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Drinking Water Taste and Odor: Compound Identification and Treatment

by

Trevor Satchwill

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ABSTRACT

This thesis has investigated the impact of algal derived taste and odor (T/O) on water quality, with particular attention to the chrysophyte species which predominate the Glenmore Reservoir. In this research, the linkage of a specific algae to the production of particular T/O compounds was established (e.g. *Uroglena* with E2,Z4-heptadienal, *Dinobryon* with E2,Z4,Z7-decatrienal) Other major findings include the differential response of algal derived aldehydes to water treatment processes. Volatile organic compounds (VOCs) derived from two significant algae in the Glenmore Reservoir, E2,Z4,Z7-decatrienal (*Dinobryon*) and E2,Z4-heptadienal (*Uroglena*), have very different impacts on water quality. Both aldehydes impart a fishy odor to the source water, however only heptadienal persists through the Water Treatment Plant to cause T/O problems in finished drinking water. Additionally, it was found that the algal derived VOCs are highly unstable. Both the fatty acid precursors and the unsaturated aldehydes or hydrocarbons are subject to rapid biotic and abiotic degradation.

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I dedicate this thesis to:

My children, Emily and Jane.

Everyday you make proud with your wisdom and kindness.

Emily, you see the good in everything.

Jane, you fill the world with your joyous spirit.

You guys are my heroes.

And:

Shaunna Leigh McCauley.

You brought back the light.

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LIST OF ABBREVIATIONS

amu	Atomic Mass Unit
CGS	Conventional Gravity Sedimentation
CW	Carbowax
DAF	Dissolved Air Flotation
DOC	Dissolved Organic Carbon
DVB	Divinylbenzene
FPA	Flavor Profile Analysis
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
GWTP	Glenmore Water Treatment Plant
HSPME	Headspace Solid Phase Micro Extraction
ISTD	Internal Standard
MDL	Method Detection Limit
m/z	Mass-to-charge ratio
OTC	Odor Threshold Concentration
PA	Polyacrylate
PDMS	Polydimethylsiloxane
PFBHA	*o*-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine-HCl
PUFA	Polyunsaturate Fatty Acid
RSD	Relative Standard Deviation
SPME	Solid Phase Micro Extraction
T/O	Taste and Odor
TOC	Total Organic Carbon
VOC	Volatile Organic Compound

CHAPTER 1: INTRODUCTION

1.1 Drinking Water Aesthetics

The palatability of drinking water in terms of its clarity, taste and odor (T/O) is a consumer's only direct indication of water quality [1-5]. For this reason, both suppliers and consumers place great emphasis on these organoleptic properties of drinking water [2, 4, 6-9]. For surface water supplies, the primary water treatment processes of chlorine disinfection and coagulant assisted filtration generally yield water of good clarity and free of pathogenic organisms [2, 10]. Treatment and removal of taste and odor compounds can be more difficult.

Identification, quantitation and treatment of these volatile organic compounds (VOC) is important to the water treatment industry [11-13]. Specific knowledge of the sources and composition of T/O compounds is needed for utility operators to maintain public confidence in the safety of municipal drinking water supplies. Drinking water T/O problems are extensive and pervasive [7]. T/O complaints are reported on a global scale, and they account for the largest single class of consumer complaints to water utilities [14].

In order to verify that a particular algae bloom is the source of T/O event, several criteria should be met [7, 15]. Ecological evidence of both the organism and the odor is needed. After isolation, the organism should be assessed to verify that it produces the same odor as was observed in the environment. Finally, chemical identification of the odor compound is needed. Without all these facets, proof of algal related odor events is circumstantial rather than conclusive.

1.2 Research Goals

In this research, a useful analytical procedure was developed, based on sample concentration by solid phase microextraction (SPME), with detection by gas chromatography-mass spectrometry. This procedure was then applied to several synthetic and natural systems (cultures, mesocosms, surface waters) to identify the odor causing compounds derived from various algae known to produce T/O compounds. This information was used to study the dynamics of T/O production under various conditions. Finally, the effect of drinking water treatment on these T/O compounds was examined with bench and pilot scale experiments.

1.3 Taste And Odor

There are four basic tastes that can be perceived [10]. These are sweet, sour, salty and bitter. Sour and salty tastes are evoked by simple ionic compounds, while sweet and bitter are generally caused by organic compounds (e.g. sucrose, denatonium benzoate). In drinking water, tastes are generally caused by inorganic compounds, as these compounds are present at much higher concentrations than organic compounds ($mg \cdot L^{-1}$ vs. $\mu g \cdot L^{-1}$). In contrast to these four basic tastes, odor is a much more complex phenomenon, with a wide range of descriptors and sensations. These are often classified into major categories (e.g. earthy, musty, swampy, fragrant, grassy, chemical), each with several addition descriptors (e.g. potato bin, bell pepper, rooty, wet cardboard for earthy). Odor stimulants are diverse in their chemistry and elicit a wide range of descriptors [2, 10]. In addition, odor perception can change with temperature and concentration [16].

Human perception of tastes and odors differs from instrumental detection methods. Human odor perception occurs on a logarithmic scale, in contrast to the linear response of most instrumental chemical detection systems. In addition, stereoisomers may differ in both their odor intensity and character [8]. As a result of the interaction of taste and odor, it is often difficult to distinguish tastes from odors, and the two senses are often reported together. This combined sensation is referred to as flavor [7].

T/O compounds may enter water supplies through a variety of means (urban, agricultural runoff, sewage, industrial or municipal discharge) [17, 18], but the immediate source of T/O is most frequently algal. Drinking water supplies often are affected by seasonal T/O events caused by volatile organic compounds (VOC) from algal blooms [19, 20]. Chemical and structural information on the T/O compounds relevant to this study are compiled in Appendix A.

While T/O events can occur in both nutrient rich (eutrophic) and nutrient-poor (oligotrophic) reservoirs, the majority of research has been directed towards eutrophic systems in which blue-green algae are the dominant species. In the case of blue-green algae blooms, two terpenoid compounds geosmin (E1,10-dimethyl-E9-decalol) and 2-methylisoborneol (2-MIB; 1,2,7,7-tetramethyl-exo-bicyclo[2.2.1]heptan-2-ol) are the most important T/O compounds. In less eutrophic surface waters, algae other than blue-greens dominate. In the case of the oligotrophic Glenmore Reservoir (Calgary, AB), T/O events are linked with *Dinobryon spp.* and *Uroglena americana*, two chrysophyte algae [13, 20, 21]. The odor compounds associated with these algae are derived from the oxidation of cellular polyunsaturated fatty acids (PUFA), which produces a range of unsaturated aldehydes [11, 13, 19].

1.4 Algae

1.4.1 Definition of Algae

The term algae comes from the Latin word for seaweed, *alga*. Algae or phytoplankton are a very diverse group of organisms [22], and have been variously described as:

> "any of a large group of mostly aquatic organisms that contain chlorophyll and other pigments and can carry on photosynthesis, but lack true roots, stems, or leaves; they range from microscopic single cells to very large multicellular structures; included are nearly all seaweeds."
> Academic Press, Dictionary of Science and Technology.

Van den Hoek *et al.* [23] define algae as:

> "... very diverse photosynthetic plants that have neither roots nor leafy shoots, which also lack vascular tissues; certain algae are not able to photosynthesise but are nevertheless classified as algae because of their close resemblance to photosynthetic forms."

A more inclusive definition of algae suggests:

"Algae are a very diverse group of mostly photosynthetic, mostly aquatic organisms. They include both photosynthetic prokaryotes (Cyanobacteria) and several distinct eukaryotic lineages, generally separate from fungi or protozoa. They are single or multicellular and range in size by several orders of magnitude (<1um to >45 m (giant kelp)), sessile, planktonic or motile, with varying degrees of heterotrophic activity. They inhabit a range of environments from freshwater-marine water, ice, air, aquatic substrate (epiphytes), terrestrial substrate (rock, soil, bark) and include many symbiotic (e.g. lichens) and parasitic forms (e.g. some dinoflagellates)." S. Watson, personal communication.

While a detailed taxonomic description of algae is beyond the scope of this research, some of the characteristics of relevant algae will be presented. Algae traditionally are classified based on aspects such as cellular organization, pigmentation, storage products and flagellation. The algae found in the Glenmore Reservoir fall under 7 major divisions: *Cyanophyta* (blue-green), *Chlorophyta* (green), *Euglenophyta* (euglenoids), *Pyrrhophyta* (dinoflagellates), *Cryptophyta* (cryptomonads), *Haplophyta* (haplophyte, prymnenophyte) and *Chrysophyta* (golden-brown). Of particular interest are the three important classes of *Chrysophyta*, *Chrysophyceae* and *Synurophyceae* (chrysophytes) and *Bacillarophyceae* (diatoms) [13, 21].

At present, chrysophytes are among the dominant algal species in the Glenmore Reservoir, although diatoms are important in the spring and autumn populations. Chrysophytes associated with taste and odor problems in Glenmore include *Dinobryon cylindricum*, *Dinobryon divergens*, *Synura petersenii* and *Urogelena americana* (Table

1.1). The diatom *Asterionella formosa* can also cause taste and odor problems [24]. Small populations of filamentous blue-green algae such as *Anabaena* and *Oscillatoria* are also present. While these algae are important causes of T/O in eutrophic waters, they have minimal impact on low nutrient systems such as the Glenmore Reservoir.

Table 1.1 Taxonomic data for algae associated with odor.

Algae	Form	Individual cell size µm	Major Odor Compound	Odor Characteristic
Chrysophytes				
Dinobryon spp.	Colonial	36 x 10	2,4-decadienal 2,4,7-decatrienal	Fishy
Uroglena americana	Colonial	7 x 5	2,4-heptadienal	Fishy
Synura petersenii	Colonial	30 x 10	2,6-nonadienal	Cucumber
Mallomonas sp.	Unicellular	10 x 8		
Diatoms				
Asterionella formosa	Colonial	75 x 5	1,3,5-octatriene 1,3,6-octatriene	Geranium, Fishy
Cyanobacteria				
Anabaena spp.	Filamentous	3.5 x 3.5	Geosmin, 2-MIB	Earthy-musty
Oscillatoria spp.	Filamentous	(3-7) x 2	Geosmin, 2-MIB	Earthy-musty

1.4.1.1 Blue-Green Algae

The Cyanobacteria or blue-green algae are the class of algae most often associated with surface water T/O events. These algae are photosynthetic prokaryotes with a robust cell wall structure. They are the dominant algal species in highly productive (eutrophic) waters, and often grow in filamentous mats. These noxious blooms detract from the aesthetic quality of the water as a result of the unpleasant filamentous mats and the offensive T/O compounds. In addition, several toxic compounds (e.g. anacystin, microcystins) may be produced. These have been implicated in the deaths of dogs, cattle

as well as humans [25, 26, 27]. While low nutrient mountain fed rivers such as the Elbow River are not impacted significantly by blue-greens, other prairie surface water sources such as dug-outs, sloughs and irrigation systems are often eutrophic and suffer from blooms of these algae [27].

Geosmin and 2-MIB are the T/O compounds commonly produced by blue-green algae. The other important source of these compounds is the soil bacteria *Actinomycetes* [15]. These compounds are produced in the isoprenoid pathway as side-products of pigment synthesis (carotenoids, chlorophyll)[28]. As such, they are secondary products of primary metabolism. It has been proposed that geosmin and 2-MIB are formed as overflow products, serving as a mechanism to dissipate excess carbon. Recent studies into the production of 2-MIB by *Oscillatoria perornata* and *Pseudanabaena articulata* suggest that formation of this compound reflects carbon accumulation as a result of increased cellular metabolism [28].

Both geosmin and 2-MIB are potent T/O agents, with very low odor threshold concentration (OTC) of 4 – 10 and 9 – 42 $ng \cdot L^{-1}$, respectively [12, 16]. These earthy-musty odor compounds are responsible for the majority of reported T/O events in surface waters. Importantly, these compounds are environmentally stable and can undergo long range transport, affecting areas distant from where the compounds are produced [3, 5]. There are natural mechanisms for the breakdown of these compounds, and argosmin, the non-odorous dehydration product of geosmin [29], is often detected especially during cell die-off when there is increased bacterial activity. However, these processes are slow relative to transport and production. Geosmin and 2-MIB are produced throughout the

cell's lifecycle, can accumulate intracellularly, or be released into the environment either through cell leakage or cell lysis.

1.4.1.2 Chrysophytes

The three chrysophytes of particular interest in the Glenmore Reservoir are *Uroglena americana* and *Dinobryon divergens* and *cylindricum*. They are both naked flagellates with fragile cell membranes. *Dinobryon* is encased in an open-ended cellulose tube or lorica. *Uroglena* produces a loose gelatinous matrix, which aids in holding the colonial form together. Both are photosynthetic mixotrophs and can extract both organic (e.g. bacteria) and inorganic resources (sunlight, dissolved nutrients). This adaptation allows them to sustain periods of high growth rates at low nutrient levels. These chrysophytes typically have high cell lipid content (30-40% dry weight) with a large composition of PUFA (>60%)[13]. The fatty acids are used both to form the lipid bilayer of the cell membrane and as storage products. *Mallomonas* and *Synura* are other T/O producing chrysophytes, however their impact on the Glenmore Reservoir is minor [21].

1.4.1.3 Diatoms

The diatom *Asterionella formosa* is another commonly observed algae in the Glenmore Reservoir. It is encased in a durable silica case or frustule. While this structure is porous, to allow exchange of the cell with the environment, it can impede the diffusion of products. In addition to the fatty acids in the cell membrane, *Asterionella* produces lipid storage vacuoles.

1.5 Algal Derived Chemicals

1.5.1 PUFA Oxidation

An important difference between these eukaryotic algae and the blue-greens is that the associated T/O compounds are not produced through the cells life cycle. Rather, they are formed following the loss of cellular integrity through death, senescence or mechanical disruption. After the cell membrane fails (death, senescence, induced lysis), catabolic enzymes are activated which break down the PUFA, initially to hydroperoxides. Instead, these odor compounds are stored as precursors in the form of triglycerides of PUFAs (linolenic, linoleic, arachidonic acids; Appendix A3). The cellular function of these PUFAs are for the storage of chemical energy, and to form the cell membrane (lipid bilayer). Upon lysis, lipoxygenases are activated which initiate the breakdown of fatty acids through the formation of C_9 or C_{13} hydroperoxides [19, 32, 33]. These degradation products are cytotoxic, and are unlikely to be stored as intracellular materials [30, 31]. The general scheme is shown in Figure 1.1.

1.5.2 Formation of Unsaturated Aldehydes

Subsequent enzymatic action by hydroperoxide lysases further oxidizes the products into a variety of compounds, including several unsaturated aldehydes. These unsaturated aldehydes are associated with fishy odors and off-flavors in both environmental and food samples. Autoxidation of mackerel oil produces 2,4,7-decatrienal, a fishy off-flavor compound [35], while the lipoxygenase products of linolenic acid can form 2,4-decadienal and 2,4,7-decatrienal [36, 37].

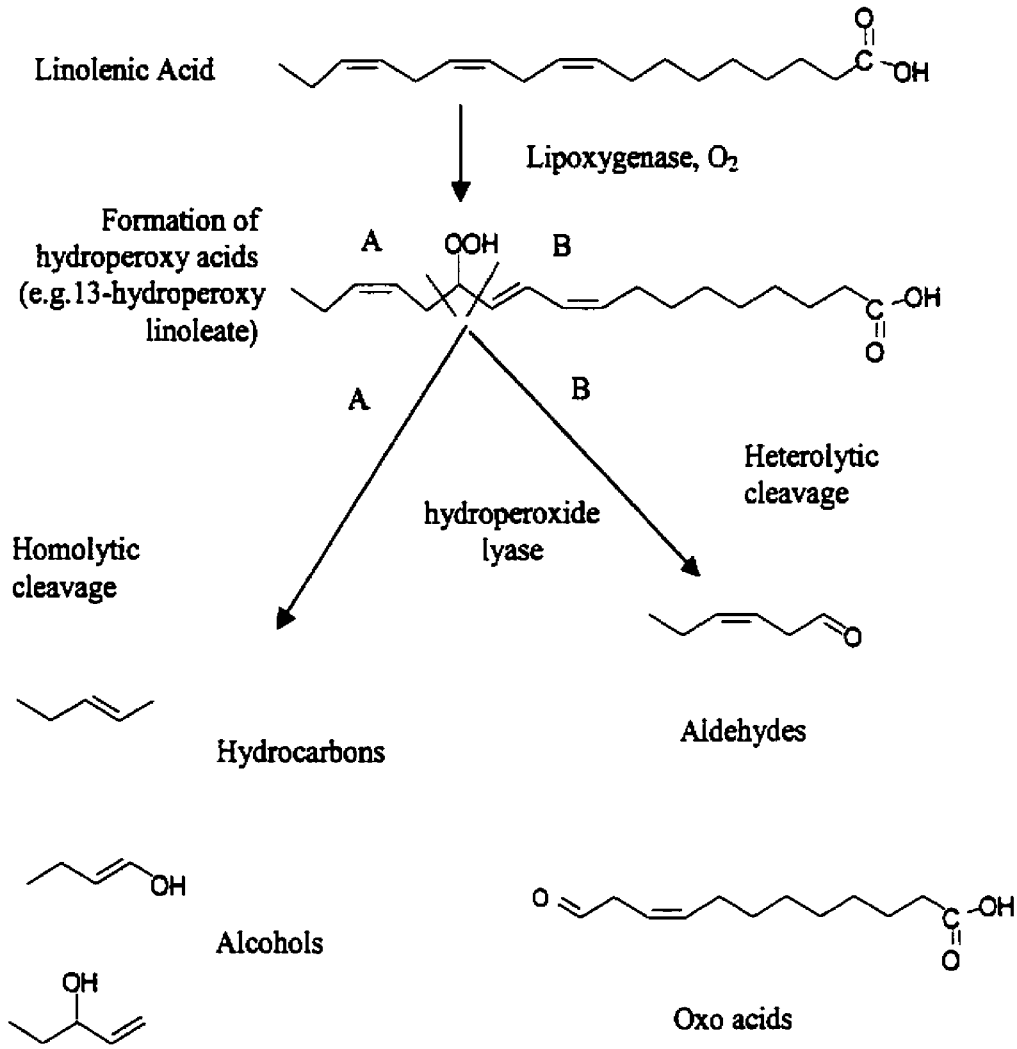

Figure 1.1 Enzymatic oxidation of PUFA to form hydrocarbons, alcohols, aldehydes and oxo-acids. After Jüttner [19].

The T/O compounds produced by the PUFA catabolism include E2,Z4-heptadienal, E2,Z4-decadienal and E2,Z4,Z7-decatrienal. These compounds are associated with the chrysophytes *Mallomonas, Uroglena americana, Dinobryon divergens* and *Dinobryon cylindricum. Synura petersenii* is the only chrysophyte known

to produce the cucumber odor E2,Z6-nonadienal, although this is common metabolite of plants [37] The structures of these and other related compounds are compiled in Appendix A. Where the isomeric designation is not important, these compounds will be referred to in the text as heptadienal, nonadienal, decadienal and decatrienal.

1.5.3 Formation of Hydrocarbons

In addition to unsaturated aldehydes, oxidation of algal PUFAs also results in the formation of algal pheromones. This was first identified in 1971 when Muller *et al.* isolated the male gamete attractant, ectocarpene from the marine brown algae *Ectocarpus siliculous* [38]. Since then several other C_{11} and C_8 semiochemicals have been isolated [39- 41]. Their function includes synchronization of mating through controlled release of the male spermatozoids and attraction to the female cells. Additionally, these compounds can serve as a chemical defence mechanism. The threshold biological activity for these compounds range from 1-1000 pmol (ca. 0.1 – 100 $ng \cdot L^{-1}$).

1.5.4 Biotic and Abiotic Degradation of Algal Metabolites

The complex formation and instability of these PUFA derivatives has serious implications for their chemical analysis. The fatty acid precursors are unstable and subject to chemical and enzymatic oxidation. Losses which occur as a result of sample collection and storage are difficult to detect. The enzymatic formation of the aldehydes is in competition with several other reactions, such as oxidation to hydrocarbons, alcohols and oxo-acids [19]. Upon formation, there are several efficient means for the abiotic

degradation of these chemicals. These pathways include further oxidation by $^1O_2$ (singlet oxygen) and ·OH (hydroxyl radical) [39, 40].

Therefore, the mass and product distribution of PUFA byproducts produced from a sample will be dependent on the conditions of cell lysis and enzyme activation, and the interval between cell lysis and sample analysis. What is formed and measured in a laboratory setting may over or underestimate aldehyde production in the environment.

1.5.5 Isomerization

Other analytical complications arise from the facile isomerization of the unsaturated aldehydes from E,Z to E,E forms [42, 43]. These geometric isomers have distinct physical and physiological properties. Chromatographic analyses of these aldehydes will often resolve the different isomers, so that two or more retention times need to be monitored for each compound. The degree of isomerization increases with sample storage time, where sufficient time for thermal and photolytic isomerization processes (Figure 1.2) has elapsed.

1.6 Analytical Requirements

1.6.1 Introduction

These biogenic T/O compounds are potent, and can be perceived at very low ($\mu g \cdot L^{-1}$ - $ng \cdot L^{-1}$) concentrations. In order to identify and follow the biological formation of these T/O compounds in surface and drinking water supplies, a suitable method is needed. With the ability to identify and quantitate the odor compounds, there is additional

capability to study biological processes and their response to environmental change (eutrophication, resource limitation).

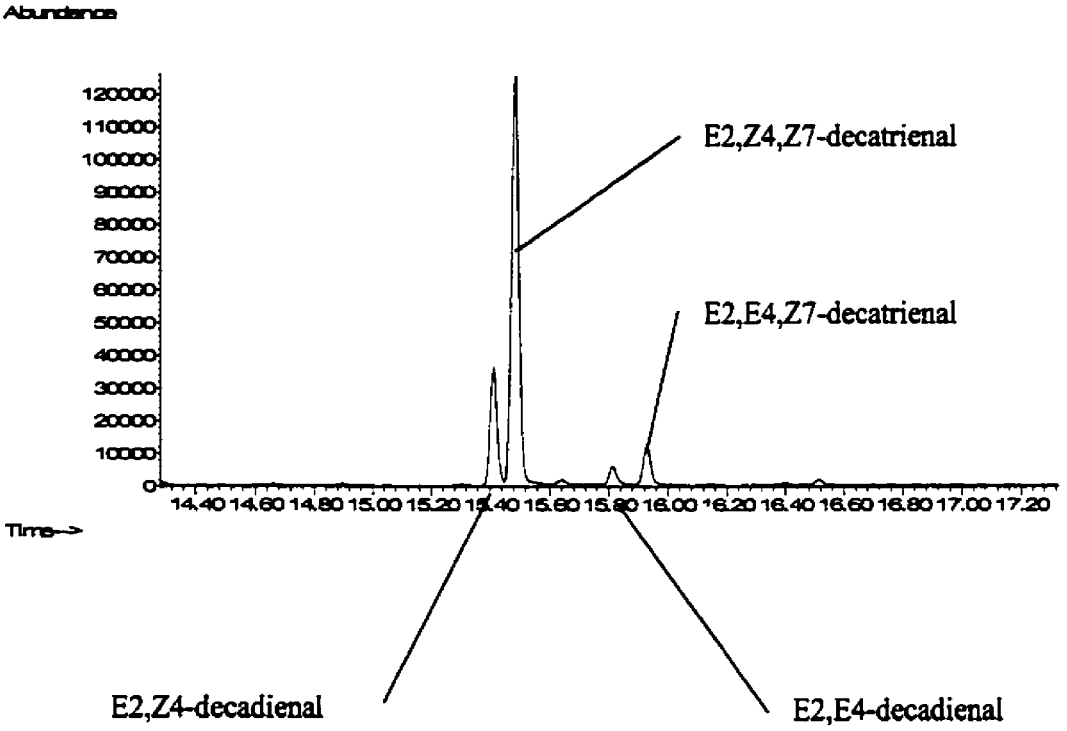

Figure 1.2. Extracted ion (m/z 81) chromatogram of a mixed algal culture. Two major isomers of the C_{10} aldehydes are present. The biologically formed E,Z and E,Z,Z are the major peaks. The more stable E,E and E,E,Z isomers are ca. 10% of total mass. The sample was analyzed immediately after collection to minimise isomerization.

1.6.2 Analytical Resources

This research was conducted in collaboration with the City of Calgary Waterworks Division. As part of their contribution, access was provided to the

instrumental and analytical resources of the City of Calgary's Water Quality Laboratory, located at the Glenmore Water Treatment Plant. Of particular value was the physical access and proximity to the reservoir for sample collection, and the well-equipped laboratory. Instruments used for this research include a Hewlett-Packard 6890/5972 gas chromatograph-mass spectrometer (GC-MS), a Shimadzu TOC-5000 Total Organic Carbon (TOC) analyzer, a Zeiss inverted microscope, as well as instrumentation for nutrient and physical tests (pH, turbidity, major ions).

1.7 The Glenmore Reservoir and Water Treatment Plant

1.7.1 The Reservoir

The Glenmore Reservoir (Calgary, Alberta) is an artificial impoundment of the Elbow River, created in 1933 by construction of the Glenmore Dam (Figure 1.3) [44]. The reservoir was created to provide a source of high quality water for Glenmore Water Treatment Plant, built at the same time. The Elbow River drains a 1200 km^2 watershed in the Rocky Mountains, west of Calgary. The depth of the Reservoir at crest ranges from 1 m at the inflow to 21 m at the dam face, with an average depth of 7.4 m. The reservoir covers an area of 383 ha, with a storage capacity of 22.1 x 10^6 m^3. The average inflow to the reservoir is 0.7 x 10^6 $m^3 \cdot d^{-1}$, with 0.1-0.5 x 10^6 $m^3 \cdot d^{-1}$ withdrawn by treatment plant for the production of potable water. Minimum downstream flows are maintained to preserve the important fish habitat in the lower Elbow River. Water retention time in the reservoir can vary from 2-60 days. Typical inflow rates are low; however spring run-off and storm events can increase the flow from < 10 to 50 – 250 $m^3 \cdot s^{-1}$

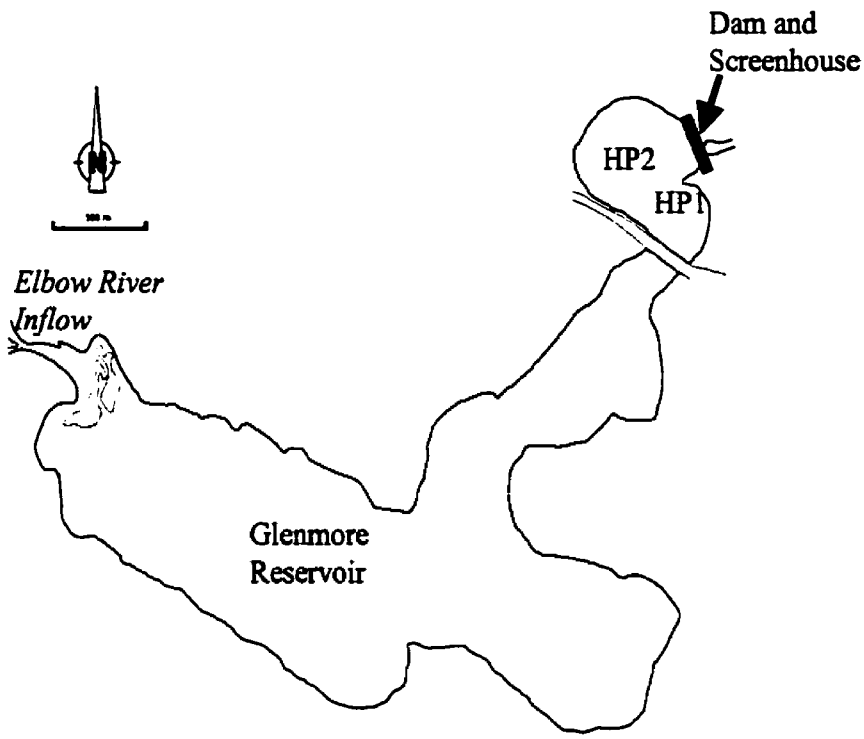

Figure 1.3 The Glenmore Reservoir (Calgary, AB). Headpond sample locations (HP1, HP2), dam and screenhouse are indicated.

Important long term limnological data for the Glenmore Reservoir are presented in Table 1.2, and general water quality characteristics are presented in Table 1.3. The results in Table 1.3 are from single measurements from samples collected at the start of each month

The reservoir is a high quality, low nutrient (oligo-mesotrophic) water supply. However, long term trends indicate that water quality parameters such as turbidity, phosphorus and coliforms (total and fecal) are increasing. This degradation of water quality may lead to increased occurrence of T/O events [44].

Table 1.2 Long term seasonal means in Glenmore Reservoir, water column average 1991-1999.

Parameter	Average
Residence time (days)	33
Secchi depth (m)	3.5
Silica ($mg \cdot L^{-1}$)	4.1
Total Phosphorus ($mg \cdot L^{-1}$)	0.012
Total Nitrogen ($mg \cdot L^{-1}$)	0.730
Heterotrophic Plate Count ($CFU \cdot mL^{-1}$)	330
Chlorophyll-*a* ($mg \cdot L^{-1}$)	0.0013
Total phytoplankton biomass ($mg \cdot L^{-1}$)	0.240

1.7.2 The Glenmore Water Treatment Plant

The City of Calgary's Glenmore Water Treatment Plant is located on the Glenmore Reservoir, and provides drinking water to half of the city's current population of 860,000 [44, 45]. The plant production is rated at 450 $ML \cdot d^{-1}$. The Bearspaw Water Treatment Plant, which draws water from the Bow River provides the remainder of potable water for Calgary. Both plants utilize similar conventional treatment processes for disinfection and particle removal [46, 47]. At Glenmore, source water is withdrawn from the Reservoir via a low lift pumping station located at the dam. The disinfection process consists of free chlorination to a concentration of approximately 1 $mg \cdot L^{-1}$ at the point where water enters the plant headworks, with an additional free chlorine application after filtration. A residual chlorine concentration of at least 0.5 $mg \cdot L^{-1}$ is maintained throughout the treatment plant. The post-filtration chlorine dose is trimmed to provide sufficient free chlorine to maintain a disinfectant residual of at least 0.2 $mg \cdot L^{-1}$ throughout the water distribution system. Particle removal is achieved by aluminum sulfate (alum) assisted flocculation and coagulation. This is followed by gravity

sedimentation and filtration through mixed bed filters comprised of 300 mm of 1.0 mm diameter anthracite over 0.5 mm sand. Following treatment, water is distributed to customers via a network of 3,900 km of water mains [45]. Consistent water pressure (350 – 550 kPa) is maintained by 36 secondary pump stations. Potable water is stored in 18 strategically located reservoirs with a combined capacity of 450 ML. The *per capita* use of water in Calgary is approximately 530 $L \cdot d^{-1}$.

Table 1.3 Glenmore Reservoir general water quality data (1999).

	UNITS	January	February	March	April	May	June	July	August	Septembe	October	Novembe	December
Color	TCU[1]	<2	<2	<2	5.7	2.0	6.4	3.5	4.9	5.7	3.9	2.7	2.5
Turbidity	NTU[2]	0.5	0.6	0.9	2.1	2.1	1.3	2.5	1.5	0.8	1.0	0.8	0.6
Temperature	°C	2.7	2.3	2.8	4.6	7.2	19.1	13.1	19.8	14.0	10.7	5.8	1.8
Conductivity	µS/cm	466	479	468	425	372	392	363	337	379	383	392	426
pH		8.1	8.1	8.1	8.5	8.4	8.5	8.4	8.6	8.4	8.4	8.4	8.4
Hardness as $CaCO_3$	mg·L^{-1}	238.9	275.7	227.5	207.2	178.0	194.9	187.4	175.6	201.2	189.0	202.7	214.8
Total Alkalinity as $CaCO_3$	mg·L^{-1}	181.5	181.5	170.7	150.5	129.8	137.0	134.4	138.0	151.7	151.8	152.3	162.6
Carbonate	mg·L^{-1}	0.0	0.0	0.0	4.1	1.3	0.0	2.1	11.0	2.5	1.6	2.4	3.2
Bicarbonate	mg·L^{-1}	221.3	221.3	208.2	175.2	155.8	167.0	159.6	146.2	179.9	181.8	180.8	191.8
TDS[3]	mg·L^{-1}	293.0	296.4	292.0	261.0	226.0	248.2	231.0	206.0	227.0	231.8	242.4	260.8
TOC[4]	mg·L^{-1}	0.87	0.71	0.68	0.71	0.99	1.42	1.26	2.75	2.34	1.61	1.30	0.98
Ammonia	mg·L^{-1}	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Bromide	mg·L^{-1}		<0.1			<0.01			<0.01			<0.01	
Calcium	mg·L^{-1}	65.3	75.5	61.6	58.1	49.0	51.8	50.3	47.5	53.4	50.5	54.0	58.5
Chloride	mg·L^{-1}	4.2	4.2	4.1	3.3	3.3	3.3	2.2	2.0	2.7	2.5	2.7	3.1
Cyanide	mg·L^{-1}		<0.001						<0.001				
Fluoride	mg·L^{-1}	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Magnesium	mg·L^{-1}	18.4	21.2	17.9	15.1	13.5	15.9	15.0	13.8	16.5	15.3	16.5	16.7
Nitrate	mg·L^{-1}	0.1	0.1	0.1	0.1	0.1	0.0	<0.002	<0.002	0.2	0.0	0.0	0.0
Nitrite	mg·L^{-1}	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
Phosphorus	mg·L^{-1}	0.080	0.002	0.004	0.005	0.005	0.006	0.006	0.005	0.005		<0.002	<0.002
Potassium	mg·L^{-1}	0.9	1.3	0.9	0.9	1.5	0.8	0.8	0.7	0.9	0.8	0.9	1.0
Silica	mg·L^{-1}	4.4	4.6	4.0	3.0	3.0	3.0	4.1	4.4	4.3	4.1	4.2	3.7
Sodium	mg·L^{-1}	4.2	4.7	4.2	3.7	3.8	3.6	2.8	2.9	3.9	3.4	3.5	3.9
Sulfate	mg·L^{-1}	66.3	68.2	69.5	69.0	59.8	65.5	53.5	36.7	42.5	48.0	52.3	59.0
TKN[5]	mg·L^{-1}	0.1	<0.05	0.1	0.2	<0.05	0.1	0.3	0.2	0.2	0.1	0.1	0.1
Heterotrophic Plate Count	CFU/ml	48	16	14	24	25	2900	430	32	540	530	1010	1520
Total Coliform	MPN[6] /100 mL	1	2	1	1	4	58	91	25	50	53	43	37
E. Coli	MPN /100 mL	0	0	0	0	1	3	5	4	9	12	0	4
Giardia	CYSTS /100 L	9.0	1.7	22.0	1.7	0.4	0.3	2.2	14.5	0.2	1.7	3.0	5.7
Cryptosporidium	OOCYSTS /100 L	0.4	0.8	2.0	0.8	0.4	0.1	1.1	2.9	0.2	0.3	0.4	0.1

[1] TCU – True Color Units
[2] NTU – Nephlometric Turbidity Units
[3] TDS – Total Dissolved Solids
[4] TOC – Total Organic Carbon
[5] TKN – Total Kjehldal Nitrogen
[6] MPN – Most Probable Number

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CHAPTER 2: ANALYTICAL METHODS

2.1 Introduction

One of the major goals of this research was to develop a useful analytical procedure for the chemical analysis of T/O compounds. The T/O compounds of interest are low molecular weight (70 – 250 amu) hydrocarbons, alcohols and aldehydes [1]. Generally, the compounds are well suited to gas chromatographic separation. The developed procedure would need to be amenable to a wide range of sample types (low and high particulate surface waters, cultured algae, bacterial isolates on agar, particulate matter). As the T/O compounds are often potent, and can impart an offensive odor in water at $ng \cdot L^{-1}$ concentrations, a highly sensitive procedure was required [1, 2].

Several different analytical techniques are available for the analysis of volatile and semi-volatile algal T/O compounds in water. These include, but are not limited to, the closed-loop stripping apparatus developed by Grob, steam distillation-extraction, continuous liquid-liquid extraction, resin absorption and liquid or solid phase extraction [1, 3, 4]. These procedures are generally suited to a particular matrix and concentration of analyte, e.g. liquid extraction of aqueous phases, static headspace sampling for $mg\text{-}\mu g \cdot L^{-1}$concentrations.

Solid Phase Micro Extraction (SPME) is a relatively new sample concentration procedure that has earned significant attention [5]. It is an easily adaptable procedure which can be applied to both liquid or headspace analysis of a wide range of compounds [6, 7]. It is designed for use with gas chromatography, and it is a highly efficient sample concentration procedure. Therefore, SPME was selected for sample extraction and concentration. Gas chromatography–mass spectrometry was selected to provide the

separation and detection capacity as it is a highly sensitive procedure (sub-ng), and it can provide exact compound identification, based on mass fragmentation spectra.

Other analytical methods used in this research include physical tests for water turbidity, free chlorine residual in water, Total and Dissolved Organic Carbon (TOC, DOC). Limnological tests included nutrient analysis for silica and phosphorous, chlorophyll-*a*, and taxonomic assessment of algae by inverted microscopy. Water treatment tests were conducted with the aid of Conventional Gravity Sedimentation (CGS) and Dissolved Air Flotation (DAF) jar test apparatuses [8].

2.2 Sold Phase Micro Extraction (SPME) Method Development

2.2.1 Introduction

Based on both experimental and literature research, different sample conditions for SPME analysis were evaluated in terms of their effect on method detection limits, time required to process a sample, and range of applicability. SPME adsorbent phase, sample phase (liquid or headspace), sample volumes, sample temperature and salt addition were considered. Samples were stirred in all cases to keep the aqueous phase equilibrated.

2.2.2 SPME

SPME uses a small (1 cm) piece of fused silica, on which a thin (7-100 μm) liquid adsorbent phase has been coated. The fiber is supported on a stainless steel needle. The mechanical integrity of the needle is protected by a stainless steel tube. Sample concentration is conducted by inserting the SPME fiber into the liquid or headspace

phase of a sample. When the sample extraction period is complete, the fiber is withdrawn into the holder, the needle is injected into the heated inlet of a gas chromatograph, the fiber is extended and the sample is then thermally desorbed (200-300 °C).

SPME is not based on exhaustive extraction of the sample, but on an equilibrium between the compound concentration in the sample and that in the solid adsorbent phase [9, 10]. For liquid phase sampling, the distribution constant, K, is defined as:

$$K = \frac{C_s}{C_{aq}} \qquad 2.1$$

Where C_s is the analyte concentration in the solid phase, C_{aq} is the concentration in the aqueous phase, and V_{aq} is the liquid volume. Under equilibrium conditions, the number of moles extracted n_s is defined by:

$$n_s = C_0 V_{aq} - C_\alpha V_{aq} \qquad 2.2$$

Where C_0 and C_α area the initial and equilibrium concentrations of the compound in the sample, respectively [9].

In the case of headspace sampling, three phases are involved with an equilibrium established between the headspace (gas phase), the solid adsorbent phase and the sample matrix. In this case the number of moles extracted by the fiber at equilibrium is defined by [10, 11]:

$$n_s = \frac{K_1 K_2 C_0 V_1 V_2}{K_1 K_2 V_1 + K_2 V_3 + V_2} \qquad 2.3$$

While compound recovery is maximized at the equilibrium condition, quantitative analyses can be conducted under non-equilibrium conditions [11-14].

2.2.3 SPME Adsorbent Phases

SPME fibers with several different adsorbent phases are commercially available for a variety of applications (Supelco, Oakville, ON). The phases are based on synthetic polymers such as divinylbenzene (DVB), polyethylene glycol (carbowax™, CW) and polydimethylsiloxane (PDMS). By altering the composition and thickness of the adsorbent phase, the SPME fiber can be tuned for particular applications, such as for volatile or semi-volatile headspace sampling, or liquid sampling for non-volatile constituents. PDMS phases are appropriate for non-polar compounds and CW for polar compounds. Polymers can be combined to improve efficiency and range of applicability of a fiber, as with the PDMS/DVB phase. Other phases include a proprietary carbon based phase (Carboxen™) and polyacrylate (PA). Phase thickness ranges from 7 μm to 100 μm At this time, the only phase which is chemically cross-linked to the fiber is the thin 7 μm PDMS. The other compositions are either non-bonded or only partially cross-linked. As such, they are subject to bleed.

For this project, three phases suitable for volatile, semi-volatile and polar compounds were evaluated. These included 100 μm PDMS, 85 μm PA, and the 65 μm PDMS/DVB. The PDMS/DVB fiber was selected as it afforded superior adsorption for the range of target compounds. Compared to the PDMS fiber, the relative response of PDMS/DVB was 12, 4, and 4 times greater for 2-MIB, geosmin and biphenyl-d_{10}. Recovery by the PA fiber was 1-2 times greater than the PDMS. Detailed studies by other

researchers [1, 4, 5] have also reported superior overall performance by the PDMS/DVB fiber.

2.2.4 SPME Sampling Phases

In general, either the gas or a liquid phase of a sample can be analyzed. Gas phase sampling can be applied to gas, liquid or solid samples provided the analytes in the sample have sufficient volatility to be partitioned into the gas phase. The mass required in the vapor phase for the useful application of SPME will very much depend on the sensitivity of the detection system and the length of sample extraction time available. For quantitative gas phase analyses, the sample volume must be known or controlled, typically by the use of gas-tight sample containers. In the case of liquid or solid headspace sampling, a three-phase partition is established between the condensed and gas phase, and between the gas and adsorbent SPME phase [11, 12].

In the case of liquid phase sampling, the analytes are partitioned between the liquid phase and the adsorbent SPME phase. In practice this can be complicated by additional phases contributed by the matrix, such as dissolved and particulate organic material [15].

In this work, both liquid and headspace sample procedures were evaluated. Initially liquid phase sampling was employed as it provides the greatest exposure of analyte to the SPME fiber. However, liquid phase sampling was complicated by a number of practical factors, especially when applied to natural water samples or biological cultures. The SPME fiber may become coated by any dissolved, colloidal or particulate organic material in the sample. This inhibits the absorption of analytes. During

sample desorption, compounds are rapidly released from the fiber by a high temperature thermal process (200-300 °C). Any non-volatile organic matrix material (e.g. humic and fulvic acids, colloids) irreversibly bakes onto the fiber and drastically reduces the fiber performance in terms of extraction efficiency, reproducibility and lifetime. In situations where salts are used in the sample preparation, the salts crystallize in the fiber pores and irreversibly damage the mechanical integrity of the fiber. These issues reduced the fibers' useful lifetime to fewer than ten analyses [3, 16].

In headspace SPME (HSPME) sampling, many of these problems are avoided by not directly contacting the sample matrix. The headspace sampling is a much cleaner procedure, and the useful fiber lifetime is extended to greater than 100 extractions. In addition, it was determined that analyte recovery by headspace sampling was 5-10 times more efficient than liquid phase sampling [16].

2.2.5 SPME Sample Conditions

2.2.5.1 Volume

Based on the practical considerations in the development of a method suitable for field, cultures and laboratory analyses, the sample volume was kept as low as possible. Often culture material or field material is very limited in volume (< 250 mL) and aliquots are needed for other analyses (nutrients, species enumeration, organic carbon). Fortunately, SPME is well suited to low volume samples due to the geometry of the fiber and the high efficiency of the procedure. For this study, standard 43 mL volume septum capped glass vials with Teflon™ faced silicone rubber septa were used (I-Chem SB36-0040, New Castle DE). Samples were collected and stored headspace free, and then 13

mL were volumetrically withdrawn via pipette, leaving 30 mL of sample. A stir bar and NaCl were then added, which increased the condensed volume to 35 mL, leaving a headspace volume of 8 mL. These volumes were selected to provide the maximum sample volume and allow sufficient freeboard for the 1 cm long SPME fiber. The final headspace/liquid ratio therefore is 1:5.26. Different headspace volumes were not evaluated in this research; however Bao *et al.* [1, 4] report that sample recovery and headspace volume are inversely related.

2.2.5.2 Salt Addition and Sample Temperature

Salting out is a common procedure to assist with the partition of dissolved organic constituents from the aqueous phase into another phase (adsorbent, organic liquid, vapor) [14]. The maximum solubility for NaCl in water is 0.39 $g \cdot cm^{-3}$ of water at 100 °C [17]. To ensure complete dissolution of the salt and good mixing from the stir bar, a 20% w/v concentration was selected which is equivalent to 6 g NaCl in 30 mL of sample. In addition to the salting out effect, NaCl is used to induce cell lysis and to activate lipoxygenase enzymes [18, 19]. This process is essential to the formation of algal VOCs from PUFA.

The partition of volatile compounds from a condensed phase into the headspace phase can be assisted by raising the sample temperature. Excessively high temperatures can impair recovery by shifting both the sample–headspace and the fiber–headspace equilibrium to favor the headspace phase [3]. Additionally, temperatures close to the boiling point of the bulk matrix (i.e. water) can lead to condensation on the fiber and increase the pressure inside the vial. Condensation on the fiber results in a new sample-

liquid-fiber interface, which alters the extraction equilibrium. Increased vial pressure can lead to leakage through the cap, septum or past the needle puncture.

For this study, a sample temperature of 65 °C was selected as it afforded a balance between improved recovery and detrimental effects. At first a stirrer-hotplate was used to mix and to heat the sample in a water bath. Temperature control of this apparatus was difficult as the heat load (water bath volume) changed with time. To achieve consistent conditions for sample extraction, a Pierce ReactiTherm (Supelco, Oakville, ON.) was modified to accommodate the sample vials in an aluminum block. This maintained the sample temperature within 2 °C of the set point.

The effects of salt addition and sample temperature on compound recovery were compared. The conditions were: salt addition at 0 and 20% w/v, and sample temperature at 25 and 65 °C. Four combinations of the two temperatures and salt addition were evaluated. The results of triplicate analyses are summarized in Table 2.1. For comparative purposes, the response was normalized to the baseline results for 0% salt and 25 °C. The separate effects of salt or sample heating improved compound recovery by 1.85 – 13 times. The combined treatments improved compound recovery by 4.5 – 21.1 times with no detrimental effect.

2.2.5.3 Extraction Time

SPME is based on equilibrium sampling rather than a quantitative extraction procedure such as closed loop stripping, liquid or solid phase extraction [9]. The time to reach equilibrium conditions can be determined experimentally by measuring the time at which no further increase in compound recovery is observed. The equilibrium time will

Table 2.1 Effect of heating (20 and 65 °C) and NaCl on relative compound recovery. All compounds were added to reagent water at a concentration of 100 $ng \cdot L^{-1}$; Biphenyl-d_{10} is the internal standard.

Treatment	**Relative Recovery (mean values, n=3)**			
Heated	No	No	Yes	Yes
Salted	No	Yes	No	Yes
2-MIB	1.00	5.35	5.37	15.67
Geosmin	1.00	4.81	12.97	21.13
Biphenyl-d_{10}	1.00	1.85	4.12	4.49

differ for each compound, based upon the respective chemical and physical properties. However, equilibrium conditions are not required for quantitative analysis [3, 12-14, 20]. As with conventional headspace analysis, quantitative analyses of volatile constituents can be achieved provided consistent and reproducible conditions are used. Therefore, the sampling time can be adjusted to balance sample extraction time with sample throughput.

Various sample extraction times, from 10-240 minutes were evaluated for the four unsaturated aldehydes. The time intervals increased from 10 minutes initially to 170 minutes in order to observe rapid initial changes and slower changes as the system approached equilibrium. The other sample conditions were not altered from the standard procedure (section 2.2.5.7, below). The results (Table 2.2) show that compound recovery improves with extraction time. Heptadienal reached equilibrium after approximately 120 minutes while the amount recovered for the other components continued to increase past 240 minutes. However, improved sensitivity and sample throughput can be competing factors in the selection of optimal extraction times. The use of a 30 minute extraction

time afforded reasonable balance of the two requirements. A similar study of 2-MIB and geosmin, which are less polar compounds, showed that under the same conditions equilibrium was reached within 2 h, but by 1 h 75-80% of the maximum response had been achieved. In addition, the 30 minute extraction time minimized the effects of chemical degradation that occurs in natural samples [19]. In biological samples, bacterial and enzymatic processes can lead to rapid isomerization or loss of the unsaturated aldehydes. As discussed Section 2.5, these losses can range from 64-100%.

Table 2.2 Effect of extraction time on relative analyte recovery. Compounds were prepared in reagent water at a concentration of 5.0 $\mu g \cdot L^{-1}$.

SPME Extraction Time (min)	Heptadienal	Nonadienal	Decadienal	Decatrienal
	$\mu g \cdot L^{-1}$			
10	1.0	1.0	1.0	1.0
20	4.4	1.6	1.4	2.4
40	6.5	10.0	6.9	9.9
90	7.6	9.4	8.5	18.6
120	19.3	23.9	13.7	32.2
170	21.9	35.1	20.6	53.8
240	22.3	42.4	26.1	75.7

2.2.5.4 Recovery After Repeated Extractions

A repeated extraction series was performed on a standard solution which contained a mixture of heptadienal, nonadienal and decadienal. The standard solution was subjected to 8 sequential 30 minute HSPME extractions and GC-MS analyses. As a result of the dynamic sample conditions, these analyses were not corrected by internal standard recovery. Instead, results were plotted against the unadjusted detector response (Table

2.3, Figure 2.1). The amount recovered with each additional sample declined monotonically. Decadienal was depleted quickly, with recovery in the second extraction of 22% and 0% by the fifth extraction. Nonadienal had a slower rate of depletion, with

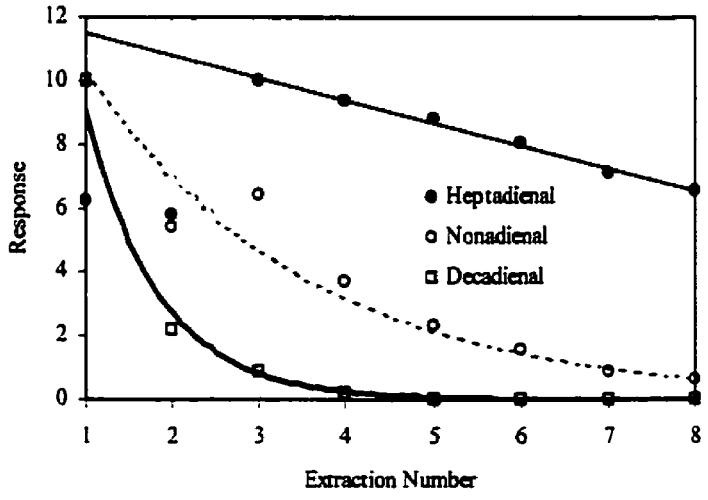

Figure 2.1 Effect of repeated HSPME extraction on compound recovery. Compounds were prepared in reagent water at a concentration of 10.0 $\mu g \cdot L^{-1}$. Internal standard correction was not applied.

Table 2.3 Data summary of repeated extraction experiment.

	Relative Recovery		
Repetition	**Heptadienal**	**Nonadienal**	**Decadienal**
1	10.00	10.00	10.00
2	9.30	5.40	2.20
3	16.10	6.43	0.85
4	15.10	3.70	0.21
5	14.20	2.31	0.01
6	12.90	1.57	0.01
7	11.41	0.89	0.01
8	10.54	0.62	0.01

6.2% of the original amount recovered after the eighth extraction. Heptadienal, which has the lowest recovery under the HSPME sample conditions, had a very shallow depletion curve, which is consistent with little change in the sample concentration. A first order approximation of the theoretical response curve for repeated extractions follows the general form of:

$$n_s = (1 - E)^e \qquad 2.4$$

where n_s is the amount of sample recovered, E is the efficiency of a single extraction and e is the number of repetitions. In general, the data are in agreement with this model (Figure 2.1). The initial two data points for heptadienal do not follow the predicted curve. As heptadienal has the lowest estimated rate of recovery for a single extraction (1.85%, below), small variations in the mass recovered will have a disproportionately large effect on the estimated amount recovered by HSPME. As well, the effect of variable fiber performance could not be compensated for by internal standard correction.

2.2.5.5 SPME Sample Efficiency

The efficiency of the HSPME for the target compounds was assessed by analyzing a known volume (1.4 μL) of 10 $\mu g \cdot mL^{-1}$ standard solution by GC-MS. Then, a standard 2.0 $\mu g \cdot L^{-1}$ sample was extracted via the HSPME procedure and analyzed under the same GC-MS conditions. The peak areas from each analysis were calculated, and then normalized by the mass in each sample (14 and 60 ng for the liquid and HSPME injections, respectively). The data (Table 2.4) indicate that heptadienal in particular has a lower rate of recovery at 1.8 vs. 7.4 – 13.0 % for the other compounds. This is consistent

with results from the repeated extraction experiments, where heptadienal has the slowest uptake under heated conditions and the best recovery at 25 °C, relative to the other aldehydes.

A similar experiment was conducted to assess the recovery of 2-MIB, geosmin and biphenyl-d_{10} by HSPME. The recoveries were 27-31% for 2-MIB, 40-43% for geosmin and 40-65% for biphenyl-d_{10}. The high rate of recovery from samples contributes to the good method detection limit of 1 $ng \cdot L^{-1}$ for these compounds by this method.

Table 2.4 Efficiency of HSPME recovery, based on mass.

Compound	Extraction Efficiency %
Heptadienal	1.9
Nonadienal	8.3
Decadienal	13.0
Decatrienal	7.4
2-MIB	29.0
Geosmin	41.5
Biphenyl-d_{10}	53.9

SPME is an equilibrium sampling process, and complete recovery of all analytes from the sample phase is not possible, such as can be achieved by liquid or solid phase extraction. However, SPME is a very efficient sample extraction process as 100% of the sample recovered is injected onto the analytical instrument (GC, HPLC). In contrast, only a small portion (0.1-1%) of a sample concentrate can practically be injected for analysis after conventional solid or liquid extraction procedures. Aggressive sample concentration steps can lead to loss of volatile analytes, and irreversible adsorption on glassware. To

achieve similar levels of sensitivity, larger sample volumes or more sensitive instrumentation must be employed.

2.2.5.6 Analyte Desorption

After the extraction period, samples were immediately desorbed at 250 °C in the split/splitless injector of the gas chromatograph for 90 s. The splitless (purge off) time was set to 120 s to provide sufficient time to insert and withdraw the fiber prior to inlet purge valve switch. The fact that good chromatographic peaks are observed, free of distortion such as tailing is a good indication that desorption from the fiber is rapid. Periodically, the fiber is desorbed a second time to determine if there is any carry-over from a previous sample. Typically, >99% of the analytes by mass are desorbed. Carry-over can interfere in situations where a very concentrated sample is analyzed. In these cases, the fiber is baked for an additional period of time. An unused, heated injection port with inert carrier gas supply was used for this purpose.

2.2.5.7 Final HSPME Protocol

The standardized sample conditions which were adopted are summarized in Table 2.5. The conditions were selected to balance analytical sensitivity, sample throughput and sample stability. Biological samples were stored without preservation as viable specimens where possible. Storage was in an appropriate lighted incubator (12-15 °C) or alternatively, at 4 °C in a lighted refrigerator. Analysis was conducted as soon as possible to minimize any change or degradation of the sample. Immediately prior to analysis, the sample was gently mixed to suspend particles and algae, and subsamples were collected

in 43 mL glass vials. The sample was adjusted to 30 mL by the volumetric removal of water via pipet (VWR Calibra 832, Mississauga, ON). The internal standard solution was added to a concentration of 100 $ng \cdot L^{-1}$, followed by 6 g of NaCl and a 12 mm stirbar. The NaCl was pre-combusted overnight at 410 °C to remove any organic contaminants. After addition of the stirbar and salt, the liquid and headspace volumes were 35 and 8 mL, respectively. For non-aqueous samples (particulate, bacterial cultures), a 20% w/v NaCl solution was added to yield the same headspace volume. This was done to provide a consistent sample matrix, and to induce cellular lysis and enzyme activation. The vial was sealed and mixed to dissolve the NaCl. The septum was pierced with a disposable syringe, and the SPME needle was inserted into the vial. The vial was placed in the 65 °C block heater/stirrer with the SPME holder supported by a clamp. After visual inspection to ensure the sample was stirred, the SPME fiber was extended and the headspace was sampled for 30 minutes with a 65 μm PDMS/DVB fiber. The heater block temperature was monitored with a NIST traceable digital thermometer and the SPME sample time

Table 2.5 Standard HSPME sample protocol used for thesis research.

Sample Phase	Headspace
Vial Volume	43 mL
Sample Size	30 mL
Headspace:Liquid Ratio	1:5.26
Salt addition	6 g (20% w/v)
Sample Temperature	65 °C
Sample Mixing	Stir bar to vortex
Extraction Time	30 min
SPME Fiber	PDMS/DVB
Injection	Splitless, 250 °C
Desorbtion	250 °C, 60 s
GC column	DB-1701
Oven temperature profile	40(5)-240@ $8°C \cdot min^{-1}$
MS	Scan 40-300 m/z; 2.7 $scan \cdot s^{-1}$

was measured with the gas chromatograph's system timer. After each analysis, the fiber performance was assessed based on noise and internal standard recovery. In addition, the fiber was examined for obvious signs of wear or damage.

The fiber was desorbed for 1 minute at 250 °C and injected onto a HP 6890 gas chromatograph coupled to a 5972 mass spectrometer (Agilent Technologies, Mississauga, ON) in splitless mode. The sample was analyzed in full scan mode from 50–300 m/z.

2.2.5.8 GC-MS Operational Parameters

The Agilent 6890 GC-MS used for the analysis and identification of the taste and odor compounds is highly sensitive and can provide full scan mass spectroscopic data from less than 1 ng of organic compound. From the acquired mass spectrum, compound identification can be performed using library search (commercial or in-house), structural elucidation. This provides compound identification based on structure in addition to identification based on retention time matching.

Two analytical columns were used for the chemical analysis. A general purpose screening column (DB-5ms polydimethylsiloxane-5% phenyl silicone, 20 m x 0.18 mm id x 0.1 μm phase thickness; J&W, Folsom CA) was used initially. This column was not suitable for the separation of C_{10} unsaturated aldehyde isomers, and the similarities in the mass spectrum of these compounds made it impossible to separate the signals by extracted ion analysis. A more polar column provided adequate separation of these compounds (DB-1701 14% cyanopropylphenyl-methyl polysiloxane, 30 m x 0.25 mm x 0.25 μm phase thickness; J&W, Folsom CA). The oven temperature profile used was an initial temperature of 40 °C, 5 minute hold, ramp at 8 °C·min^{-1} to 240 °C.

Acquisition was conducted both in scan mode (40 – 300 m/z) and in selected ion monitoring for diagnostic ions. In certain analyses, such as for geosmin and 2-MIB, selected ion monitoring was used successfully to reduce background noise and achieve very low (1 $ng \cdot L^{-1}$) detection limits. The quantitation ions (m/z 112 and 95, respectively) are free of background interference. The same technique was applied to the unsaturated aldehydes, which have base peaks at m/z 81 (2,4-dienals, 2,4,7-decatrienal) and 69 (2,6-nonadienal). However, there was significant background noise from coincident ions and background bleed. Additionally, several geometric isomers of each compound exist (i.e. E2,E4-heptadienal and E2,Z4-heptadienal), each with different chromatographic retention times. Therefore, aldehyde samples were analyzed in full scan mode and compound identification was confirmed by mass spectrometry. While the absolute method sensitivity was decreased, this was necessary to provide accurate compound identification. An additional benefit was that other volatile compounds with biological significance could be identified from a single analysis.

Along with the periodic inlet maintenance procedures (replacement of septum, liner, inlet seals), 5 – 15 cm of column was cut from the inlet end. Due to the larger bore of the SPME needle compared to a conventional syringe, septum fragments accumulated at the front of the column. Together with column phase oxidation and SPME fiber particles that detached during desorbtion, chromatographic performance would become degraded. The mass spectrometer was tuned with the automatic maximum sensitivity autotune, with perfluoro-*tert*-butyl amine as the reference compound. The mass resolution and abundance was tuned based on the ions m/z 69, 219 and 502.

2.2.6 Fiber Performance

2.2.6.1 Fiber Bleed

The adsorbent phase of the SPME fiber is based on polymers similar to those of gas chromatographic capillary columns. As with the PDMS based GC columns, the phase will bleed. Phase bleed is directly proportional to the amount of phase and exponentially proportional to temperature [21], and occurs due to the degradation of the polymer. While thin polymer films (e.g. 7 μm PDMS) can be chemically crosslinked and deactivated to minimize bleed, few of the commercially available SPME phases and loadings are available in fully cross-linked forms. As a result, there is a significant amount of background bleed as well as discrete polymer peaks from the SPME fiber. These undesirable characteristics affect the overall utility of the procedure as they obscure sample peaks and impair sensitivity through the increased background noise. When applied to trace level analyses, fiber bleed is the major contributor to the chromatogram. The effects of bleed can be compensated for by selected ion ejection, in the case of ion trap mass spectrometers [22], use of detectors which are insensitive to the silica based bleed (i.e. flame ionization and electron capture detectors), or by selected ion monitoring in the case of quadropole or magnetic sector mass spectrometers. In many situations, however, it is essential to acquire full-scan data to confirm compound identity, especially if the compound of interest has few distinctive ions which can be used as qualifiers.

The mass spectrum of the PDMS/DVB fiber used is characterized by substituted benzene compounds (C_1-C_3 benzene, m/z 91) and $Si(CH_3)_3$ (m/z 73). The PDMS contributes discrete peaks from polymer fragments throughout the chromatogram. A typical chromatogram with the m/z 73 extracted is shown in Figure 2.2.

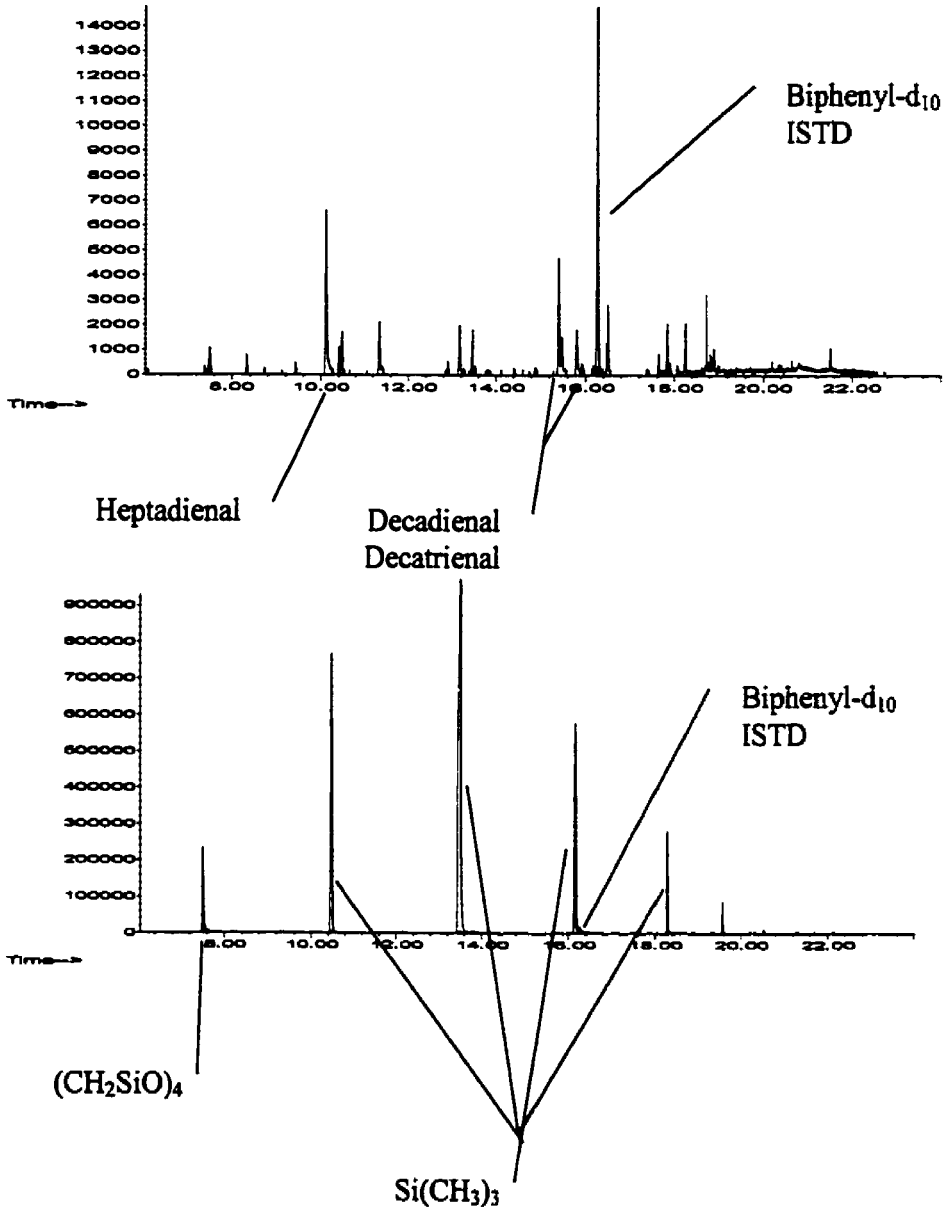

Figure 2.2 Extracted ion chromatograms (Glenmore Reservoir, January 13, 2000). Upper panel: algal VOCs (m/z 81); lower panel: fiber bleed (m/z 73, 281). The same chromatogram is used in both cases, with the internal standard peak indicated (ISTD). Y-axis scaled to largest peak.

2.2.6.2 HSPME Precision

The reproducibility of replicate SPME analyses was found to vary greatly, with relative standard deviations (RSD) of 35.7% in terms of absolute recovery for chemical standards. The integrated peak area count data from five HSPME and five liquid injection analyses of the internal standard compound biphenyl-d_{10} are presented in Table 2.6. For the conventional liquid injection analyses, the RSD is 5.2%. External sources of error such as detector fluctuation, sample preparation were evaluated and controlled through standard quality assurance measures such as the routine analysis instrument performance samples, analytical solutions. The use of an appropriate internal standard solution to correct for fiber variability was essential in generating quantitative results. The HSPME analysis was especially sensitive to 'first run of day' events, and an initial work-in analysis was beneficial. Nonetheless, calibration and procedure was very robust, and quality assurance samples run over several months to years time would remain in acceptable analytical limits.

Table 2.6 Comparison of replicate analyses by HSPME and by liquid injection. Biphenyl-d_{10} was used as the reference compound.

	Integrated Peak Area Counts	
Replicate	**HSPME**	**Liquid Injection**
1	277662	3243395
2	621990	3558574
3	705000	3672357
4	434694	3482841
5	394316	3298129
Average	486732	3451059
Standard Deviation	173849	178923
%RSD	35.72	5.18

2.3 HSPME Method Validation

2.3.1 Limit Of Quantitation

The quantitative method detection limits (MDL) were calculated based on established analytical procedures [23, 24]. The MDL is determined by analyzing 9 replicate standards at 1-5 times the estimated detection limit. The MDL is calculated by multiplying the standard deviation times the student's t value appropriate for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom. This statistical method provides a conservative estimate of detection limit. In certain cases a lower detection limit was established at the lowest calibration point, based on acceptable recovery of matrix spike and calibration sample. Separate calibration and detection limit studies were performed for the unsaturated aldehydes and the terpene compounds. The aldehydes were calibrated in the range of 500 – 10,000 $ng \cdot L^{-1}$, and the detection limit study was conducted at 1000 $ng \cdot L^{-1}$. The two terpenoids, 2-MIB and geosmin were calibrated over the range of 2 – 100 $ng \cdot L^{-1}$, and the detection limit study was conducted at 100 $ng \cdot L^{-1}$. The calibration curves for the T/O compounds are plotted in Figures 2.4 and 2.5. The detection limit and calibration data are presented in Appendix B, and summary data is presented in Table 2.7.

The estimated quantitation limits for the aldehydes were 508, 622, 512 and 545 $ng \cdot L^{-1}$ for heptadienal, nonadienal, decadienal and decatrienal, respectively. The quantitation limit for 2-MIB was 24 $ng \cdot L^{-1}$ and for geosmin 22 $ng \cdot L^{-1}$. The detection limit data were collected from the on-going quality assurance samples analyzed with each analytical series.

Table 2.7 Summary of detection limit statistics for HSPME method.

Compound	% Minimum Recovery	% Maximum Recovery	Standard Deviation ($ng \cdot L^{-1}$)	Estimated Detection Limit ($ng \cdot L^{-1}$)
2,4-Heptadienal	75	129	175	508
2,6-Nonadienal	75	141	215	622
2,4-Decadienal	89	139	177	512
2,4,7-Decatrienal	83	143	188	545
2-MIB	88	114	8	24
Geosmin	89	110	8	22

The method was calibrated for each of the T/O compounds from the estimated detection limit up to the maximum expected sample concentration. For 2-MIB and geosmin, the method was calibrated from 2-100 $ng \cdot L^{-1}$. The unsaturated aldehydes were calibrated from 500 – 10,000 $ng \cdot L^{-1}$. The calibration curve was constructed based on the method of internal standards. The fully deuterated biphenyl-d_{10} was added as an internal standard to all samples and standards at a level of 100 $ng \cdot L^{-1}$. Peak integration was automatically performed by the chromatography software. The least squares linear regression data for the calibration (Appendix B) had excellent linearity, with R^2 values which ranged from 0.984 for 2-MIB to 0.998 for decadienal and decatrienal.

2.3.2 Split Sample Method Validation

The applicability of the HSPME procedure was validated in an inter-laboratory split sample study between Calgary (City of Calgary) and Burlington (Canadian Center for Inland Waters). Each laboratory conducted independent analyses, based on their own calibration procedures. In this study, fortified Sibbald Lake water containing algal derived geosmin and 2-MIB was diluted with Glenmore Reservoir water to two concentration ranges, one low range near the OTC, and one at ca. 5 times higher

concentration. Five replicates of each sample were analyzed by both laboratories and the results (Table 2.8) were evaluated with a two-way analysis of variance. There was no statistical differences in the results obtained by the two laboratories ($P < 0.0001$). The excellent agreement in results between the two laboratories demonstrates the repeatability of the HSPME procedure.

Table 2.8 Replicate analysis of split samples (laboratories in Calgary, AB and Burlington, ON).

	Level	Procedure	N	Mean ($ng \cdot L^{-1}$)	Std. Dev.	%RSD
Geosmin	High	A	5	223.9	19.1	8.5
		B	5	228.6	5.1	2.2
	Low	A	5	37.0	4.3	11.6
		B	5	44.1	1.1	2.3
2-MIB	High	A	5	151.6	16.2	10.7
		B	5	150.4	8.5	5.7
	Low	A	5	24.9	3.3	13.3
		B	5	29.0	1.7	5.9

2.4 Analytical Standards

2.4.1 Introduction

Where available, authentic chemical standards were purchased from commercial sources. Several of the conjugated E,Z compounds are unstable, and subject to isomerization. As a result, the more stable E,E isomer was used for calibration. The natural isomer was identified by mass spectrometry, and the calibration data was used for both isomers. Other compounds (2,4,7-decatrienal, 1,3,5-octatriene and 1,3,6-octatriene) were not commercially available [25]. Dr. F Jüttner (Institute für Pflanzebiologie,

Universität Zürich) kindly donated a sample of synthetic E2,E4,Z7-decatrienal. Access to this chemical standard was of invaluable help to this research, as it allowed for instrument calibration, and for chemical dynamics to be studied. In the case of octatriene, no standard was available and these compounds were assigned an arbitrary response factor. Where present, the sum of all geometric isomers was reported. The initial samples of 2-MIB and geosmin were kindly donated by Dr. Brian Brownlee (Canadian Centre for Inland Waters, Burlington). The chemical standards are listed in Table 2.9.

Table 2.9 Chemical reference standards.

Compound	CAS Registry	Molecular Formula	Molecular Mass	Source
E2,E4-heptadienal	4313-03-5	$C_7H_{10}O$	110.2	Aldrich
E2,Z6-nonadienal	209-178-6	$C_9H_{14}O$	138.2	Aldrich
E2,E4-decadienal	25152-84-5	$C_{10}H_{16}O$	152.2	Aldrich
E2,E4,Z7-decatrienal	13552-96-0 (E,Z,Z)	$C_{10}H_{14}O$	150.1	F. Jüttner
Geosmin	19700-21-1	$C_{12}H_{22}O$	182.1	Wako
2-Methylisoborneol	2371-42-8	$C_{11}H_{20}O$	168.3	Wako
Biphenyl-d_{10}	1486-01-7	$C_{12}D_{10}$	164.3	Aldrich

2.4.2 Aldehyde Standard Preparation

Primary standards were prepared by the gravimetric dilution of the neat chemical in methanol to yield individual stock solutions of 2-10 $mg \cdot mL^{-1}$. A mixed solution containing all 4 aldehydes was prepared by volumetric dilution of the stock to yield a 10.0 $\mu g \cdot mL^{-1}$ solution in methanol. Calibration and matrix spike samples were prepared by volumetric dilution of the 10.0 $\mu g \cdot mL^{-1}$ solution in reagent water or matrix water in the range of 500-10,000 $ng \cdot L^{-1}$.

2.4.3 Geosmin and 2-MIB Standard Preparation

These were purchased as a 1.0 $mg \cdot mL^{-1}$ solution in methanol from Wako Chemicals. The working solution was volumetrically diluted to 1.0 $\mu g \cdot mL^{-1}$ in methanol. Calibration and matrix spike samples were prepared by volumetric dilution of the 1.0 $\mu g \cdot mL^{-1}$ solution in reagent water or matrix water in the range of 2-100 $ng \cdot L^{-1}$.

2.4.4 Internal Standard Preparation (Biphenyl-d_{10})

This was gravimetrically prepared 5 $mg \cdot mL^{-1}$. This was volumetrically diluted to 0.050 $\mu g \cdot mL^{-1}$ in methanol. Thus the addition of 60 µL to 30 mL sampled yielded a final concentration of 100 $ng \cdot L^{-1}$.

2.5 Sample Storage Evaluations

2.5.1 Storage Of Biological And Chemical Samples

Geosmin and 2-MIB in natural and synthetic samples were found to be stable for at least 28 days, as were chemical standards of the unsaturated aldehydes. These samples were prepared in filtered reagent water, free of biological contaminants. Natural samples which contained geosmin and 2-MIB in biological matrices (cultures, surface waters) were also stable for extended periods [3]. However, the unsaturated aldehydes in biological matrices were degraded rapidly and acceptable storage procedures were difficult to develop. Both the fatty acid precursors and the resultant unsaturated aldehydes are subject to rapid chemical and biological degradation [20]. Several storage options were evaluated for the long term storage of algal samples, and for the chemical analysis of volatile aldehydes.

Wendel and Jüttner examined inhibition of enzyme degradation by two procedures [18]. Lipoxygenase activity was inhibited when air and molecular oxygen were removed from algal solutions. Under these strict anoxic conditions, no aldehydes or hydrocarbons were formed after activation of the enzyme by salt addition, demonstrating the dependence of this reaction on molecular oxygen. Radical scavengers such as 3-*tert*-butyl 4-hydroxyanisole and propyl gallate were also effective at inhibiting the enzymatic formation of aldehydes and hydrocarbons. While these interventions were successful inhibitors of the lipoxygenases, and present an attractive sample storage option, procedures to re-activate the enzymes were not successful. In the former case, re-introduction of oxygen only partially restored activity, and after 40 minutes without oxygen, no VOCs were liberated. In the latter case, no viable means of sequestering the radical scavenger was found.

2.5.2 Low Temperature Storage

Storage at –20 °C was evaluated. As the freezing process would disrupt the cellular integrity and release the cellular contents, it was desirable to freeze the samples in gas tight containers, and then thaw them prior to analysis in the same container. The same 43 mL glass septum vials were evaluated, however it was found that the expansion of the water on freezing was uneven, the vials would break even when 50% volume was left for expansion. In cases where the sample survived storage, significant degradation of the sample was observed when compared to duplicate samples analyzed freshly. Plastic vials (30 mL polyethylene) were also evaluated, using standard solutions of aldehydes, as they would be more resistant to breakage. In this case, it was found that the plastic

strongly adsorbed the volatile organic compounds, and even short term storage led to drastically reduced recovery.

2.5.3 Salt Addition

Headspace free sample storage in glass vials with 20% w/v NaCl added was also examined. This was attempted in an effort to minimize any bacterial degradation and provide a uniform storage matrix. However, as with the sample analysis protocol, salt addition also induced the loss of cellular integrity and activation of enzymes. Within 48 h 100% of heptadienal, nonadienal and decatrienal, and 94% of decadienal were eliminated from the sample, even when the storage samples were spiked with synthetic chemical standards. By contrast, 81.6 and 70.7% of geosmin and 2-MIB remained. It is postulated that the enzymes released from the algal cells continued their action on the aldehydes, and subsequent oxidation occurred.

The storage of analytes spiked in reagent water, and in a biological matrix comprised of untreated Glenmore Reservoir water and algal culture material was evaluated after 0 and 14 days (Table 2.10). In reagent water, the 2,4-aldehydes were stable over 14 days while 100% of nonadienal and 22% of decatrienal were lost. In matrix water (mixture of reservoir water and algal culture), the unsaturated aldehydes were stable through the first day of storage when the time 0 analyses were conducted. After 14 days, 0-7.7% of these compounds were recovered. Nonadienal was not detected in either the reagent or the matrix water samples. By contrast, geosmin and 2-MIB were stable under both storage conditions (Table 2.11).

Table 2.10 Recovery of aldehydes after storage (0, 14 days) in reagent water and biological matrix.

Sample		Compound ($ng \cdot L^{-1}$)			
		Heptadienal	**Nonadienal**	**Decadienal**	**Decatrienal**
MQ	Avg (n=4)	2301.8	2228.8	2355.5	477.8
0 day	Std Dev	474.8	80.0	84.3	24.9
MQ	Avg (n=4)	2832.3	0.0	1997.3	105.7
14 day	Std Dev	61.1	0.0	31.6	6.7
% Change on storage		+23	-100	-15.3	-77.8
Matrix	Avg (n=4)	2195.5	2455.8	2357.0	557.5
0 day	Std Dev	212.4	142.6	108.2	24.2
Matrix	Avg (n=4)	169.8	0.0	100.5	39.5
14 day	Std Dev	227.9	0.0	119.3	45.7
% Change on storage		-92.3	-100	-95.7	-92.9

Table 2.11 Recovery of geosmin and 2-MIB after storage (0, 14 days) in reagent water and biological matrix.

Sample		Compound ($ng \cdot L^{-1}$)	
		2-MIB	**Geosmin**
MQ	Avg (n=4)	151.3	106.5
0 day	Std Dev	1.7	3.0
MQ	Avg (n=4)	150.7	162.0
14 day	Std Dev	3.2	4.0
% Change on storage		-0.3	+52.1
Matrix	Avg (n=4)	153.8	105.5
0 day	Std Dev	6.1	2.4
Matrix	Avg (n=4)	165.8	176.3
14 day	Std Dev	2.8	4.6
% Change on storage		+7.8	+67.1

Further investigation of the short term (5 h) storage effects was conducted on algae cultured material (Table 2.12). Triplicate samples were analyzed fresh (0 h), after 5 h storage, and after 5 h storage with 5% w/v NaCl added. The recovery of the algal derived hydrocarbon octatriene increased, both with storage and storage with NaCl. For the unsaturated aldedhydes, little change was observed over 5 h storage. With the addition of NaCl, however, the salt induced lysis and enzyme activation reduced aldehyde recovery to 9-36%.

Table 2.12 Recovery of algal VOCs after short term (5h) storage, with and without the addition of 5% w/v NaCl.

	Relative recovery after treatment			
Treatment	**Octatriene**	**Heptadienal**	**Decadienal**	**Decatrienal**
0 h (control)	1.00	1.00	1.00	1.00
5 h	2.33	1.12	0.91	1.06
5 h, NaCl	5.05	0.36	0.09	0.27

2.5.4 Chemical Derivativization

Oxime derivatization based on the method Glaze and Weinberg [23, 26] was also evaluated. In this procedure the oxime derivatives are formed by the addition of *o*-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine·HCl (PFBHA). As the oxime derivatives are chemically stable, this may afford a useful long-term storage solution for algal derived unsaturated aldehydes.

For algal samples, lysis was induced by the addition of salt and the released fatty acids underwent natural enzymatic degradation for 30 minutes. The derivatizing reagent

was then added, and the samples were incubated at 40 °C for 2 h in a GC oven. The samples were then quenched with dilute H_2SO_4, extracted into a 2 mL hexane, and a 1 μL volume was injected and analyzed by GC-MS.

Initial results at high concentration were promising; however sensitivity was lost due to the less efficient solvent extraction step vs. HSPME. For the liquid injection, only 0.05% of the total extract volume is injected. In the case of HSPME, 100% of the extracted sample is injected onto the instrument. The use of HSPME to concentrate the hexane extract is not practical due to competitive adsorption of the solvent. However, Martos and Pawliszyn [27] report the successful use of HSPME to recover PFBHA derivatives of acetone directly from aqueous solution.

The oxime derivatives are formed with an equal proportion of E and Z isomers from each aldehyde, leading to a more complex chromatographic signal [23]. This feature complicates interpretation, as closely spaced isomers of the aldehydes exist in natural samples. Additionally, the mass spectrum of derivatives are dominated by the C_6F_5-CH_2^+ ion, and a relatively weak molecular ion. For trace analysis using electron ionization, compound identification would have to be based on retention time. If available, negative chemical ionization may have yielded more molecular information and greater sensitivity as it is ideally suited to electron capture compounds, such as the PFBHA derivatives.

2.6 Ancillary Analytical Methods

2.6.1 Liquid Microextraction Procedure

In chemical oxidation studies at $mg \cdot L^{-1}$ concentrations, a micro liquid-liquid extraction procedure was employed, based on accepted procedures [24]. In this case a

small volume of sample (5-10 mL) was extracted by shaking with 2 mL of methyl-*tert*-butyl ether in a sealed culture tube. The extract was analyzed directly by gas chromatography-mass spectrometry. Samples were injected via a liquid autosampler (Hewlett-Packard 7673, Mississauga, ON). Chlorooctane was used as an internal standard. This was a convenient and rapid method for the analysis of samples in chemical oxidation and adsorption studies.

2.6.2 Physical Tests

Turbidity, free chlorine and TOC were measured as described in Standard Methods [23]. Turbidity was analyzed using a Hach 2100N turbidimeter (Loveland, CO) and results are expressed as Nephelometric Turbidity Units (NTU), free chlorine was measured with a Hach pocket colorimeter using N,N,-diethyl-*p*-phenylenediamine as an indicator. TOC was measured as non-purgeable organic carbon on a Shimadzu TOC-5000 (Mandel Scientific, Guelph, ON) using catalytic assisted combustion at 680 °C with infrared detection. Samples for DOC were filtered through a 0.45 μm polyvinyldifluoride syringe filter immediately prior to analysis.

2.6.3 Limnological Tests.

Samples for chlorophyll-*a* were filtered through Whatman GF/F filters under low vacuum (<200 kPa) and frozen. Filters were later ground, acetone extracted and measured for fluorescence on a Sequoia-Turner model 450 fluorometer, corrected for pheophytin (APHA 1991). Silica (Si) was determined from 125 mL subsamples by a UV-Visible spectrophotometric method. Samples were reacted with ammonium molybdate at pH 2.1,

and measured on a Hach DR/400 (Loveland, CO) spectrophotometer (APHA 1995). Total and dissolved phosphorous were determined from 150 mL samples by a standard persulfate-ammonium molybdate colorimetric technique, modified for analysis on a Technicon Autoanalyzer II (Pulse Instrumentation, Saskatoon SK).

2.6.4 Phytoplankton

Samples for phytoplankton enumeration and biomass analysis were preserved with Lugol's iodine and stored under low light. Biomass was estimated from the biovolume by the inverted microscope technique [28]. In this method, the average biomass is calculated from average species cell dimensions, with a density of 1.0 $g \cdot mL^{-1}$ assumed. Detailed taxonomic analysis of the phytoplankton was conducted on sizes larger than 5 μm. Algal taxa were identified and enumerated with a minimum count of 200 of the most abundant species from 5 or 10 mL settled aliquots.

2.6.5 Jar Test

2.6.5.1 Dissolved Air Flotation Jar Test

Aluminum sulphate solutions were prepared at a strength of 0.8% and 1.5%, respectively, such that each 1 mL addition provided 5 $mg \cdot L^{-1}$ aluminum sulphate. The DAF jar tester used volumes of 0.80 L, while the CGS jar tester used 1.50 L volumes. The DAF jar test unit (Aztec Environmental Control, Didcot UK) was operated with a rapid mix time of 2 minutes at 400 rpm, equivalent to a velocity gradient, G = 350 s^{-1}, based on the 0.80 L sample size. Aluminum sulphate coagulant was added by syringe 10-20 s into the rapid mix phase. At the end of the rapid mix, air saturated water was injected

at the base of each jar for 10 s at a rate 6 mL s^{-1} to give a recycle ratio of 7.5%. A flocculation period of 10 minutes followed, with a paddle speed of 100 rpm to achieve a velocity gradient of G = 75 s^{-1}. After a 10 minutes of quiescent flotation, samples were collected through a port located on the base of each jar [29].

2.6.5.2 Conventional Gravity Sedimentation Jar Test

Aluminum sulfate solutions were prepared as above. The CGS jar test (Magne-Drive model 73, Coffman Industries, Westford MA) was operated with a rapid mix of 2 minutes at 100 rpm (G = 215 s^{-1}) followed by a 13 minute flocculation at a paddle speed of 30 rpm (G = 35 s^{-1}). Aluminum sulphate coagulant was added by syringe 10-20 s into the rapid mix phase. Samples were collected after a 1 h period by carefully drawing the clarified layer of water off with a 50 mL syringe. The syringe was fitted with a 90° angle adapter to facilitate sample collection from the clarified surface layer .

2.7 Laboratory Quality Assurance Practices

2.7.1 Introduction

Standards of Good Laboratory Practice such as the maintenance and periodic assessment of equipment, instrumentation, consumable supplies were practiced. Results and experimental observations were recorded in bound notebooks or carefully maintained electronic formats (spreadsheet, instrument record files). Quality assurance and quality control procedures (analysis of method blanks, check samples) were adhered to and records of these measures were kept.

2.7.2 Analytical balance

Sartorius MC-1 electronic balance, accurate to 0.1 mg (VWR, Mississauga, ON). The balance was autozeroed and the performance checked against certified 0.200 g and 2.000 g masses. The results were recorded as part of the laboratory internal quality assurance procedures.

2.7.3 Refrigerators

Samples, reagents and standards were stored as appropriate at 4 °C in Sanyo Medicool laboratory coolers (Caltec Scientific, Edmonton, AB). The chamber temperature was recorded daily with NIST traceable thermometers (VWR Mississauga, ON).

2.7.4 Pipettes

Eppendorf adjustable pipettes (100, 1000 µL) and a Calibra macropipete (10 mL) were used for the volumetric delivery of reagents and solutions (VWR Mississauga, ON). Disposable polyethylene tips were used once only, and evaluated for contamination through assessment of procedure blanks. Accuracy was checked gravimetrically at low, mid and high ranges (i.e. 20, 50, 100%).

2.7.5 Syringes

For low volume delivery of under 50 µL, glass microliter syringes were used (Hamilton, Reno NV). Syringes were washed 10 times with reagent grade methanol before and after use.

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CHAPTER 3: BENCH SCALE EXPERIMENTS

3.1 Introduction

In order to both accurately measure and effectively treat the T/O compounds for drinking water treatment purposes, or for ecological investigations of cellular biology and chemistry, it is important to know how algae and algal-derived compounds respond to different processes. The effects of sample storage conditions, laboratory analyses and plant treatment processes on algae need to be characterized.

A series of bench scale experiments were conducted to better understand the chemistry of algal T/O processes. In one series of experiments, the chemical stability of the T/O compounds in the treatment process were evaluated, using synthetic chemical standards of the stable isomeric forms (E2,E4-heptadienal, E2,Z6-nonadienal, E2,E4-decadienal, E2,E4,Z7-decatrienal). In a more extensive series of experiments, mixed algal cultures were subjected to water treatment and laboratory processes. These included treatments with chlorine, alum coagulation, filtration and short term storage. In addition, the flavor profile and odor threshold concentration of the synthetic aldehydes were evaluated.

3.2 Chlorination Stability Experiments

3.2.1 Introduction

Chlorine is an oxidant and disinfectant commonly used by the drinking water treatment industry in treatment plants, with typical application rates of 1–5 $mg \cdot L^{-1}$. Chlorine is used in variety of forms, such as elemental chlorine (Cl_2), chlorine dioxide (ClO_2) and monchloramine (NH_2Cl). Elemental chlorine forms hypochlorous acid

(HOCl) when dissolved in water. Chlorine dioxide, a more powerful oxidant is often used, although chlorite by-product formation is a concern [1, 2]. Monochloramines, formed by the reaction of free chlorine with ammonia is commonly used in distribution systems as a secondary disinfectant. Although monochloramine is a weak oxidant, it is well suited to maintain a disinfectant residual in treated water since it is very stable and persistent. Non-chlorine based oxidants include ozone, hydrogen peroxide combined with ultraviolet irradiation and potassium permanganate. These non-chlorine oxidants are often applied as pretreatment processes to inactivate chlorine resistant pathogens (e.g. *Giardia* cysts, *Cryptosporidium* oocysts), treat dissolved organic carbon or color, or to control nuisance organisms such as zebra mussels.

There are several studies on the effect of chemical oxidation on T/O compounds in water supplies. These often focus on more common compounds such as geosmin and 2-MIB, pyrazines or chloroanisoles. The oxidation of five odor compounds including geosmin and 2-MIB was studied by Layezerly *et. al* [3]. They report that oxidation of geosmin and 2-MIB with chlorine, chlorine dioxide, ozone and potassium permanganate at reasonable water treatment application rates (1-10 $mg \cdot L^{-1}$) achieved only modest reductions of <20%. Even at much higher rates (10-100 $mg \cdot L^{-1}$) and long contact times (16-40 h), oxidative removal of these compounds was <60%. Burlingame [4] reports on the treatment of water from the Delaware River which contained up to 106 $ng \cdot L^{-1}$ of algal derived nonadienal. In treatment plants where chlorine dioxide or chloramine were used, the odor compound persisted through the treatment process. With 12 h of contact time, low (0.6 $mg \cdot L^{-1}$) applications of chlorine were ineffective while increased applications (2.4 $mg \cdot L^{-1}$) eliminated the offensive odor. It was not reported if the other treatments

were able to attenuate the concentration of nonadienal, or if they were entirely ineffective.

3.2.2 Experimental

In this study, the effect of free chlorine on the stability of odorous aldehydes was evaluated, as this is the practice at the Glenmore Water Treatment Plant [5]. Synthetic samples of T/O aldehydes were prepared in reagent water for stability experiments. One batch contained E2,Z6-nonadienal, E2,E4-heptadienal and E2,E4-decadienal at 1.0 $mg \cdot L^{-1}$ concentrations, prepared directly in reagent water from neat chemicals. A second series of samples containing E2,E4,Z7-decatrienal at 1.0 $mg \cdot L^{-1}$ were prepared from a dilute solution in acetone (1.0 $mg \cdot L^{-1}$). This was done to minimize unwanted interaction between chlorine and the acetone solvent. Samples were chlorinated with a $Ca(OCl)_2$ solution to provide a free chlorine residual of 1.6-1.8 $mg \cdot L^{-1}$. This concentration of the aldehydes at ca. 1000 times higher than would normally be encountered in natural surface waters was selected as it allowed for rapid analysis of the samples and was high enough to observe the treatment effects.

All samples were stored without headspace in 10x13 mm culture tubes with Teflon™ lined caps, at room temperature and protected from light. The sample volume in the culture tubes were adjusted from 9 to 7 mL by the volumetric removal of 2 mL. Samples were extracted with 1 x 1 mL methyl *tert*-butyl ether, with 1-chlorooctane added as an internal standard at 10 $\mu g \cdot L^{-1}$. The samples were extracted manually by shaking for 90 s. The organic phase was removed by Pasteur pipette and analyzed by GC-MS, with a scan range of 40 - 300 m/z.

For the first batch of aldehydes (heptadienal, nonadienal, decadienal), the oxidation reaction was monitored over a 24 h period. Unchlorinated samples were retained for analysis at 0 h and at 24 h to confirm stability in the absence of chlorine. All analyses were conducted in triplicate. In case of decatrienal, chlorine oxidation was monitored over a 24 h period with no sample headspace, and over 8 h with sample headspace. Due to the limited availability of this standard, triplicate analyses were not conducted on all samples.

3.2.3 Results and Discussion

Figure 3.1 compares the stability of the aldehydes in chlorinated water over a 24 h period while stored with zero headspace in culture tubes. The two treatments evaluated the stability of the aldehydes in water with and without chlorine at 1.6 - 1.8 $mg \cdot L^{-1}$. The concentration of conjugated aldehydes, heptadienal and decadienal in the presence of chlorine declined slightly from the initial concentration of 1.0 to 0.85 and 0.86 $mg \cdot L^{-1}$, respectively. By comparison, the unchlorinated samples had measured concentrations of 0.97 and 1.02 $mg \cdot L^{-1}$. The concentration of nonadienal declined monotonically by 82% from the initial value of 1.0 to 0.12 $mg \cdot L^{-1}$ after 24 h while the concentration of the unchlorinated sample remained largely unchanged.

The data for E2,E4,Z7-decatrienal is charted in Figure 3.2. Chlorinated and unchlorinated decatrienal was stored headspace-free and consequently protected from atmospheric oxygen. Over the 8 h monitoring period, decatrienal concentrations declined from an initial value of 1.0 to 0.92 $mg \cdot L^{-1}$ in the unchlorinated samples and to 0.77 $mg \cdot L^{-1}$ in the chlorinated sample. A second set of samples was stored uncapped and

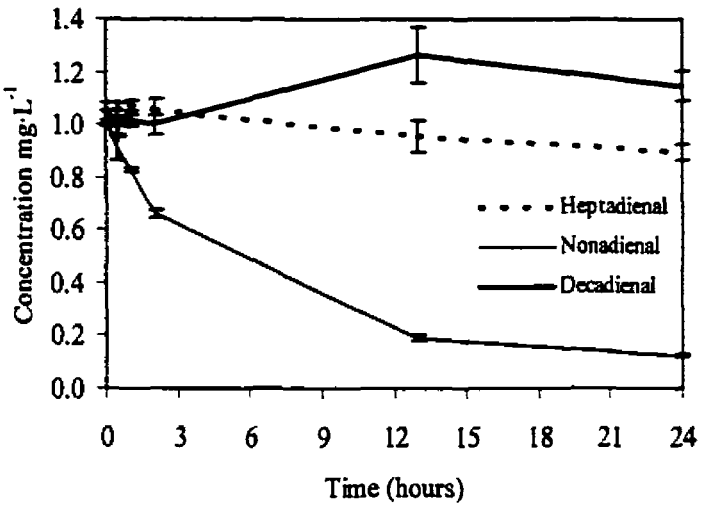

Figure 3.1 Effect of chlorine contact on aldehyde concentration, samples stored with headspace. The initial aldehyde concentration was 1.0 $mg \cdot L^{-1}$ and the applied chlorine treatment was 1.8 $mg \cdot L^{-1}$.

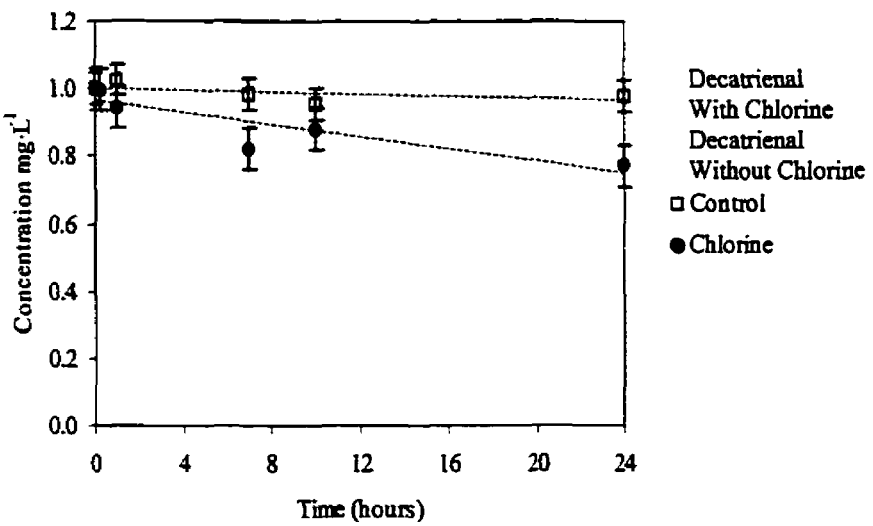

Figure 3.2 Effect of chlorine contact on decatrienal concentration, samples stored without headspace. The initial aldehyde concentration was 1.0 $mg \cdot L^{-1}$ and the applied chlorine treatment was 1.8 $mg \cdot L^{-1}$.

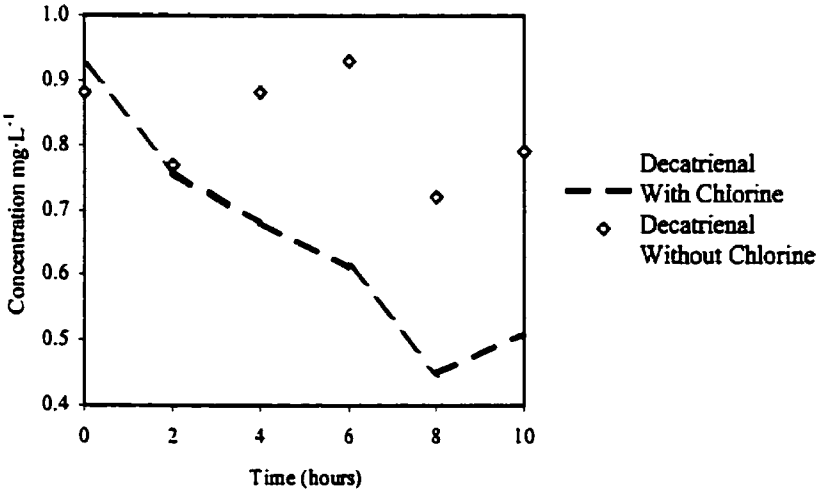

Figure 3.3 Effect of chlorine contact on decatrienal concentration, samples stored with headspace. The initial aldehyde concentration was 1.0 $mg \cdot L^{-1}$ and the applied chlorine treatment was 1.8 $mg \cdot L^{-1}$.

monitored at hourly intervals for 8 h (Figure 3.3). In this case the chlorinated samples decreased to 0.47 $mg \cdot L^{-1}$ while the unchlorinated sample contained 0.92 $mg \cdot L^{-1}$.

It is hypothesized that the conjugated aldehydes are more resistant to electrophilic attack by chlorine due to the carbonyl group which withdraws electron density from the carbon atoms, and renders this system significantly less nucleophillic. In hypochlorous acid, (HOCl) there is a formal positive charge on chlorine ($HO^- Cl^+$), making this an electrophilic species [2]. In the case of 2,6-nonadienal, the unconjugated C_6 double bond is susceptible to electrophilic attack by chlorine and is thus rapidly degraded. Similar losses were observed 2,4,7-decatrienal, which has the C_7 double bond out of conjugation.. The implication of these results is that some algal derived T/O compounds, such as 2,6-

nonadienal may be treated easily in a conventional treatment situation, whereas other compounds such as 2,4-decadienal and 2,4-heptadienal may be highly resistant to removal after they are introduced into the treatment plant.

3.3 Liquid – Solid Separation Processes

3.3.1 Introduction

Physical removal of particulate and colloidal material through coagulation is a standard water treatment process [1, 5]. Trivalent metal ions (Al^{3+} and Fe^{3+}) from stable formulations such as aluminum sulfate, polyaluminum chloride or ferric chloride typically are used to promote coagulation through charge destabilization of suspended and colloidal material present in the water. Historically, simple gravity sedimentation has been used to remove solids from water in the treatment stream. While this is efficient for dense, particulate material such as inorganic silts and clays, buoyant particles such as algal cells can be more difficult to remove. In these cases, dissolved air flotation (DAF) has been employed successfully [6, 7]. The initial metal ion assisted coagulation step in DAF is similar to the gravity sedimentation process. In the solid separation step, a stream of air saturated water is introduced into the process stream after coagulation. As the bubbles rise, they attach to particles, and they float to the surface. The dirty froth layer is removed by a mechanical skimmer, and the clarified water is drawn from the subsurface.

The potential advantage to DAF treatment for algal derived T/O situations is that it provides a means for the gentle removal of intact cells [6]. The efficiency of DAF treatment for algae should be enhanced as it works in concert with the natural buoyancy of the algae. The algal T/O compounds are derived from intracellular PUFA. Thus, if the

cells are removed intact, this provides a means to remove a potential source of T/O compounds. Studies on the effect of chemical coagulants on the viability of algae (*Aphanizomenon flos-aquae*) after treatment have shown minimal effect. Chlorine, which is applied often in concert with coagulants, was physiologically toxic and caused extensive cellular damage to the same species [8].

3.3.2 Experimental

Experiments were conducted on natural surface water samples drawn from the Glenmore Reservoir on January 2-5, 2000 during an under-ice bloom of *Dinobryon* and *Asterionella,* which are know producers of T/O compounds. An increase in the FPA value for the source water coincided with the algae bloom, which reached an annual maximum value of 9 (intense fishy) on December 29, 1999 compared to the annual average value of 3.7.

The efficacy of DAF and CGS were evaluated on bench scale jar test equipment. The treatments were compared in terms of turbidity, TOC and biomass reduction. In addition, the T/O composition of the untreated water was determined by HSPME. Additional experiments utilizing cultured algal material mixed with reservoir water were conducted. These cultures were primarily composed of *U. americana* and *D. cylindricum*, two common chrysophytes found in the Glenmore Reservoir, also known producers of T/O compounds. *U. americana* is linked with the production of heptadienal, while *Dinobryon spp.* produce decadienal and decatrienal in addition to heptadienal [9].

3.3.3 Results and Discussion

Phytoplankton removal was consistently more efficient by DAF than by CGS. DAF treatment at 10 $mg \cdot L^{-1}$ coagulant removed between 46.7 and 92.2% of the total algal biomass. At the same treatment level, gravity sedimentation removed 41.5%. Biomass removal by gravity sedimentation in the GWTP had similar performance at 47.7%. (Table 3.1). Figure 3.4 compares phytoplankton removals for DAF and CGS over the coagulant dose range of 0 – 20 $mg \cdot L^{-1}$. The reduction in algal biomass was effective at removing the fishy odour causing compounds. It is unlikely that significant concentrations of free aldehydes are stored in the cells as these compounds are cytotoxic [10]. Since the precursors of the odour causing aldehydes are intracellularly stored PUFAs, removal of the intact phytoplankton cells effectively removes the odour compounds. This was demonstrated by filtering untreated water samples through glass fibre filters and measuring both the aldehyde concentration and the biomass in the filtrate and on the filter media. Over 95% of the algal biomass was retained through gentle filtration under low vacuum, with small flagellates under 5 μm in size comprising 98% of the unretained biomass. This procedure was able to capture virtually all of the odor compounds, and no detectable concentrations of aldehydes were found in the filtrate. These data from a sample collected on February 1, 2000 are summarized in Table 3.2. Microscopic examination of samples taken after coagulant addition indicated that algal cells were not harmed and their membranes remained intact. In samples collected from the treatment plant following chlorination, significant disruption of the algal cells was noted.

Table 3.1. Biomass reductions in Glenmore Reservoir water (January 2-5, 2000) by DAF (Dissolved Air Flotation) and CGS (Conventional Gravity Sedimentation). Coagulant dose of 10 $mg \cdot L^{-1}$ aluminum sulphate applied in all cases. GWTP-Settled refers to samples from the full scale plant, CGS and DAF are jar test samples.

	Biomass $\mu g \cdot L^{-1}$			
	Total	**Chrysophyceae**	**Bacillariophyceae**	**Cryptophytes**
Untreated	500.1	347.3	0.0	152.8
GWTP - Settled	261.5	223.3	0.0	38.2
% reduction	**47.7**	**35.7**	-	**75.0**
Untreated	296.3	239.6	1.7	58.7
CGS-10	173.3	146.9	27.9	0.0
% reduction	**41.5**	**38.7**	-	**100.0**
Untreated	271.7	197.2	37.0	37.4
DAF-1	53.4	58.3	2.2	1.0
% reduction	**80.3**	**70.4**	**94.1**	**97.4**
Untreated	271.7	197.2	37.0	37.4
DAF-2	21.1	18.1	3.0	0.0
% reduction	**92.2**	**90.8**	**91.9**	**100.0**
Untreated	194.6	185.0	19.7	0.0
DAF-3	103.7	83.8	19.4	0.0
% reduction	**46.7**	**54.7**	**1.3**	-

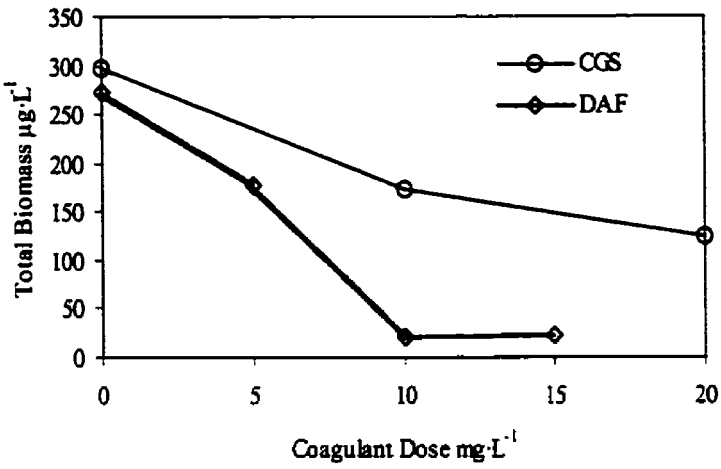

Figure 3.4 Comparison of total biomass reduction by DAF and CGS jar test treatments (alum coagulation).

Table 3.2 Comparison of dienal and biomass concentrations in raw water samples before and after filtration through 934-AH filter. Taxonomic assessment of the raw water was performed on a subsample (February 1, 2000).

	Aldehyde Concentration, µg·L^{-1}				Total Biomass µg·L^{-1}
	Heptadienal	**Nonadienal**	**Decadienal**	**Decatrienal**	
Raw	-	-	-	-	381.2
Raw (Filtered)	1.135	0.003	0.180	0.402	-
Filtrate	0.000	0.000	0.000	0.000	17.4

The DAF experiments with the cultured material were assessed in terms of aldehyde concentration and turbidity. The DAF treatment with a coagulant application was 20 mg/L aluminum sulfate reduced the water turbidity by 75.7% from 3.40 to 0.82

+/-0.17 (n=4) NTU. Taste and odor compounds were reduced significantly by this treatment. Heptadienal and decadienal were reduced from 1386 and 58 $ng \cdot L^{-1}$, respectively, to below detection. Decatrienal, the major T/O constituent by mass, was reduced by 83.3% from 10,477 to 1750 $ng \cdot L^{-1}$. These results are consistent with previous experiments where the reduction in algal biomass and turbidity resulted in decreased T/O concentrations (Table 3.3).

Table 3.3 Removal efficacy of DAF for algal VOC precursors and turbidity.

Alum		Turbidity NTU	Heptadienal	Decadienal	Decatrienal
			$ng \cdot L^{-1}$		
0 $mg \cdot L^{-1}$	Average (n=3)	3.4	1386	58	10,477
	Std Dev		393.1	14.1	1275
20 $mg \cdot L^{-1}$	Average (n=4)	0.825	0	0	1750
	Std Dev	0.173	0	0	587
% Reduction		75.7	100	100	83.3

3.4 Filter Concentration Experiments

3.4.1 Introduction

A series of related experiments with mixed culture assemblages was conducted to evaluate a filter concentration procedure. In the case of low algal density, the concentration of algal VOCs liberated is below the method detection limit of ca. 250 $ng \cdot L^{-1}$. As the aldehyde precursors are stored as intracellular fatty acids, a series of experiments was conducted to evaluate the efficacy of pre-concentrating algae on glass fiber filters. This would allow an efficient means to increase the sample volume without

otherwise altering the HSPME protocol. The goal was to develop a convenient sample preparation step that could be widely applied to lab and field settings.

3.4.2 Experimental

In this procedure, the algal biomass was concentrated via filtration of 500 – 1000 mL sample volumes using precombusted glass fiber filters (934-AH, Whatman Clifton, NJ; nominal pore size 1.8 μm) and low vacuum (< 140 kPa) and immediately sealed in a 43 mL sample vial. Samples were stored at –20 °C, or analyzed immediately. Prior to analysis, enzymatic action was initiated by the addition of 30 mL 20% w/v NaCl. Apart from the concentration step, all aspects of the HSPME protocol were the same. A mixed culture of two T/O causing chrysophytes, *Uroglena americana* and *Dinobryon cylindricum* was used as experimental material. The procedure was evaluated by comparing triplicate analyses of the stock culture by the standard protocol, freshly filtered 500 mL aliquots and 500 mL filtered aliquots stored overnight at –20 °C. Low temperature sample storage was evaluated as a potential means of sample preservation. The filtrate was also analyzed for leakage products. Triplicate analyses were performed, and results were normalized to 30 mL for comparative purposes.

3.4.3 Results And Discussion

The procedure was effective at concentrating algal samples, however there was significant under-recovery of the algal VOCs. The procedure was evaluated on two batches of samples with similar algal composition. For the first batch of freshly filtered samples, the average recovery was 44.4 and 32.7% for heptadienal and decatrienal,

respectively. After 24 h storage at –20 °C, the recovery for heptadienal decreased to 9.7%, and 3.4% for decatrienal. Decadienal was present at below the normal detection limit, and was only measured in the freshly filtered samples (Table 3.4). No leakage of heptadienal was found in the filtrate; however measurable amounts of decatrienal were found.

Table 3.4 Comparison of filter concentration and storage (-20 °C) on aldehyde stability and recovery, batch 1.

Treatment		Heptadienal	Decadienal	Decatrienal
		$ng \cdot L^{-1}$		
Vial 30 mL Stored 0 h	Average (n=3)	990.3	0.0	2287.0
	Std. Dev.	542.9	0.0	207.2
Filtrate 30 mL Stored 0 h	Average (n=3)	0.0	0.0	50.0
	Std. Dev.	0.0	0.0	17.1
Filtered[1] Stored 0 h	Average (n=3)	440.0	6.6	748.1
	Std. Dev.	40.9	2.2	187.4
Filtered[1] Stored 24 h Frozen	Average (n=3)	96.4	0.0	78.8
	Std. Dev.	55.2	0.0	65.8

[1] 500 mL sample normalized to 30 mL

In the second batch of freshly filtered samples, heptadienal, decadienal and decatrienal recoveries were 82.4, 30.4 and 8.7%, respectively (Table 3.5). Recovery from the samples frozen for 24 h were again lower, at 57.6% for heptadienal, 18.3% for

decadienal and 1.7% for decatrienal. The only compound present in the filtrate was decatrienal, at 33.1% of the concentration in the control sample

Better recovery was observed for heptadienal, which is produced primarily by *Uroglena americana*. Decatrienal production is associated with *Dinobryon divergens*, suggesting the differential recovery may be related to the algal taxa. While both these colonial algae are naked flagellates with fragile cell membranes, the *Uroglena* cells may be more resistant to the mechanical stress of filtration. In results from the DAF experiments, T/O compounds from both algae were removed efficiently by (>90% for decatrienal). Based on this, the observed leakage was due to mechanical stress, and that leakage of cellular constituents (PUFA) is not normal.

Table 3.5 Comparison of filter concentration and storage (-20 °C) on aldehyde stability and recovery, batch 2.

Treatment		Heptadienal	Decadienal	Decatrienal
		$ng \cdot L^{-1}$		
Vial 30 mL Stored 0 h	Average (n=3)	3902.3	69	6111.3
	Std. Dev.	1806.0	33.6	627.2
Filtrate 30 mL Stored 0 h	Average (n=3)	0.0	0.0	2026
	Std. Dev.	0.0	0.0	238.1
Filtered[1] Stored 0 h	Average (n=3)	3215.0	21.4	534.6
	Std. Dev.	1450.1	17.5	536.5
Filtered[1] Stored 24 h Frozen	Average (n=3)	2250.6	12.6	102.1
	Std. Dev.	1096.5	6.53	35.0

[1] 500 mL sample normalized to 30 mL

The performance data of the filtration procedure as presented is highly conservative, as it was evaluated on samples where the cell density was very high. This led to longer times (ca. 20 minutes vs. 1 min for surface water) to filter the 500 mL sample volume and higher associated differential pressures across the filter. Despite these unresolved issues, filter concentration was usefully employed on a number of occasions to identify algal VOCs in low density samples, and to estimate concentrations.

3.5 Effect of Sample Temperature

3.5.1 Introduction

Using similar algal composition and synthetic standard solutions, the effect of sample temperature was evaluated. The goal was to verify that exposure of the algal samples to elevated heat (65 °C) did not impair the enzymatic formation of the unsaturated aldehydes. The genesis of these aldehydes is through a complex series of biochemical reactions which involves the release of cellular constituents (PUFA, enzymes) and activation of the enzymes [11]. For laboratory analyses, this was achieved through the addition of NaCl. Heat could potentially affect the product distribution, rate and extent of aldehyde performance.

3.5.2 Experimental

A mixture of *Uroglena americana* and *Dinobryon cylindricum* cultures were prepared in a flask and temporarily stored at 4 °C in a glass fronted and illuminated refrigerator. The flask was swirled to mix, and fresh aliquots were taken for analysis. Analyses were conducted in triplicate on the algal culture at room temperature (22 °C)

and at 65 °C. None of the other sample analysis conditions was altered. Two calibration series covering the range of 500 – 10,000 $ng \cdot L^{-1}$ were prepared and analyzed, one at room temperature and one at 65 °C. The results were calculated based on the method of internal standard in all cases; however, it should be noted that sample temperature affects the recovery of the biphenyl-d_{10} internal standard. The average recovery of the internal standard at room temperature is 65% that of a heated sample. Since a calibration series was run at both temperatures, the effect of heat on aldehyde formation can be discriminated from the overall analytical results.

3.5.3 Results and Discussion

Standard samples analyzed at 65 °C had 2.1-2.3 times the recovery over the cold (22 °C) standards for heptadienal, nonadienal and decadienal (Table 3.6). Recovery of decatrienal increased by 4.2 times. For both the hot and cold calibration series, linear calibration curves were constructed, although the lower two levels for heptadienal and the lowest level for nonadienal were not found in the cold standards. For comparative purposes all sample results were calculated based on the hot (65 °C) standard series. The data are presented in Table 3.7. The algal mix samples were characterized prior to this experiment, and were known to produce abundant quantities of heptadienal and decatrienal in addition to small amounts of decadienal. Only decatrienal was detected in the algal mixture sample analyzed at 22 °C. When analyzed hot, all three aldehydes were found in the mixed culture samples. As the difference in recovery between hot and cold standards is approximately 2 times for heptadienal and 4 times for decatrienal, the effect of sample temperature on formation and recovery can be estimated. The average

concentration of decatrienal in hot sample was 2310 and 456 ng·L^{-1} for the cold sample. This result is close to what is expected in the absence of any influence on formation. By contrast, heptadienal was detected but not quantified in the cold sample. The concentration in the heated samples was 962 ng·L^{-1}. The expected concentration in the cold sample would be ca. 450 ng·L^{-1}, which is well within the performance capabilities of the analytical method. This suggests that temperature influences the formation or recovery of heptadienal, but not decatrienal.

Table 3.6 Effect of sample temperature (20 and 65 °C) on algal VOC recovery. Samples were prepared in reagent water from stable chemical standards.

Temp. (°C)	Cal. Level (ng·L^{-1})	Heptadienal	Nonadienal	Decadienal	Decatrienal	Biphenyl-d_{10}
		Detector Response				
25	0.5	0	0	55	85	354560
	1	0	104	219	174	387294
	2	388	634	774	473	393845
	5	3116	2653	2449	1488	202222
	10	4448	4596	4179	2411	255117
65	0.5	525	291	374	463	277662
	1	774	863	925	905	622078
	2	1801	2177	2174	2184	704346
	5	5891	5477	5206	5536	433538
	10	9683	9729	9717	10197	402629

Table 3.7 Effect of sample temperature (20 and 65 °C) on algal VOC recovery. Samples were prepared from a mixed assemblage of *D. cylindricum and U. americana.*

Temperature (°C)	Heptadienal	Decadienal	Decatrienal
		$ng \cdot L^{-1}$	
20	0.0	0.0	425
	0.0	0.0	487
	0.0	0.0	455
Average	0.0	0.0	455.7
Std dev	0.0	0.0	31.0
65	878	0.0	2375
	1027	0.0	2335
	430	0.0	2060
	1514	0.0	2466
Average	962.3	0.0	2309.0
Std dev	446.8	0.0	174.8

3.6 Water Treatment Process Effects

3.6.1 Introduction

One of the goals of this research was to study the effect of conventional water treatment processes on the algal derived aldehydes. The primary unit processes of in-plant storage, chlorine addition, aluminum sulfate coagulation were evaluated. A modified algal mixture comprised of *Asterionella formosa*, *Mallomonas* and *Synura* in addition to *Uroglena americana* and *Dinobryon cylindricum* was used. Based on earlier sample stability investigations, it was determined the sample preparation and storage time needed to be carefully controlled in order to minimize uncontrolled variability.

3.6.2 Experimental

Each replicate was prepared and stored separately in 100 mL volumetric flasks. Treatment chemicals (Chlorine, NaCl, Alum) were added to the partially (70-85%) filled flask and mixed. The flask was filled to the top (headspace free) and capped with a teflon™ stopper. The stopper was taped in place, and the vial was stored at 4 °C. Immediately prior to analysis a 30 mL sample was transferred to a 43 mL sample vial, and processed as per the established procedure (addition of 6g NaCl, stir bar, internal standard, capped, HSPME for 30 minutes at 65 °C). Aliquots for Lugol's, DOC and residual chlorine were collected as needed.

To minimize storage artifacts, samples were collected immediately prior to treatment or analysis, after first gently mixing algal culture. The sample collection and treatment was scheduled based on a 32 minute sample analysis cycle. The time sequence devised to evaluate in-plant treatment effects (chlorine, alum, in-plant storage) was based on a 5 h contact time, similar to the residence time in the full scale WTP. The chemical applications (chlorine, alum) selected were based on reasonable treatment practices for the GWTP. The chlorine application rate was difficult to establish due to the high organic content and chlorine demand of the culture. Typical DOC concentrations in the mixed cultures ranged from 21.6-37.5 $mg \cdot L^{-1}$, with a chlorine demand (30 minute contact) of ~ 10 mg/L. Typical DOC values for the Glenmore Reservoir are 1-3 $mg \cdot L^{-1}$, with a 24 h chlorine demand of 0.5-2 $mg \cdot L^{-1}$. Additional experiments were conducted at higher chlorine applications of 9.6 and 22.8 $mg \cdot L^{-1}$.

The sample storage and exposure times were carefully scheduled to ensure each sample was treated in a consistent and systematic fashion. The schedule is detailed in

Table 3.8. Odor production in the constituent cultures was assessed qualitatively by microscopic inspection of algal composition, and by osmic analysis after adehyde formation was induced. The volume of mixed culture was estimated, and the cultures were mixed in a single container. An aliquot was analyzed by HSPME immediately. After verification that the composition and concentration was satisfactory, the samples and treatments were prepared. The samples were decanted from the mixed algal culture every 30 minutes, rather than a batch preparation of the samples to meet the requirements of constant storage time. Two sets of 5 h storage experiments were prepared first, ahead of the "0 h – no treatment" samples. Solutions, sample containers and other reagents were set up ahead of time to minimize the analytical turn around time, and to help keep the schedule. Where necessary, synchronization intervals were inserted to correct for any lag in the schedule. An additional sample treatment of storage with NaCl was included as part of the storage investigation experiments.

3.6.3 Results

The results are summarized in Table 3.9 The short term storage conditions (4 °C, no light, headspace free) changed the concentration of VOCs measured. The variation in results was within the precision of analysis, and it was determined that the storage conditions did not influence the treatment results for the odorous aldehydes. In contrast to the aldehydes, the two octatriene isomers from *Asterionella formosa* showed a significant increase in their relative concentration of 5.2 times the control series. The potential of *A. formosa* to influence negatively or positively aldehyde concentrations, either by

Table 3.8 Sample preparation and analysis schedule, 14 July, 2000.

Time	Sample Preparation and SPME	GC-MS analysis
6:00	Prepare algal mixture	
6:30	Initial mixture analysis	
7:00	Prepare sample aliquots	
7:30		
8:00	**No Treatment, 5 h Storage**	
	Replicate 1	
8:30	Replicate 2	
9:00	Replicate 3	
9:30	**Chlorine 1.8 mg·L^{-1}, 5 h Storage**	
	Replicate 1	
10:00	Replicate 2	
10:30	Replicate 3	
11:00	**No Treatment, 0 h Storage**	**No Treatment, 0 h Storage**
	Replicate 1	Replicate 1
11:30	Replicate 2	Replicate 2
12:00	Replicate 3	Replicate 3
12:30	Synchronize time	Synchronize time
13:00	**Alum 16 mg·L^{-1}, 5 h Storage**	**No Treatment, 5 h Storage**
	Replicate 1	Replicate 1
13:30	Replicate 2	Replicate 2
14:00	Replicate 3	Replicate 3
14:30	**Alum 16 mg·L^{-1}, Chlorine 1.8 mg·L^{-1}, 5 h storage**	**Chlorine 1.8 mg·L^{-1}, 5 h Storage**
	Replicate 1	Replicate 1
15:00	Replicate 2	Replicate 2
15:30	Replicate 3	Replicate 3
16:00	**1.5 g NaCl, 5 h Storage**	
	Replicate 1	
16:30	Replicate 2	
17:00	Replicate 3	
17:30		
18:00		**Alum 16 mg·L^{-1}, 5 H Storage**
		Replicate 1
18:30		Replicate 2
19:00		Replicate 3
19:30		**Alum 16 mg·L^{-1}, Chlorine 1.8 mg·L^{-1}, 5 H storage**
		Replicate 1
20:00		Replicate 2
20:30		Replicate 3
21:00		**1.5 g NaCl, 5 H Storage**
		Replicate 1
21:30		Replicate 2
22:00		Replicate 3
22:30		
23:00		End of experiment

competitive oxidation of PUFA precursors to octatriene, or by enhancement of aldehyde concentration was studied. Analysis of algal monocultures indicated that octatriene was produced only by *A. formosa*, and that it did not produce any of the unsaturated aldehydes. The increase in PUFA oxidation products from *A. formosa* with time may be due to the slower release of cellular contents from the protective frustule.

Table 3.9 Effect of water treatment processes (storage, low chlorine, alum) on algal VOC recovery.

Treatment		Octatriene	Heptadienal	Decadienal	Decatrienal
		$ng \cdot L^{-1}$			
No Treatment Stored 0 h	Average (n=3)	0.80	3601	93	15700
	Std. Dev.	0.10	1823	80.7	2198
No Treatment Stored 5 h	Average (n=3)	4.10	1302	8	4249
	Std. Dev.	0.10	407	2.8	860
Chlorine 1.8 $mg \cdot L^{-1}$ Stored 5 h	Average (n=3)	0.70	2371	49	12345
	Std. Dev.	0.36	495	4.0	2765
Alum 16 $mg \cdot L^{-1}$ Stored 5 h	Average (n=3)	11.30	28888	467	16828
	Std. Dev.	2.21	3246	168	1698
Chlorine 1.8 $mg \cdot L^{-1}$ Alum 16 $mg \cdot L^{-1}$ Stored 5 h	Average (n=3)	5.37	7558	108	8856
	Std. Dev.	2.56	3635	25	1261

3.6.4 Discussion

3.6.4.1 Alum Coagulation

Addition of alum at treatment plant dosages (16 $mg \cdot L^{-1}$) had the unexpected effect of enhancing recovery of heptadienal eight-fold, and decadienal by six-fold over that of the control sample analyzed fresh (Figure 3.5). The measured concentrations of decatrienal in this treatment were not significantly different from the control series. This is notable, as both decadienal and decatrienal are both derived from *Dinobryon spp.* This level of alum addition was sufficient to precipitate particulate and colloidal material from solution. Unlike the jar test experiments, no biomass is removed from the sample container, and there is no loss of mass on treatment. The sample flasks were gently agitated prior to the collection aliquots. Previous studies have shown that these levels of coagulant have no deleterious effect on algae, such as lysis induced by osmotic shock. Samples collected for microscopic analysis did not show signs of damage. Two processes which affect VOC recovery are the efficiency of PUFA oxidation and competitive adsorption from the organic matrix. If enzyme activity were the primary cause, VOC recovery would be a function of storage time rather than treatment. While enzyme effects cannot be ruled out, it is more likely that recovery improved due to the removal of extracellular detritus through coagulation which reduced matrix effects. Competitive adsorption by the matrix is known to affect the recovery of analytes, especially when the extraction is an equilibrium, rather than an exhaustive process [12-14].

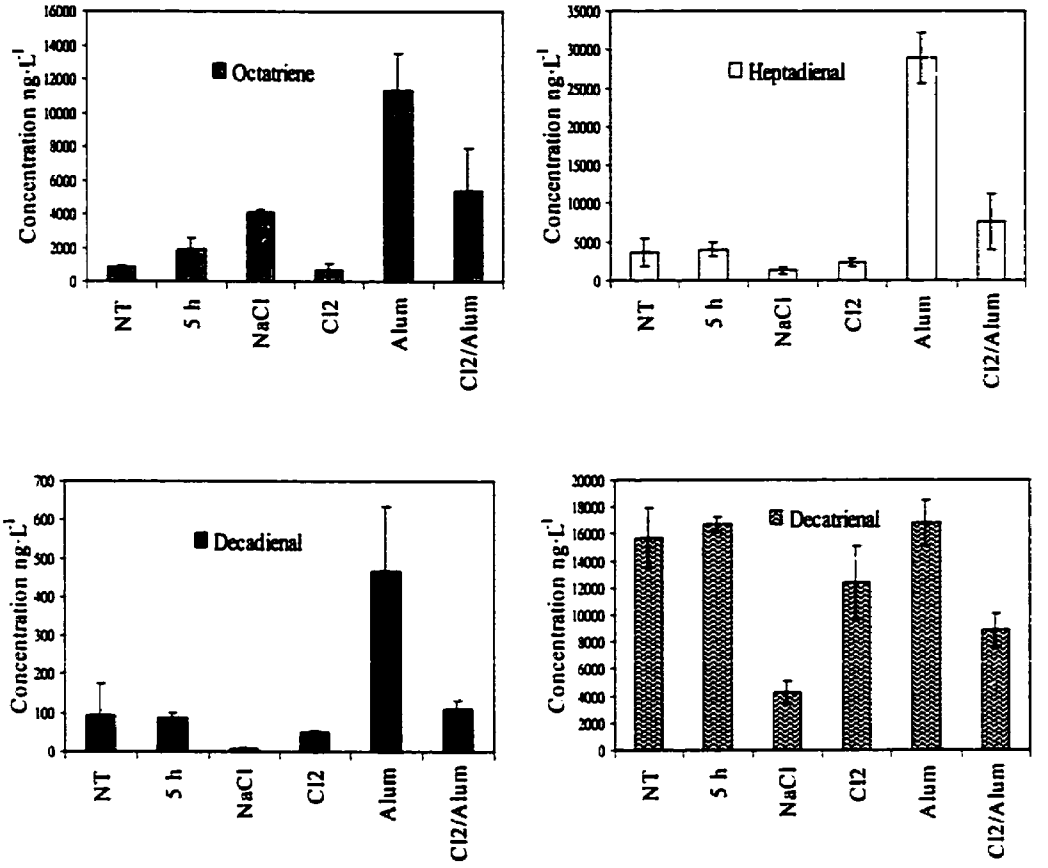

Figure 3.5 Effect of water treatment processes on algal VOC recovery for n=3 replicates. The treatments are: NT (control series, no treatment), 5h (5 hours storage), NaCl (5% w/v NaCl and 5 h storage), Cl2 (1.8 mg·L^{-1} Cl_2 and 5 h storage), Alum (16 mg·L^{-1} Alum and 5 h storage), Cl2/Alum (1.8 and 16 mg·L^{-1} Cl_2 and Alum, respectively and 5 h storage). Results shown for (A) octatriene, (B) heptadienal, (C) decadienal and (D) decatrienal. Note that y-axis scales differ.

Octatriene, which is produced by *Asterionella formosa* [15], also had a dramatic increase in concentration under this treatment regime. The measured values were 13-15 times that found in the control samples. Formation and recovery of this compound was enhanced by both increased storage time and by coagulation of organic matrix constituents.

3.6.4.2 Low Chlorine Treatment

Chlorine was added to the algal mixtures at a low concentration (1.8 $mg \cdot L^{-1}$), which was quickly consumed in the high DOC matrix (28 $mg \cdot L^{-1}$). A negative effect was observed for all of the algal derived compounds, ranging from a decrease of 12.5% for octatriene to 21.4, 34.2 and 47.3% for decatrienal, heptadienal and decadienal, respectively. The degree of attenuation was commensurate with the small amount of chlorine added relative to the organic carbon content. Statistical analysis gave a $P > f = 0.0379$ for the chlorine treatment, which is a significant effect.

3.6.4.3 Low Chlorine and Alum Treatment

The same mixed assemblage of algae was challenged with chlorine and alum at the same levels used above (1.8 and 16 $mg \cdot L^{-1}$, respectively). The chlorine solution was added first, followed by the culture and then the alum. This was done to ensure good mixing of the chlorine prior to alum assisted coagulation. At the treatment level of 1.8 $mg \cdot L^{-1}$ free chlorine, no residual chlorine was measured after 2 minutes, and no chemical oxidation occurred after this time. The concentration of decatrienal was reduced by 44% from 15,700 to 8856 $ng \cdot L^{-1}$, similar to the results obtained for the chlorine-alone

treatment. By contrast, heptadienal increased from 3601 to 7558 ng·L^{-1}. This is consistent with the alum-alone treatment, where there was a dramatic increase in the concentration of heptadienal. The two treatments had opposite influences on VOC production, with the increase from alum addition being tempered by the negative effect of chlorine addition. The measured concentrations had a similar product distribution as for the alum-only treatment. The octatriene concentration was reduced by 47% over the alum-only treatment. Heptadienal, decadienal and decatrienal were reduced by 26.2, 23.0% and 52.3%, respectively.

3.7 High Chlorine

3.7.1 Introduction

A companion experiment was conducted on a similar algal assemblage at a higher chlorine treatment of 24.5 mg·L^{-1}. In addition to the baseline samples, the treatments of storage time (5 h), chlorine addition with storage time (24.5 mg·L^{-1}, 5 h) and chlorine addition, alum coagulation and storage time (24.5 and 16 mg·L^{-1}, 5h), using the same experimental protocol as above.

3.7.2 Results and Discussion

The applied chlorine was rapidly consumed, with 6.0, 0.8 and 0.02 mg·L^{-1} present after 10, 30 and 60 minutes, respectively. The experimental results were consistent with the previous observations (Table 3.10, Figure 3.6). Short term storage of samples had no significant effect on measured aldehyde concentrations, although decatrienal had a higher average concentration of 24,025 vs. 19,092 ng·L^{-1}. As before, the octatriene derived from

Asterionella increased 41.7 %. Chlorine addition at 24.5 $mg \cdot L^{-1}$ reduced the levels of all compounds by 100-98%. Three unsaturated aldehydes were detected at trace levels (20-345 $ng \cdot L^{-1}$) while nonadienal and octatriene were not present following chlorination.

With the combined addition of coagulant and chlorine, all compounds were detected with the exception of nonadienal. Relative to the control samples, the measured concentration of octatriene, heptadienal, decadienal and decatrienal were 8.3, 5.0, 13.2 and 55.6% that of the control samples.

Table 3.10 Effect of treatment processes on (storage, high chlorine, alum) on algal VOC recovery.

Treatment		Octa-triene	Hepta-dienal	Nona-dienal	Deca-dienal	Deca-trienal
				$ng \cdot L^{-1}$		
No Treatment Stored 0 h	Average (n=3)	2.40	16651	766.7	384.3	19092
	Std. Dev.	0.53	3644	153.8	198.8	4041
No Treatment Stored 5 h	Average (n=3)	3.33	14944	1010.0	388.7	24025
	Std. Dev.	0.61	1254	41.0	46.2	1101
Chlorine 24.5 $mg \cdot L^{-1}$ Stored 5 h	Average (n=3)	0.0	310.0	0.0	19.3	345
	Std. Dev.	0.0	82.9	0.0	6.8	106
Chlorine 24.5 $mg \cdot L^{-1}$ Alum 16 $mg \cdot L^{-1}$ Stored 5 h	Average (n=2)	0.55	833.0	0.0	50.5	10688
	Std. Dev.	0.21	500.6	0.0	13.4	1636

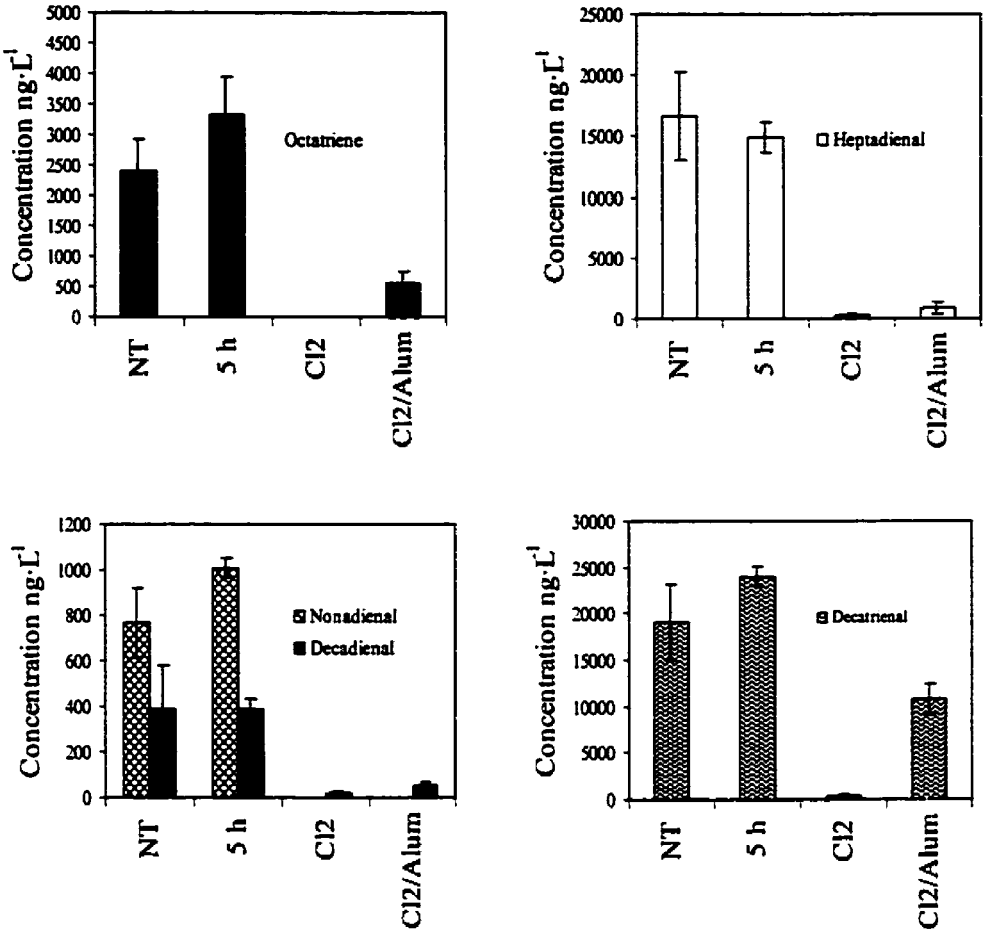

Figure 3.6 Effect of water treatment processes on algal VOC recovery for n=3 replicates. The treatments are: NT (control series, no treatment), 5 h (5 hours storage), Cl2 (24.5 mg·L^{-1} Cl_2 and 5 h storage), Alum (16 mg·L^{-1} Alum and 5 h storage), Cl2/Alum (24.5 and 16 mg·L^{-1} Cl_2 and Alum, respectively and 5 h storage). Results shown for (A) octatriene, (B) heptadienal, (C) nonadienal and decadienal and (D) decatrienal.

3.8 Time Series

3.8.1 Introduction

The effect of chlorine addition alone was evaluated at two treatment levels of 9.6 and 24.5 $mg \cdot L^{-1}$ chlorine. A similar mixed algal culture was used in this experiment, but the design was altered to time series, with single measurements for VOC and chlorine residual taken every 30 minutes. The goal was to maintain a chlorine residual through at least part of the contact time, to observe changes with time.

3.8.2 Results and Discussion (9.6 $mg \cdot L^{-1}$ chlorine).

The experimental results are presented in Table 3.11. At the 9.6 $mg \cdot L^{-1}$ treatment level the chlorine residual declined to 1.3 and 0 $mg \cdot L^{-1}$ of chlorine after 5 and 10 minutes, respectively (Figure 3.7). Four samples were analyzed for VOCs at 30 minute intervals, which is the minimum analytical cycle time. As all the chlorine was consumed within the first 30 minute interval, virtually all the change in VOC concentration occurred during that time. All measured compounds were reduced by ca. 90%, with the exception of decatrienal which was reduced to ca. 34%. This result is interesting as the previous bench scale experiments indicate the aldedhydes with unconjugated double bonds are most susceptible to chlorine oxidation. These previous experiments were conducted with a high ratio of synthetic substrate to chlorine (3:1.8 VOC:Cl_2), whereas this experiment had a low substrate to chlorine ratio (0.03:9.6 VOC:Cl_2) in the presence of a high organic matrix. In addition, decatrienal was the most abundant VOC with a measured concentration of 25,322 $ng \cdot L^{-1}$, compared to 13,850 $ng \cdot L^{-1}$ for heptadienal.

Table 3.11 Chorine oxidation time series, 9.6 mg·L^{-1}.

Time (min)	Chlorine mg·L^{-1}	Octatriene	Heptadienal	Decadienal	Decatrienal
		ng·L^{-1}			
0	9.6	4.5	13850	485	25322
2	1.3				
30	0.0	0.2	870	11	17152
60	0.0	0.2	776	11	17821
90	0.0	0.2	1216	18	16590

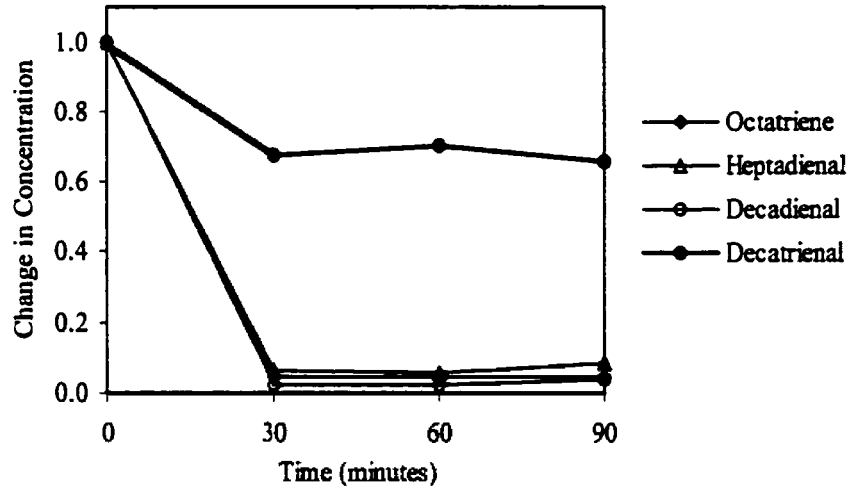

Figure 3.7 Relative change in algal VOC concentration with time. Chlorine applied at 9.6 mg·L^{-1}.

3.8.3 Results and Discussion (22.8 mg·L^{-1} chlorine)

A second time series was run under similar conditions, modified by the addition of *Synura*, which produces 2,6-nonadienal. Samples for VOCs were collected and analyzed every 30 minutes for 3 hours, and periodic chlorine measurements were taken. The initial chlorine application of 22.8 mg·L^{-1} was sufficient to leave a residual of 12 mg·L^{-1} after 3 hours. In this experiment all the algal VOCs were reduced to < 10% within

the first 30 minute interval (Table 3.12, Figure 3.8). Despite the continuous high chlorine residual and contact time, complete oxidation of the algal VOCs was achieved only for octatriene and 2,6-nonadienal. The other compounds (heptadienal, decadienal, decatrienal) were detectable throughout the time series at a concentrations of 200-500 $ng \cdot L^{-1}$.

Table 3.12 Relative lose by chorine oxidation time series, 22.8 $mg \cdot L^{-1}$.

Time (min)	Chlorine $mg \cdot L^{-1}$	Octatriene	Hepta-dienal	Nonadienal	Decadienal	Decatrienal
0	22.8	1.00	1.00	1.00	1.00	1.00
10	19.2					
20	17.2					
30	15.6	0.0	0.02	0.00	0.11	0.03
60	14.6	0.0	0.07	0.00	0.20	0.04
90	13.0	0.0	0.03	0.00	0.14	0.03
120	11.2	0.0	0.02	0.00	0.04	0.02
150		0.0	0.04	0.00	0.08	0.03
180	11.2	0.0	0.04	0.00	0.10	0.05

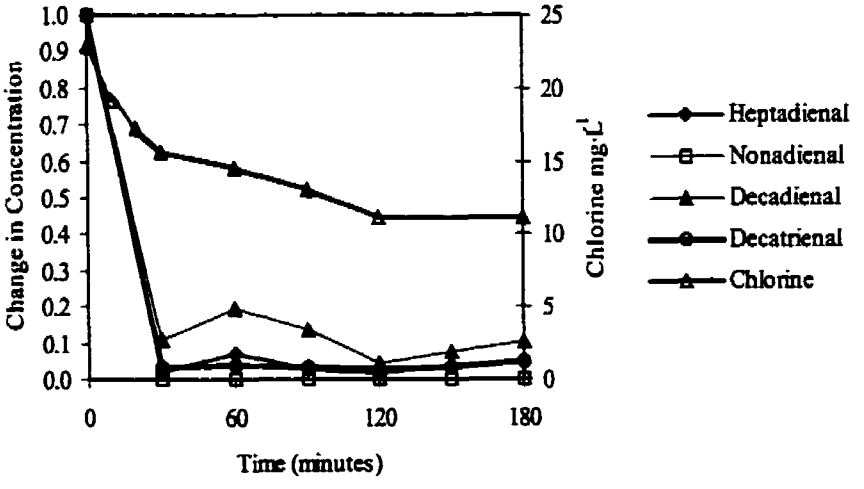

Figure 3.8 Relative change in algal VOC concentration with time. Chlorine applied at 22.8 $mg \cdot L^{-1}$.

3.8.4 Chlorination By-Products

In all the experiments where high chlorine applications were used, the formation of halogenated disinfection by-products was concomitant with the oxidation of algal VOCs. The GC-MS operational parameters allowed the identification of dibromochloromethane and bromoform, while the lighter haloforms (chloroform and bromodichloromethane) eluted during the mass spectrometer solvent delay stage. The formation of brominated haloforms is consistent with the use of NaCl in the analysis, where chlorine oxidizes bromide impurities in the salt, which results in the formation of brominated haloforms [16]. As these chlorinated by-products were not quantified, a mass balance estimate was not possible. The formation of chlorinated by-products with known health impacts [1, 16, 17] as a result of chlorination precludes the use of high chlorine applications solely for T/O control.

3.9 Sensory Assessment Methods

3.9.1 Introduction

Two standardized tests for the sensory assessment of the water quality were used in this study. These organoleptic assessments were Flavour Profile Analysis (FPA) and Odor Threshold Concentration (OTC) [17]. In the case of FPA, a panel of trained analysts evaluate the odor characteristics by sniffing a flask of the water sample. The water is warmed to a standard temperature of 25 °C, and the flask is swirled to equilibrate the dissolved organic compounds with the headspace. Both the odor quality and the intensity is evaluated by the panel. The intensity is rated on a scale of absent (0), perceptible (1-4), moderate (5-8) or intense (9-12).

For the GWTP source water the typically odor descriptors are fishy and musty, while chlorinous is the most common descriptor for finished water. Source water odor intensity is typically below 4 during the periods of cold weather, and increases to 4-8 through the spring and summer (Figure 3.9). In 1999, however, the opposite was true and the maximum recorded FPA value of 9 was on December 29. This intensely fishy odor was directly attributable to an under ice-bloom of *Dinobryon divergens*. The FPA test is conducted weekly on source and finished water at the GWTP as part of a long term water quality monitoring program at Calgary Waterworks.

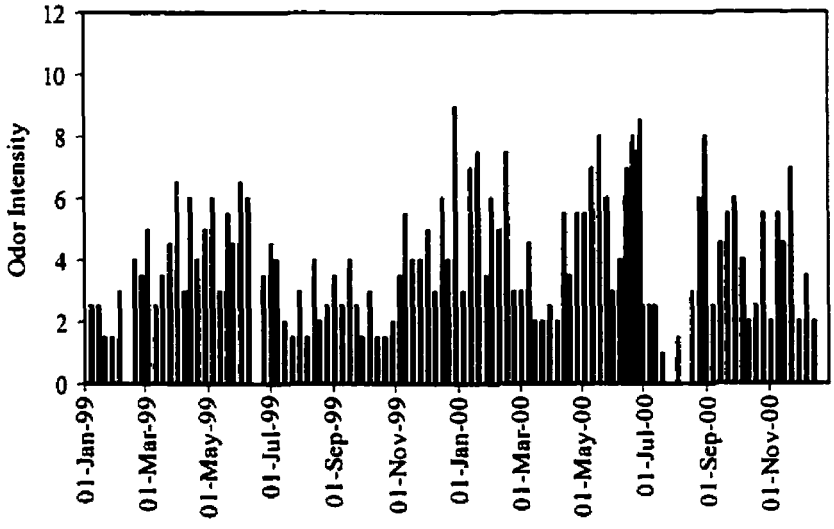

Figure 3.9 Flavor Profile Assessment data for untreated Glenmore Reservoir water, January 1999 – December 2000.

The Odor Threshold Concentration (OTC) procedure was used to estimate the odor potency of known T/O compounds. This is an important factor in assessing the impact of an odor constituent as both the concentration and the potency will determine

the overall impact. OTC studies are conducted commonly in the food and beverage industry. It can be difficult to compare OTC data from different studies as the test conditions can vary considerably in terms of matrix (water, oil) and temperature (ambient to 45 °C or above). Literature values were used where possible; however, these were not available for heptadienal, decadienal and decatrienal. As chemical standards of the biologically formed E,Z and E,Z,Z isomers were not available, the synthetic E,E and E,E,Z isomers were used. It was recognized that the chemical isomers may have different OTC and odor characteristics than the biologically formed compounds. A chemical standard of E2,Z6-nonadienal was available, and it was also included in the OTC assessment.

To prevent sensory impairment, the four compounds were evaluated in two different sets, starting with the least potent odor compounds (heptadienal, decadienal) and progressing to the more potent (decatrienal, nonadienal).

3.9.2 Experimental

Individual solutions of the four aldehydes were prepared at 1 $mg \cdot mL^{-1}$ concentrations by the volumetric addition of ca. 12.5 µL pure standard into 10.0 mL of methanol. The exact volume of chemical added was adjusted based on density. An intermediate dilution of 1.25 $\mu g \cdot mL^{-1}$ was volumetrically prepared in reagent grade water from the above solution. From this, a series of test solutions was prepared from the intermediate dilution to cover the desired range of concentrations. The 250 mL test solutions were prepared in deodorized 500 mL glass stoppered Erlenmeyer flasks. The flasks were identified only by code to prevent bias. OTC panelists were selected from the

trained pool of FPA evaluators, and asked to determine both the odor character and the flask where the odor was first perceptible. The conditions used by the OTC panel were identical to those used for FPA.

Experienced panelists from the FPA pool were selected for participation on the OTC assessment. The range of concentrations selected was based on literature reports or preliminary screening sessions.

Table 3.13 Summary of Odor Threshold Concentration experimental conditions. Biological odor character included for comparison.

Compound	Isomer	Concentrations Assessed $\mu g \cdot L^{-1}$	Odor Character
2,4-Heptadienal	E,E	5, 10, 25,40, 60	Watermelon stale
	E,Z		Fishy-Rancid
2,4-Decadienal	E,E	0.1, 0.5, 1, 5	Cucumber
	E,Z		Fishy-Rancid
2,4,7-Decatrienal	E,E,Z	0.1, 0.5, 1, 5	Cucumber
	E,Z,Z		Fishy-Rancid
2,6-Nonadienal	E,Z	0.05, 0.10, 0.15,0.25	Cucumber

3.9.3 Results and Discussion

The synthetic chemical standards of E,E and E,E,Z isomers did not have the characteristic fishy-rancid odors associated with the biologically formed E,Z, E,Z,Z isomers, and they appeared to have higher OTC than the biological species (Table 3.14). Odor sensitivity to different stereoisomers and odor character at different concentrations has been reported in the literature [18].

In addition to the OTC analysis, indirect assessment of the natural (EZ) isomers was conducted of algal monocultures known to produce specific aldehydes (heptadienal

from *Uroglena americana*, decatrienal from *Dinobryon spp.*). From the chemical analysis of algal cultures and natural samples in which fishy odors were just perceptible, the OTC for EZ and EZZ is conservatively estimated at 0.1-0.2 $\mu g \cdot L^{-1}$.

Based on OTC data, qualitative analysis and literature reports, the C_{10} aldehydes are the most important contributors by mass to fishy-rancid odors in surface water. While heptadienal can impart a similar odor, much higher concentrations are needed to have an impact.

Table 3.14 Summary of Odor Threshold Concentration analysis.

Sample	Odor Descriptor	OTC $\mu g \cdot L^{-1}$ (this study)	OTC $\mu g \cdot L^{-1}$ (literature)
Blank	None		
2,4-Heptadienal	Stale, Watermelon at higher concentrations	25	
2,4-Decadienal	Cucumber Yeasty-doughy	0.1	
2,4,7-Decatrienal	Cucumber-grassy	1.0	
2,6-Nonadienal	Cucumber-Clean	0.05	0.05
Geosmin	Earthy-musty		0.004-0.042
2-MIB	Earthy-musty		0.010-0.040

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CHAPTER 4: FIELD SURVEYS

4.1 Introduction

The analytical procedures developed were applied to several surveys of natural and synthetic systems. These include an under-ice bloom of *Dinobryon divergens*, followed by an open water bloom of *Uroglena americana* in the Glenmore Reservoir. Periphyton samples were examined from a survey of Alberta streams, and the St. Lawrence River. A similar survey for the soil bacteria *Actinomycetes* from littoral and river sources was conducted. Finally, samples of blue-green algae from Pine Lake, Alberta were analyzed in support of a nuisance algae investigation.

4.2 Under-Ice *Dinobryon* Bloom.

4.2.1 Introduction

In winter, 1999 mild weather conditions kept the reservoir (partially) ice free until December 24th and free of snow cover until January 3rd. In addition to sunlight, other resources such as TOC and bacterial levels were elevated compared to previous years (Table 4.1). TOC ranged up to 1.34 $mg \cdot L^{-1}$ compared to the long term under-ice average of 0.8 $mg \cdot L^{-1}$. Similarly the bacterial population, as measured by heterotrophic plate count, increased through the fall, with a maximum of 1970 colony forming units per mL ($cfu \cdot mL^{-1}$) on December 1, 1999. Bacterial levels declined through the remainder of the year, to an average of 456 $cfu \cdot mL^{-1}$. Other source water nutrient data as presented in Table 4.1.

Through the period of ice cover, sampling of the reservoir was conducted from the dam screen house, which provides access to the water column in front of the dam,

ahead of the raw water intakes. Samples were collected with a Kemmerer apparatus at 1, 2 and 6 m intervals. Analyses for silica, chlorophyll *a*, TOC, total and dissolved phosphorous and taxonomic examination were conducted. Nutrient analyses were performed in accordance with Standard Methods [1].

4.2.2 Results and Discussion

Through this period, both the algal biomass and FPA increased. The annual maximum FPA of 9 was recorded on Dec 29, 1999, compared to the annual average value of 3.6 (Figure 3.9). The source water odor was characterized as intensely fishy. The phytoplankton biomass developed through December until mid-January, and increased from average winter concentration of 240 to over 1000 $\mu g \cdot L^{-1}$. *Dinobryon divergens*, along with the diatom *Asterionella formosa* were the dominant algal species by mass.

Table 4.1 Glenmore Reservoir seasonal source water nutrient data. December 22, 1999 – January 19, 2000.

	Nutrients $mg \cdot L^{-1}$			
	Total Phosphorous	**Dissolved Phosphorous**	**Silica**	**DOC**
Minimum	0.0026	0.0010	3.77	0.81
Maximum	0.0234	0.0083	4.22	1.06

Chemical analysis of the water for VOC indicated that heptadienal, decadienal and decatrienal were present in the water at concentrations of 200 – 4700 $ng \cdot L^{-1}$ during this period (Table 4.2). These compounds are known to be formed by *Dinobryon spp.*

Despite the elevated algal biomass and the noticeable fishy odor, the concentration of algal VOCs was often very close to the analytical detection limit. The procedure of filter concentration (Section 3.4) was applied to enhance the detection limit. While this procedure was not quantitative, it did allow identification of odorous compounds at levels normally below the method detection limit.

In order to better assess the distribution of algae in the reservoir, under-ice samples were collected from two locations on the headpond on January 13, 2000. One site was off the main axis, midway to the east shore (HP1; Figure 1.3). The other was located on the main water channel, in line with the plant intakes and the causeway (HP2). Samples were collected from the surface water layer and from 6 m. The chemical and taxonomic results from these locations was similar in composition to that of screen house samples (*Dinobryon* algae, heptadienal and decatrienal VOCs). Samples were also collected from the source water intakes for the treatment plant.

Table 4.2 Algal VOC composition in the Glenmore Reservoir.

		Compound ($ng \cdot L^{-1}$)		
Date	**Location**	**Heptadienal**	**Decadienal**	**Decatrienal**
31 Dec, 1999	Plant Intake	110	60	500
10 Jan, 2000	Screen House	870	170	100
13 Jan, 2000	Screen House	4700	1250	700
13 Jan, 2000	HP1	3140	410	250
19 Jan, 2000	Screen House	1800	400	140
27 Jan, 2000	Plant Intake	1140	180	400

Throughout the source water T/O episode, the characteristic fishy odor was never detected in the finished drinking water, either by the weekly FPA panel, or by customer complaints. On one occasion (January 31, 2000), samples were collected through the plant process train in order to determine the effect of treatment. In-plant samples were collected after chlorine addition, coagulation and sedimentation. Taxonomic assessment indicated that 43.1% of the total algal biomass was removed (Table 4.3), and the remaining algae had suffered cellular disruption as a result of chlorine addition. Chemical analysis for algal VOCs found no aldehydes present in either the in-plant or the finished water sample. As cell lysis and PUFA oxidation had already been initiated, the filter concentration procedure was not applicable.

Table 4.3 Taxonomic data and biomass removal by sedimentation.

January 31, 2000 Species	**Raw Water** Biomass $\mu g \cdot L^{-1}$	**Post Sedimentation** Biomass $\mu g \cdot L^{-1}$	% Reduction
Total Biomass	490.4	279.2	43.1
Asterionella formosa			
Dinobryon divergens	208	139.4	33.0
Chrysochromulina parva	22.5	5.9	73.8
misc. flagellates 5 < µm	10.6	7.1	33.0
Cryptomonas rostratiformis	56.5		100
Pseudopedinella elachista	102.4	59.8	41.6
Katablepharis ovalis	16.8	16.8	0
Ochromonas cf granularis		11.1	
Rhodomonas minuta	79.5	21.4	73.1
Chrysococcus rufescens			
Spiniferomonas trioralis	3.8		100
Synedra acus v angustissima	0.5	0.5	0

In the course of these surveys, marked variability was noted in both the taxonomic and chemical results over the course of a single day. As these fluctuations (± 100% algal

biomass, ±10,000% VOC) were too large and rapid to be the result of a biological process, other factors were examined.

Standard practice for the Glenmore plant is to increase production through periods of low electrical demand, and decrease production when electrical demand is high. The result of this peak shaving is a diurnal cycle. To explore the effect of this on algae VOCs, 1 L samples were collected in intervals before, during and after the transition on January 31, 2000. Filter concentration was used to enhance the HSPME sensitivity.

Table 4.4 Short term variability in source water VOCs (January 31, 2000).

Time	Algal VOCs ($ng \cdot L^{-1}$)		
	Heptadienal	**Decadienal**	**Decatrienal**
07:30 h	7.7	1.1	2.8
12:00 h	22.3	3.4	15.7
15:00 h	250.7	49.5	260.3

As shown in Table 4.4, Figure 4.1, there is an inverse relation between plant production and algal VOCs. It appears that high plant flow rates deplete the algae populations at the raw water intakes. As coordinated under-ice sampling was not feasible, the extent of this depletion is not known. The local population was replenished rapidly, and the measured VOC concentrations increased from < 50 to over 500 $ng \cdot L^{-1}$ within the span of 8 h.

The levels of heptadienal and decadienal measured were correlated significantly to algal biomass, especially that of the dominant species *Dinobryon* [2]. While this algal bloom affected the source water T/O, it had very little impact on the finished drinking

water quality. This can be attributed to the facile oxidation of decatrienal by chlorine, even at low ($mg \cdot L^{-1}$) applications. It is hypothesized that the fishy

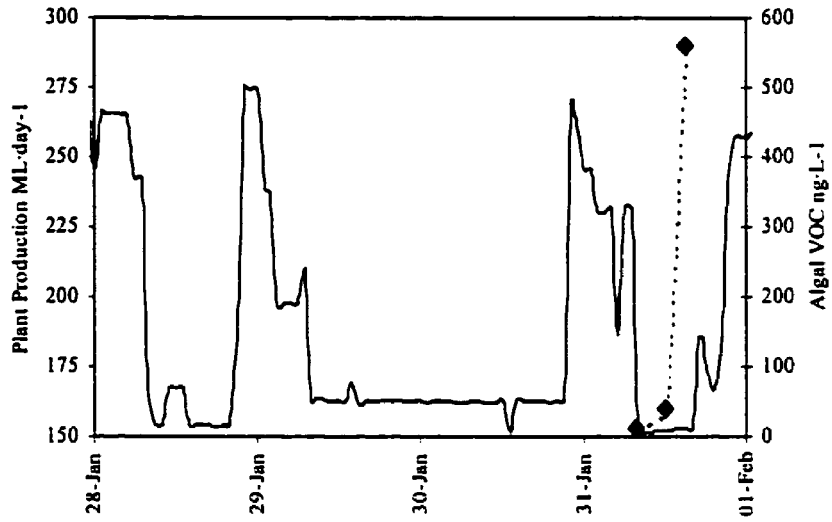

Figure 4.1 Treatment plant production (solid line) and source water algal VOCs (dashed line). Midnight is indicated by the date.

source water odor was due largely to decatrienal derived from *Dinobryon*. Decatrienal can be oxidized by chlorine under normal plant operating parameters of ca. 5 h retention time and chlorine dosages of 0.5-2.0 treatment process.

Even though heptadienal was detected at higher concentrations than decatrienal, it has a lower odor potency and therefore less effect on aesthetic water quality (Table 3.14). Filtration was used to concentrate the algae in source water samples, and thus achieve a

lower detection limit. Algal VOCs were not detected in finished water by either HSPME or by FPA.

4.3 Open Water *Uroglena* Bloom.

4.3.1 Introduction

The under-ice T/O event in the Glenmore Reservoir was followed by a subsequent algal bloom in June, 2000. The weather preceding the event was clear and warm, and there was no apparent odor in the finished water. This was followed by an intense local thunderstorm on June 8th. By the next day, several complaint calls had been received regarding fishy-metallic tastes and odors in the drinking water. Samples collected from the reservoir and raw water intakes were subjected to FPA, taxonomic assessment and algal VOC determination.

4.3.2 Results and Discussion

Samples collected by integrated tube sampling to 6m depth were found to contain 750 – 1,500 ng·L^{-1} of heptadienal and 100 – 1,200 ng·L^{-1} of decatrienal. The dominant algal species was identified by microscopic assessment as *Uroglena americana*, which is known to produce heptadienal and decatrienal through the oxidation of PUFA [3].

Plankton net tow samples were collected from the reservoir headpond and analyzed for algal VOCs as well. A qualitative assay was conducted to estimate the relative proportion of aldehydes produced by the enzymatic oxidation. The estimated ratio of heptadienal:decatrienal was 0.48:1. Decadienal comprised 2.8% of the total VOC composition by mass.

Immediately preceding the *Uroglena* bloom, raw water FPA ranged from 3-4. The FPA increased to 6-8.5 through the period of June 12-26, 1999. The finished water FPA had a moderate intensity of 2.5 – 4, with odor descriptors of fishy and metallic. By June 28, 1999, the bloom was over, the source water FPA had declined to 2.5 and no fishy-metallic odors were present in the finished water. Estimates of *Uroglena* cell counts were conducted. The peak density of 1137 cells·mL^{-1}, reached on June 22, rapidly declined to 142 cells·mL^{-1} by June 26, 1999.

4.4 Discussion of Bloom Events

Both the *Dinobryon* and *Uroglena* bloom events have similar dynamics to a succession of Glenmore Reservoir blooms which occurred in 1992 [4]. Through May to August of 1992, there were two distinct bloom events which produced fishy-rancid odors in the source water. The first was a bloom of *Uroglena americana* which increased to a maximum biomass 1400 μg·L^{-1} on May 26, 1992. This fishy odor persisted through the treatment process and resulted in many finished water odor complaints through the four week bloom, until June 17. This was followed by a bloom of *Dinobryon spp.* which lasted from July 15 to August 12, 1992, with a maximum algal biomass of 520 μg·L^{-1} on July 22. Again, the source water had an intense fishy-rancid odor; however, no offensive odors were present in the finished water.

The two recent blooms were not as intense as in 1992; however, the results were similar. The under-ice *Dinobryon* bloom affected the source water but not the finished water, while both *Uroglena* events imparted an offensive, fishy odor to the finished drinking water. As a result of research into the taste and odor dynamics of these algae, it

is now known that the important odor compound associated with *Dinobryon* blooms is decatrienal. This compound is effectively removed by the water treatment process through chlorine oxidation. While *Uroglena* can produce decatrienal, it also produces large quantities of heptadienal [3]. Based on the chemistry and biology of these blooms, it is known that heptadienal is relatively stable to chlorine oxidation, and can cause offensive taste and odor problems in drinking water. With this knowledge and understanding of the bloom dynamics, the City of Calgary was able to issue the following press release on June 13, 2000. Based on customer response, the press release was effective in allaying public concerns about municipal drinking water quality.

> Date: Tue Jun 13 12:39:03 2000
> Subject: Algae Growth Generates Drinking Water Taste and Odor Complaints
> From: Peter Brodsky -----BEGIN MD5
> CITYBEAT - CITY OF CALGARY PRESS RELEASE
> CALGARY - The City of Calgary Waterworks has ascertained that a harmless, yet odorous algae is growing in the Glenmore Reservoir. The algae, *Uroglena americana*, is effectively removed by the drinking water treatment process. However, citizens in some parts of the city, particularly in the south, may notice a distinct metallic or fishy odor in the drinking water as a result of the algae and the water treatment process used. "We have received several complaints that the water has a metallic flavor. It has been our experience that complaints about the aesthetic quality of the water are associated with chlorine dosing changes or increased biological activity in the Glenmore Reservoir and not the level of safety of the drinking water," states Allyn Humber, Acting General Manager of Waterworks. "Our scientists have responded to the complaints, have conducted tests on all aspects of the drinking water and have confirmed that it is well within the Canadian Drinking Water Guidelines," says Humber. It is not unusual for various species of algae to bloom in lakes, ponds and reservoirs in spring and summer under the right conditions. Waterworks is monitoring the development of the algae bloom and is adjusting the water treatment process accordingly. Chlorine is added in the treatment process to destroy micro-organisms to make the water safe for consumption. As the quality of water drawn from the reservoir changes, the amount of chlorine and other chemicals used to treat the water fluctuates. As a result, the taste and odor of the drinking water changes. "I want to reinforce to Calgarians that the drinking water is safe to drink and poses no health threat. The integrity of our drinking water is protected and this is a temporary situation," assures Humber. It is too early to determine how long the algal growth

cycle will last or to predict the intensity of the taste and odor. However, judging from the outcome of a similar situation in 1992, the anticipated spring run-off from the mountains or a significant rain event may play a significant role in the elimination of the algae bloom in the reservoir.

4.5 Periphyton Survey

In a related study, the HSPME was applied to other samples types in an effort to better understand the dynamics of T/O production in general. In one series of samples, periphyton were collected in triplicate from streams of different nutrient status in northern Alberta streams and the St. Lawrence River. These samples were analyzed for algal VOCs The periphyton (sessile attached algae) were identified as *Hydrurus foetidus*.

This survey was qualitative in that the algae mass was not estimated. Rather, the samples were collected by scraping the attached algae from the rocks and placing them in glass sample jars, along with overlying water. The samples were refrigerated for storage and transport. Immediately prior to chemical analysis, cell lysis and enzyme activation was induced through salt addition as previously described.

In addition to odorous aldehydes, several biologically active compounds were identified in some of the samples. These compounds were first identified in marine brown algae, but have since been isolated in fresh water algae such as diatoms. These pheromones are also common to a large number of plants via several different synthetic pathways [5].

These pheromones are produced in nature through the beta oxidation of PUFA, similar to that of the odorous aldehydes. It is noteworthy that all these pheromones are oxidation products of primary products, and they are not synthesized by intracellular processes. The compounds produced are either C_8 or C_{11} polyunsaturated hydrocarbons.

In this survey, octatriene, dictyotene and ectocarpene were positively identified by GC-MS. Authentic standards were not available; however, structural information from the fragmentation pattern and excellent library matches provided a means of identification. Other related compounds were also recovered but their structure was not fully characterized.

4.6 Pine Lake

Samples were collected from Pine Lake, AB for analysis. This is a eutrophic lake that supports a variety of blue-green algae, and which is affected by both odor and swimmers itch complaints. Swimmers' itch can result from parasitic organisms, or from dermal irritation caused by algal toxins. The taste and odor compounds geosmin and 2-MIB are also produced by blue-green algae.

Samples from Pine Lake were collected by Alberta Environment staff on July 19 and 26, 2000 and shipped to Calgary in insulated coolers. Taxonomic analysis confirmed the dominant species to be the blue green algae *Gleotrichia*, which is known to produce T/O compounds. Chemical analysis by HSPME confirmed the presence of large quantities of geosmin in addition to several unidentified compounds. The geosmin concentration in these samples was estimated at 105,000 $ng \cdot L^{-1}$. The unsaturated aldehydes heptadienal and decatrienal were present at 22,600 and 14,300 $ng \cdot L^{-1}$ respectively. One compound was identified as argosmin, the dehydration product of geosmin [6]. Other major components included a series of $C_{15}H_{24}$ hydrocarbons such as β-cyclocitral and copane.

Subsequent analysis of the sample by HSPME after 14 days storage indicated significant bacterial activity had taken place. This was evidenced by the formation of several thiol compounds which were not initially present. These are formed through the bacterial degradation of cellular amino acids. This can exacerbate T/O events as the initial algal derived odors of geosmin, 2-MIB are followed by sulfur based compounds from bacterial activity. The sulfur compounds dimethyl trisulfide and dimethyl tetrasulfide were positively identified by GC-MS.

4.7 Analysis of *Actionomycetes*

4.7.1 Introduction

Actinomycetes are a class of soil bacteria which are a known and important source of geosmin and 2-MIB [7]. This additional biological origin of these compounds has complicated T/O investigations as it expands the potential source to include bacterial and soil-wash, as well as algal-aquatic process.

In this preliminary survey, samples were collected from locations along the Credit River and grown in culture. Isolates were then taken and grown under standardized conditions on defined media. Samples of the isolated colonies were cut from the agar plates and placed in VOC sample vials along with 30 mL of 20% w/v NaCl and analyzed by HSPME.

4.7.2 Results and Discussion

It was found that several strains of *Actinomycetes* were present in different locations. As well, the different strains differed in the production of geosmin and 2-MIB.

It is not clear whether this is the result of environmental factors such as life cycle or resource limitations or metabolic capacity (lack of synthetic pathways to form geosmin or 2-MIB). Preliminary studies are underway to further understand the dynamics of odor production by this organism. This initial investigation has already clearly established that odorous compound production can vary greatly between samples and species of *Actinomycetes*.

4.8 References

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CHAPTER 5: FUTURE DIRECTIONS

5.1 Introduction

This project studied the dynamics of algal derived volatile in terms of their formation, measurement and impact on drinking water supplies. As the study progressed, it became evident that the biogenesis and degradation of these compounds would require significant study to understand the chemistry and biology of the processes involved properly.

5.2 Relation of Cellular Fatty Acids to VOC Production

One area for further study is the biochemistry of PUFA formation, and their subsequent oxidation to biologically active compounds which include semiochemicals such as pheromones, allomones, and T/O compounds. As these compounds are typically breakdown products of primary metabolites, the cellular formation and storage of these compounds will have a strong determination on the product distribution.

It would be useful to study both the lipid composition of algae and the volatile constituents which are derived from PUFA oxidation following cellular disruption. Elucidation of the linkages between specific fatty acids, enzymes and the resultant VOC would yield significant insight into the biochemistry of algal T/O in particular and semiochemical processes in general. These semiochemicals are produced by the extracellular breakdown of primary storage materials, such as fatty acids [1]. In the natural communities, the release of these compounds during predation may be favorable to the rest of the colony, if the released chemicals deter further grazing [2]. Unsaturated aldehydes such decatrienal have been shown to reduce predation in other species such as

insects [3]. While these detailed mechanistic studies were not undertaken, these processes are a potentially rich area of study. Some specific questions are why *Asterionella*, which stores polyunsaturated fatty acids and produces large quantities of the hydrocarbon octatriene, but apparently no unsaturated aldehydes [4]. Similarily, the chrysophyte *Synura petersenii* is the only known algal source of the potent cucumber odor compound, E2,Z6-nonadienal [5]. These observations may be the result of either enzymes or fatty acids which are specific to, or absent from these algae.

Related studies into changes of PUFA content and VOC production in relation to growth phase would be of value. As the algae go through their life cycles, changes in fatty acid composition occur. The influence of fatty acid composition on VOC products and concentration is not clearly understood.

5.3 Sample Storage And Stability

Standard sample preservation techniques appropriate to chemical samples are not amenable to algal assemblages. Refrigeration, acid preservation, filtration (this work) or storage under inert gas are either ineffective at slowing biochemical processes, or stop them completely [6]. Conversely, biological sample preservation such as formalin or Lugol's iodine destroy desirable biochemical processes (e.g. PUFA oxidation) and interfere with subsequent chemical analysis. In this study, algal samples were stored in a viable form until analysis whenever possible. To minimize any effects from laboratory storage, samples had to be analyzed as soon as possible, and the sample collection to

analysis cycle was carefully coordinated. In some cases this imposed restrictions on experimental design.

Several options to facilitate sample storage have been considered. Ideally, a means to suspend and reactivate the natural enzymatic catabolism process is sought. Due to the complex and varied enzymatic processes involved, this would be an intricate endeavor. An alternative may be to inactivate the natural enzymes, isolate the constituent fatty acids form the sample for storage, and then subject them to a standard enzyme mixture.

A more conventional approach is to derivatize the aldehydes after first inducing cell lysis and enzymatic breakdown of the fatty acids. While the preliminary studies with *o*-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine·HCl to prepare oxime derivatives show promise, the method lacked sufficient sensitivity and did not provide satisfactory structural information.

5.4 Oxidation Kinetics

With respect to the amelioration of the algal T/O compounds, it would be beneficial to extend the knowledge of oxidation kinetics, particularly in regards to 2,4,7-decatrienal. In this work, the experimentation conducted was limited by the availability of the authentic chemical standard. As the material was prepared in solvent, there was competition between the solvent and the aldehyde for chlorine oxidation. It would be beneficial to conduct additional studies at different chlorine:substrate ratios, and with

other oxidants used in drinking water treatment such as chloramine, ozone, and UV-peroxide.

5.5 References

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4. Jüttner, F. and Muller, H. Excretion Of Octadiene And Octatrienes By A Freshwater Diatom. *Natuwissenschaften*, 1979. **66**, p. 363-364.

5. Wee, J., Harris, S., Smith, J., Dionigi, C. and Millie, D. (1994). Production Of The Taste/Odour Compound, *trans*-2, *cis*-6-Nonadienal Within The Synurophyceae. *Journal of Applied Phycology* 1994. **6**, p. 365-369.

6. Wendel, T. and Jüttner, F. Lipoxygenase-Mediated Formation Of Hydrocarbons And Unsaturated Aldehydes In Freshwater Diatoms. *Phytochem.* 1996. **41**, p. 1445-1449.

APPENDIX A: GC-MS RESULTS OF ALGAL VOC ANALYSIS

A.1 Introduction

While the formation of these compounds all proceeded by the enzymatic oxidation of PUFAs, different species of algae tended to produce different compounds, even when grown under similar conditions. These differences may result from variation in PUFA formation, enzyme content or carbon utilization based on environmental influences. Literature reports have already established certain associations, such as heptadienal with *Uroglena americana*, nonadienal with *Synura petersenii* and octatriene with *Asterionella formosa*. With the exception of *Synura petersenii*, which is the only known algal producer of nonadienal, the other compounds are produced in varying degrees by other algae.

Some of the important algae VOC and producer species are listed in Table A2. Molecular data such as chemical name, structure, Chemical Abstracts Registry numbers, mass spectra and representative chromatograms are presented. Table A2 summarizes basic chemical information. Structural information for the same is provided in the following figures.

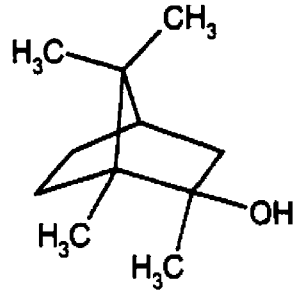
Table A1 VOCs from the oxidation of PUFA and associated algal species.

COMPOUND	**Species**
PUFA derivatives	
E2,Z4-decadienal E2,E4-decadienal	*Dinobryon divergens;D. cylindricum; Mallomonas papillosa; S. uvella; S. petersenii;* cf. *Syncrypta* sp.; *U. americana; Uroglena sp.*(UTCC276).
E2,Z4,Z7-decatrienal E2,E4,Z7-decatrienal	*D. divergens;D. cylindricum; M.papillosa; S. petersenii; U. americana; Uroglena sp.* (UTCC276); *Melosira varians*
E2,Z4-heptadienal E2,E4-heptadienal	*D. divergens;D. cylindricum; S. uvella; M. papillosa; U. americana;*(UTCC276); *Fragilaria crotonensis; M. varians*
n-heptadecane	*S. petersenii*
E2,Z4-octadienal	*A. granulata; F. crotonenesis; M. varians*
1,3-octadiene	*A. formosa; D. cylindricum; S. uvella*
1,E3,Z5-octatriene	*A. formosa; S. uvella*
Ectocarpene (butenyl-cyloheptadiene)	*Gomphonema parvulum; Phaeodactylum tricornutum; Amphora venetia*
Dictyopterene A (hexenyl-vinyl-cyclopropane)	*G. parvulum; P tricornutum; A. venetia*
Dictyopterene C′ (6-butylcycloheptadiene)	*G. parvulum; P. tricornutum; A. venetia*

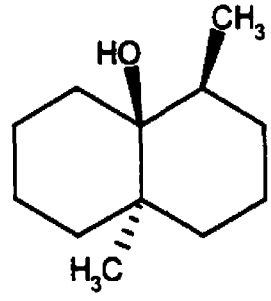
Table A2 Chemical names, registry numbers, formulas and molecular mass data for taste and odor, and other related compounds identified in this research.

Compound	CAS Registry	Molecular Formula	Molecular Mass	Source
E2,E4-heptadienal	4313-03-5	$C_7H_{10}O$	110.2	Aldrich
E2,Z6-nonadienal	209-178-6	$C_9H_{14}O$	138.2	Aldrich
E2,E4-decadienal	25152-84-5	$C_{10}H_{16}O$	152.2	Aldrich
E2,E4,Z7-decatrienal	13552-96-0 (E,Z,Z)	$C_{10}H_{14}O$	150.1	F. Jüttner
Geosmin (E1,10-Dimethyl-E-decalin-9-ol)	19700-21-1	$C_{12}H_{22}O$	182.1	Wako
1,3E,5Z-octatriene	400087-61-4	C_8H_{12}	108.1	Survey
6-[Z1-butenyl]-1,4-cycloheptadiene (ectocarpene)	33156-93-3	$C_{11}H_{16}$	148.1	Survey
2-Methylisoborneol (1,2,7,7-tetramethyl-exo-bicyclo[2.2.1] heptan-2-ol)	2371-42-8	$C_{11}H_{20}O$	168.3	Wako
Biphenyl-d_{10}	1486-01-7	$C_{12}D10$	164.3	Aldrich
Dimethyl trisulfide	3658-80-8	$S_3C_2H_6$	125.9	Survey
Dimethyl tetrasulfide	5756-24-1	$S_4C_2H_6$	157.9	Survey

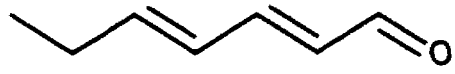
COMPOUND	CAS REGISTRY NUMBER	MOLECULAR FORMULA	MOLECULAR MASS	SOURCE
2-Methylisoborneol	2371-42-8	$C_{11}H_{20}O$	168.3	Wako



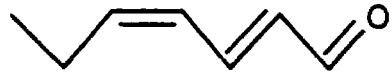
COMPOUND	CAS REGISTRY NUMBER	MOLECULAR FORMULA	MOLECULAR MASS	SOURCE
(-) Geosmin (1,10-Dimethyl-decalin-9-ol)	19700-21-1	$C_{12}H_{22}O$	182.1	Wako



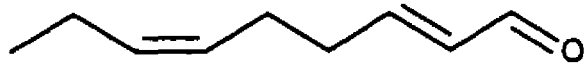
COMPOUND	CAS REGISTRY NUMBER	MOLECULAR FORMULA	MOLECULAR MASS	SOURCE
E2,E4-heptadienal	4313-03-5	$C_7H_{10}O$	110.2	Aldrich



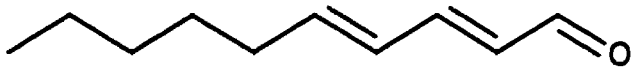
E2,Z4-heptadienal



COMPOUND	CAS REGISTRY NUMBER	MOLECULAR FORMULA	MOLECULAR MASS	SOURCE
E2,Z6-nonadienal	209-178-6	$C_9H_{14}O$	138.2	Aldrich



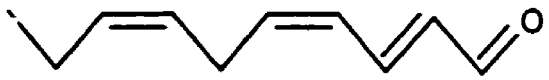
COMPOUND	CAS REGISTRY NUMBER	MOLECULAR FORMULA	MOLECULAR MASS	SOURCE
E2,E4-decadienal	25152-84-5	$C_{10}H_{16}O$	152.2	Aldrich



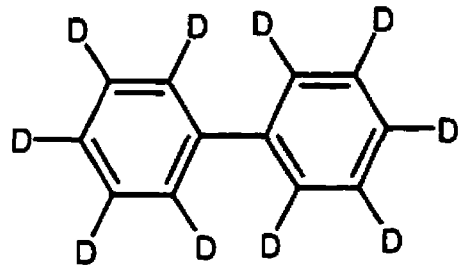
E2,Z4-decadienal

COMPOUND	CAS REGISTRY NUMBER	MOLECULAR FORMULA	MOLECULAR MASS	SOURCE
E2,E4,Z7-decatrienal	13552-96-0 (E,Z,Z form)	$C_{10}H_{14}O$	150.1	F. Jüttner

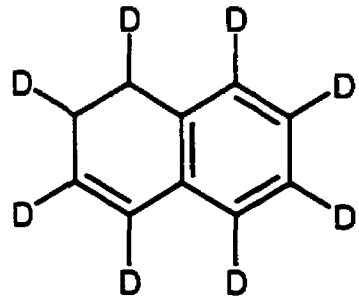
E2,Z4,Z7-decatrienal



COMPOUND	CAS REGISTRY NUMBER	MOLECULAR FORMULA	MOLECULAR MASS	SOURCE
Biphenyl-d_{10}	1486-01-7	$C_{12}D_{10}$	164.3	Aldrich

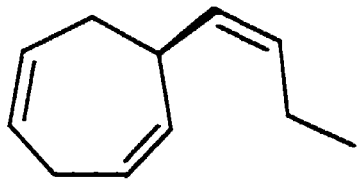


COMPOUND	CAS REGISTRY NUMBER	MOLECULAR FORMULA	MOLECULAR MASS	SOURCE
Naphthalene-d_8	1146-65-2	$C_{10}D_8$	136.24	Aldrich

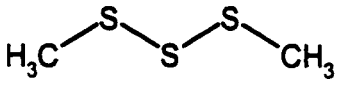


COMPOUND	CAS REGISTRY NUMBER	MOLECULAR FORMULA	MOLECULAR MASS	SOURCE
1,3E,5Z-octatriene (fucoserratene)	400087-61-4	C_8H_{12}	108.1	survey

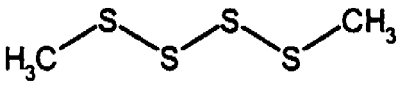
COMPOUND	CAS REGISTRY NUMBER	MOLECULAR FORMULA	MOLECULAR MASS	SOURCE
6S-[Z1-butenyl]-1,4-cycloheptadiene (ectocarpene)	33156-93-3	$C_{11}H_{16}$	148.1	survey



COMPOUND	CAS REGISTRY NUMBER	MOLECULAR FORMULA	MOLECULAR MASS	SOURCE
Dimethyl trisulfide	3658-80-8	$S_3C_2H_6$	125.9	survey



COMPOUND	CAS REGISTRY NUMBER	MOLECULAR FORMULA	MOLECULAR MASS	SOURCE
Dimethyl tetrasulfide	5756-24-1	$S_4C_2H_6$	157.9	survey



A.2 Mass Spectral Data

A.2.1 Introduction

Representative mass spectra of the T/O compounds identified in this research are presented. Where possible, a library reference spectrum (National Institute of Science and Technology, Gaithersburg, MD) is provided for comparison.

The mass spectrum of each taste and odor compound has a characteristic fragmentation pattern. Electron impact ionization at 70 eV was used to generate spectra which could be matched to standard reference libraries using probability based matching. Compounds such as the internal standard ($biphenyl_{d10}$) and 2,4,7-decatrienal not present in commercial libraries were added to a custom library.

A2.2 Mass Fragmentation Spectra

Although there are several geometric isomers of the polyunsaturated aldehydes are found in samples, the mass spectra of these isomers were identical under the acquisition conditions.

For the 2,4 unsaturated aldehydes, the mass spectrum is dominated by the base peak ion at m/z 81. As this is not an ion common to interfering peaks, this diagnostic ion was used for quantitation. This ion results from the $C_5H_5O^+$ fragment which can rearrange into a resonance stabilized form, as shown in Figure A1.

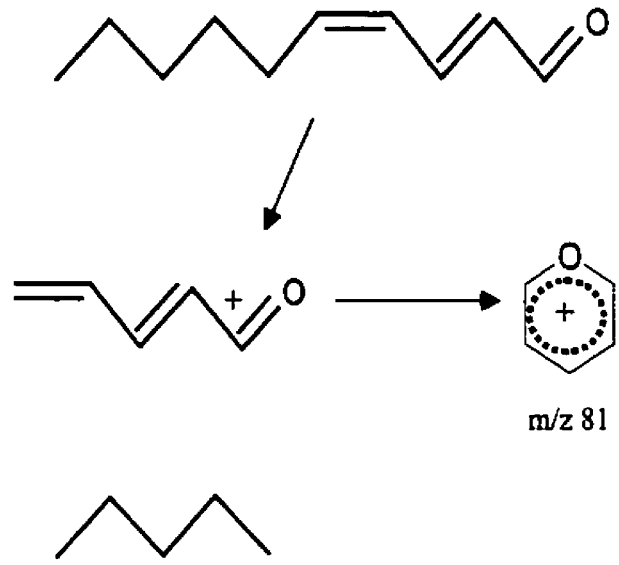


Figure A1 Proposed scheme for the fragmentation of 2,4-dienals under standard (70 eV) ionization conditions.

The triply unsaturated 2,4,7-decatrienal has a more complex spectrum, with many strong ions present. The molecular ion at m/z 150 is weak, and the spectrum is dominated by a cluster of strong peaks at m/z 77, 79, 81. These are likely $C_6H_5^+$, $C_6H_7^+$ $C_5H_5O^+$ ions which can cyclize to resonance stabilized forms.

The unconjugated aldehyde 2,6-nonadienal has base peaks of similar intensity at m/z 69,70. This may result from cleavage between the 4 and 5 carbons yielding $C_5H_9^+$, $C_4H_6O^+$ fragments. A strong m/z 41 ion is also observed.

A2.3 Mass Spectra of Taste and Odor, and Related Compounds

A2.3.1 Introduction

The following figures follow the format where the top panel presents the mass spectrum acquired from the indicated sample, and the lower panel is the reference or library spectrum. Where no reference spectrum was available, the presumptive identification is provided.

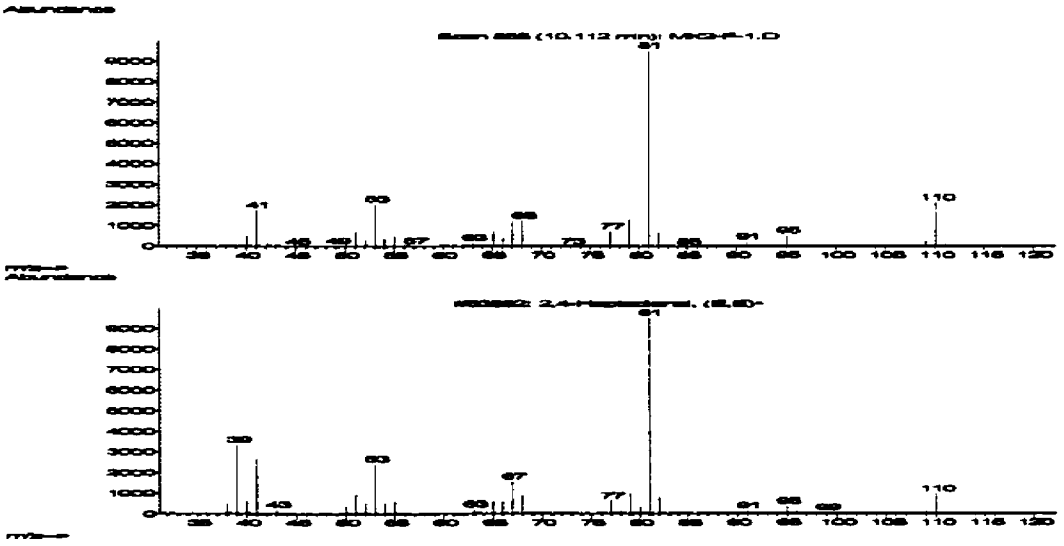


Figure A2 Mass spectrum of E2,Z4-heptadienal from a mixed algal culture of *Dinobryon* and *Uroglena*.

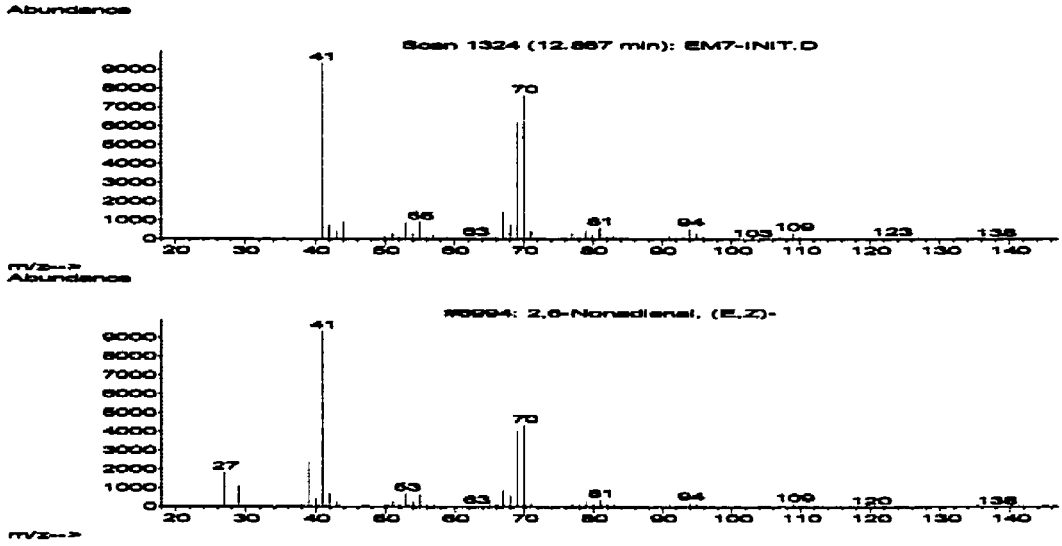


Figure A3 Mass spectrum of E2,Z6-nonadienal from a mixed algal culture containing *Synura petersenii.*

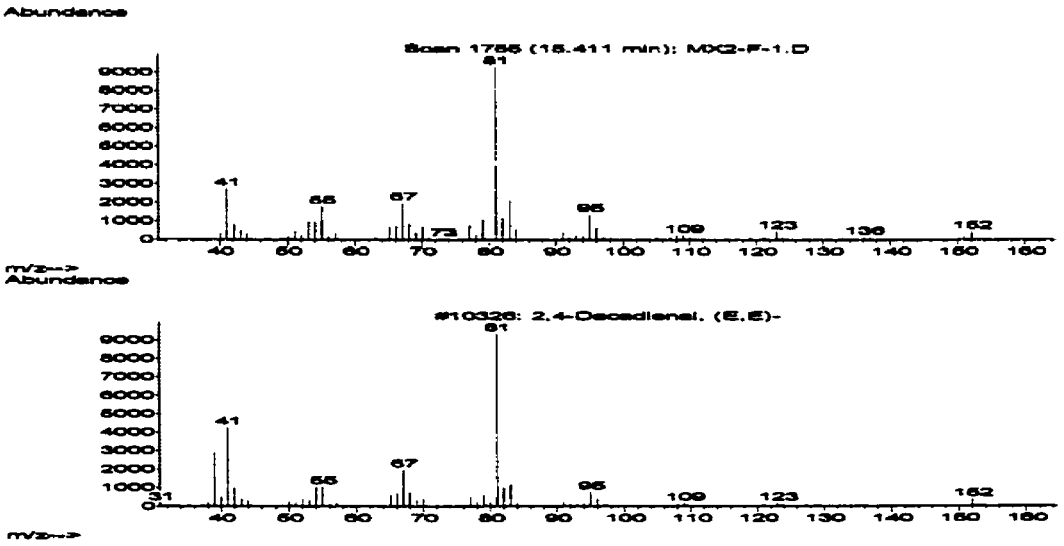


Figure A4 Mass spectrum of E2,Z4-decadienal from a mixed algal culture of *Dinobryon* and *Uroglena.*

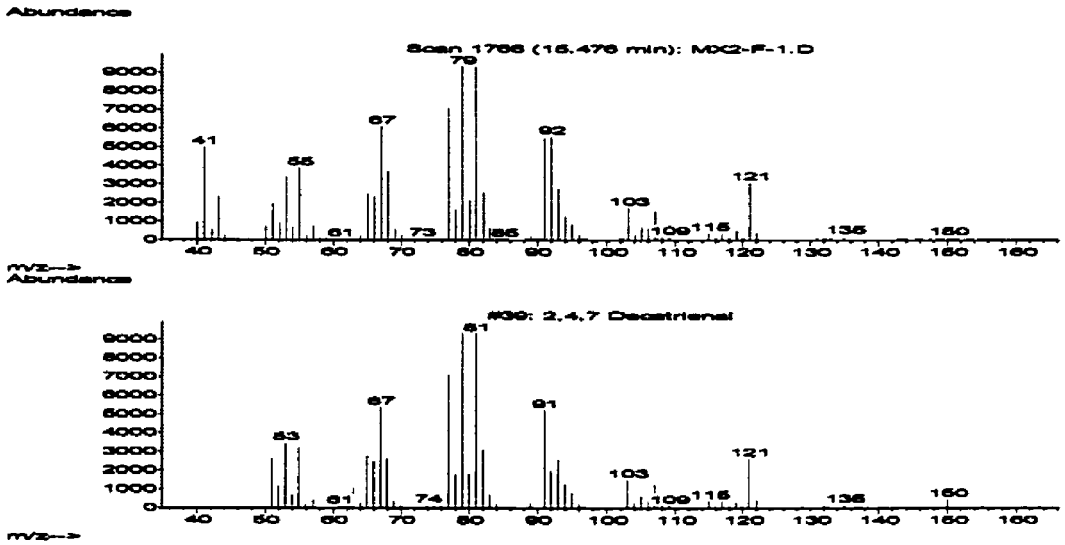


Figure A5 Mass spectrum of E2,Z4,Z7-decatrienal from a mixed algal culture of *Dinobryon* and *Uroglena.*

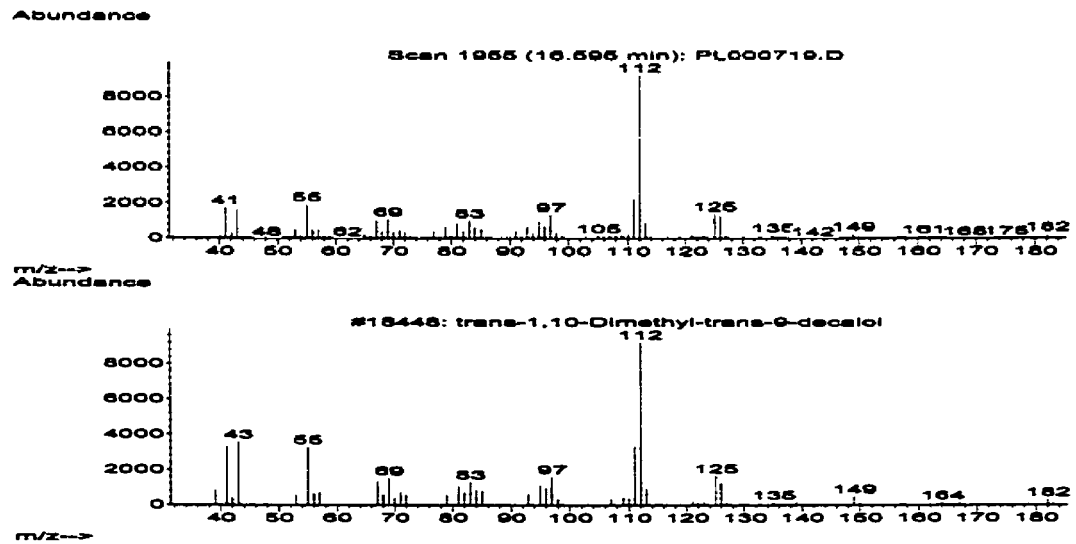


Figure A6 Mass spectrum of geosmin from Pine Lake, AB.

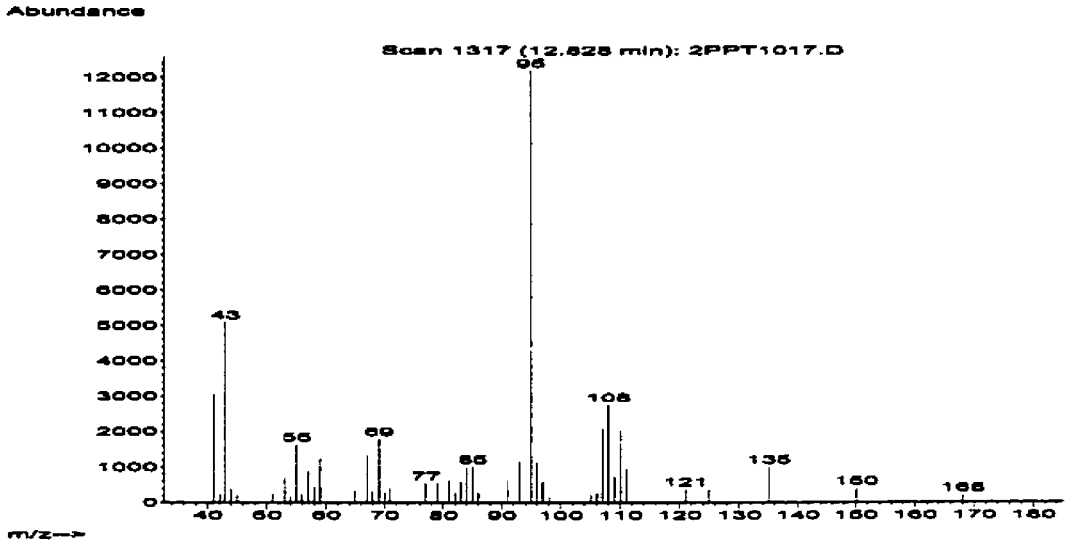


Figure A7 Mass spectrum of 2-MIB from a chemical standard.

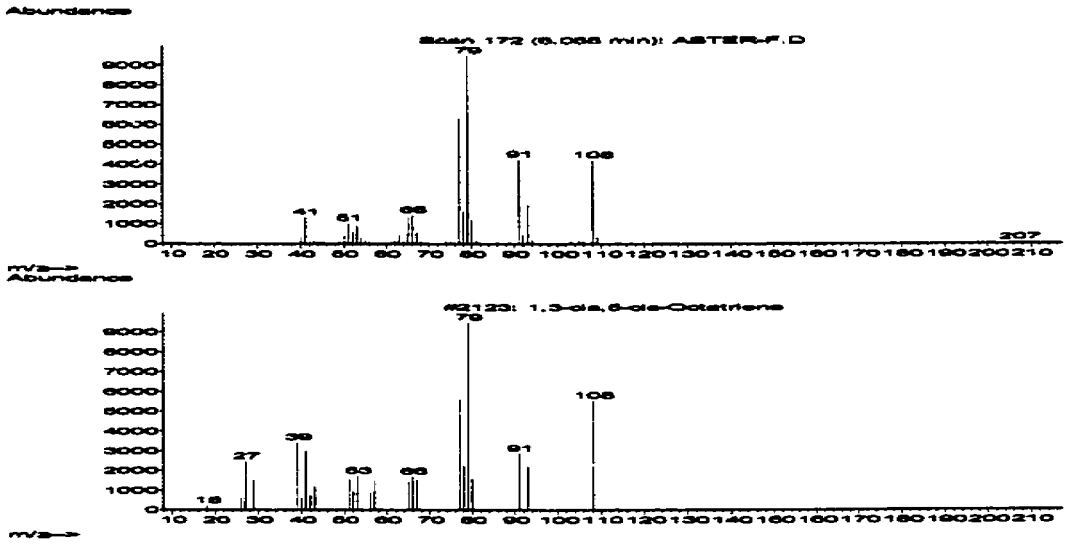


Figure A8 Mass spectrum of 1,3,5-octatriene from a culture of *Asterionella formosa*.

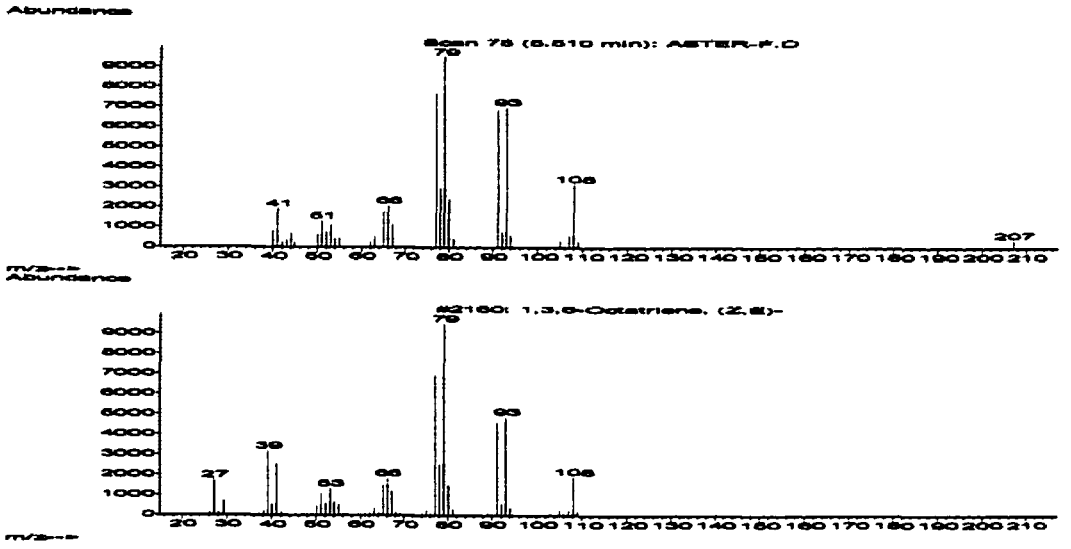


Figure A9 Mass spectrum of 1,3,6-octatriene from a culture of *Asterionella formosa.*

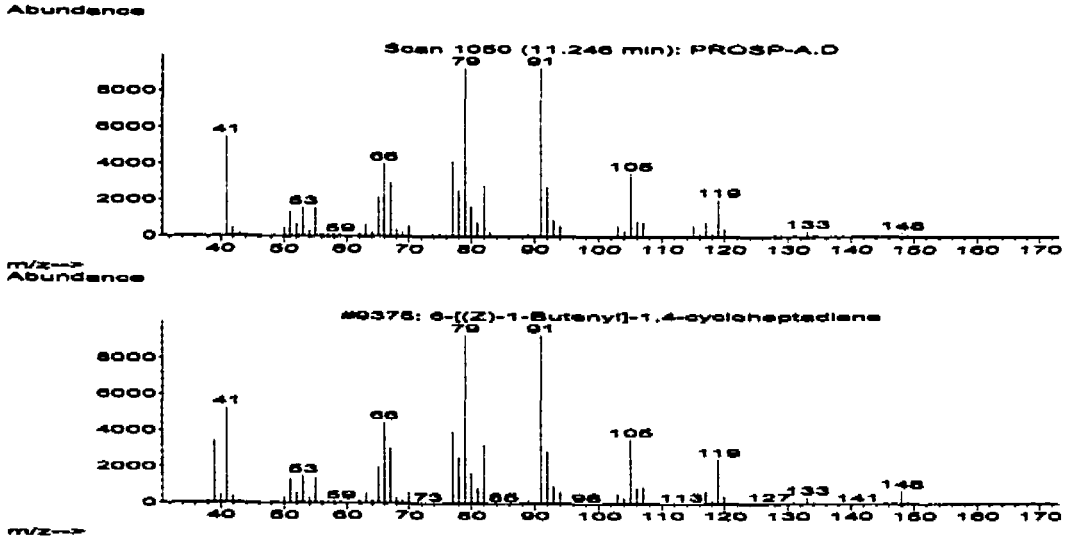


Figure A10 Mass spectrum of ectocarpene from a survey sample containing *Hydrurus foetdus.*

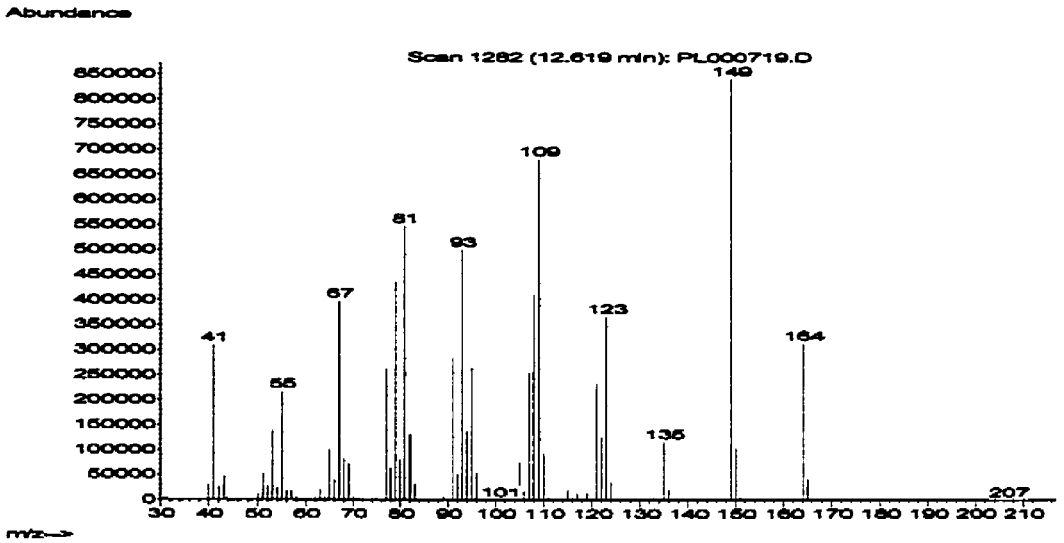


Figure A11 Mass spectrum of argosmin, a dehydration product of geosmin, from a survey sample of Pine Lake, AB.

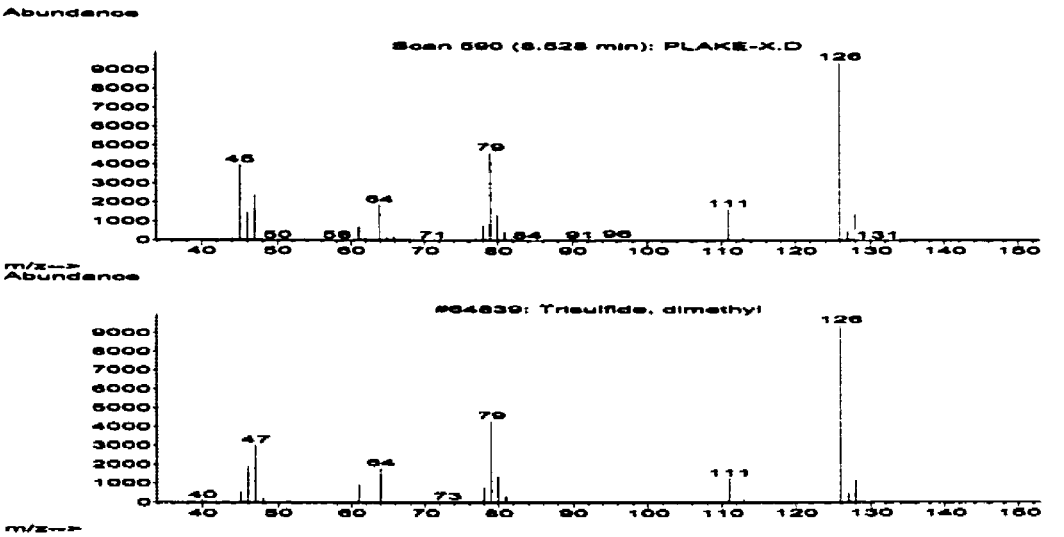


Figure A12 Mass spectrum of dimethyl trisulfide, a bacterial degradation product, from a stored (14 day) survey sample of Pine Lake, AB.

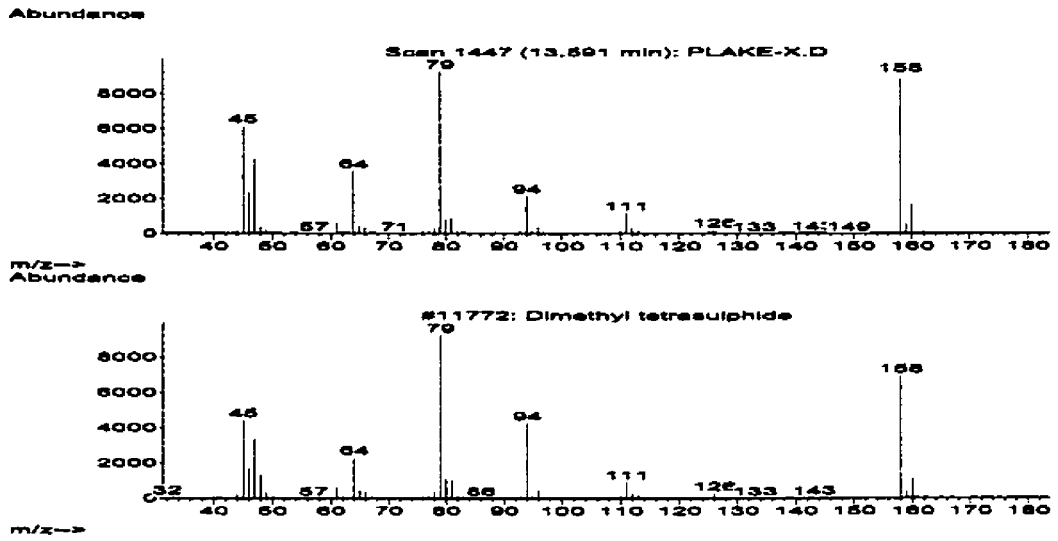


Figure A13 Mass spectrum of dimethyl tetrasulfide, a bacterial degradation product, from a stored (14 day) survey sample of Pine Lake, AB.

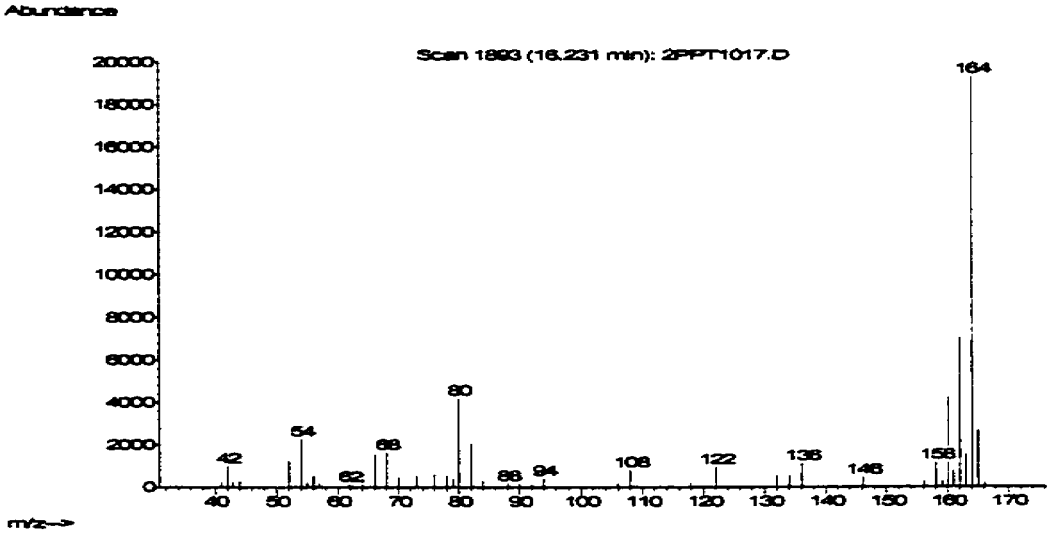


Figure A14 Mass spectrum of biphenyl$_{d10}$, the internal standard compound used for HSPME analysis.

A3 Gas Chromatographic Results

A.3.1 Introduction

The chromatographic separation and mass spectroscopic analysis of the algal VOCs was a major component of this work. This appendix presents representative chromatographic data from chemical standards, monocultures, mixed assemblages and natural water samples (Figures A15 – A29). The chemical compounds and algal species are identified where possible.

As several geometric isomers of the unsaturated aldehyes are formed, with similar mass spectrums (i.e. E2,Z4-heptadienal and E2,E4-heptadienal), it was important to chromatographically separate the isomers in order to achieve proper identification and quantitation. Representative gas chromatograms are presented in.

The mass spectra were acquired in full scan mode (m/z 40 –300). The sample source and compounds are identified in the figure titles. HPSME was used to concentrate samples. In order to remove background noise, extracted ion chromatograms are presented for clarity. $Biphenyl_{d10}$ is added as an internal standard at 100 $ng \cdot L^{-1}$ in most cases.

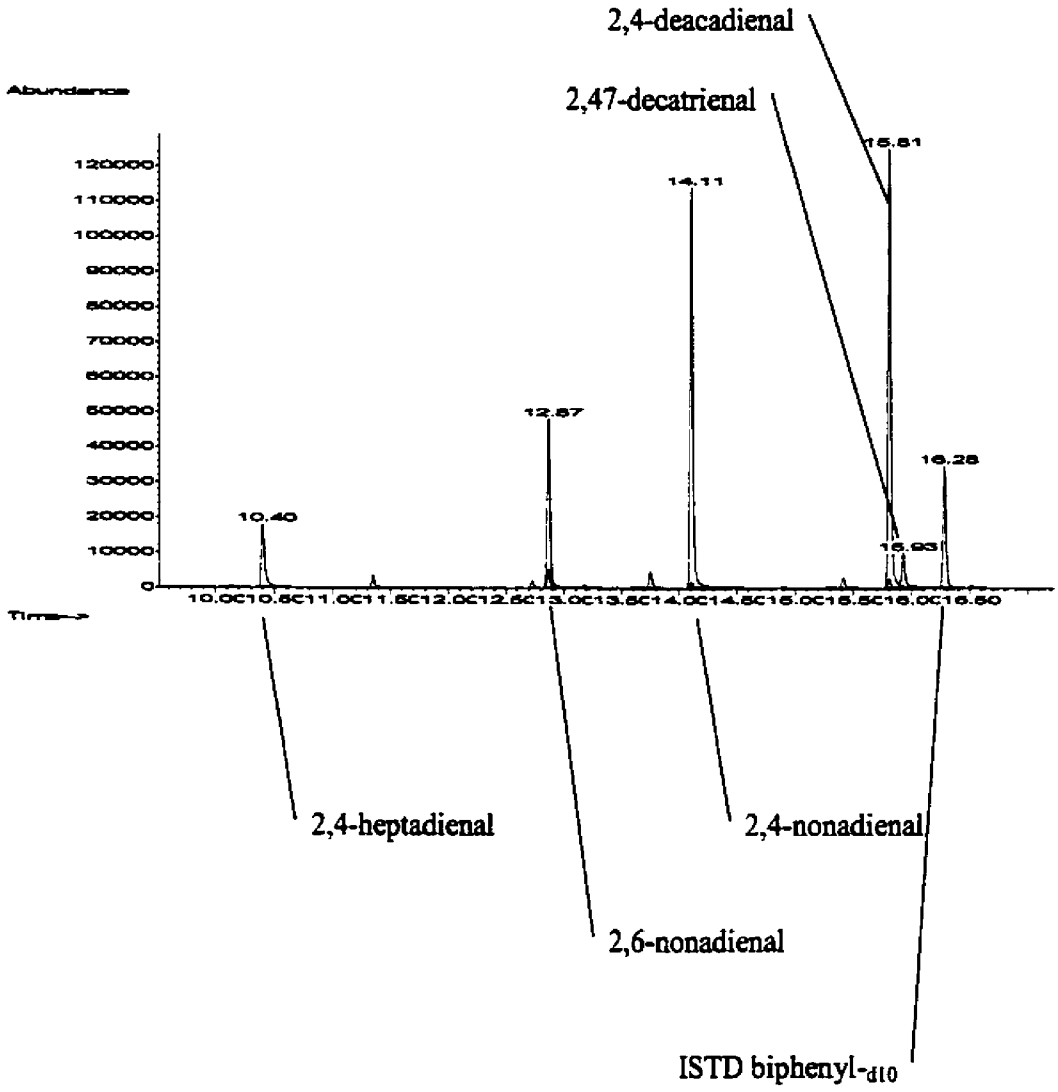


Figure A15 Extracted ion chromatogram of (1000 ng·L^{-1} Standard). Quantitation ions used: heptadienal, 2,4-nonadienal, decadienal, decatrienal m/z 81; 2,6-nonadienal m/z 69; biphenyl-d_{10} m/z 164.

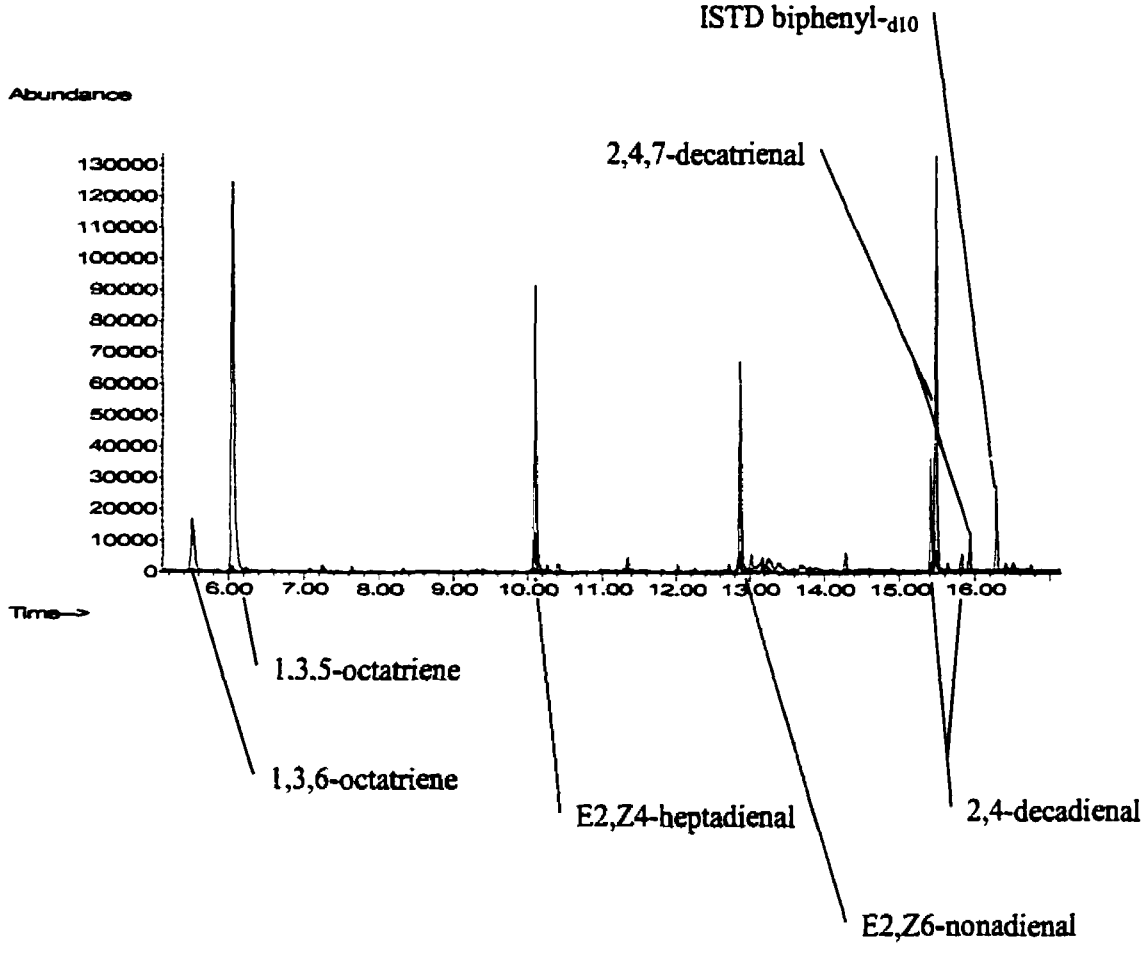


Figure A16 Extracted ion chromatogram of an algal mixture. Quantitation ions used octatriene m/z 79; nonadienal m/z 69; heptadienal, decadienal, decatrienal m/z 81; biphenyl-d_{10} m/z 164. The E,E and E,Z isomers of decadienal, and the E,Z,Z and E,E,Z isomers of decatrienal are indicated.

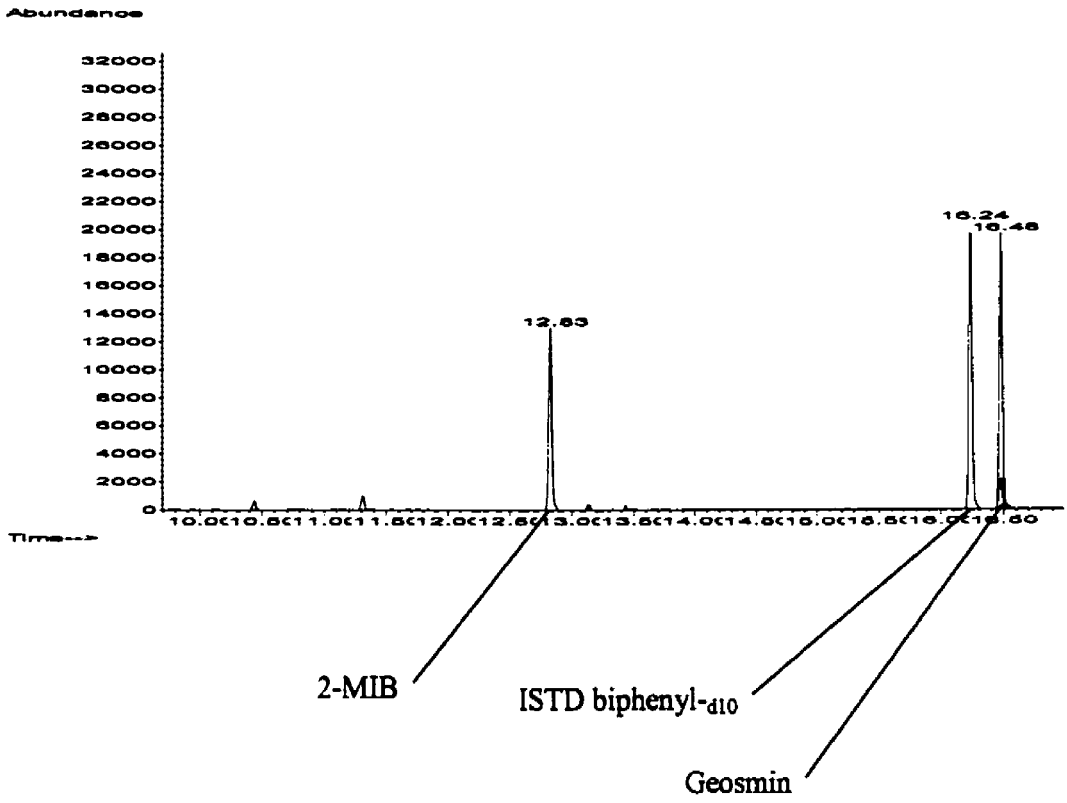


Figure A17 Extracted ion chromatogram of 100 ng·L^{-1} geosmin and 2-MIB standard. Quantitation ions used: 2-MIB m/z 95; biphenyl-d_{10} m/z 164; Geosmin m/z 112.

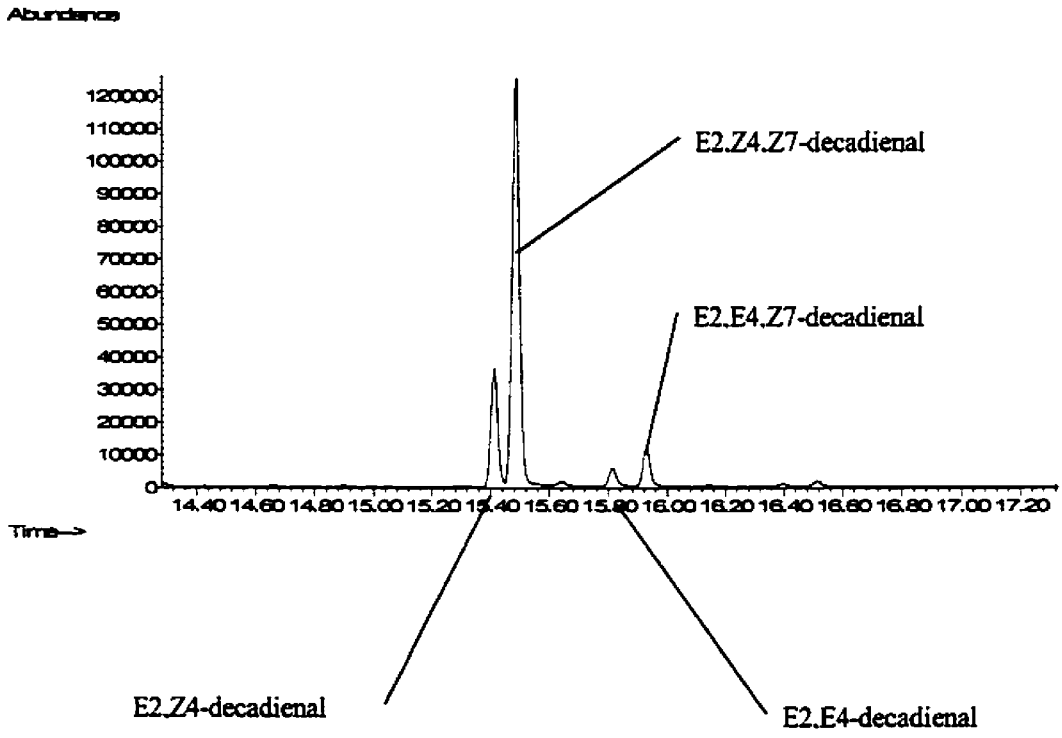


Figure A18 Extracted ion chromatogram (m/z 81) of a mixed algal culture. Two major isomers of the C_{10} aldehydes are present. The biologically formed E,Z and E,Z,Z are the major peaks. The more stable E,E and E,E,Z isomers are ca. 10% of total mass. The sample was analyzed immediately after collection to minimize isomerization. The mixed culture was comprised of *Urogelena american, Dinobryon cylindricum, Mallomonas papillosa and Asterionella formosa.*

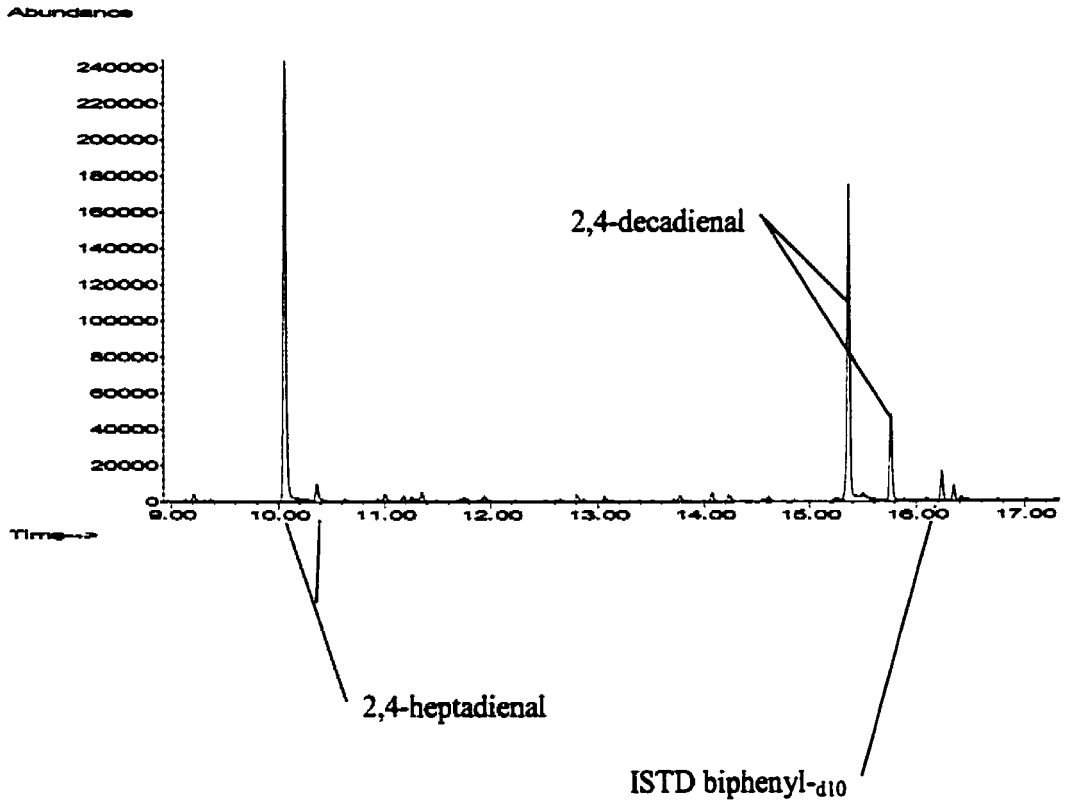


Figure A19 *Uroglena americana* culture. Major algal VOCs indentifed are 2,4-heptadienal and 2,4-decadienal (ion extracted m/z 81).

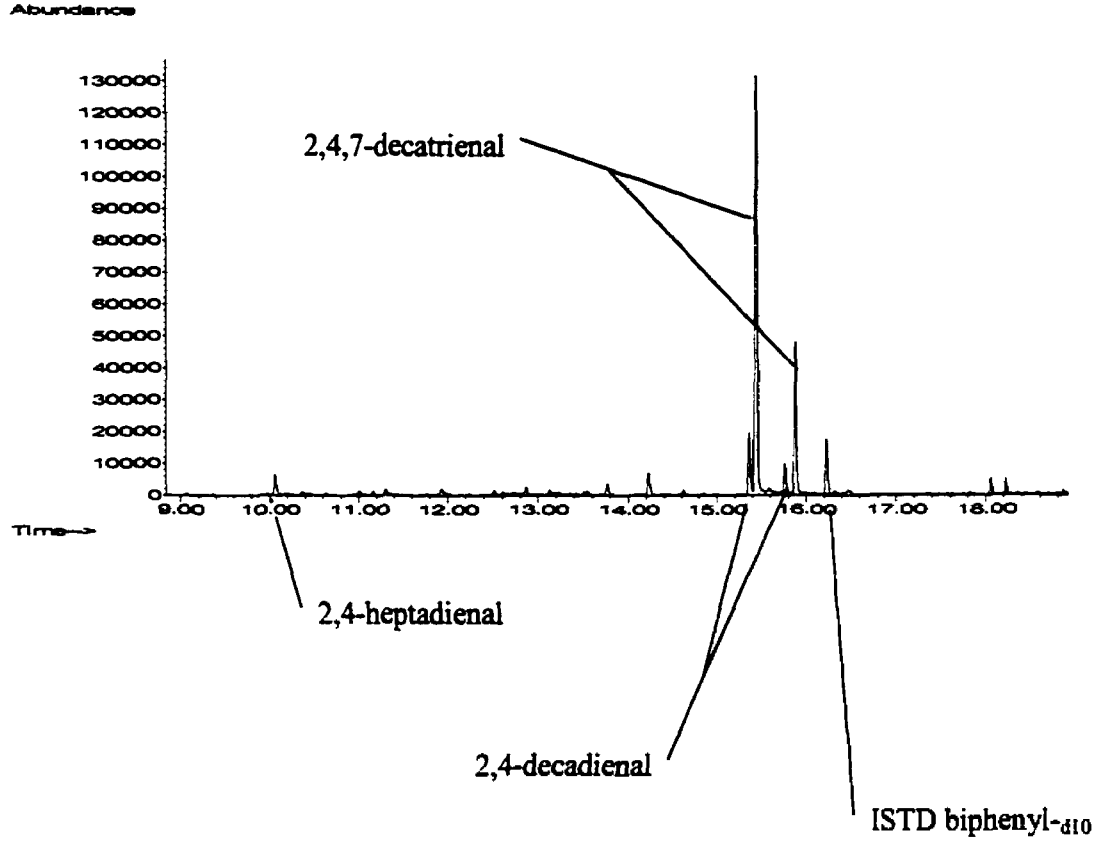


Figure A20 *Dinobryon cylindricum* monoculture. Major algal VOCs are 2,4-heptadienal, 2,4-decadienal and 2,4,7-decatrienal (extracted ion m/z 81). The E,Z and E,Z,Z isomers of 2,4-decadienal and 2,4,7-decatrienal elute first, followed by the E,E and E,E,Z isomer.

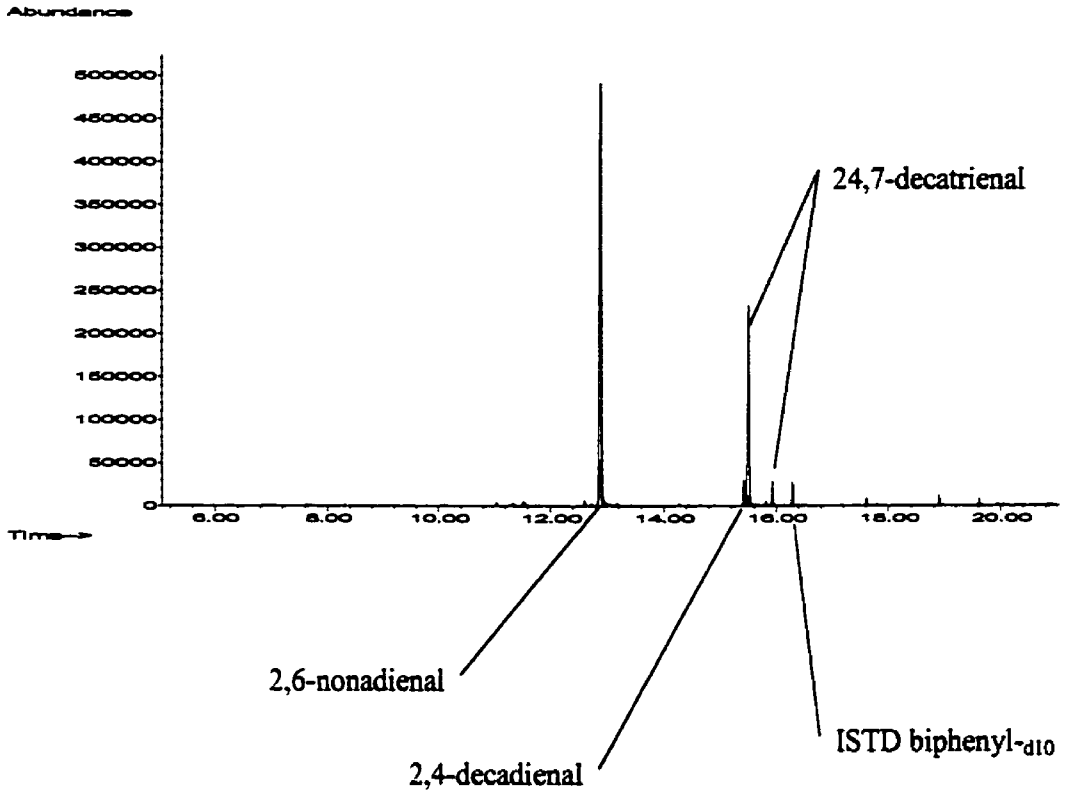


Figure A21 *Synura petersenii* monoculture. Major algal VOCs are E2,Z6-nonadienal (one isomer only, extracted ion m/z 69), 2,4-decadienal and 2,4,7-decatrienal (extracted ion m/z 81).

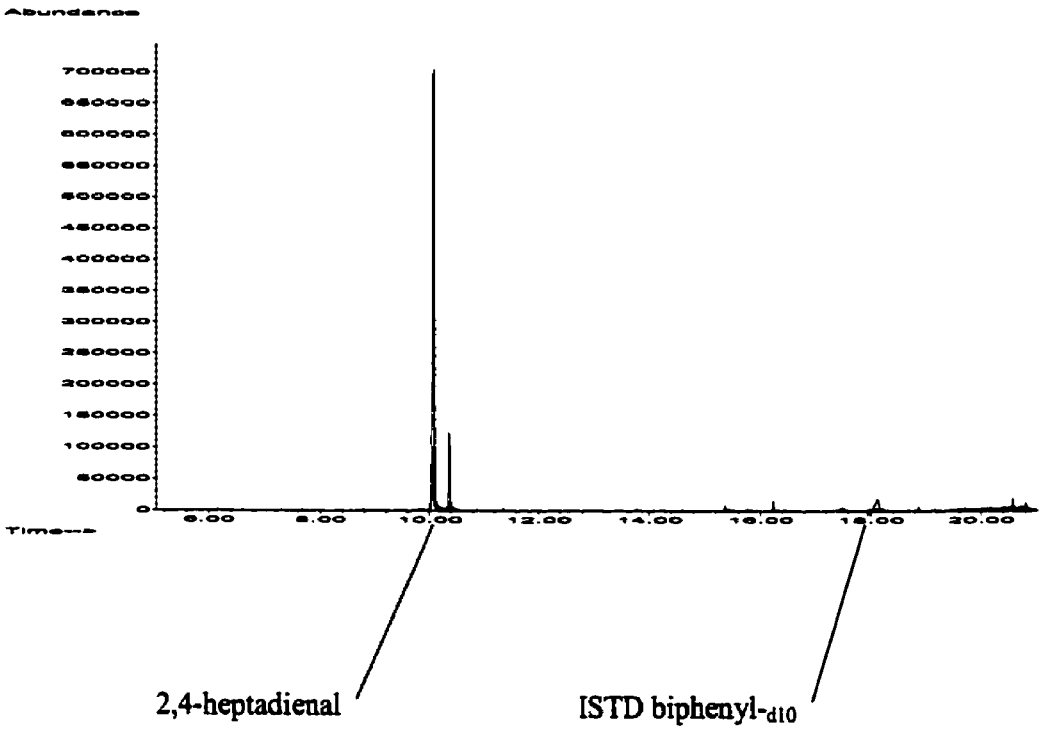


Figure A22 *Mallomonas papillosa* monoculture. The major algal VOC is 2,4-heptadienal (extracted ion m/z 81).

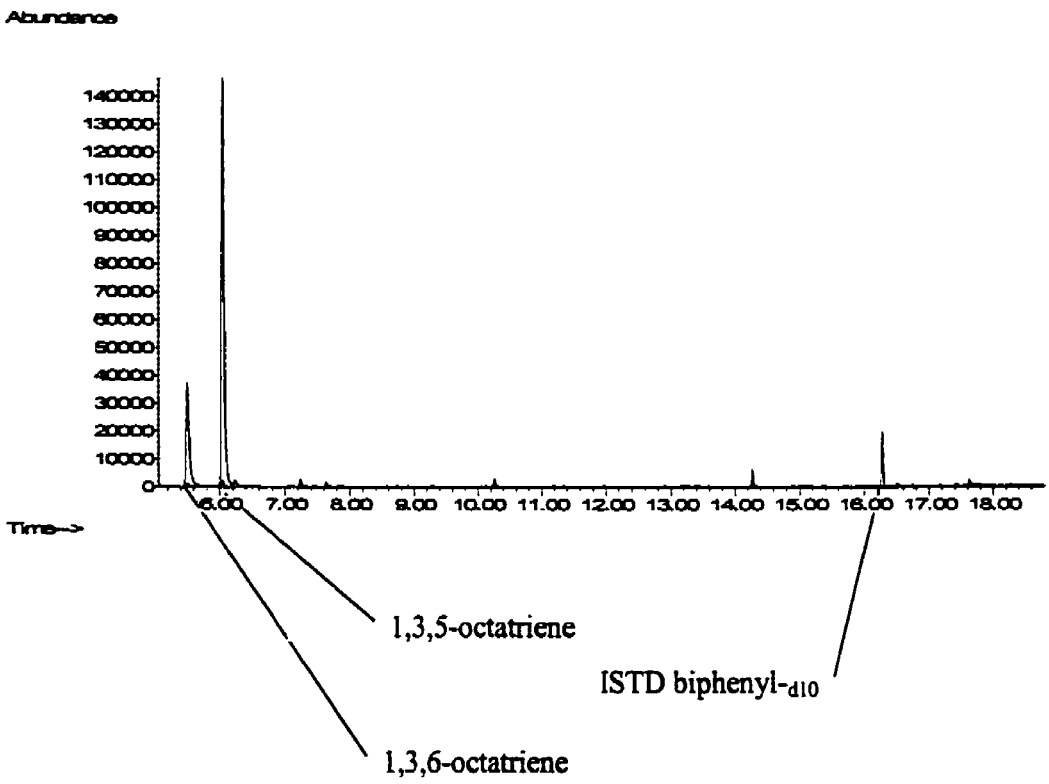


Figure A23 *Asterionella formosa* monoculture. The major algal VOC identified from this diatom were two isomers of octatriene (extracted ion m/z 79).

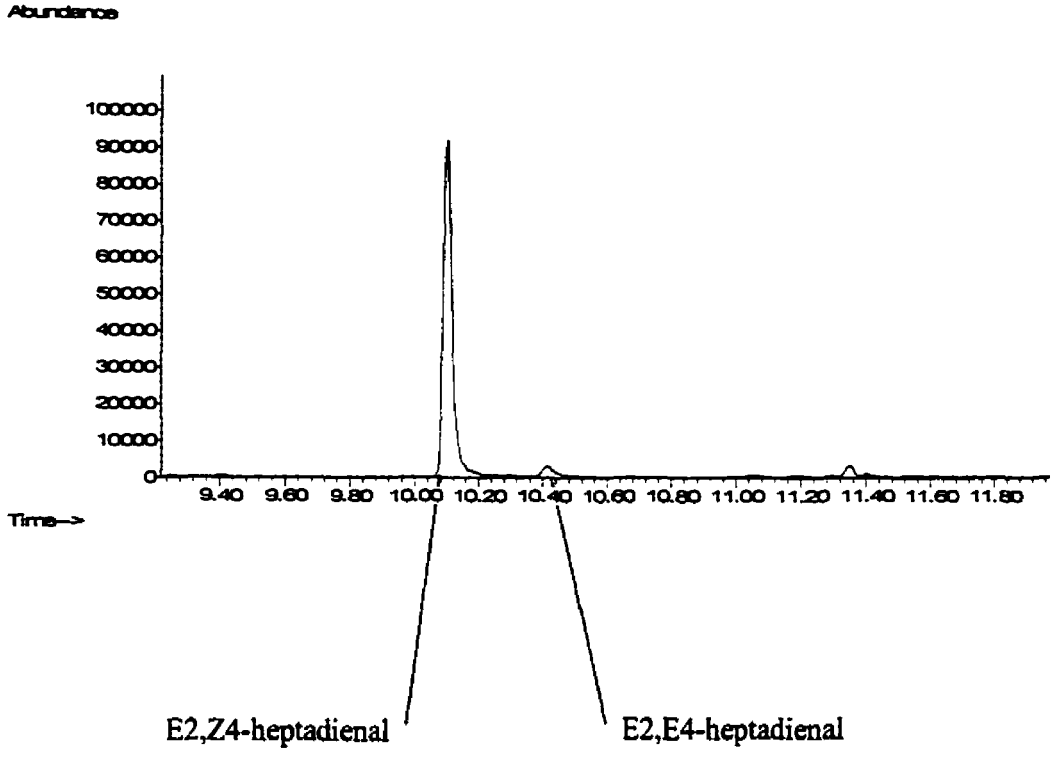


Figure A24 Extracted ion chromatogram (m/z 81) of a mixed algal culture. Two major isomers of the 2,4-heptadienal are present. The biologically formed EZ are the major peaks. The more stable EE isomer accounts for ca. 5% of total mass. The sample was analyzed immediately after collection to minimize isomerization.

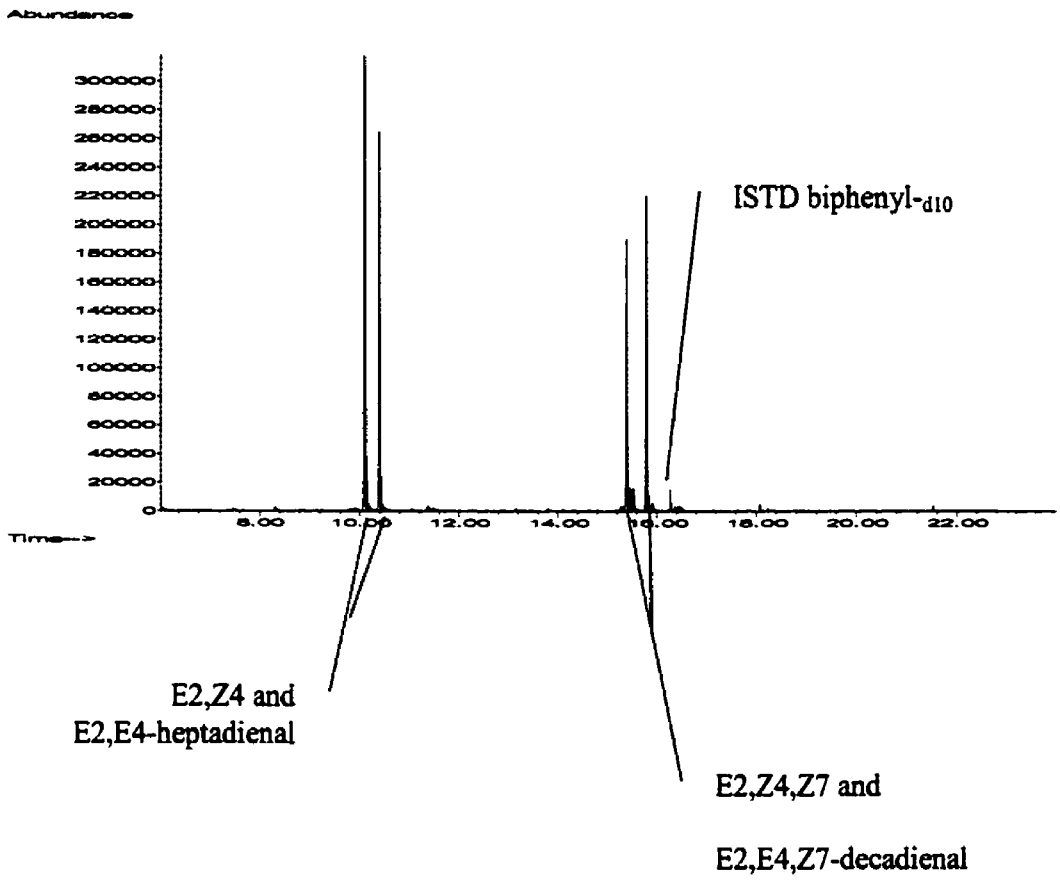


Figure A25 Under-ice sample from the Glenmore Reservoir headpond (January 13, 2000) during a bloom of *Dinobryon divergens*. A 500 mL sample was concentrated by filtration and stored frozen until analysis on January 22, 2000. Significant isomerization of the two major constituents, heptadienal and decadienal has occurred (extracted ion m/z 81).

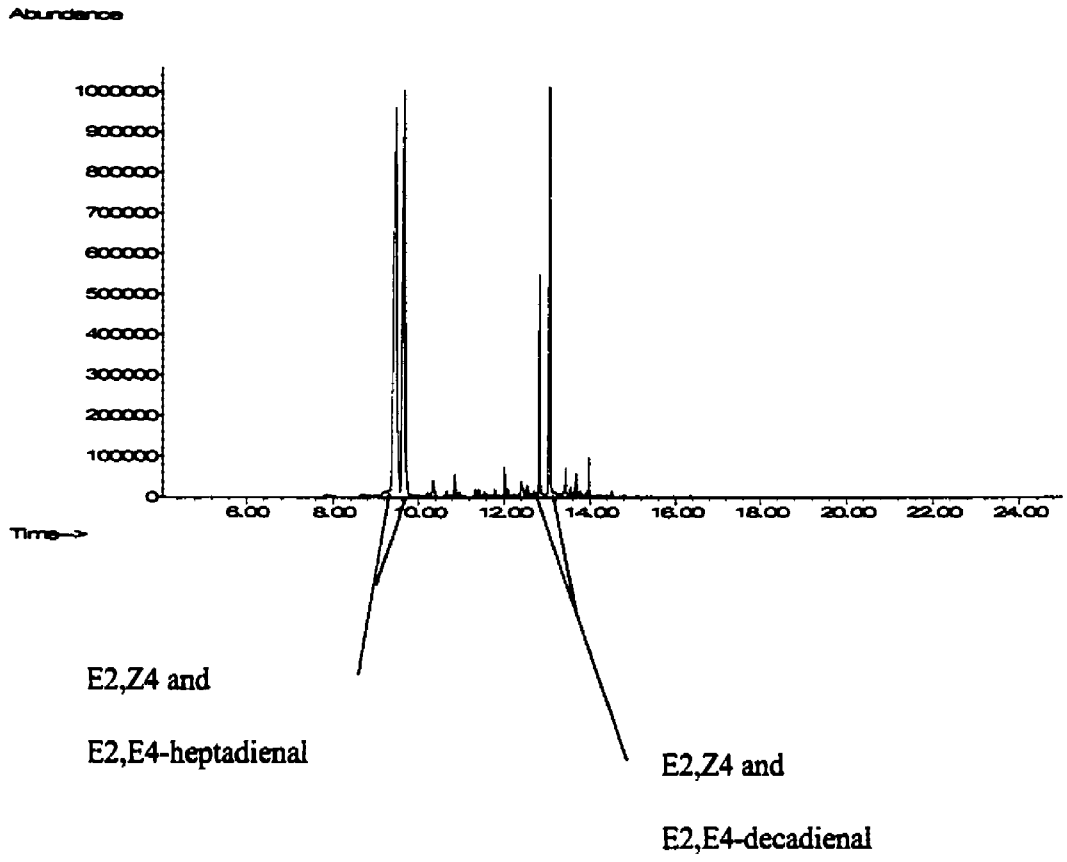


Figure A26 Phytoplankton tow-net sample collected from the Glenmore Reservoir headpond (June 14, 2000) during an open-water bloom of *Urogelena americana*. The major compounds are heptadienal and decadienal. Due to the concentrated nature of this sample, the internal standard is not resolved. Analysis was conducted on a DB5-ms column, with different chromatographic retention times than the normally used DB-1701 column (extracted ion m/z 81).

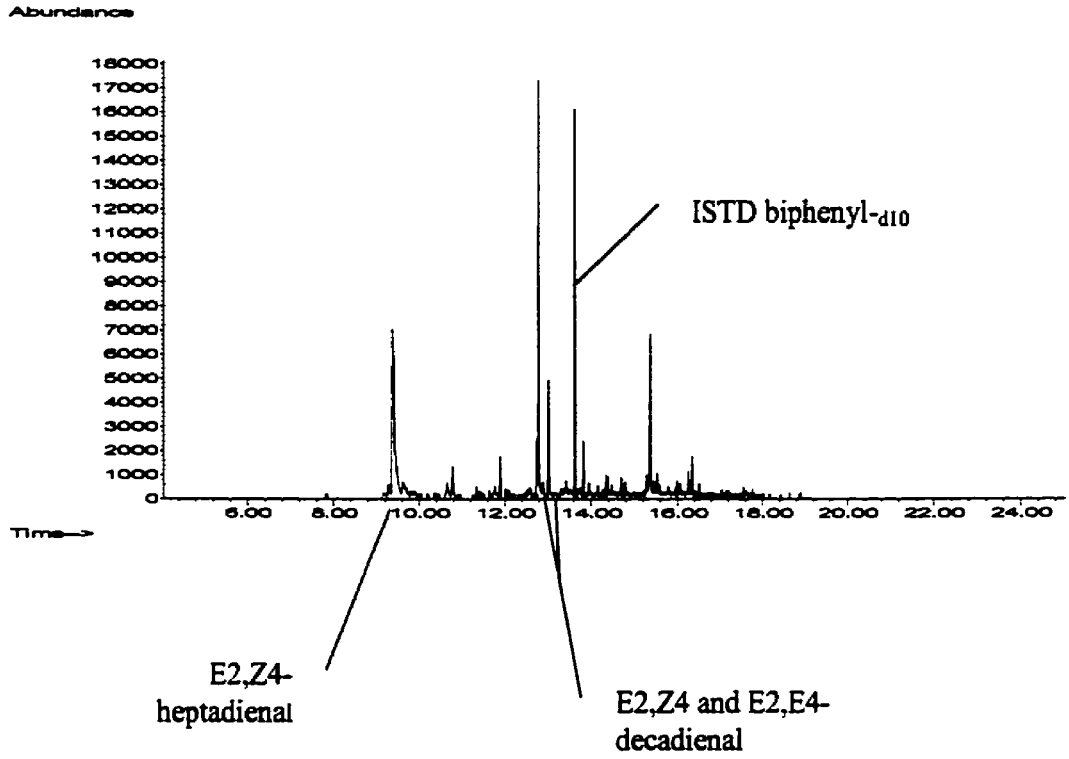


Figure A27 Integrated tube sample (10 m) collected from the Glenmore Reservoir (June 14, 2000) during an open-water bloom of *Urogelena americana*. The major compounds are heptadienal and decadienal. Analysis was conducted on a DB5-ms column, with different chromatographic retention times than the normally used DB-1701 column (extracted ion m/z 81).

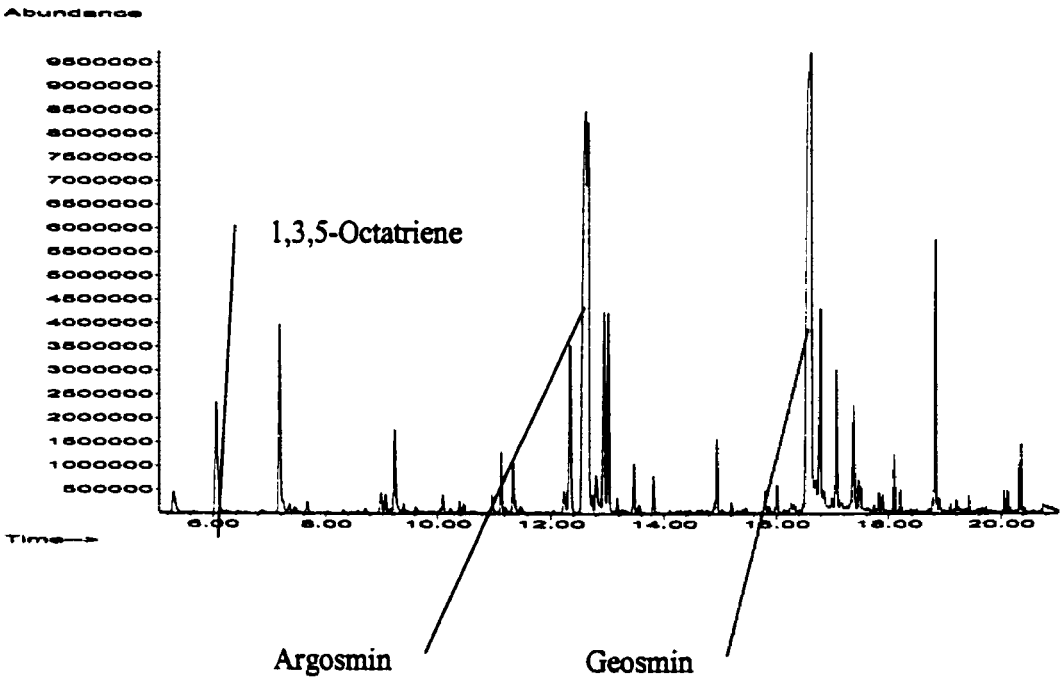


Figure A28 Sample collected from Pine Lake, AB during a bloom of the blue-green algae *Gleotrichia* (July 19, 2000). Geosmin and the geosmin dehydration product, argosmin are present (Full scan mass spectrum, m/z 40-300).

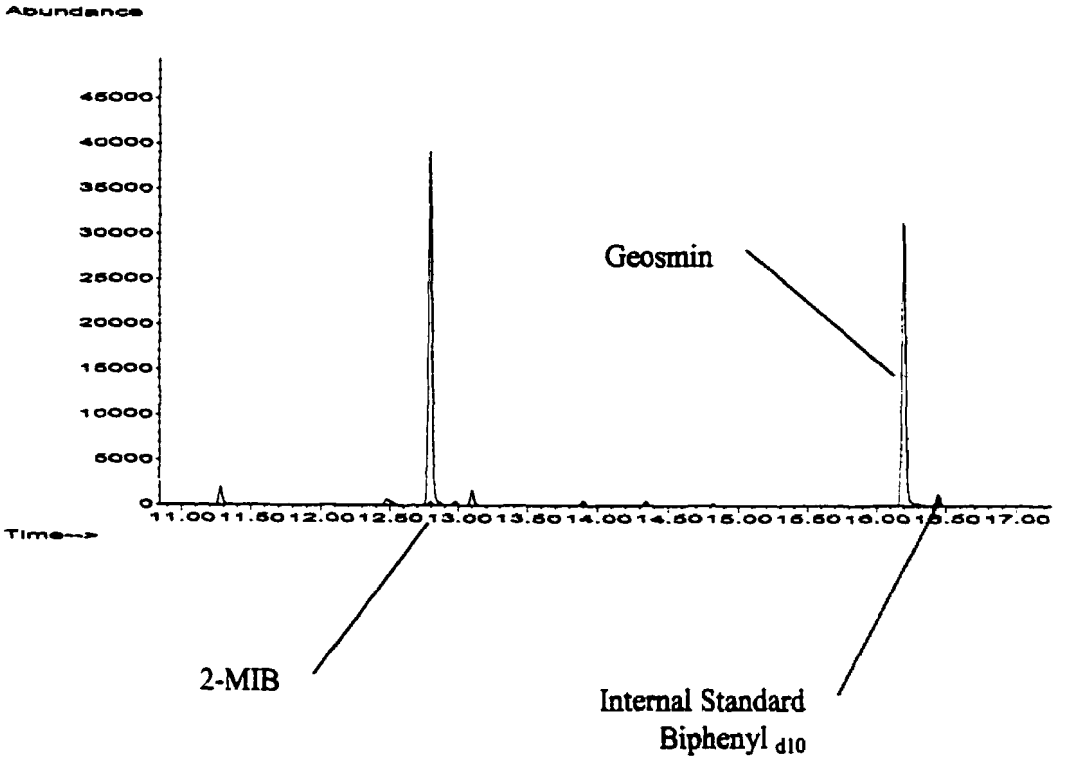


Figure A29 Cultured sample of the bacteria *Actinomycetes*. The organism produces geosmin and 2-MIB. Extracted ions m/z 112 and 95, for geosmin and 2-MIB, respectively.

APPENDIX B: CALIBRATION AND DETECTION LIMIT STUDIES

B.1 Calibration

The method was calibrated for (compounds) from the estimated detection limit up to the maximum expected sample concentration. The calibration curve was constructed based on the method of internal standards where response is corrected by internal standard recovery. Typically, the fully deuterated biphenyl-d_{10} was added to provide a concentration of 100 $ng \cdot L^{-1}$ in all samples and standards prior to analysis. Peak integration was automatically performed by the chromatography software.

B.1.1 Terpenoids

Geosmin and 2-MIB were calibrated from 2-100 $ng \cdot L^{-1}$ and analyzed by selected ion monitoring. As the OTC for these compounds is near 10 $ng \cdot L^{-1}$, it was important to calibrate to near or below the OTC.

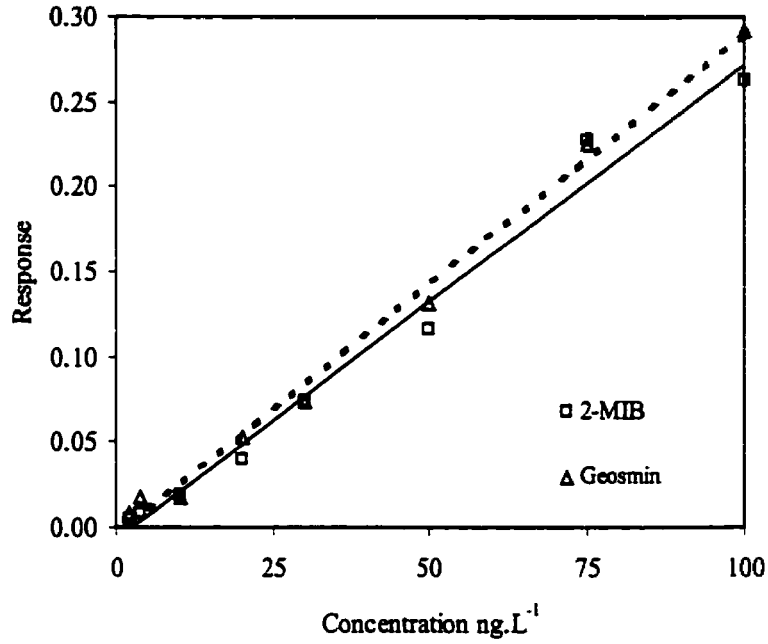


Figure B1 Calibration curve for geosmin and 2-MIB from standard solutions prepared in reagent water. Relative response corrected by internal standard recovery is plotted. The least squares regression line are shown (dashed line: geosmin, solid line: 2-MIB).

Table B1 Calibration data for geosmin and 2-MIB. Internal standard recovery was used to correct response.

Calibration Level	Compound	
	MIB	Geosmin
($ng \cdot L^{-1}$)	Relative Response	
2	0.0052	0.0078
4	0.0087	0.0178
10	0.0192	0.0175
20	0.0398	0.0526
30	0.0741	0.0737
50	0.1165	0.1314
75	0.2277	0.2251
100	0.2626	0.2924

B.1.2 Aldehydes

The four unsaturated aldehydes were calibrated from 500-10,000 $ng \cdot L^{-1}$ and analyzed by in full scan mode. The OTC for these compounds is higher (50 – 15,000 $ng \cdot L^{-1}$) then for geosmin and 2-MIB. The facile isomerization of the aldehydes neccesitated the collection of full mass spectroscopic data for compound confirmation.

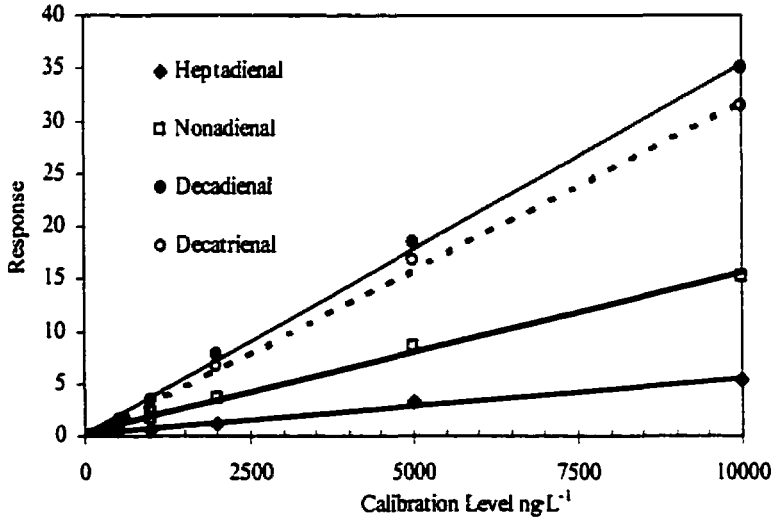


Figure B2 Calibration curve for heptadienal, nonadienal, decadienal and decatrienal from standard solutions prepared in reagent water. Relative response corrected by internal standard recovery is plotted. The least squares regression lines are shown.

Table B2 Calibration data for heptadienal, nonadienal, decadienal and decatrienal. Internal standard recovery was used to correct response.

Calibration Level ($ng \cdot L^{-1}$)	Compound			
	Heptadienal	**Nonadienal**	**Decadienal**	**Decatrienal**
	Relative Response			
500	0.494	0.8354	1.523	1.105
1000	0.631	1.7288	3.446	2.513
2000	1.145	3.6741	7.880	6.721
5000	3.214	8.6450	18.458	16.705
10000	5.316	15.324	35.083	31.362

Table B3 Linear regression data for calibration curves (Method of Least Squares).

Compound	Linear Regression Equation	R^2
Heptadienal	$y = 0.0005x + 0.2178$	0.989
Nonadienal	$y = 0.0015x + 0.4021$	0.995
Decadienal	$y = 0.0035x + 0.2599$	0.998
Decatrienal	$y = 0.0032x - 0.1459$	0.998
MIB	$y = 0.0028x - 0.0073$	0.984
Geosmin	$y = 0.0030x - 0.0051$	0.993

Table B4 Detection limit study for E2,E4-heptadienal.

Parameter:	**Theoretical Concentration**
E2,E4-heptadienal	**1000 ng·L^{-1}**
Analytical result	% Recovery
1066.0	106.6
1089.0	108.9
992.5	99.3
1065.5	106.6
750.0	75.0
1289.0	128.9
1224.0	122.4
921.0	92.1
821.0	82.1
Detection Limit	**508 ng·L^{-1}**
Standard Deviation:	175
Variance:	30728
Minimum Recovery:	75 %
Maximum Recover	129 %

Table B5 Detection limit study for E2,Z6-nonadienal.

Parameter:	**Theoretical Concentration**
E2,Z6-nonadienal	**1000 ng·L^{-1}**
Analytical result	% Recovery
927.0	92.7
924.0	92.4
977.0	97.7
1015.5	101.6
750.0	75.0
1341.0	134.1
1406.0	140.6
1074.5	107.5
870.0	87.0
Detection Limit	**622 ng·L^{-1}**
Standard Deviation:	215
Variance:	46115
Minimum Recovery:	75 %
Maximum Recover	141 %

Table B6 Detection limit study for E2,E4-decadienal.

Parameter:	**Theoretical Concentration**
E2,E4-decadienal	**1000 ng·L^{-1}**
Analytical result	% Recovery
893.0	89.3
956.0	95.6
1008.0	100.8
1020.0	102.0
970.5	97.1
1391.0	139.1
1324.5	132.5
1089.0	108.9
923.0	92.3
Detection Limit	**512 ng·L^{-1}**
Standard Deviation:	177
Variance:	31252
Minimum Recovery:	89 %
Maximum Recover	139 %

Table B7 Detection limit study for E2,E4,Z7-decatrienal.

Parameter:	**Theoretical Concentration**
E2,E4,Z7-decatrienal	**1000 ng·L^{-1}**
Analytical result	% Recovery
878.0	87.8
960.0	96.0
900.0	90.0
948.5	94.9
928.0	92.8
1429.0	142.9
1185.0	118.5
1062.5	106.3
831.0	83.1
Detection Limit	**545 ng·L^{-1}**
Standard Deviation:	188
Variance:	35425
Minimum Recovery:	83 %
Maximum Recover	143 %

Table B8 Detection limit study for 2-MIB.

Parameter:	**Theoretical Concentration**
2-MIB	**100 ng·L^{-1}**
Analytical result	% Recovery
87.8	87.8
92.6	92.6
92.9	92.9
93.4	93.4
95.6	95.6
100.0	100.0
101.5	101.5
108.8	108.8
113.7	113.7
Detection Limit	**24 ng·L^{-1}**
Standard Deviation:	8
Variance:	70
Minimum Recovery:	88 %
Maximum Recover	114 %

Table B9 Detection limit study for geosmin.

Parameter:	**Theoretical Concentration**
Geosmin	**100 ng·L^{-1}**
Analytical result	% Recovery
92.6	92.6
92.8	92.8
91.1	91.1
100.3	100.3
88.8	88.8
98.7	98.7
100.4	100.4
109.8	109.8
108.9	108.9
Detection Limit	**22 ng·L^{-1}**
Standard Deviation:	8
Variance:	57
Minimum Recovery:	89 %
Maximum Recover	110 %