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ABSTRACT

THE DETERMINATION OF 2-METHYLISOBORNEOL AND GEOSMIN IN WATER USING FLUORESCENCE SPECTROSCOPY

by
Ramesh Chandra Sharma

Geosmin and 2-Methylisoborneol are responsible for imparting earthy, musty taste and odor qualities to water. The human threshold odor concentrations (TOCs) for 2-MIB and Geosmin vary over a wide range. TOCs of 10ng/l for Geosmin and 30ng/l for 2-MIB have been reported.

Currently available methods are time consuming and require use of sophisticated technique and expensive equipment.

Fluorescence spectroscopy has been successfully used in the identification of many organic compounds. Geosmin and 2-MIB have organic origin. This research explores the possibility of using the technique of fluorescence spectroscopy in the determination of Geosmin and 2-MIB. The excitation and emission wavelengths for peak intensities have been identified for these compounds. An attempt has been made to draw a correlation between the concentration of Geosmin and 2-MIB versus fluorescent intensity.

THE DETERMINATION OF 2-METHYLISOBORNEOL AND GEOSMIN IN WATER USING FLUORESCENCE SPECTROSCOPY

by
Ramesh Chandra Sharma

A Thesis
Submitted to the faculty of
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APPROVAL PAGE

THE DETERMINATION OF 2-METHYLISOBORNEOL AND GEOSMIN IN WATER USING FLUORESCENCE SPECTROSCOPY

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CHAPTER 1

INTRODUCTION

Taste and odor problems, having a natural origin, occur seasonally in drinking and surface waters. Two compounds that are responsible for imparting earthy, musty taste and odor qualities to water have been identified from biological growths. Geosmin (trans-1, 10-dimethyl-trans-9-decalol) and 2-methylisoborneol (1,2,7,7-tetramethyl-exo-bicyclo heptan-2-ol are semi-volatile metabolites of actinomycetes [1, 2, 3, 4] and blue-green algae [5]. Geosmin and 2-MIB have also been isolated from fish in waters contaminated by these compounds [6].

Human threshold odor concentrations (TOCs) for Geosmin and 2-MIB in water can vary over a wide range of concentrations. The TOCs are a function of water temperature and residual chlorine levels and have been reported as 10ng/l for Geosmin and 30ng/l for 2-MIB [7]. Consumer complaints in treated water below these TOCs have been reported [8]. Therefore, analytical methods with detection limits in the low ng/l are essential for monitoring levels in water supplies and finished drinking water.

The most widely used method for isolating and analyzing Geosmin and 2-MIB from water is salted closed-loop stripping (CLS) followed by analysis by gas chromatography-mass spectrometry (GC-MS) with detection limits of 0.8ng/l [9]. A CLS method using isotope dilution mass spectrometry has been developed by Hwang C. J., et al [9]. Isolation of selected components from water using adsorbents is an alternative extraction method.

There is a considerable literature and operational experience to suggest that taste and odor due to algae cannot be removed by conventional treatment processes [10]. There

is evidence that the failure of conventional treatment is due to the lysis of algal cells during flocculation, releasing these compounds into the water, particularly if pre-chlorination is applied [11]. Once dissolved in the water, these compounds are not removed by flocculation and filtration alone and require additional treatment such as the use of oxidation or activated carbon, which adds significantly to both the cost and complexity of the treatment process. Air stripping is effective for substance with more than 10^{-3}/m3/atm/mol of Henry's constant, thus for Geosmin and 2-MIB which have constants less than 10^{-5}/m3/atm/mol, air stripping is not effective [12]. The rapid sand filter can not remove taste and odor causing substances, but the slow sand filter can remove minor quantity of Geosmin and 2-MIB by biological degradation [13].

Activated carbon has been used to treat water supplies to remove Geosmin and 2-MIB [17] and the adsorption kinetics of Geosmin and 2-MIB on powdered activated carbon have recently been studied by [14]. Zeolites have also been used for the removal of Geosmin and 2-MIB from water [15]. Compared several commercially available adsorbents (Amberlite XAD-2, XAD-4, XAD-8, Ambersorb XE348 and Tenax-GC) with solvent extraction methods for isolation of taste and odor causing compounds at trace levels in water. All of these adsorbents were found to be unsuitable for use because of the high artifact levels even after extensive clean–up. Taste and odor causing compounds were trapped on a Filtrasorb 300 granular activated carbon column and Geosmin and 2-MIB were recovered using Soxhlet extraction with multiple solvents [16].

Oxidation, a major treatment method for taste and odor, changes chemical characteristics of the taste and odor causing substances. Frequently used oxidants are ozone (O_3), chlorine (Cl_2), hydrogen peroxide (H_2O_2), ultraviolet rays (UV) and

potassium permanganate ($KMNO_4$). PAC is a popular medium for carbon adsorption method in temporary treatment of the taste and odor problem. Removal of intact Cynobacterial cells would significantly reduce the concentration of taste and odor metabolites present in the water. Although this would not completely negate the need for further treatment, there is no doubt that the effectiveness of treatment would be improved together with a reduction of costs by minimizing oxidant/powdered activated carbon doses and/or extension of the effective lifetime of granular activated carbon filters.

In Chapter 2, a review of literature of 2-MIB and Geosmin and their method of detection are discussed. In Chapter 3, the experimental procedure to measuring 2-MIB and Geosmin using Flourescence technique are described. The important results of this work are described in Chapter 4. Concluding remarks and suggestions for future work are given in Chapter 5.

CHAPTER 2

REVIEW OF LITERATURE OF 2-MIB AND GEOSMIN MEASUREMENT

2.1 Introduction

The earthy- musty flavors can be caused by one group of compounds, which are produced by actinomycites and certain cyanobacterias, which are particularly difficult to remove from water. Gaines and Collins [17] first isolated volatile earthy-smelling compounds from Streptomyces Odorifer, and Gerber and Lechevalier [18] attributed an earthy odor to actinomycites and named the substance geosmin. Rosen et al. [19] were the first to characterize geosmin in natural waters, and Safferman et al. [5] discovered geosmin in a cyanobacterial culture. Henley [20] characterized the species Anabaena circinalis as a geosmin producer that exclusively and consistently produces geosmin in culture. In 1984, the American Water Works Association Research Foundation conducted a survey of North American water utilities, and cyanobacteria were identified as the predominant group relevant for taste and odor problems [21]. Anabaena was identified by 46% of the respondents as the responsible genus for these problems, while benthic forms such as Oscillatoria accounted for only 16% of the associated with cyanobacteria.

One problem is the significance of growth phase on the release of geosmin. Cyanobacteria exhibit the typical growth sequence in culture; lag phase with slow growth, exponential phase, with a logarithmic increase in cell number, and stationary phase, where growth slows and eventually stops [17]. The amount of geosmin in the cells during these different stages influence the amount released to the environment [20]. When cells begin to age, two possible scenarios can be described. One is a subtle alteration in cellular membrane and subsequent loss of osmotic regulation followed by death. The other

scenario could be total lysis of cells upon reaching the end of the stationary phase. Each of these scenarios for cell death will release different amounts of Geosmin from the cells. Only in one study with cultures of Oscillatoria treated with copper sulfate was death, and subsequent release of 2-methylisoborneol (MIB), examined [22]. MIB is another earthy-musty smelling compound produced by certain microorganisms. In contrast, recent work has shown that geosmin and MIB are retained in cells after rupturing and appears to be associated with high molecular weight cellular proteins [21]. Chlorophyll was related to geosmin production, supporting the idea that geosmin is associated with thylakoids [23].

2.2 METHODS OF DETECTION

2.2.1 Standard Method 6040B

Closed-Loop Stripping, Gas Chromatographic/ Mass Spectrometric (GC/MS) Analysis is the procedure commonly used for Geosmin and MIB analysis [24]. The procedure involves stripping intermediate weight compounds from water with a recirculating stream of air and trapping the analytes on a small activated carbon filter. The analytes are eluted from the carbon with carbon di sulphide (CS_2) and injected into a GC/MS system.

2.2.2 Isotope Dilution High-Resolution Mass Spectrometry (HRMS) Method

Geosmin and 2-methylisoborneol are extracted from water onto Ambersorb 572, desorbed into dichloromethane and annualized by gas chromatography-high resolution mass spectrometry at a mass resolution of 7000 [25]. The use of the granular adsorbent Amberssorb572 allows extraction by rolling the sample bottle, isolation of the absorbency

by filtration, desorption of the analytes into DCM in the autosampler vial and injection without plugging the syringe needle.

Since there is no sample clean up, HRMS is necessary to differentiate the analytes from chemical interference when present. Isotope dilution quantitation with d_3-geosmin and d_3-2-MIB is required because the recoveries of geoamin and 2-MIB from water are highly variable. Palmentier et al [25] compared the Ambersorb 572/GC-GC- HRMS method with isotope dilution quantitation with the closed-loop stripping (CLS)/GC-low resolution mass spectrometry (LRMS) method with external standard quantitation. Increased productivity (up to 40 samples/day), faster turnaround times (48h), better between-run precision (7.7% for geosmin and 5.5% for 2-MIB) and accuracy ($\pm$11% for geosmin and $\pm$6% for 2-MIB) over the CLS/GC-LRMS method were achieved. Detection limits of 2.0ng/l for geosmin and 2.0ng/l for 2-MIB were obtained for the Ambersorb 572/GC-HRMS method. The method was successfully applied to 152 samples from lake Ontario water supplies.

2.2.3 Large Volume Purge and Trap GC/MS Method

The current standard analytical technique for Geosmin and MIB analysis, as mentioned above, is Standard Method 6040 B. The method calls for CLSA (closed-loop stripping analysis) followed by GC/MS. This method requires a special complex apparatus not common in commercial or PWS laboratories, is labor intensive, and requires the use of toxic elution solvents such as carbon disulfide.

John E. George et al [24] have successfully used the purge and trap technique. This technique is one, in which Geosmin and MIB are efficiently removed from a water

sample by purging with helium gas at a specified flow rate, sample temperature, and length of time. The analytes are trapped on a sorbent material and subsequently desorbed directly into a GC/MS system. MDLs of less than 5ppt have been obtained using this technique.

2.2.4 Ion Trap Spectrometer Method

A water sample spiked with an internal standard is placed in a washed bottle capable of holding up to 500 ml of sample. The sample is heated and purged simultaneously, effectively removing the Geosmin and MIB from the water [26]. The analytes are trapped on a 3-component trap inside of a commercially available purge-and-trap concentrator unit. A short dry purge time is added to remove excess water on the trap and in lines. The trap is heated and the compounds are transferred to GC equipped with a low dead – volume interface (LDI). The analytes are separated by a fused silica capillary column and are detected with and ion trap mass spectrometer. The quantitation ions for Geosmin and MIB are quite characteristic and can be easily extracted from the TIC (Total Ion Current) for positive identification. There is no need to operate the mass spectrometer in the SIM (Single Ion Monitoring) mode.

2.2.5 Microextraction and Gas Chromatography-Ion-Trap Detection Mass Spectrometry Method

Ming-Liang Bao et al.,[26] have determined trace levels of taste and odor compounds in water by Microextraction and Gas Chromatography-Ion-Trap Detection Mass Spectrometry.

As per Ming-Liang Bao et al., it is a simple, easy-to-perform and sensitive method for routine analysis of trace taste- and odor – causing organic compounds in natural water. The compounds investigated included geosmin, 2-methylisoborneol (MIB). One litre water samples, salted with 100g of sodium chloride, were extracted by microextraction using 2*3 ml of hexane. The extracts were analyzed by gas chromatographic-ion-trap detection-mass spectrometry (GC-ITD-MS). Mean recoveries from spiked reagent water 99±7% and 95±8% for MIB and geosmin (spiked with 5-40ng/l), respectively. For other compounds, recoveries ranged between 81 and 110%, with the exception of hexanal, heptanal and benzldehyde. The method detection limits were as low as 1ng/l for geosmin and MIB, 0.5-6ng/l for ketones and 5-25ng/l for other compounds. Recoveries and precision for most of the compounds investigated form spiked river water were similar to those obtained in reagent water.

2.3 Objective of this Research

The objective of this research is to devise a new technique using spectral fluorescent signatures (SFS) to detect 2-MIB and Geosmin in water. The method of fluorescent diagnostics is well known as an effective tool for the analysis of organics in water media[27]. The main advantages of fluorescent techniques are that 1) they have high sensitivity and 2) they allow the proximate diagnostics to be carried out without the time-consuming pretreatment of water samples, in the remote mode as well. This makes it possible to build an on-line diagnostic system based on the method of induced fluorescence. In investigation of water quality, the fluorescent techniques permit the

analysis of major organic compounds in a mixture: dissolved organic matter (DOM), Phyoplankton, and organic pollution.

The fluorescence of organic compounds in water is caused by dissolved fractions as well as by emulsion and suspended matter. The excitation and fluorescence spectra, depending on the structure of organic molecules, contain information about the complex conglomerate of water organics.

Phytoplankton: The excitation and fluorescence spectra of various micro algae differ by their characteristic features, caused by the pigment composition of the cells (chlorophylls, phycobilins, and cartenoids) and light energy transfer processes within them. This allows one to use phytoplankton pigments as a natural fluoroindicator of the taxonomic groups and assess water quality by analyzing the pigment composition in a mixed micro algae population.

DOM: The spectral characteristics of DOM are in general due to a dissolved portion of humic substance (HS), consisting mainly of fulvic acids. HS is subject to spatial and seasonal variability, which leads to changes in fluorescence spectra [27].

Chemical Pollution: The fluorescent characteristics of oil and oil products, Phenols, and their derivatives depend on polyaromatic hydrocarbons (PAH), which effectively absorb light in the ultraviolet (UV) region.

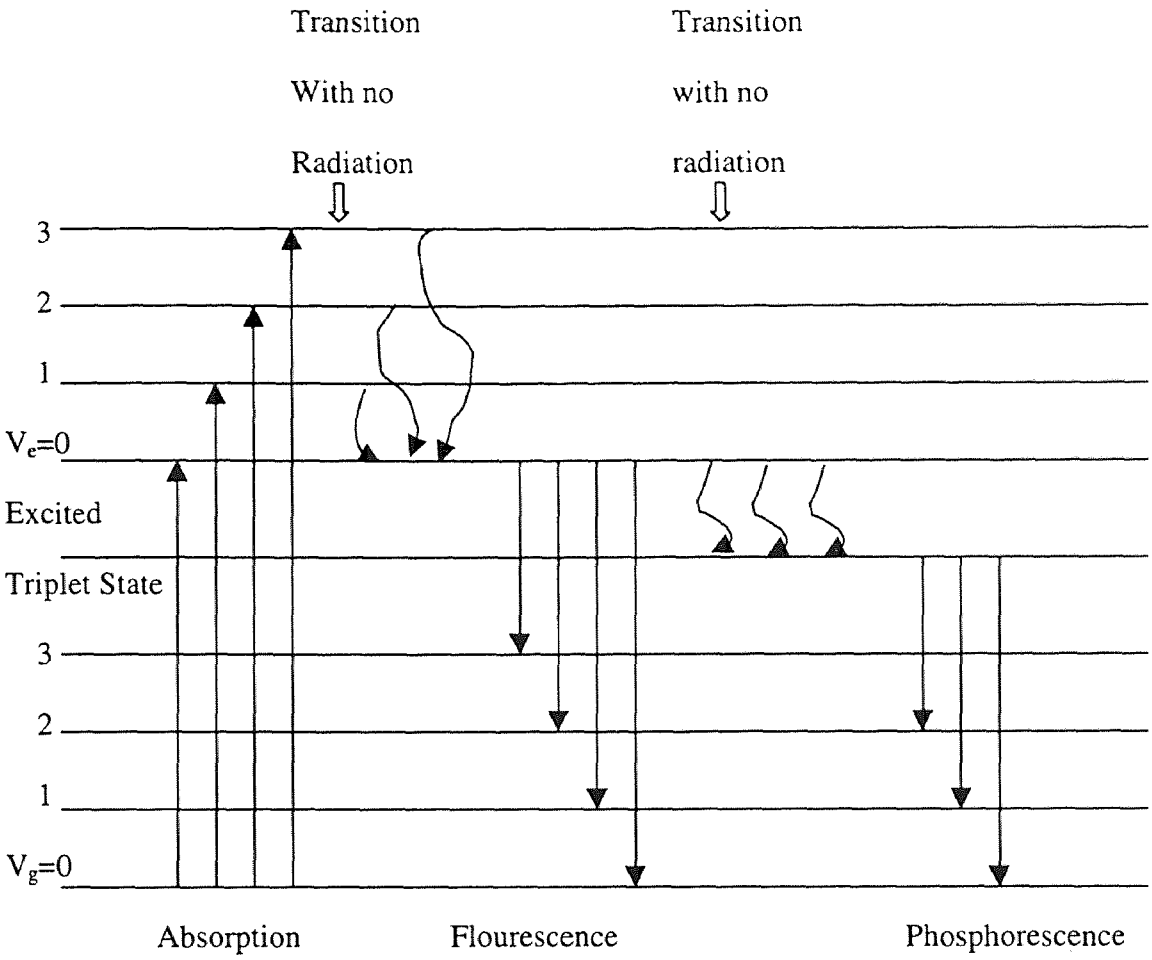
As origin of geosmin and 2-MIB is also organic it is expected that this method would be applicable in their determination.

CHAPTER 3

EXPERIMENTAL PROCEDURE

3.1 INTRODUCTION

The following Figure 3.1 illustrates the process where a substance absorbs light and then emits light, taking an organic molecule as an example.



V_e = Excited State, V_g = Ground State

Figure 3.1 Energy Level of Typical Organic Molecule [28]

At the ground state, the molecule absorbs light and transits to the excited state. The molecule loses a portion of the exciting energy as vibration energy, etc., 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" [17].

The molecule which transits without emitting radiation to the triplet state also emits optical energy when it is returned to the ground state. This optical energy is called "phosphorescence" [28].

Since transition form the triplet state to the ground state is inhibited by the selection law, phosphorescence has a lifetime longer than 10^{-4} second, while the lifetime of fluorescence is generally on the order of 10^{-8}~10^{-9} sec [28].

Since a portion of the light absorbed by a substance is lost by vibrational relaxation, etc., the fluorescence emitted from the substance has a longer wavelength than the excitation light (Stoke's law).

3.2 Fluorescence Intensity

The ratio of the optical energy absorbed by a substance relative to the total fluorescence energy emitted from the substance is called "quantum efficiency". It can generally be said that a substance having higher quantum efficiency is more fluorescent [28]. The intensity of the fluorescence emitted from the substance is proportional to the optical energy absorbed.

When diluted solutions are measured as samples, therefore, it is possible to represent the fluorescence intensity by the following formula [28]:

$F = KI_0CLEQ$

Where F.....Fluorescence intensity

K.....Instrumental constant

I_o..... .Intensity of excitation beam

C.....Concentration of sample

L..... Effective optical path length of cell

E..... Molar absorptivity

Q..... Quantum efficiency

3.2.1 Advantages of Fluorescence Analysis

In order to explain the merits of fluorescence analysis, the limit of the absorption method in analysis of a low-concentration sample is described below:

Let us assume a sample, which has transmittance of 99%, compared with a blank solution. Let us further assume an error factor (unavoidably accompanying absorbance measurement) of 0.1%.

The error factor is added to both the blank solution and sample.

Transmittance of blank solution: $100.0 \pm 0.1\%$

Transmittance of sample : $99.0 + 0.1\%$

Difference (value depending: $1.0 \pm 0.2\%$ on sample concentration)

In the example shown above, an error as high as $\pm$20% is involved in concentration measurement.

In contrast, the fluorescence analysis in which difference form the zero level generally corresponds to sample concentration to be measured is as follows:

Output signal level in sample measurement: $100\pm0.1\%$

Value corresponding to blank : ±0.1

Difference (proportional to sample concentration) : $100\pm0.2\%$

As is understood from the above example, the fluorescence analysis is based on a principle which assures error percentage will be very low especially in the case of low concentration [21].

Though certain error factors are enhanced at very low concentrations in actuality, the fluorescence analysis can generally be said to permit measurements at concentrations at least 10^{-3} below those measurable by the absorption method.

In addition to sensitivity described above, the fluorescence analysis provides more information, since it can provide emission spectra in addition to excitation spectra, which correspond to absorption spectra obtainable by the absorption method.

The fluorescence analysis enables quantitative and qualitative determinations of a sample consisting of multiple components by adequately selecting two wavelengths or recording emission spectra at excitation wavelengths selected properly (or vice versa).

3.2.2 Remarks on Fluorescence Analysis

3.2.2.1 Temperature Dependence of Fluorescence Intensity: It is said that intensities of fluorescence emitted from most types of samples are reduced 1 to 2% for sample temperature rise of 1°C [28]. For measuring samples which have high dependence on temperature, it is recommended to keep them at constant temperature by circulating thermostated water through the cell holder.

3.2.2.2 Sample Variation Due to Light: Certain types of samples are chemically affected by excitation light [16]. For measuring fluorescence intensity of such samples, the excitation slit is set as narrow as possible and a wide slit width is selected on the emission side to make up for light intensity.

For preventing samples from being chemically affected by excitation light, it will also be effective to close the shutter to cut off excitation light till start up of measurement[28].

When a variation due to light is observable even with the preventive measures described above, record output while feeding the recorder chart and determine signal level at the start position by extrapolation based on signal level variation condition[28].

3.2.3 Selection of Slit Width, Response and Scan Speed: Data of higher S/N can be obtained at wider slit width and slower response. However, slit width must be selected considering steepness of peaks to be observed, interference due to fluorescence emitted from concomitant substances and so on. Response and scan speed matched with slit width must be selected for recording a spectrum [28].

3.3 Raman Scattering

Optical emission different form fluorescence may be observed during fluorescence analysis. It may be Rayleigh scattering which appears at the same wavelength as the excitation light and Raman scattering which appears at a little longer wavelength.

A peak produced by fluorescence remains at the same location, but its height changes when excitation wavelength is varied, where as one produced by Raman scattering shifts to a different wavelength when excitation wavelength is varied [28].

Both Rayleigh scatter and Raman scatter are emitted from solvents [21]. Care should be taken to not to mistake peaks produced by such scatter for those by fluorescence.

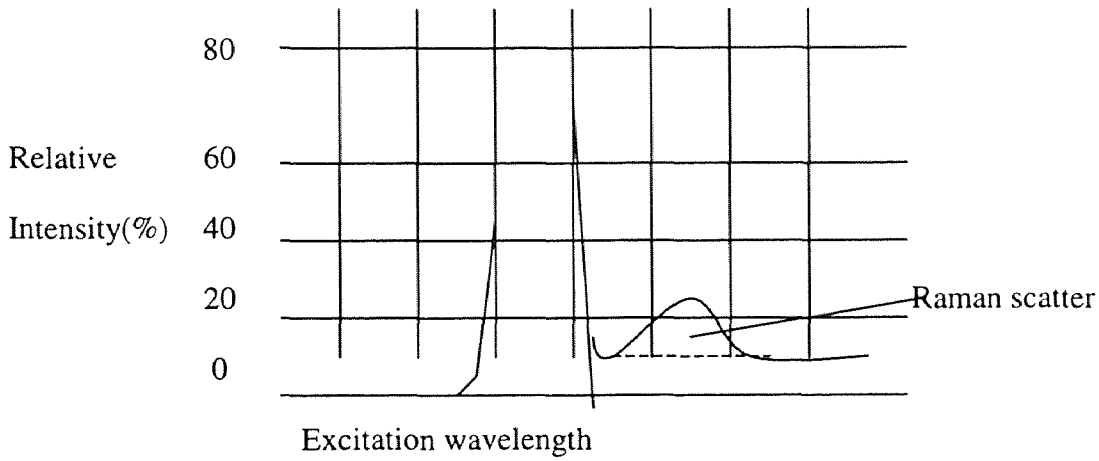


Figure 3.2 Raman Spectrum of Water [28]

3.4 Handling of High-Concentration Sample

A sample at too high a concentration entails various factors of error. The greatest error factor is absorption of excitation light occurring in the vicinity of the cell inlet which prevents the excitation light from reaching the cell center [28].

Figure3.3 illustrates an extreme case where fluorescence emitted in the vicinity of excitation light inlet cannot reach the emission monochromator.

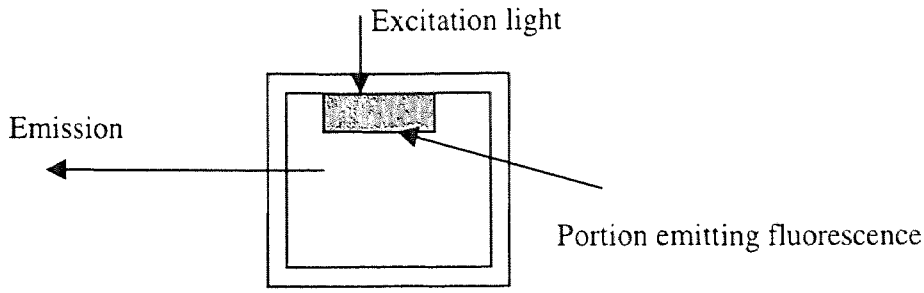


Figure 3.3 Sample Concentration too high [28]

When fluorescence is emitted from the excitation light inlet only, it is necessary to dilute the sample at an adequate ratio.

A second error factor is "concentration extinction" which hinders activation due to interaction of molecules [28].

A third error factor is reabsorption of fluorescence. It occurs at a location where the shorter wavelength end of emission spectrum is overlapped with the longer wavelength end of excitation spectrum as shown in fig 3.4.

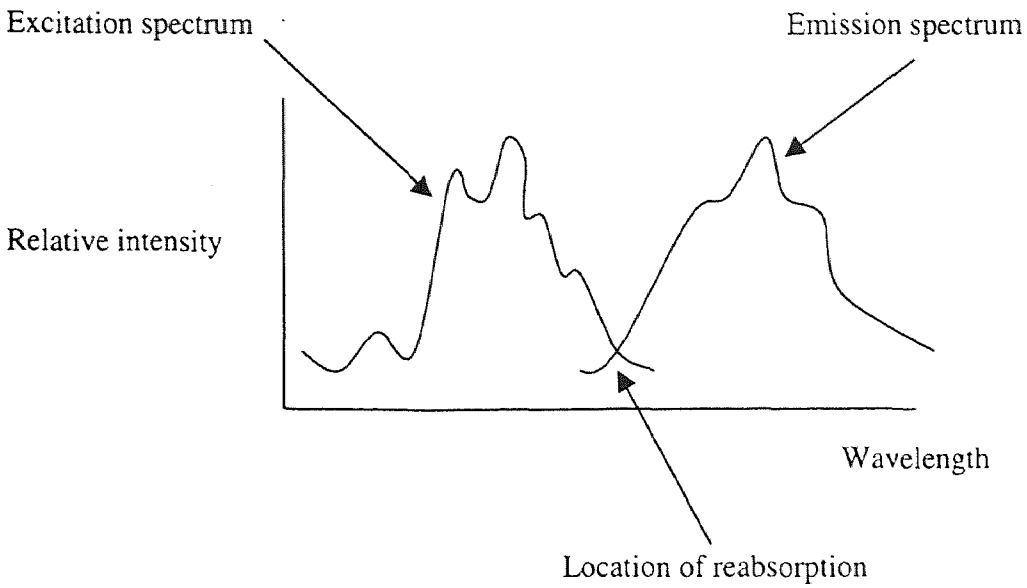


Figure 3.4 Reabsorption [28]

Hence, it appears as if the emission spectrum is shifted slightly toward longer wavelengths. However, this factor will not significantly hinder quantitative analyses of ordinary samples.

In a case where measuring error is caused due to an excessively high sample concentration, dilute it at a proper ratio or measure surfacial fluorescence by using a solid sample holder.

3.4.1 Second Order Ray

As mentioned above Raman peak which appears when excitation wavelength is close to emission wavelength. When excitation wavelength is far from emission wavelength, in contrast, the analyst must pay attention to second and third-order rays of scattered light.

The second and third- order rays appear at wavelengths two and three times as long as the excitation wavelength. When excitation wavelength is set at 240nm, for example, the second and third- order rays appear at 480nm and 720nm respectively.

To eliminate these rays, insert the short wavelength cut filter to cut off the scattered light on the emission side.

Particularly when the second and third-order rays are expected to cause a problem, it is recommended to use the optional filter set.

3.5 Contamination of Cell

Since the fluorescence spectrophotometer has high sensitivity, slight contamination on the cell must be handled properly after completing measurement.

Special care should be taken to not to leave the cell filled with a sample solution. The sample may adhere to the cell wall and become unable to be washed off after the solvent is evaporated for measuring a sample at a very low concentration, contamination not only on the inside wall but also on the outside wall may affect analytical results. If a sample solution adheres to the outside wall of the cell upon filling it with the sample, wipe it off with tissue paper before setting the cell into the cell holder.

3.6 Analytical Technique

The experiment was carried out in two stages. In first stage the fluorescence on the standards of 2-MIB and Geosmin is carried out. A plot of concentration versus fluorescent intensity is prepared.

Standard Solution Preparation: Solutions of various Concentrations are prepared using the standards of Geosmin and 2-MIB. The chemicals used were neat standards purchased from Wako Pure Chemical Industries Ltd. Organic free distilled water is used for solution preparation. Sample is stored at a temperature of 4 °C.

The Hitachi (Tokyo, Japan) Model F-3010 Spectrofluorometer will be used for measuring both excitation and emission spectra over a range of 225nm to 635nm. Band width of 12nm was used. The minimum excitation wavelength was limited to 225nm as wavelengths lower than that may result in destruction of chemical structure of the organic molecule.

Results of the experiment are presented in the next chapter.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Scope of Experiments

The results of the experiments discussed in Chapter 3 are presented here in the following sections. The first of these is a discussion of the development of spectral fluorescent signatures for Geosmin and 2-MIB standards.

4.2 Geosmin and 2-MIB Standards

The first step in this investigation was to investigate the fluorescent properties of geosmin and 2-MIB standards. By developing the SFSs for these standards, it will be possible to visually identify the types of odor producing compound in a water sample.

The first standard to be analyzed was a organic free distilled water blank. This was done to determine a flourescence baseline and to locate areas where scattering peaks might appear. There are two areas in the water SFS where scattering is seen to be a concern. This SFS contains both Raman and Second-order Ray scattering. The Raman scatter appears where the emission wavelength is approximately 30nm longer than the emission wavelength, and the Second-order Ray scatter where the emission wavelength is equal to twice the excitation wavelength is equal to twice the excitation wavelength.

Due to non-availability of any previous data the standards were scanned over a wide range. The excitation wavelength used varied from 225nm to 535nm, the emission also varied in the same range. After locating the approximate area for the characteristic peak a manual search for the exact location of the peak was done.

The location of highest intensity points for 2-MIB was at an excitation of 225nm and an emission of 297nm. Whereas for Geosmin standard the peak intensity was at an excitation wavelength of 225nm and emission wavelength of 340nm. The results obtained by drawing a regression curve for the relationship between the concentrations of the two odor causing compounds and their concentration are presented below in table 1for 2-MIB and table 2 for Geosmin respectively.

Table 1 Relationship Between Concentration of 2-MIB and Fluorescence Intensity

Concentration Range	Wavelength (Ex, Em) in nm	Relationship	Number of Samples	R^2
1-20 ppm	(225, 297)	$Y=-0.0003x^4 +0.0116x^3- 0.1244x^2+ 0.7617x+1.156$	30	0.9996
0.005-50 ppb	(225, 297)	$Y= 1.2844x^{.0473}$	20	0.9635

Table 2 Relationship Between Concentration of Geosmin and Fluorescence Intensity

Concentration Range	Wavelength (Ex, Em) in nm	Relationshi	Number of Samples	R^2
1-20 ppm	(225, 340)	$Y= 1.4245x^{0.191}$	30	0.8116
0.005-50 ppb	(225, 340)	$Y= 0.6789x^{.0264}$	30	0.9634

The concentration ranges have been studied separately to see as to how sensitive the method of detection was to the concentration of the 2-MIB and Geosmin. Due to the low concentrations of these compounds encountered in nature it was extremely important to see if the method could be applicable in that range. Preliminary results in that direction have been encouraging. The details of the above results are enclosed as Annexures A and B in this report.

CHAPTER 5

CONCLUSIONS AND NEED FOR FUTURE RESEARCH

5.1 Overview

The objective of this research was to investigate the applicability of using fluorescence spectroscopy as a tool for determining 2-MIB and Geosmin. At this preliminary level of the investigation this was accomplished. The examinations allowed preliminary quantitative relationships to be developed between fluorescence and concentration of 2-MIB and Geosmin.

5.2 2 -MIB and Geosmin Standards

The following conclusions can be drawn from the examination of the 2-MIB and Geosmin standards:

- 2-MIB and Geosmin exhibit fluorescence properties and have distinct spectral fluorescence signatures. These signatures have peak for both the compounds at an excitation of 225nm. The emission peak for 2-MIB is 297nm and 340nm for Geosmin
- There is a strong correlation between the concentration of 2-MIB and Geosmin versus fluorescence intensity. The correlation is stronger when the intensity is measured at an (225, 297) wavelength for 2-MIB and (225, 340) for Geosmin. This could be made use in using the fluorecence technique in determining these compounds. With an extensive library of Signatures it could be an extremely quick, economical and accurate method of detection.

5.3 Need for Future Research

5.3.1 Scope of Current Work

As mentioned earlier the literature search in this area did not yield any research done using this technique. The scope of the present work was therefore to carry out a preliminary study in this direction. The results as discussed in Chapter 3 have been encouraging.

5.3.2 Future Research

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. The following needs to be investigated and confirmed.

- The results need to be verified by actual field sample tests.
- To quantify the concentration accurately it is necessary to investigate presence of any overlapping peak. This would be especially helpful when the actual raw water samples are examined. Further investigations are required to determine these organic substances. Hence the SFS library should be made as extensive as possible.
- Research is required in the area of developing analytical tools for accurate quantification.

APPENDIX A

CORRELATION BETWEEN CONCENTRATION OF GEOSMIN AND FLUORESCENCE INTENSIY

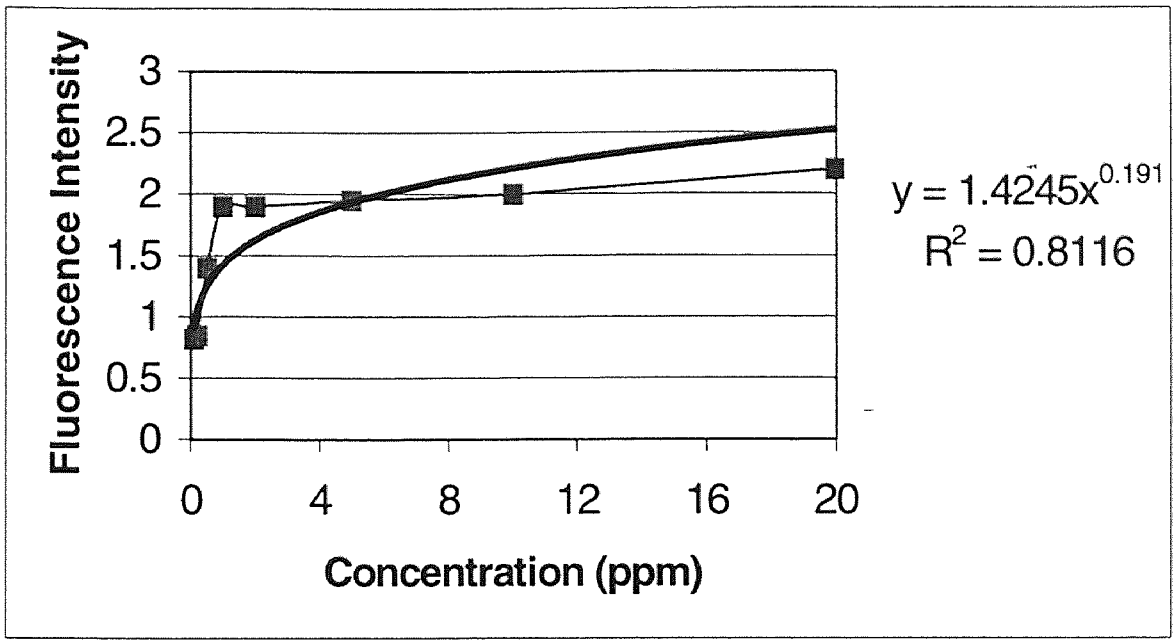


Figure A1 Relationship Between Concentration of Geosmin and Fluorescence Intensity

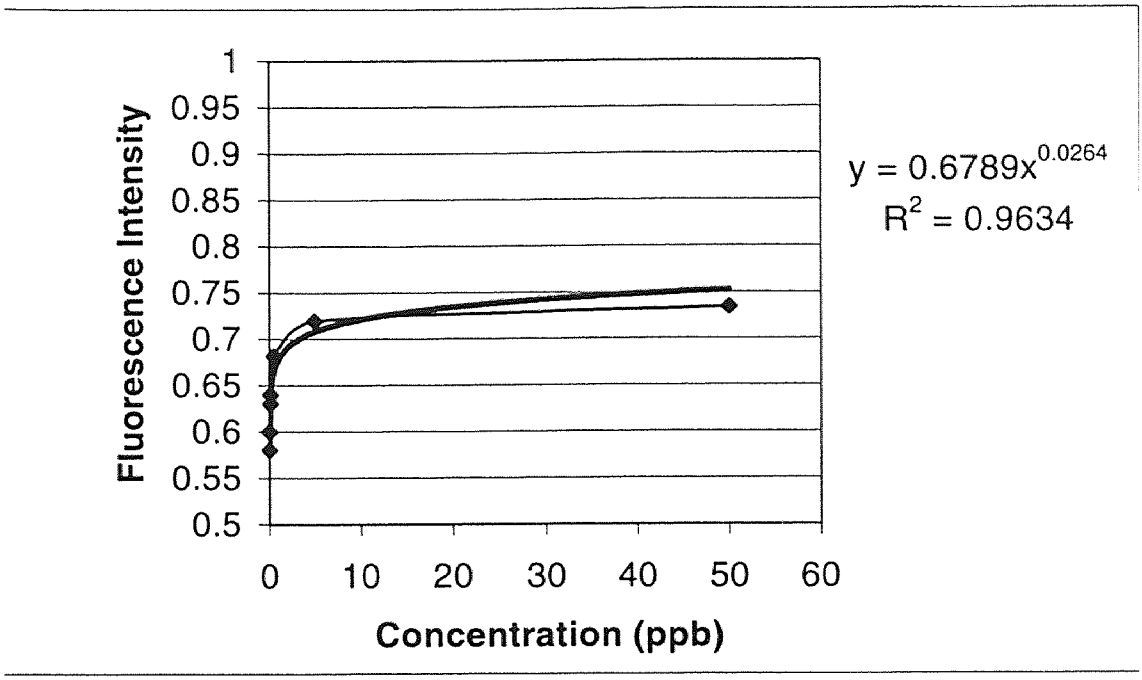


Figure A2 Relationship Between Geosmin Concentration and Fluorescence Intensity

CORRELATION BETWEEN CONCENTRATION OF 2-MIB AND FLUORESCENCE INTENSITY

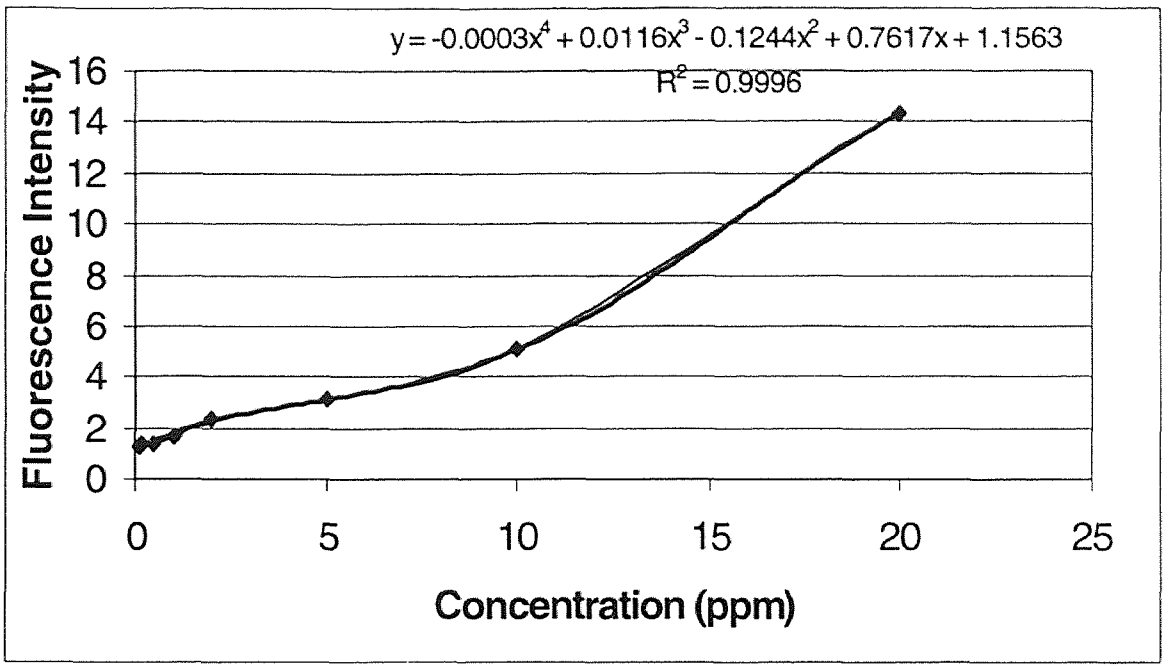


Figure A3 Relationship Between 2-MIB Concentration and Fluorescence Intensity

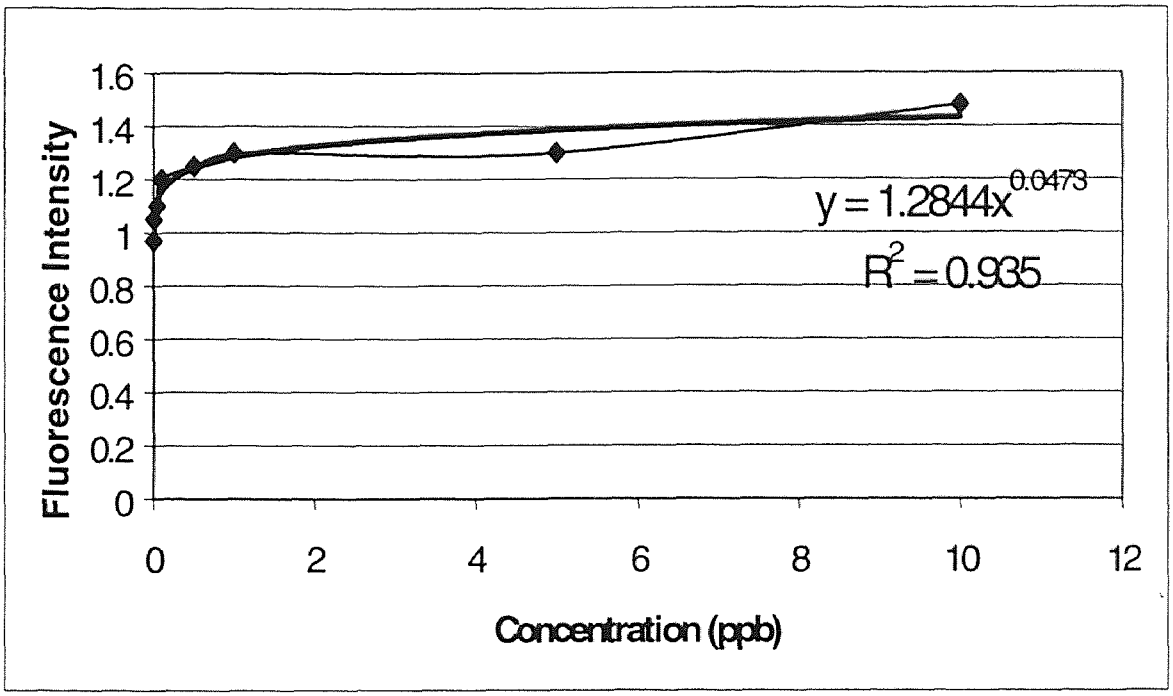


Figure A4 Relationship Between Concentration of 2-MIB and Fluorescence Intensity

APPENDIX B

GEOSMIN AND 2-MIB STANDARD SFS

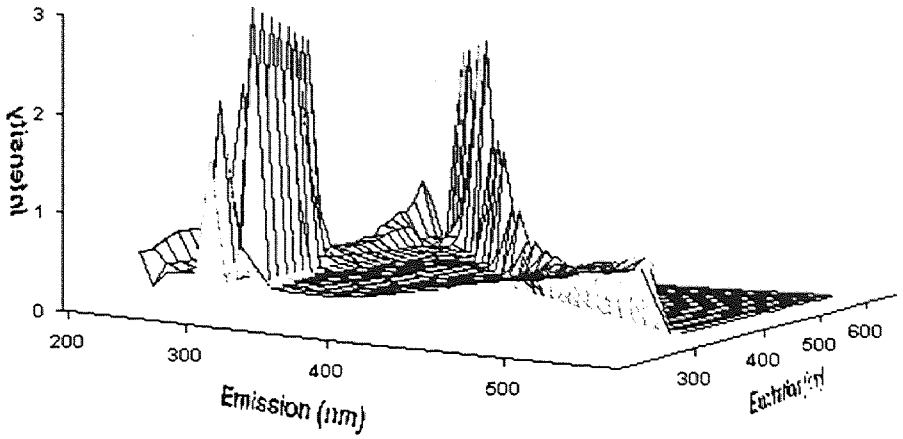


Figure B1 2-MIB(10PPB)

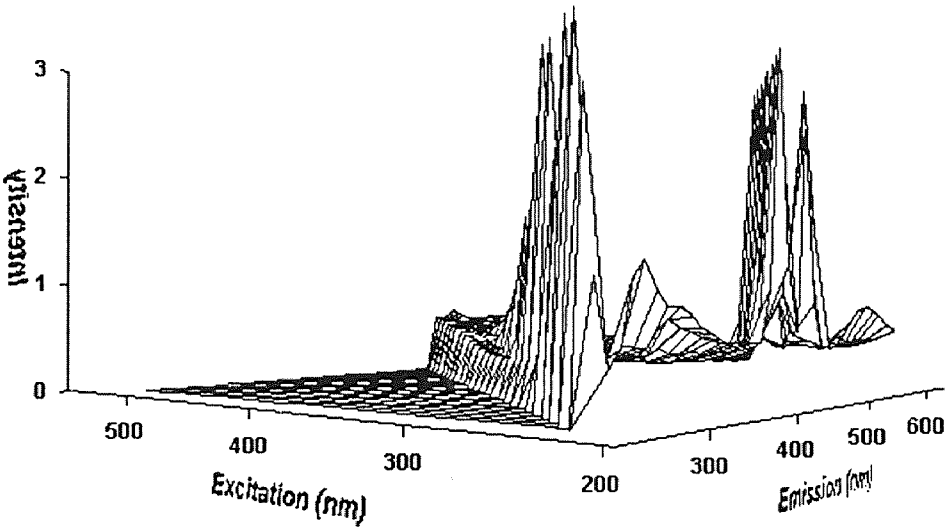


Figure B2 2-MIB(1PPB)

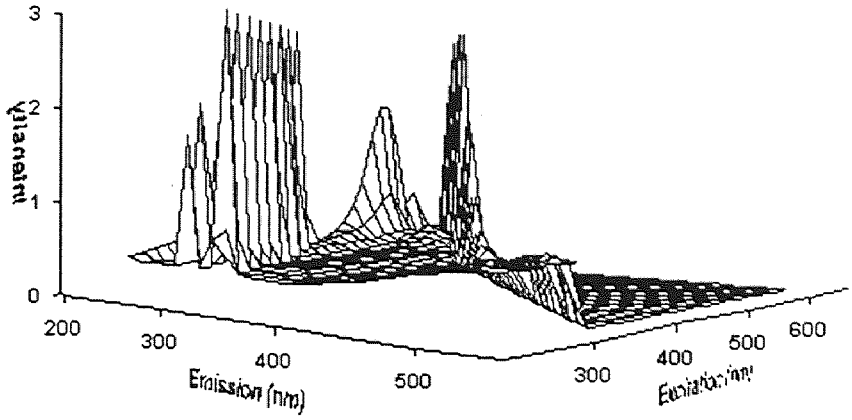


Figure B3 GEOSMIN(10PPB)

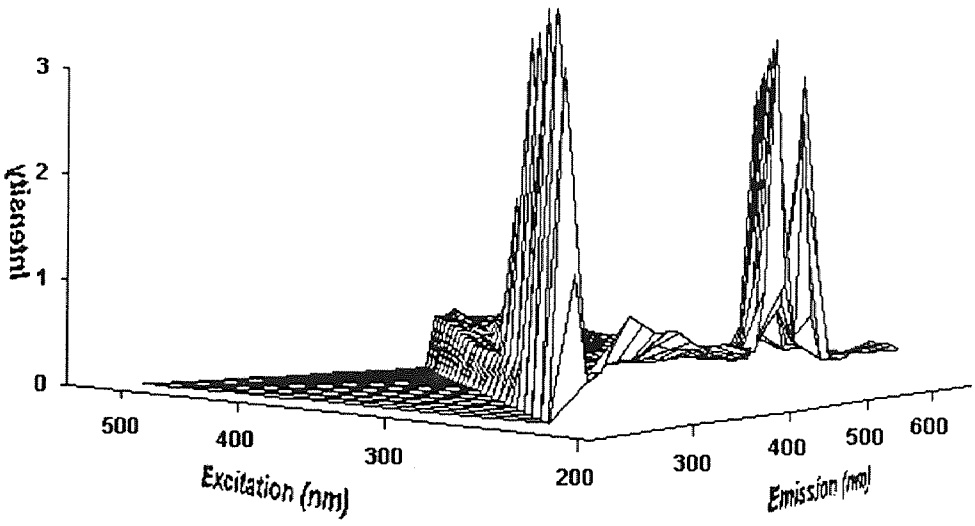


Figure B4 GEOSMIN(1PPB)

REFERENCES

1. Medsker L. L., Jenkins D. and Thomas J. F., *Odorous Compounds in Natural Waters: An Earthy-Smelling Compound Associated with Blue–Green Algae and Actinomycetes,* Environ. Sci. Tech. **2**, 461-464, 1968.

2. Medsker L. L., Jenking D. and Thomas J. F., *Odorous Compounds in Natural Water: 2-Exo-Hydroxy-2-Methylbornane, the Major Odorous Compound Produced by several Actinomycetes*. Environ. Sci. Tech. **3**, 476-477, 1969.

3. Gerber N. N., *Volatile Substances from Actinomycetes: Their Role in the Odor Pollution of Water,* CRC Crit. Rev. Microbiol. **7**, 191-214, 1979.

4. Gerber N. N., *Volatile Substances from Actinomycetes Their Role in the Odor Pollution of Water,* Wat. Sci. Tech., **15**, 115-125, 1983.

5. Safferman R. S., Rosen A. A., Mashni, C.I. and Morris, M.E., *Earthy-Smelling Substance from a Blue-Green algae,* Envir. Sci. Technol., **1**, 429-430,1967.

6. Martin J. F., McCoy C. P., Greenleaf W. and Bennett L., *Analysis of 2-Methylisoborneol in Water, Mud and Channel Catfish (Ictalurus Punctatus) from Commercial Culture Ponds in Mississippi,* Can. J. Fish Aquat. Sci., **44**, 909-912, 1987.

7. Ito T., Okumura T. and Yamamoto M., *The Relationship Between Concentration and Sensory Properties of 2-Methylisoborneol and Geosmin in Drinking Water,* Wat. Sci. Tech., **20**, 11-17, 1988.

8. Means E. G. III. and McGuire M. J., *An Early Warning Systems for Taste and Odor Control,* J. Am. Wat. Works Assoc., **78**, 77-83, 1986.

9. Hwang C. J., Krasner S. W., McGuire M. J., Moylan M. S. and Dale M. S., *Determination of Subnanogram per Litre Levels of Earthy-Musty Odorants in Water by the Salted Closed-Loop Stripping Method,* Environ. Sci. Tech. **18**, 535-539, 1984.

10. Montell A. J., *Muncipal Drinking Water Treatment Procedures for Taste and Odor Abatement – A Review,* Wat. Sci. Tech., **15**, 279-289, 1983.

11. Ando A., Miwa M., Kajino, M. and Tatsumi, S., *Removal of Musty-Odorous Compounds in Water and Retained in Algal Cells through Water Purification Processes,* Wat. Sci. Tech., **25(2)**, 299-306, 1992.

12. Lalazary, S. et al., *Air Stripping of Taste and Odor Compounds from Water,* AWWA,**76(3)**, 83, 1984.

13. Yagi, M. et al., *Odor Problems in Lake Biwa,* Wat. Sci. Tech, **15(6/7)**,311, 1983.

14 Huang C. J., Van Benchoten J.E. and Jensen J.N., *Adsorption Kinetics of MIB and Geosmin,* J. Am. Wat. Works Assoc. **88**, 116-128, 1996.

15. Ellis J. and Korth W., *Removal of Geosmin and Methylisoborneol from Drinking Water by Adsorption on Ultrastable Zeolite-Y,* Wat. Res., **27**, 535-539, 1992.

16. Hrudey S. E., Rector D. and Motkosky N., *Characterization of Drinking Water Odor Arising from Spring Thaw for an Ice- Covered Upland River Source,* Wat. Sci. Tech., **25**, 65-72, 1992 .

17. Gaines, H. D. and Collins, R.P., *Volatile Substances Produced by Streptomyces Odorifer,* Lloydia, **26**, 247-253, 1963.

18. Gerber N.N. and Lechevalier H. A., *Geosmin, and Earthy-Smelling Substance Isolated from Actinomyeetes,* Appl. Microbiol., **13**, 935-938, 1965.

19. Rosen A. A., Mashni C. I. and Safferman R.S., *Recent Developments in the Chemistry of Odour in Water: The Cause of Earthy/Musty Odour*, Water Treat & Exam., **19**, 106-119, 1970.

20. Henley D.E., *Odorous Metabolite and other Selected Studies of Cyanophyta,* Texas State Univ., Denton, Texas, 1970.

21. Barnett R.H., *Research on Control of Taste and Odor Producing Algae in Surface Reservoirs,* Proc. Am. Water Works Assoc., Water Qual. Tech. Conf., Denver, Colo.,1984.

22. Izaguire G., Hwang C. J., Krasner S.W. and Mcguire M. J., *Production of 2-MIB by Two Benthic Cynaophyta.,* Water Sci.Tech., **15**, 6/7, 211-220, 1983.

23. George III. J. E., Payne G., Conn D., Ward G. and Thoma J., *Cost- Effective Low-Level Detection of Geosmin and MIB in Water by Large Volume Purge- and-Trap GC/MS* Water Sci. Tech., **18**,3/4, 110-112, 1996.

24. Rosen B. H., MacLeod B. W. and Simpson M. R., *Accumulation and Release of Geosmin During the Growth Phases of Anabaena Circinalis(Kutz,) Rabenhorst,* Wat. Sci. Tech, **25**, 2,185-190, 1990.

25. Palmentier J. P. F. P., Taguchi Y. P., Steve W. D., Jenkins, Wang D. T., Kim-Phuong and Robinson D., *The determination of Geosmin and 2-Methylisoborneol in Water*

using Isotope Dilution High Resolution Mass Spectrometry, Water Sci. Tec., **34**, 6, 127-130, 1993.

26. Bao M-L., Barbieri M, Burrini D., Griffini O. and Pantani F., *Determination for Trace Levels of Taste and Odor Compounds in Water By Microextraction and Gas Chromatography-Ion-Trap Detection Mass Spectrometry,* Wat. Res. **33**, 545-547,1993.

27. Babichenko S., Orlov Y., Persiantsev I, Poryvkina L. and Rebrik S., *Diagnostics of Organic Substances in Water by Spectral Fluorescent Signatures (SFS),* Am. Env. Lab., **22**, 33-37,1993.

28. Hitachi, *Instruction Manual for Model F-3010 Fluorescence Spectrophotometer,* Hitachi Ltd ., Tokyo, Japan, 1988.