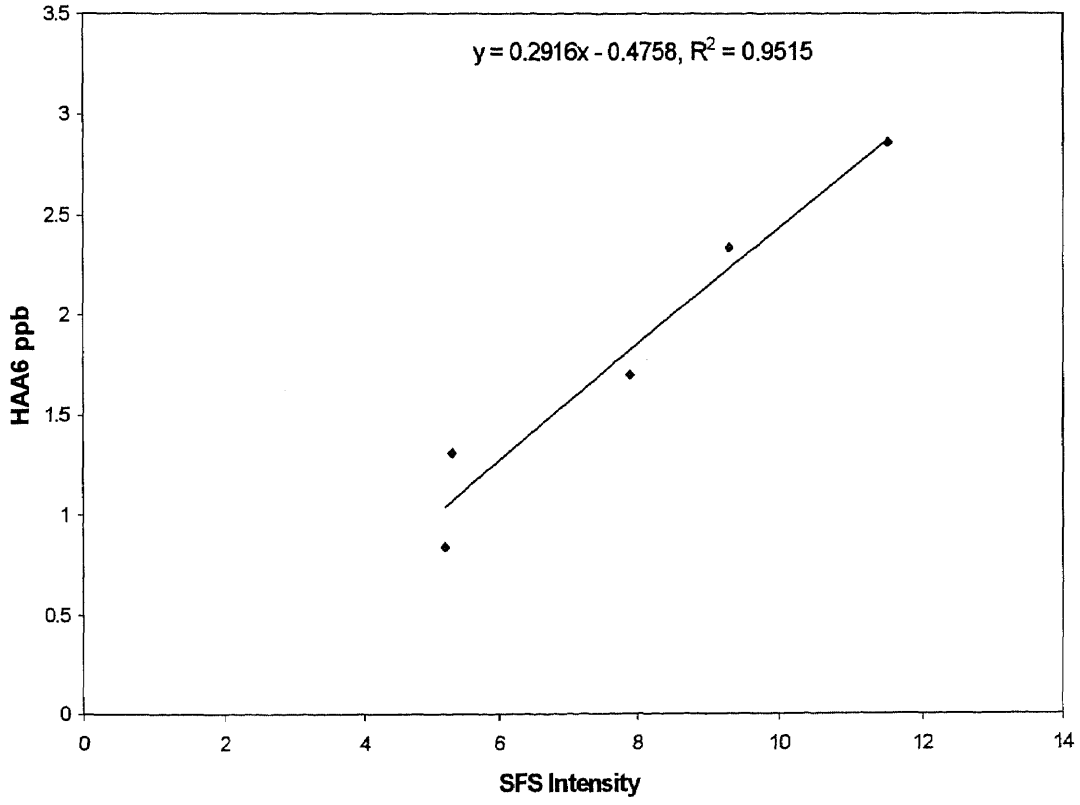


Figure F.9. HAA6 vs. SFS, River HA measured at Excitation Wavelength 250 (Major Peak)

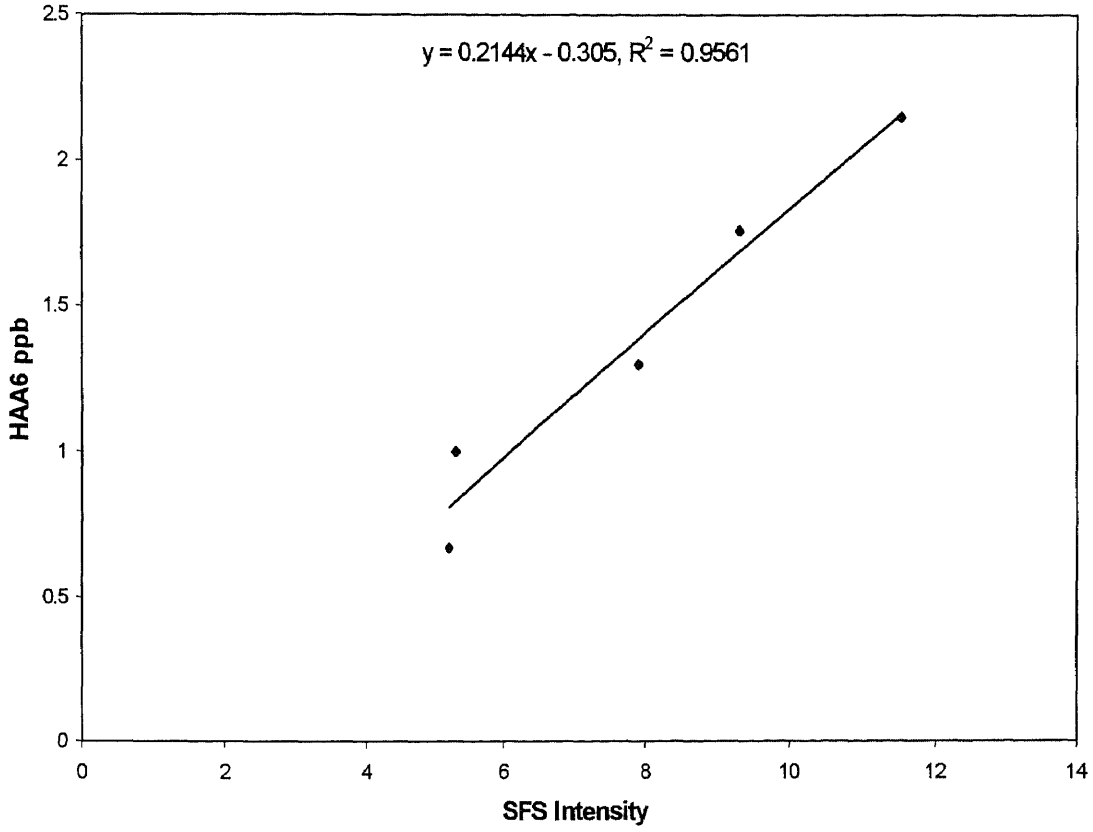


Figure F.10. HAA6 vs. SFS, River HA measured at Excitation Wavelength 350 (Minor Peak)

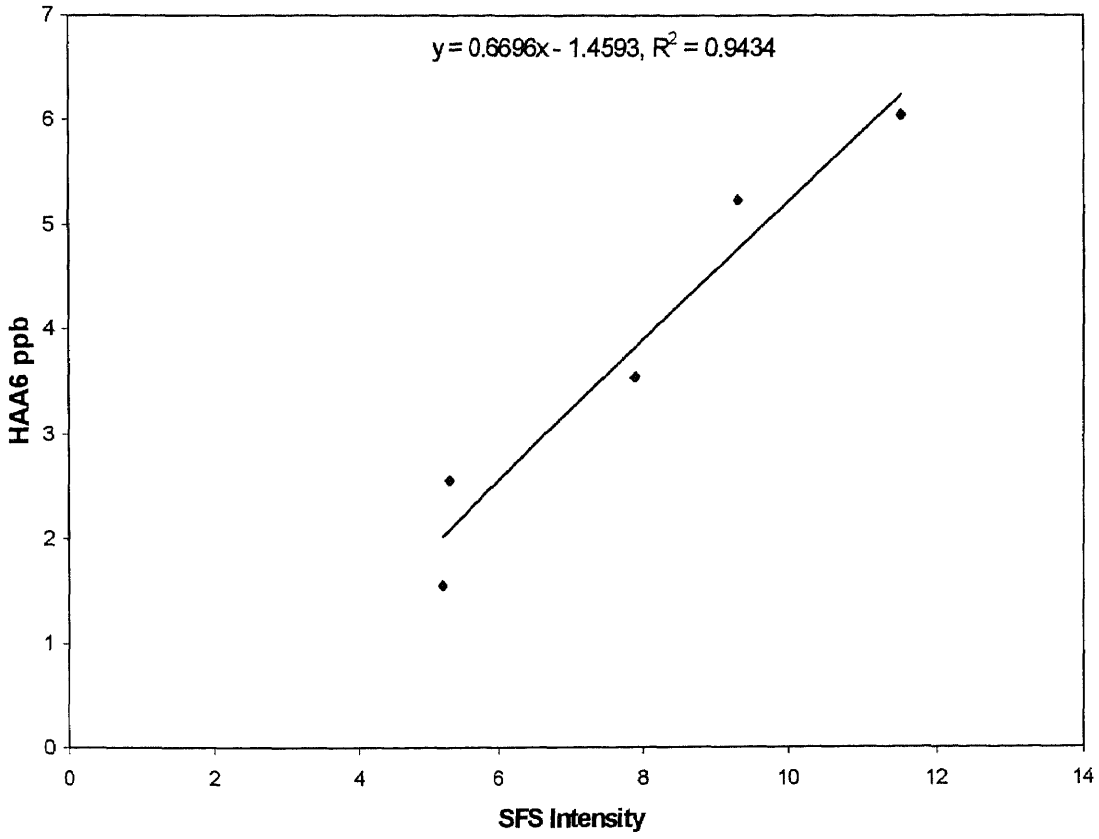


Figure F.11. HAA6 vs. SFS, River FA measured at Excitation Wavelength 250 (Major Peak)

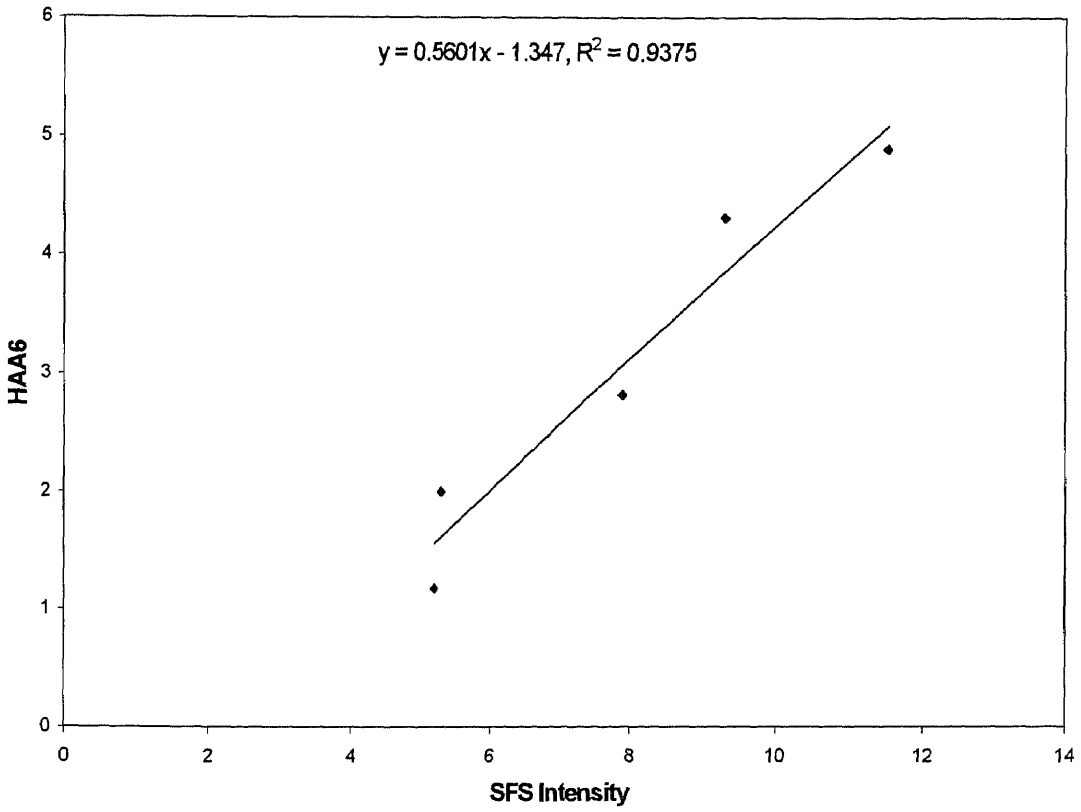


Figure F.12. HAA6 vs. SFS, River FA measured at Excitation Wavelength 350 (Minor Peak)

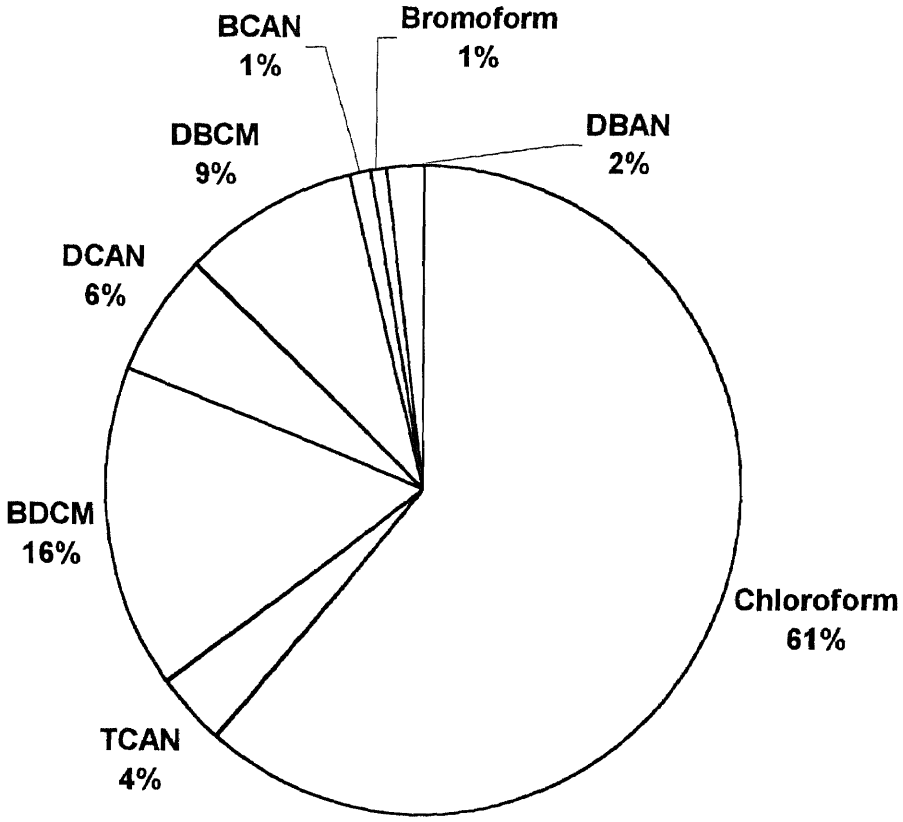


Figure F.13. Individual percentages of DBPs, River HA 10 ppm

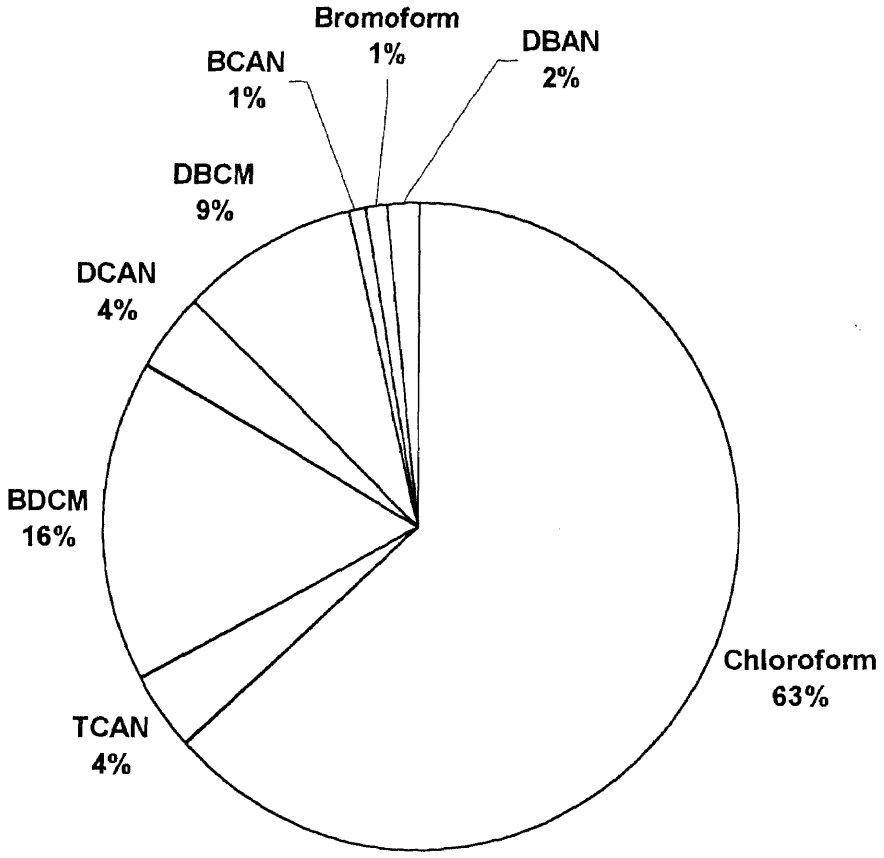


Figure F.14. Individual Percentages of DBPs, River FA 10 ppm

APPENDIX G

DBPFP VS. TOC, UV-254, SPECIFIC ABSORBANCE GRAPHS

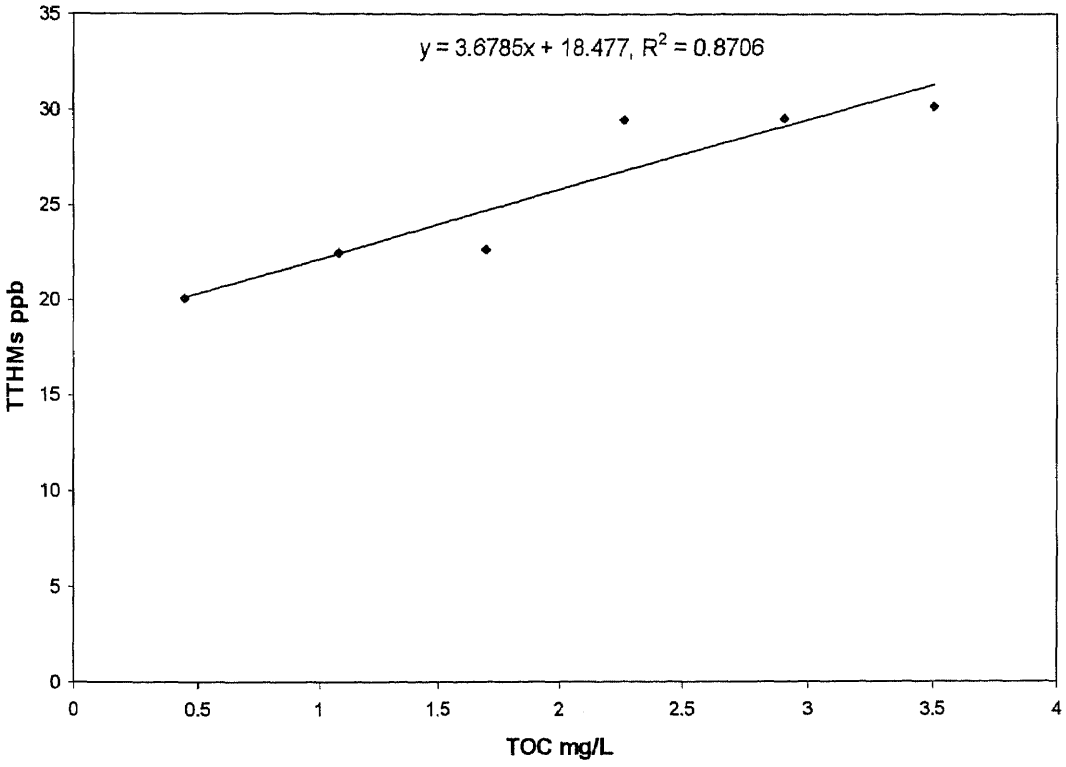


Figure G.1. TTHMs vs. TOC, River HA

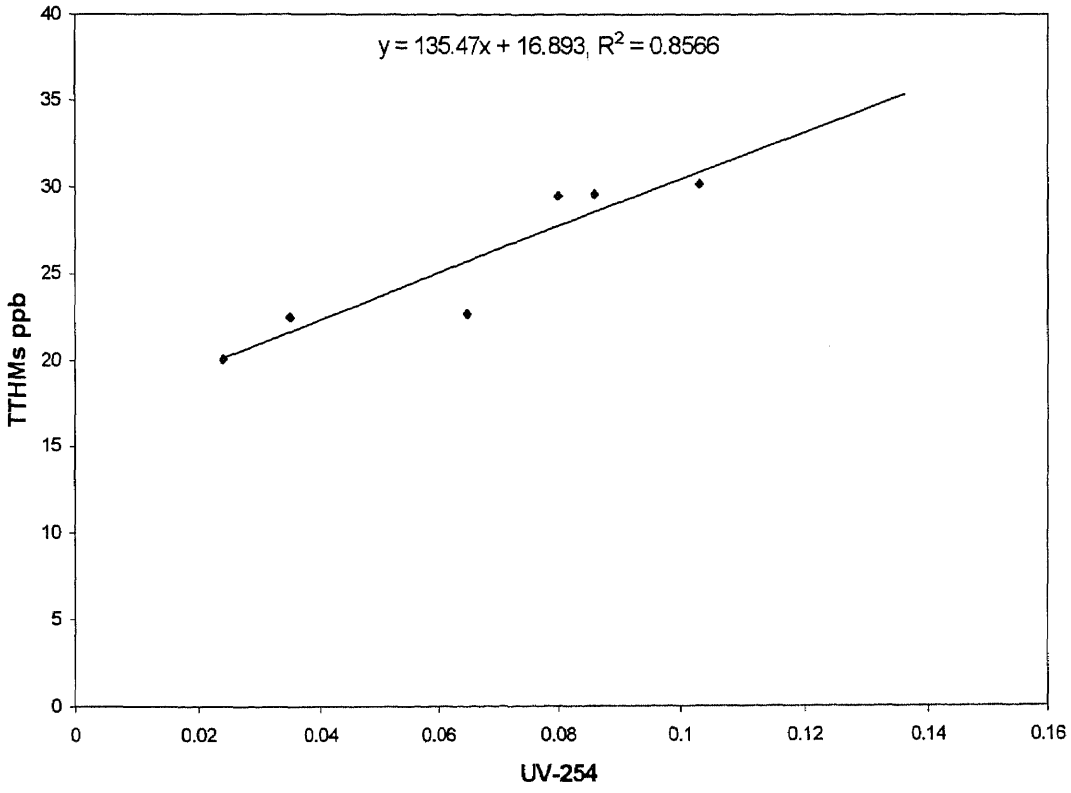


Figure G.2. TTHMs vs. UV-254, River HA

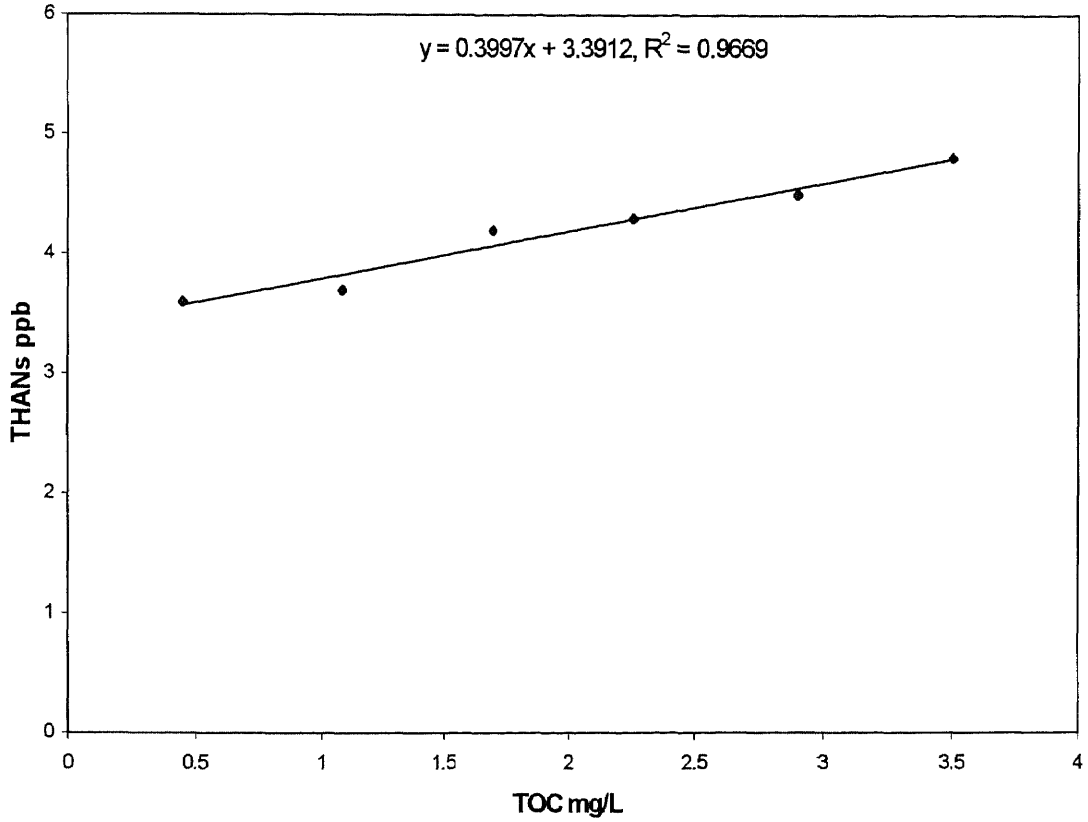


Figure F.3. THANs vs. TOC, River HA

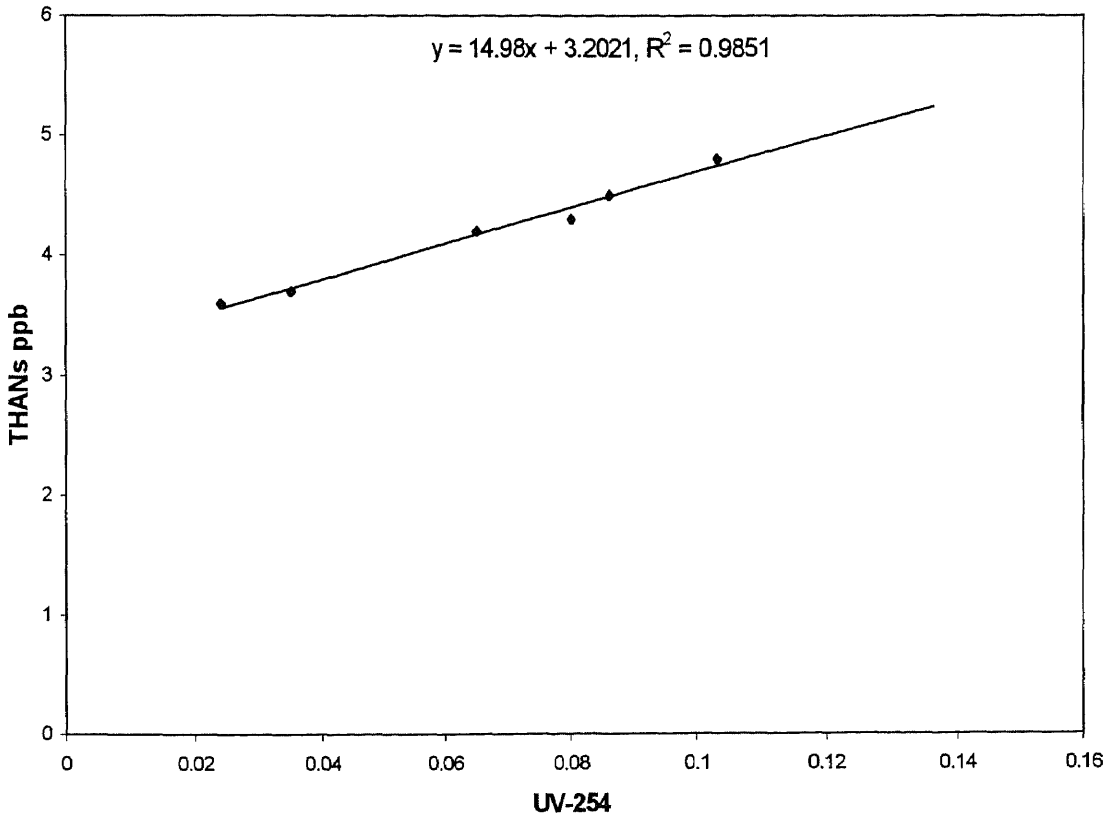


Figure G.4. THANs vs. UV-254, River HA

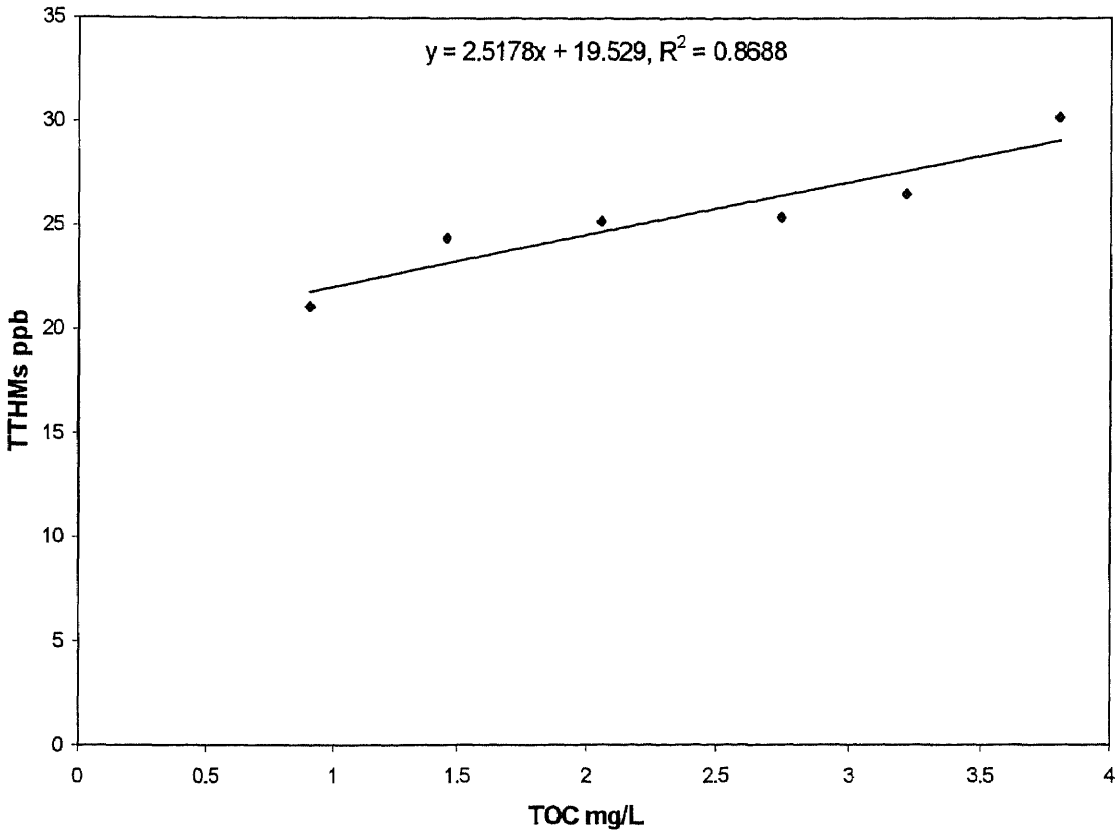


Figure G.5. TTHMs vs. TOC, River FA

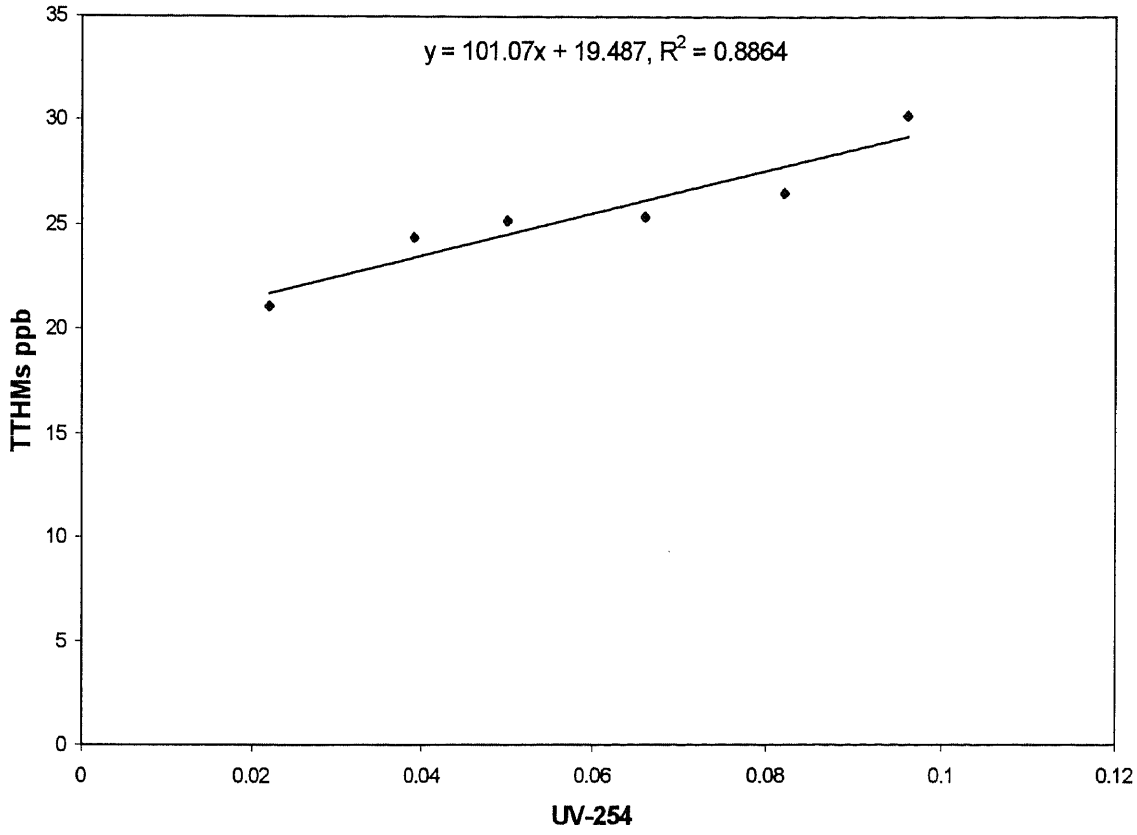


Figure G.6. TTHMs vs. UV-254, River FA

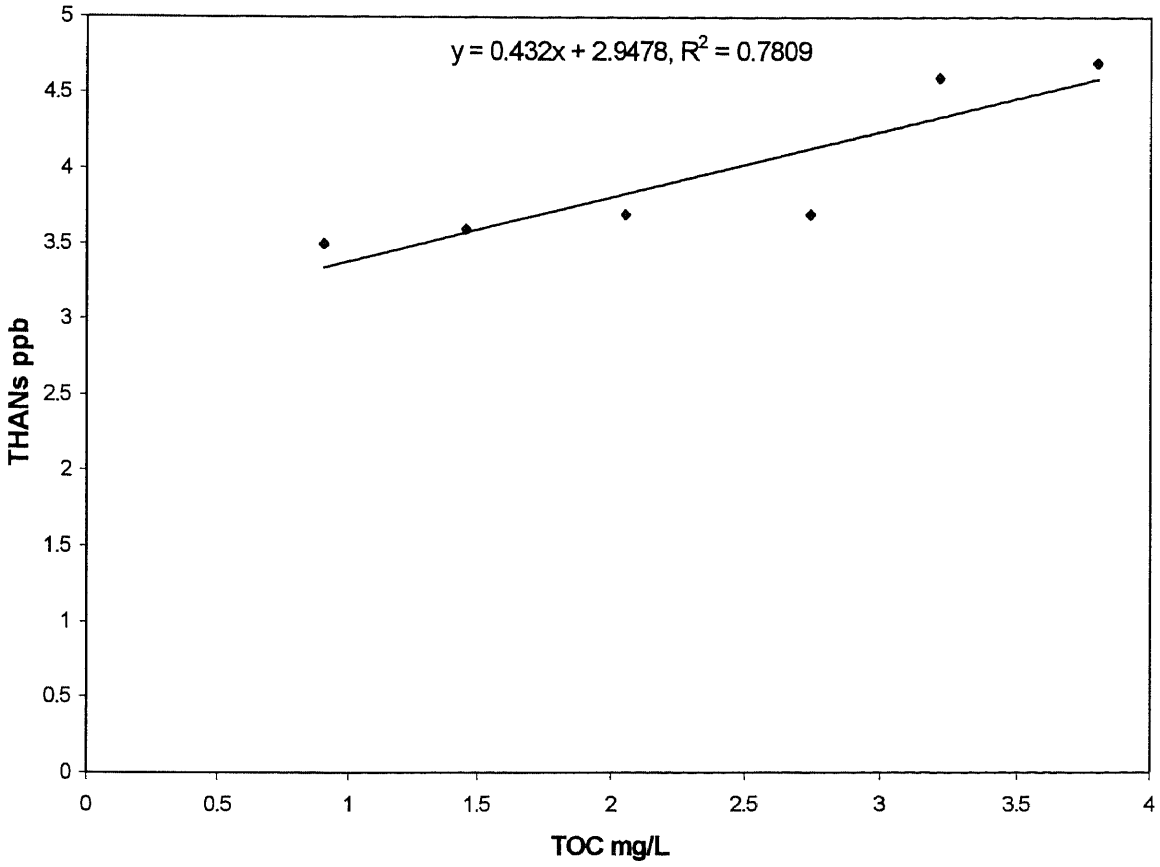


Figure G.7. THANs vs. TOC, River FA

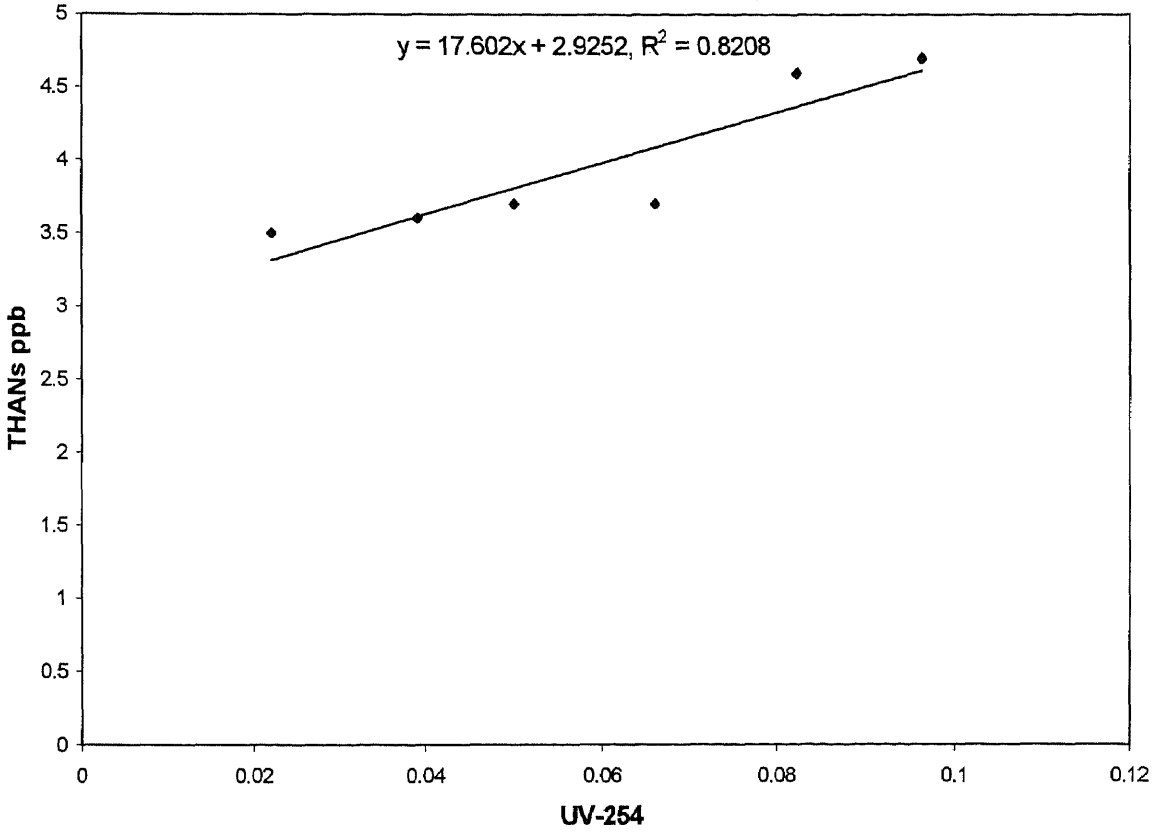


Figure G.8. THANs vs. UV-254, River FA

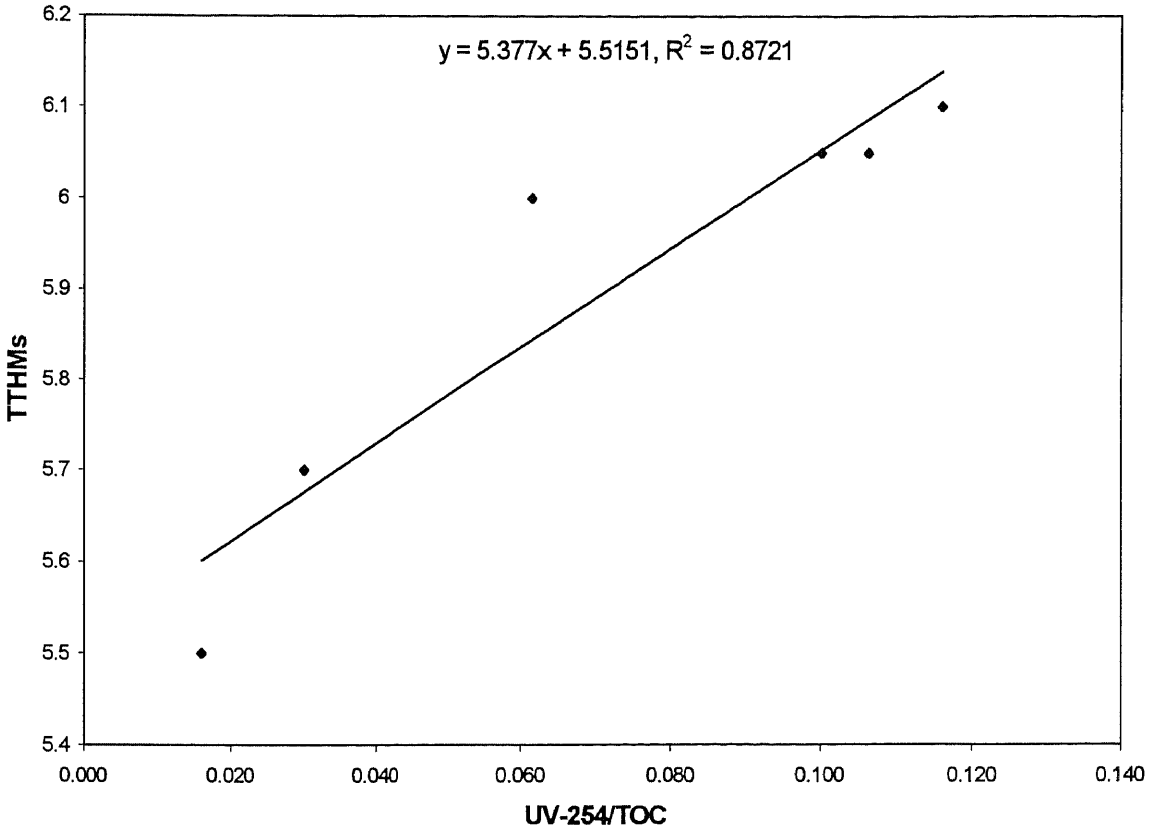


Figure G.9. TTHMs vs. UV-254/TOC (Specific Absorbance), Soil HA

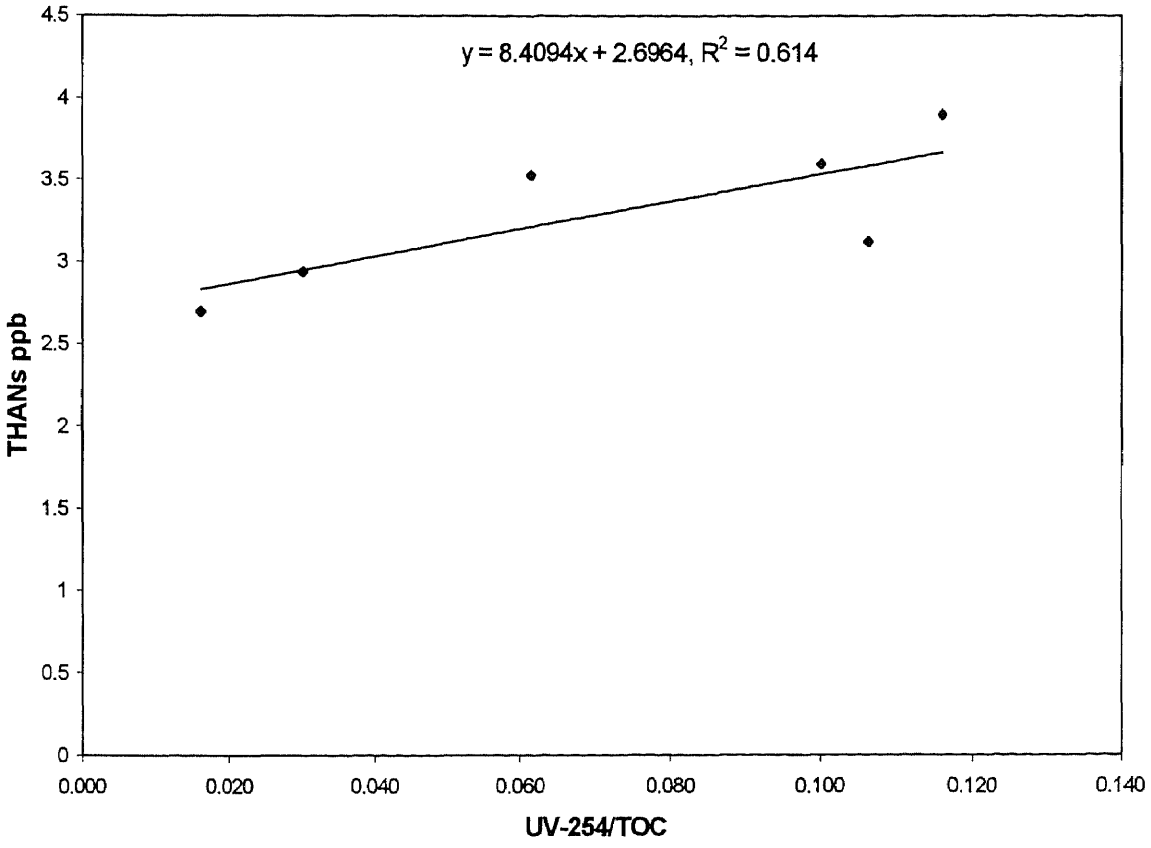


Figure G.10. THANs vs. UV-254/TOC (Specific Absorbance), Soil HA

APPENDIX H

DBPFP VS. FLUORESCENCE GRAPHS

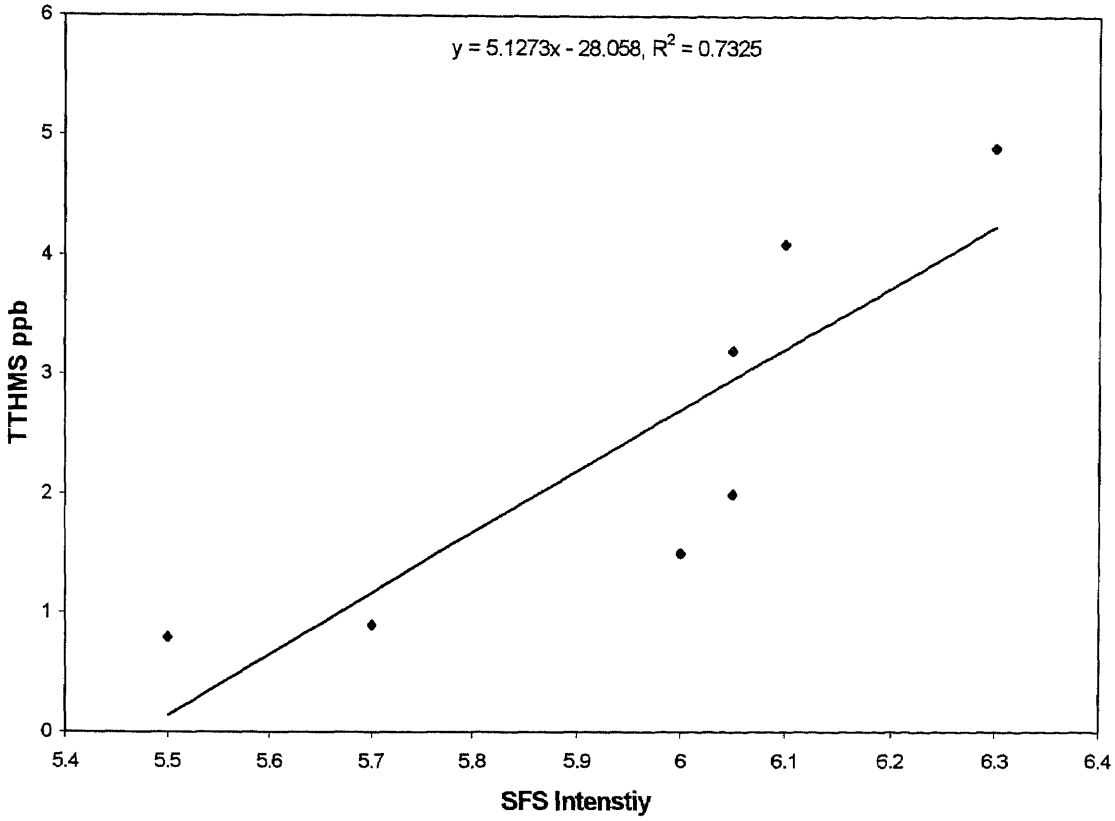


Figure H.1. TTHMs vs. SFS, Soil HA measured at Major Peak

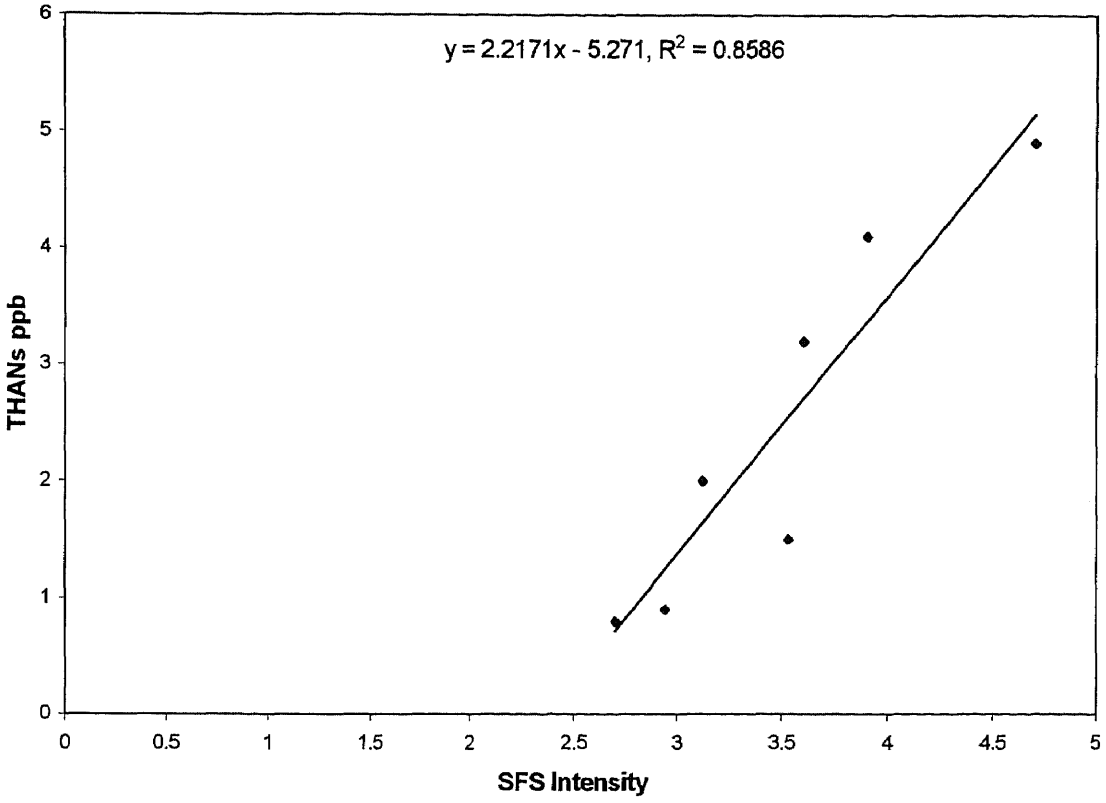


Figure H.2. THANs vs. SFS, Soil HA measured at Major Peak

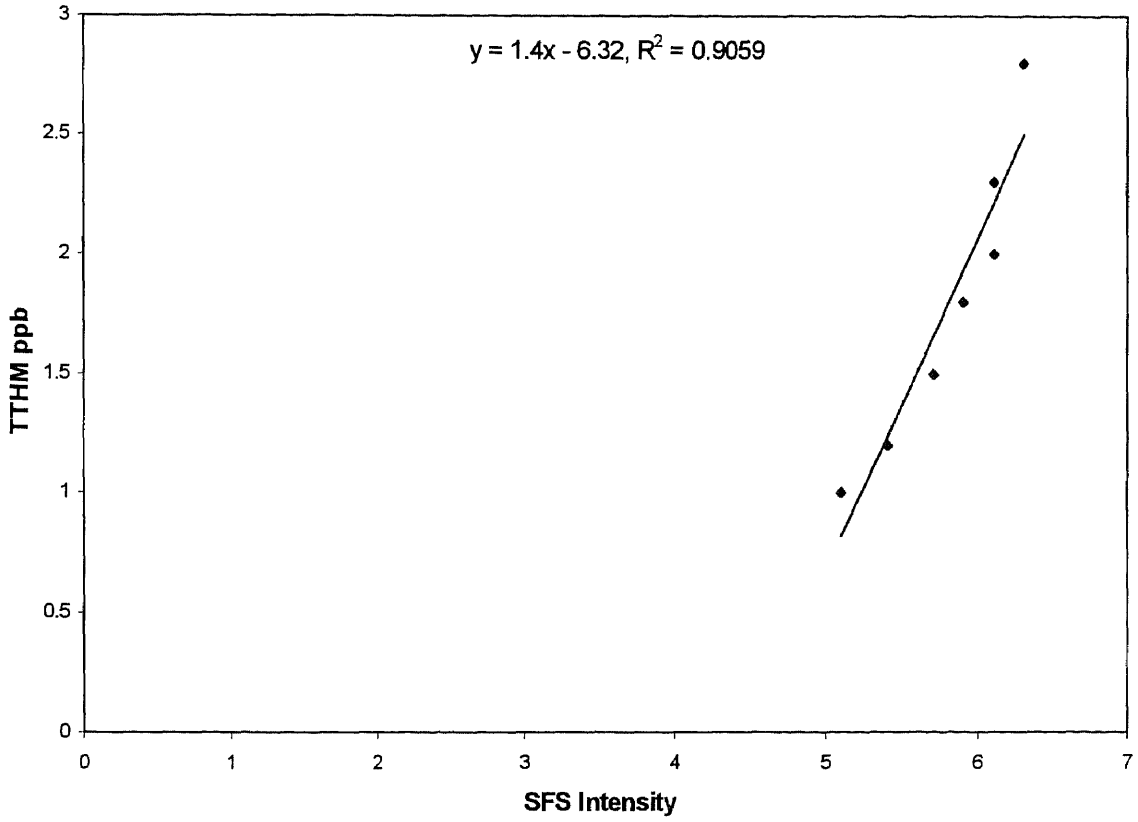


Figure H.3. TTHMs vs. SFS, Soil FA measured at Major Peak

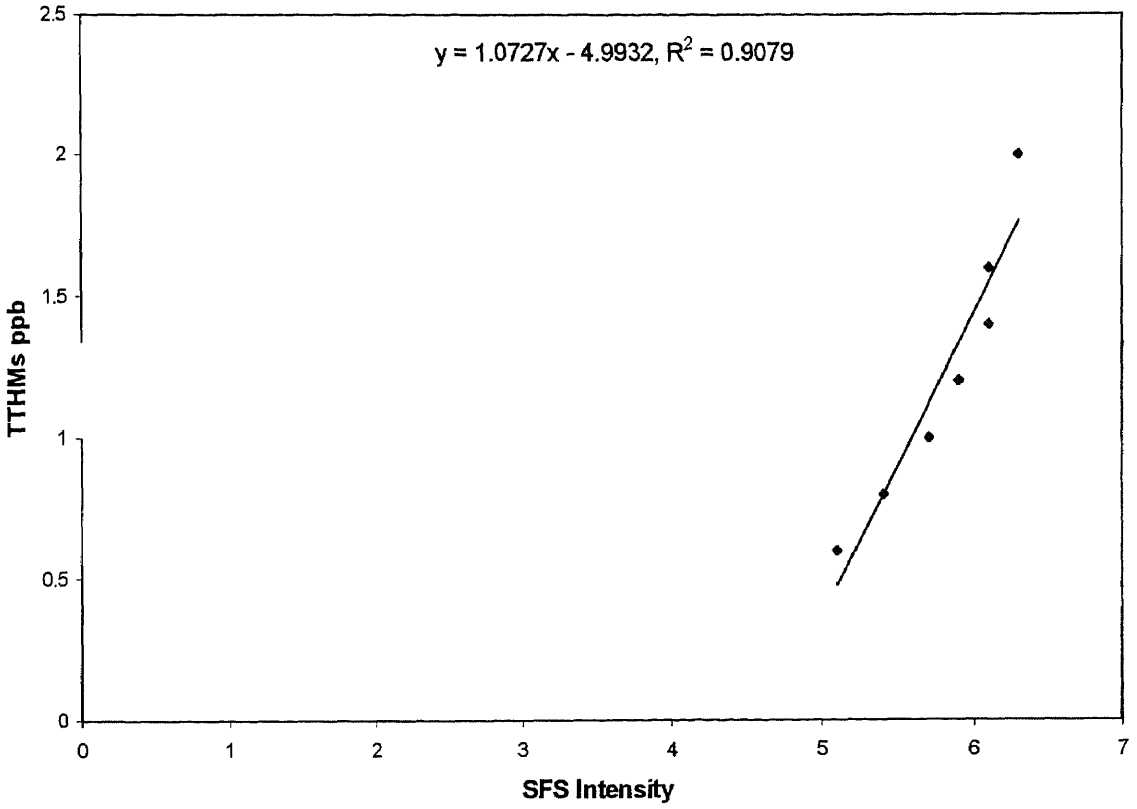


Figure H.4. TTHMs vs. SFS, Soil FA measured at Minor Peak

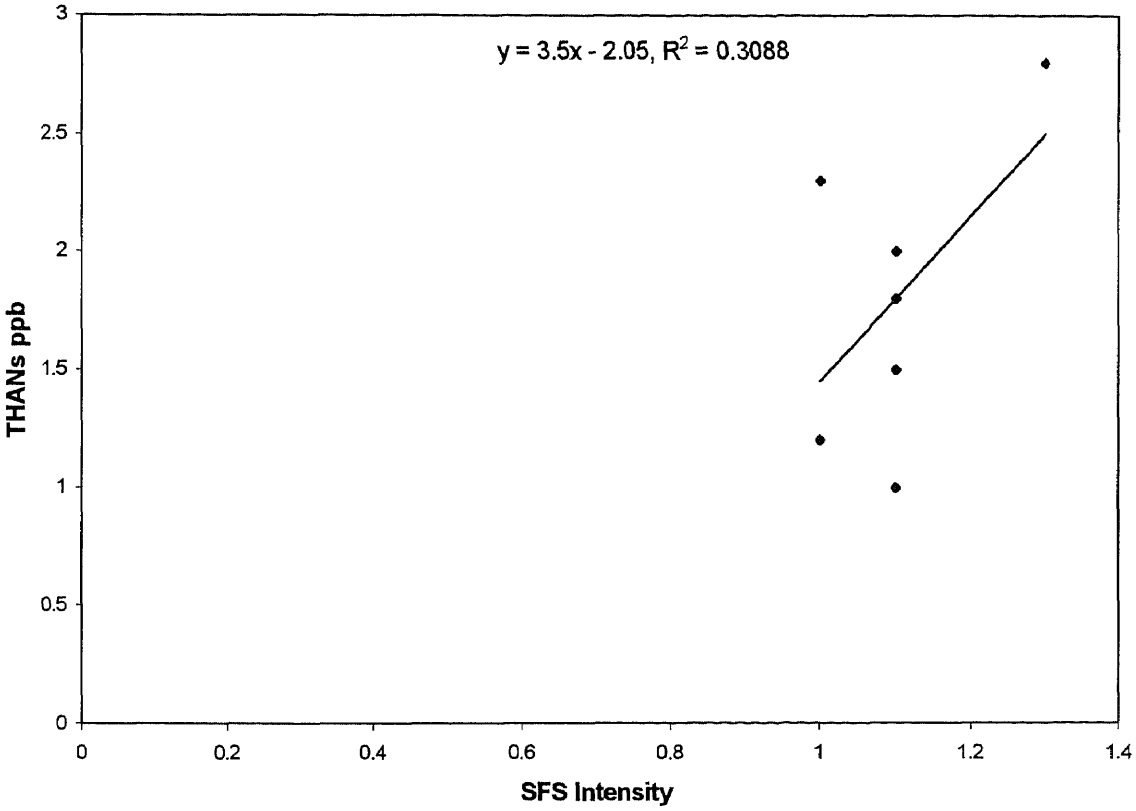


Figure H.5. THANs vs. SFS, Soil FA measured at Major Peak

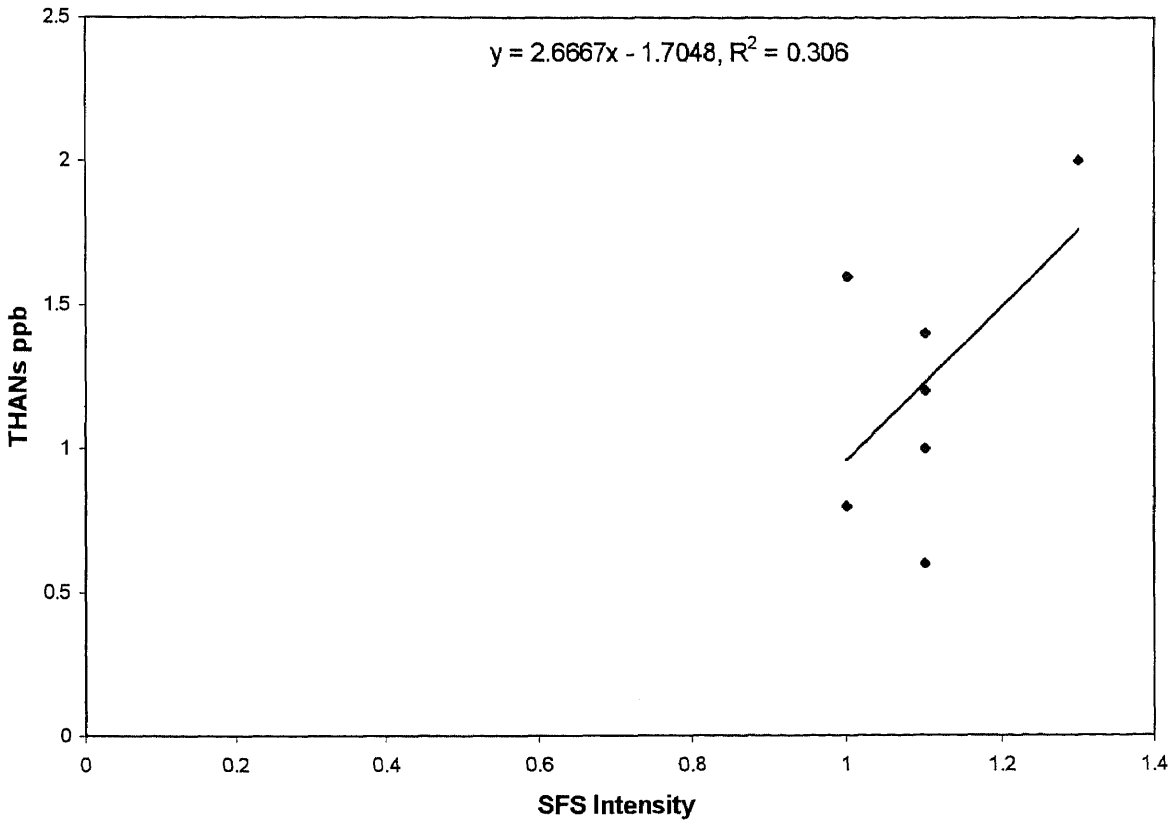


Figure H.6. THANs vs. SFS, Soil FA measured at Minor Peak

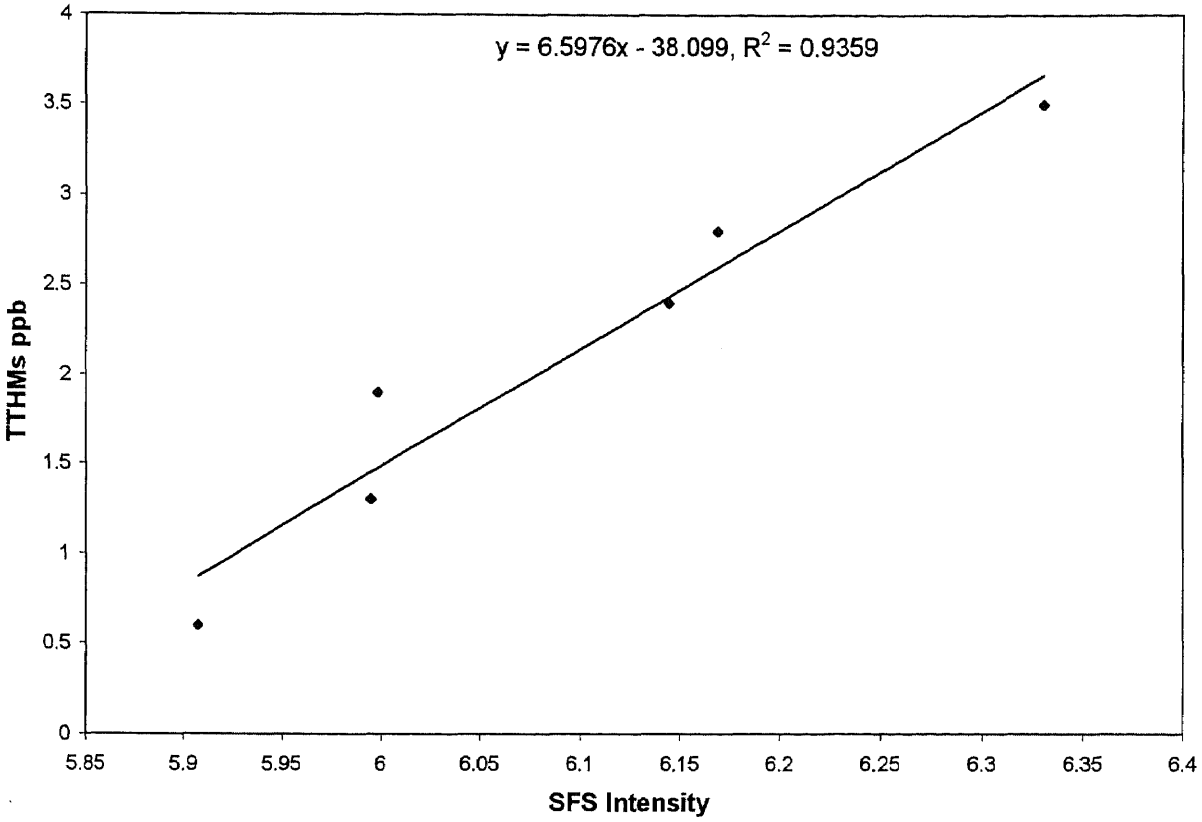


Figure H.7. TTHMs vs. SFS, Peat HA measured at Major Peak

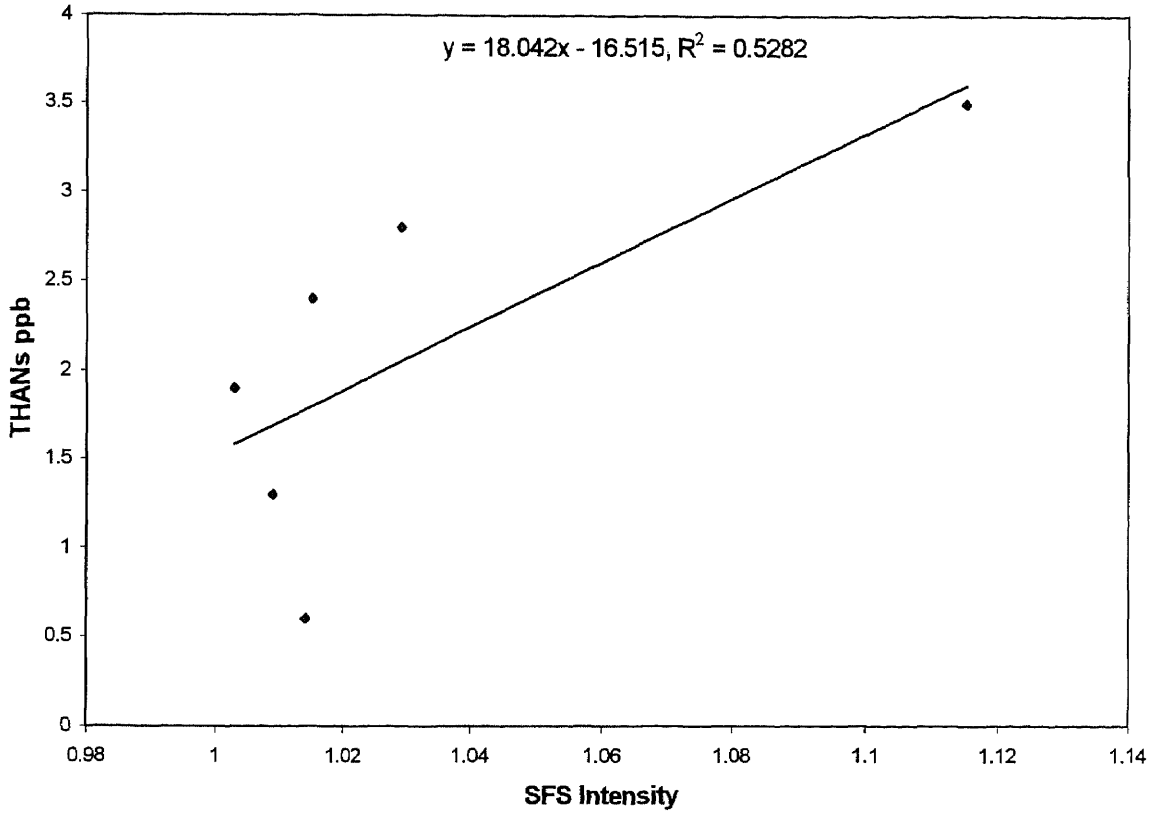


Figure H.8. THANs vs. SFS, Peat HA measured at Major Peak

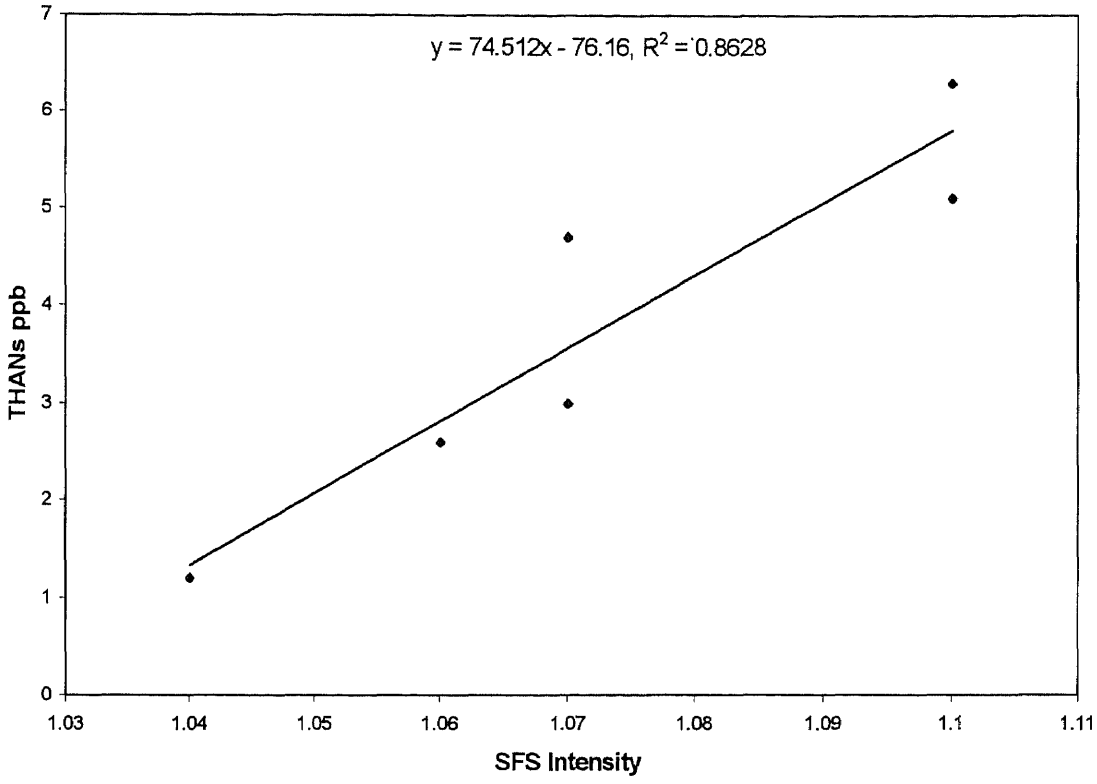


Figure H.9. THANs vs. SFS, Peat FA measured at Major Peak

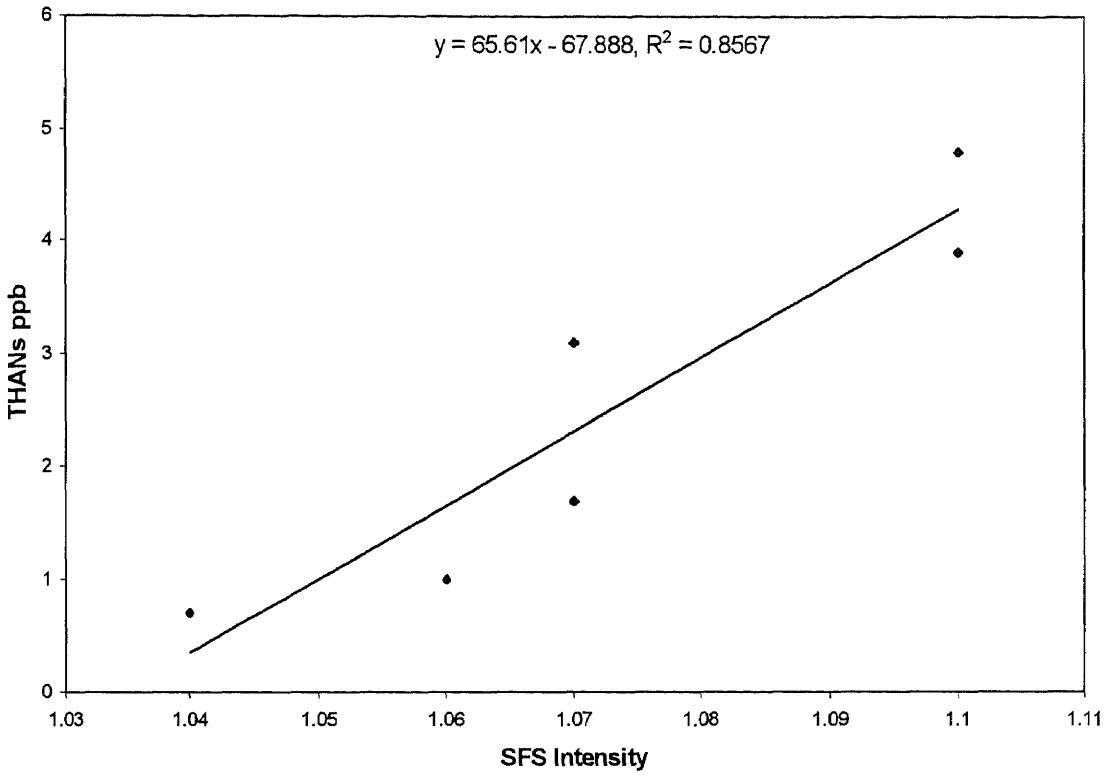


Figure H.10. THANs vs. SFS, Peat FA measured at Minor Peak

APPENDIX I

PRE- AND POST-CHLORINATION SFS

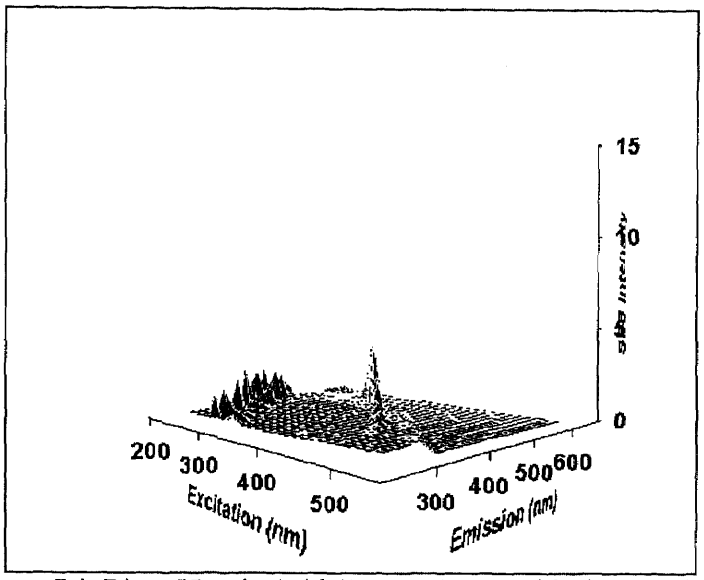


Figure I.1. River Humic Acid (0.5 ppm), Pre-Chlorination SFS

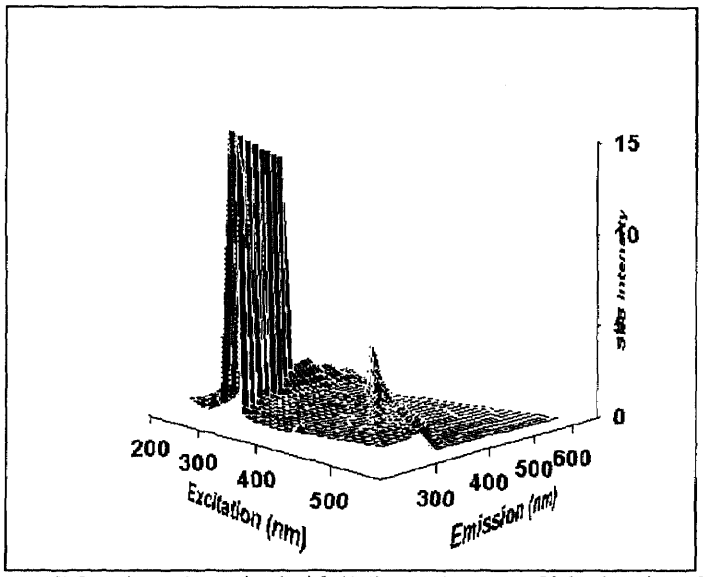


Figure I.2. River Humic Acid (0.5 ppm), Post-Chlorination SFS

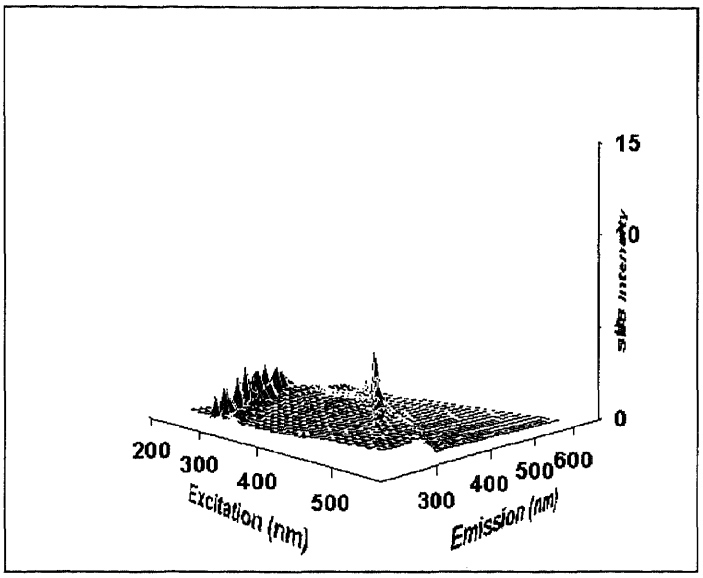


Figure I.3. River Humic Acid (1 ppm), Pre-Chlorination SFS

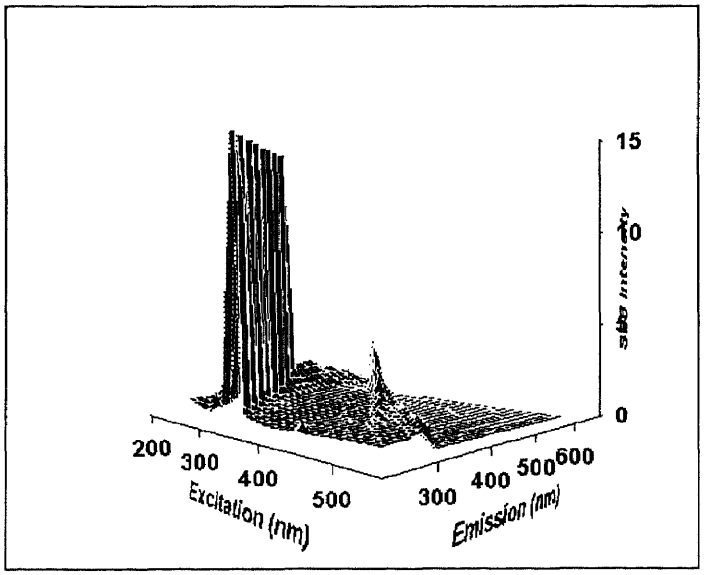


Figure I.4. River Humic Acid (1ppm), Post-Chlorination SFS

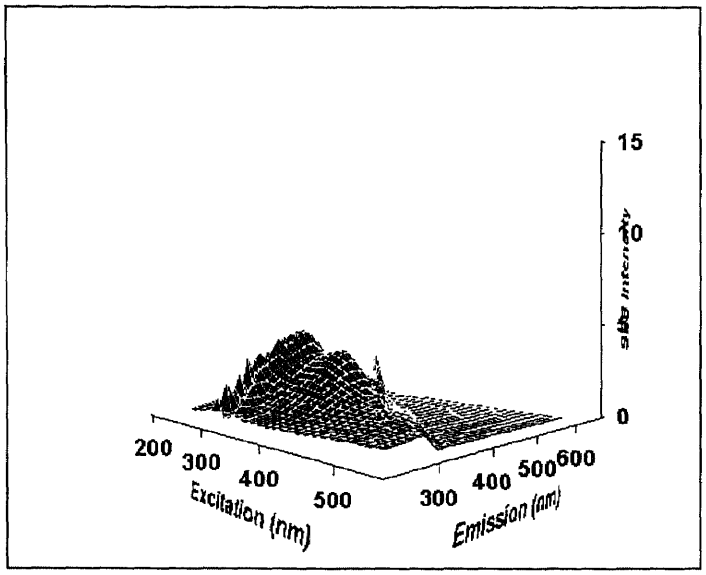


Figure I.5. River Humic Acid (2 ppm), Pre-Chlorination SFS

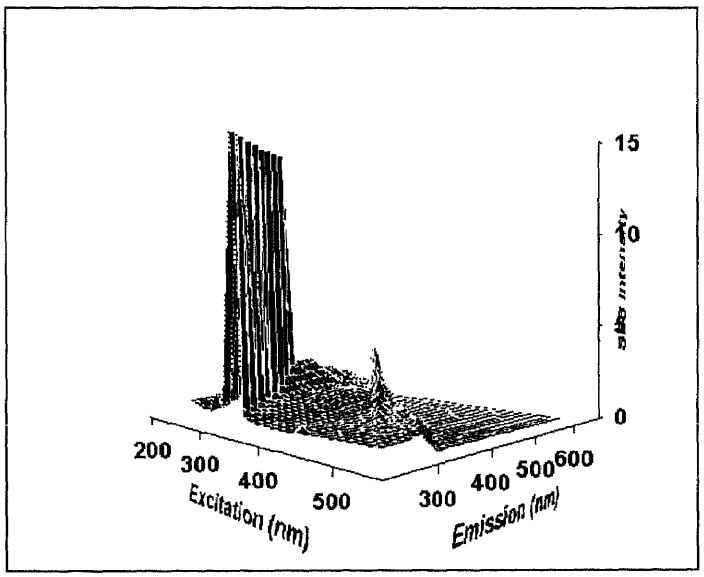


Figure I.6. River Humic Acid (2 ppm) Post-Chlorination SFS

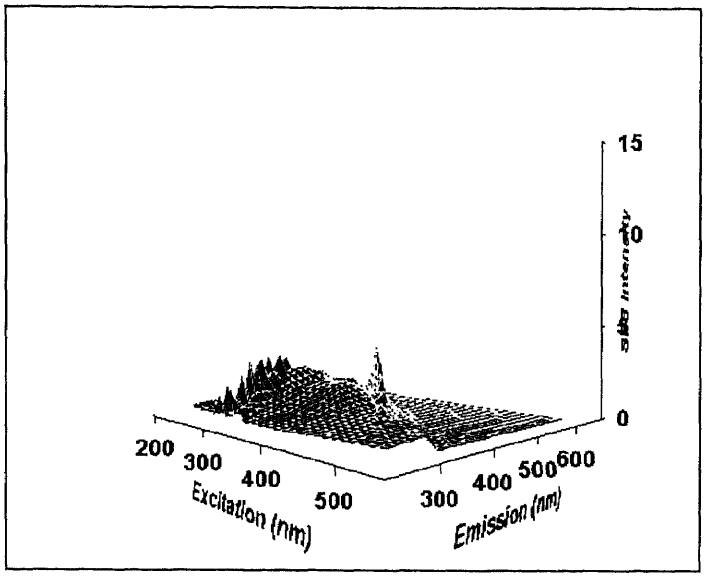


Figure I.7. River Humic Acid (2.5 ppm), Pre-Chlorination SFS

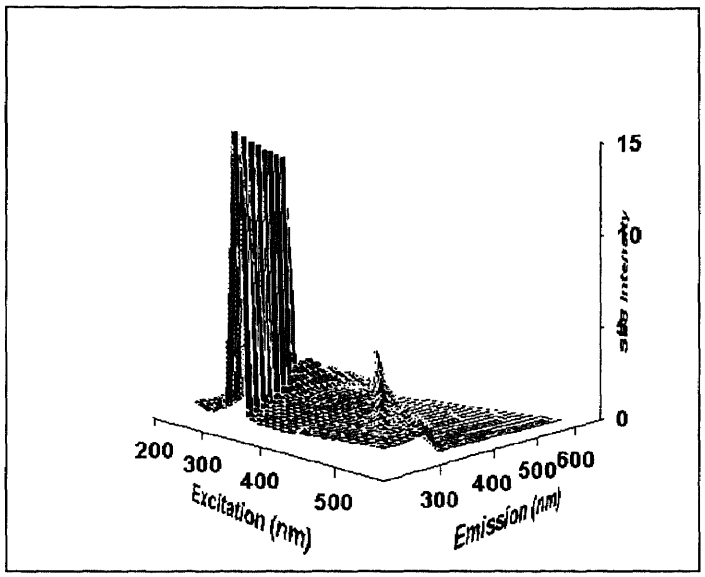


Figure I.8. River Humic Acid (2.5 ppm), Post-Chlorination SFS

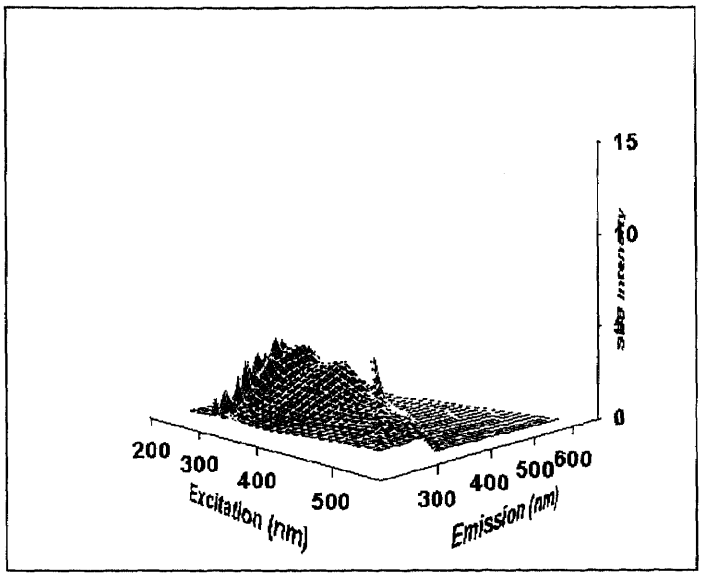


Figure I.9. River Humic Acid (4 ppm), Pre-Chlorination SFS

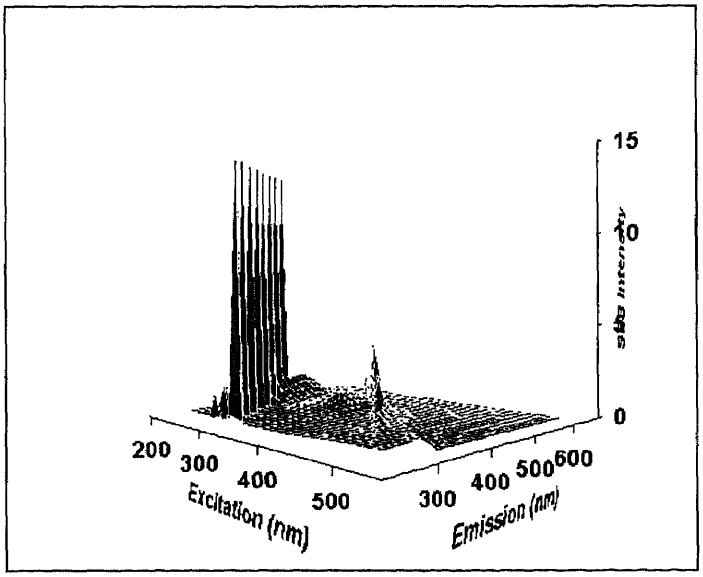


Figure I.10. River Humic Acid (4 ppm), Post-Chlorination SFS

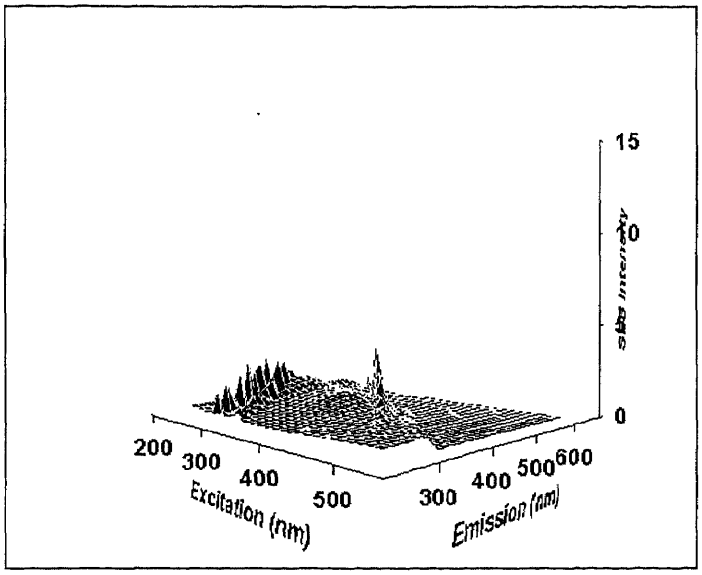


Figure I.11. River Fulvic Acid (0.5 ppm), Pre-Chlorination SFS

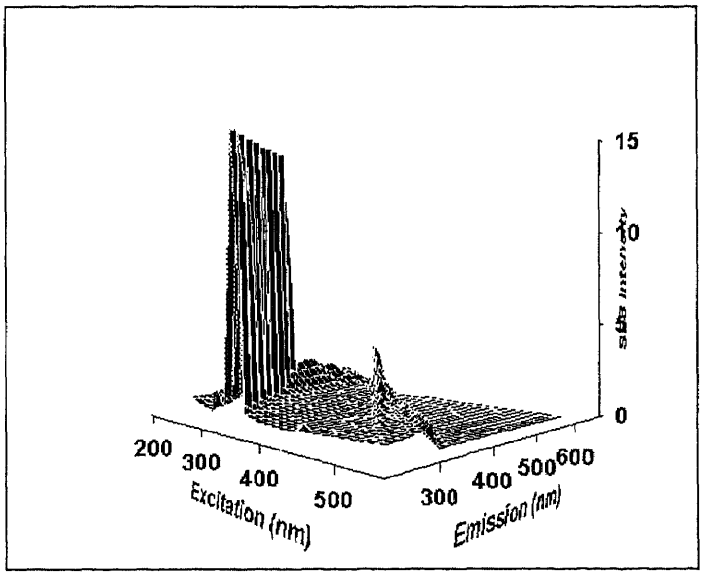


Figure I.12. River Fulvic Acid (0.5 ppm), Post-Chlorination SFS

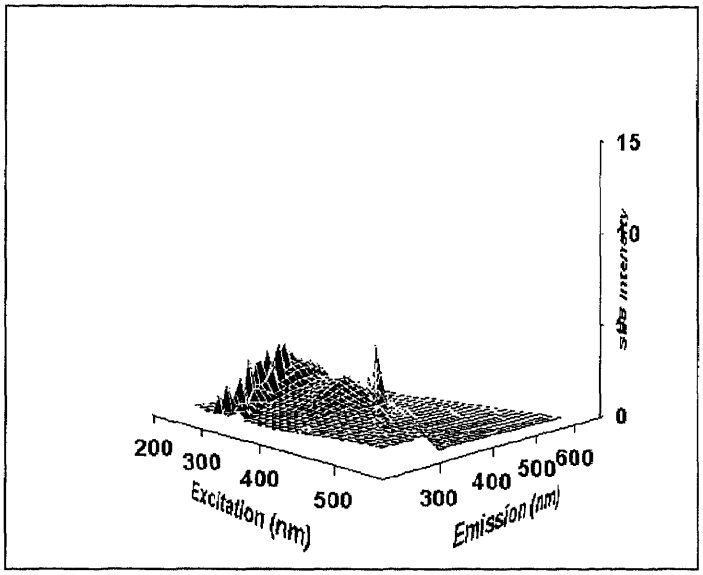


Figure I.13. River Fulvic Acid (1 ppm), Pre-Chlorination SFS

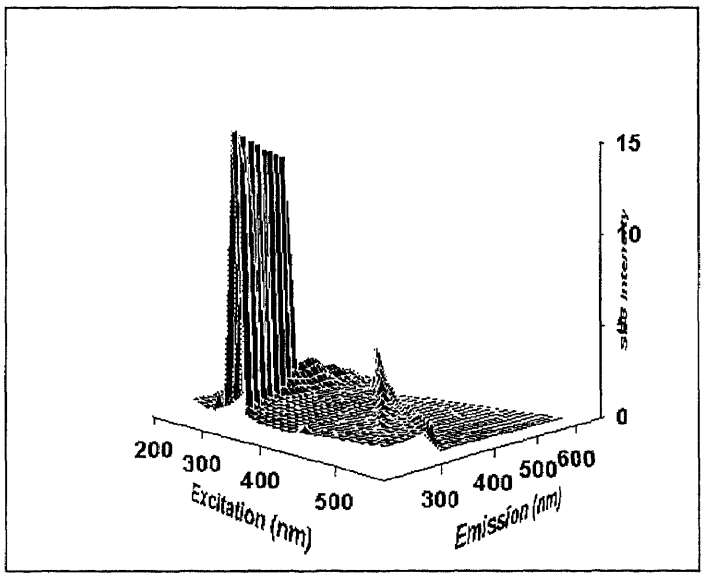


Figure I.14. River Fulvic Acid (1 ppm), Post-Chlorination SFS

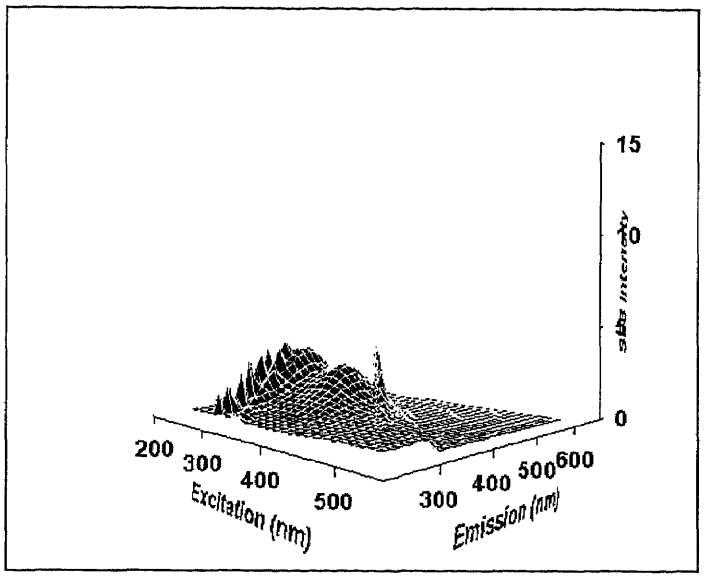


Figure I.15. River Fulvic Acid (1.5 ppm), Pre-Chlorination SFS

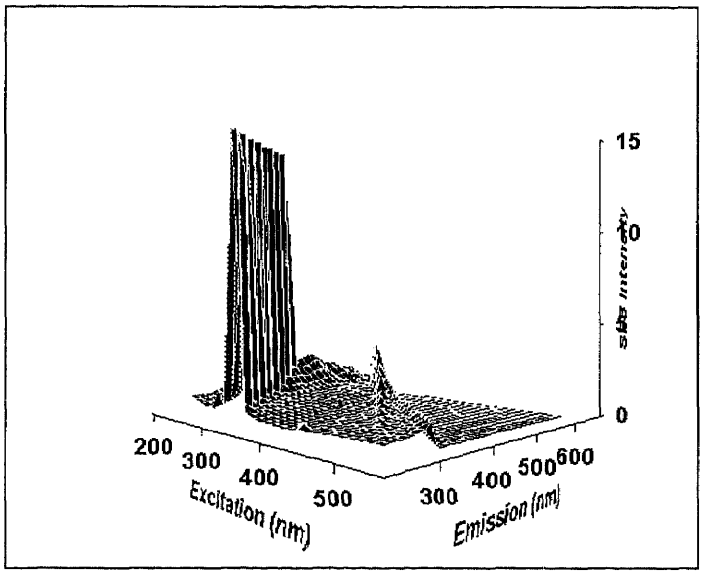


Figure I.16. River Fulvic Acid (1.5 ppm), Post-Chlorination SFS

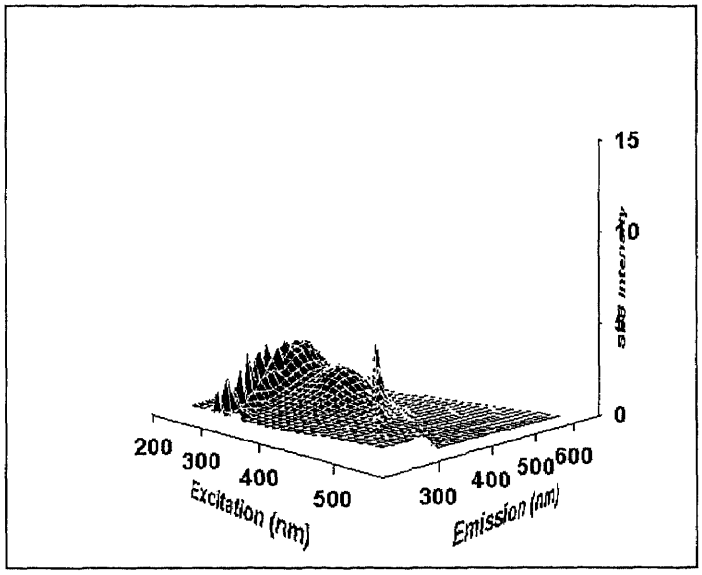


Figure I.17. River Fulvic Acid (2 ppm), Pre-Chlorination SFS

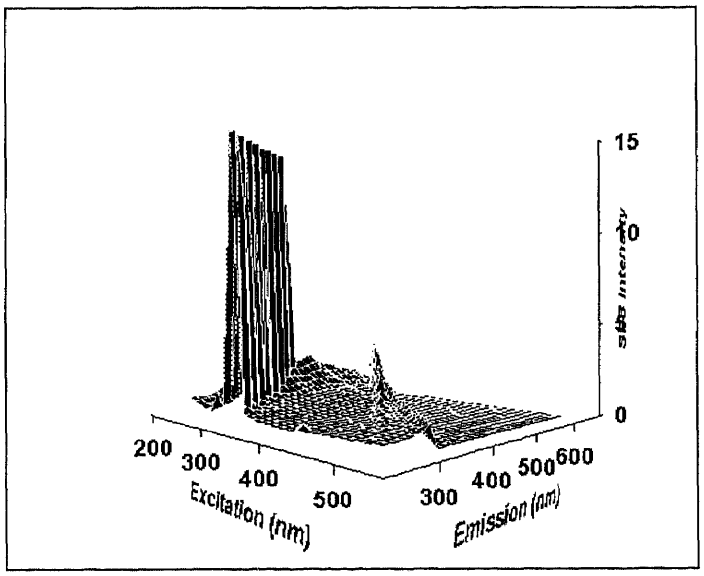


Figure I.18. River Fulvic Acid (2 ppm), Post-Chlorination SFS

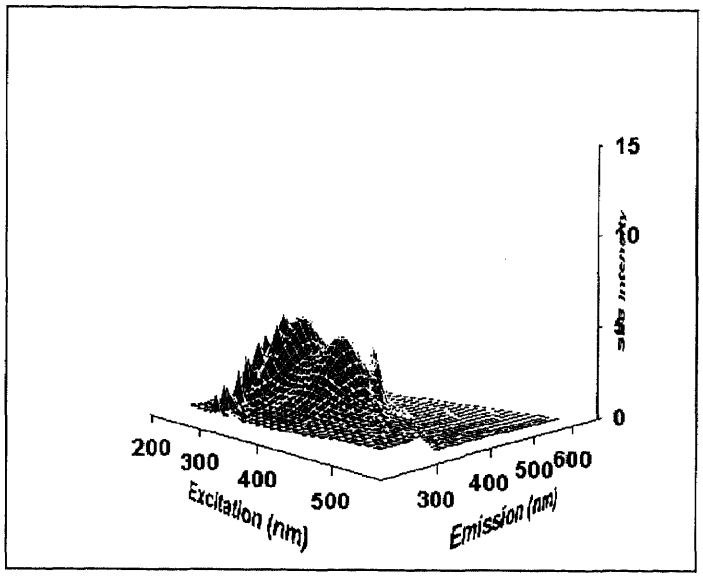


Figure I.19. River Fulvic Acid (2.5 ppm), Pre-Chlorination SFS

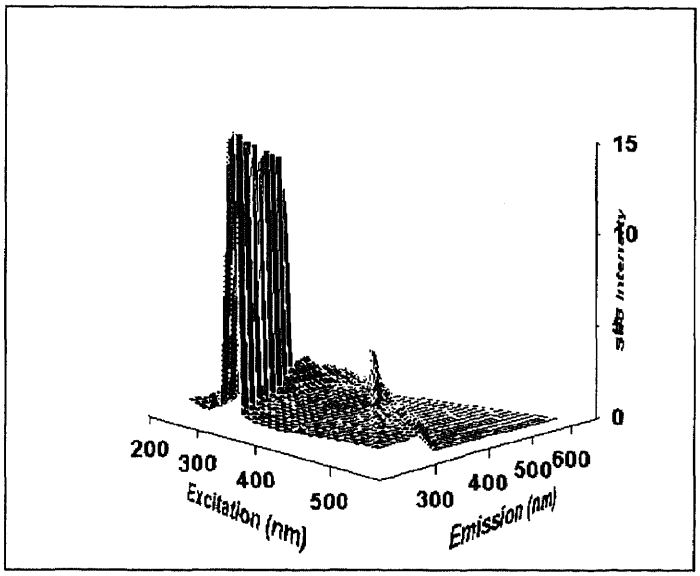


Figure I.20. River Fulvic Acid (2.5 ppm), Post-Chlorination SFS

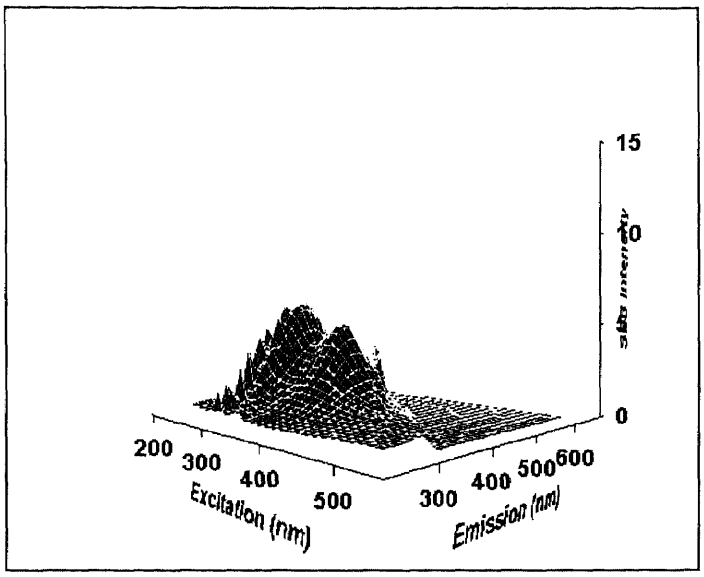


Figure I.21. River Fulvic Acid (3 ppm), Pre-Chlorination SFS

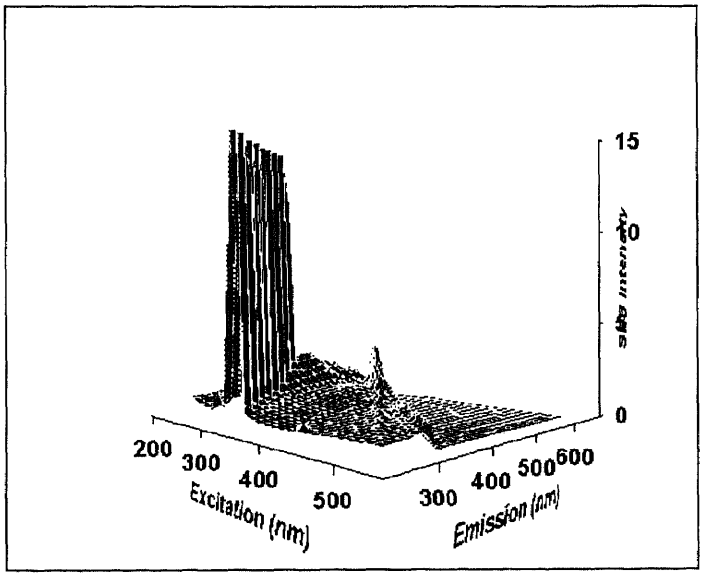


Figure I.22. River Fulvic Acid (3 ppm), Post-Chlorination SFS

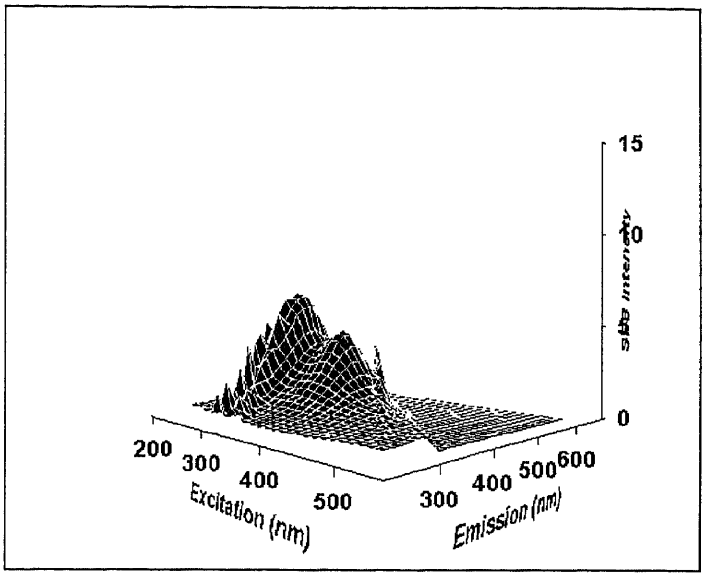


Figure I.23. River Fulvic Acid (4 ppm), Pre-Chlorination SFS

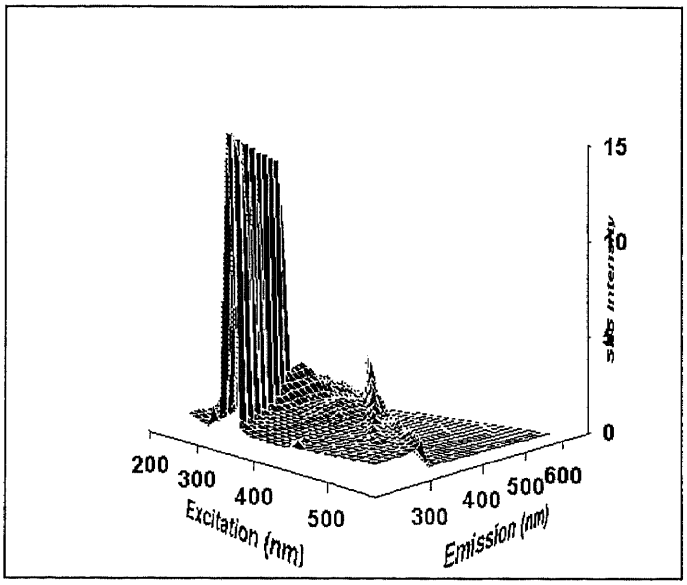


Figure I.24. River Fulvic Acid (4 ppm), Post-Chlorination SFS

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