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EFFECT OF HIGH IRRADIANCE AND IRON ON VOLATILE ODOUR COMPOUNDS IN THE CYANOBACTERIUM *MICROCYSTIS AERUGINOSA*

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Key Word Index—*Microcystis aeruginosa*; cyanobacterium; heptadecane; iron; irradiance; photoxidation; terpenoids; volatile odour compounds.

Abstract—The cyanobacterium, *Microcystis aeruginosa* was exposed to direct sunlight for 3, 6 or 9 h in media containing either low or high concentrations of iron, in order to determine any effects on the composition of volatile odour compounds (VOCs) released under photooxidative conditions. The most abundant VOCs detected included aliphatic hydrocarbons (C_{15}–C_{21}), naphthalene and the terpenoid compounds, β-cyclocitral, and β-ionone. Exposure to sunlight and low iron concentrations resulted in a decrease in β-cyclocitral, β-ionone, heptadecane and the total VOCs concentration after 9 h with respect to the control cultures. Six VOCs detected in the low iron cells were not detected in any of the high iron cells. However, those VOCs present in the high iron cells, in general, occurred at higher concentrations than the equivalent low iron cells after exposure to the sunlight conditions. Consequently, it was concluded that exposure to both high irradiance and high iron concentrations influenced the VOCs composition in cyanobacteria and this was interpreted to represent a cellular change during the photooxidation-promoting conditions.

INTRODUCTION

The presence of cyanobacteria in Australian waters is often implicated in the production of unpleasant tastes and odours, whether through excretion of volatile odour compounds (VOCs), such as geosmin or 2-methylisoborneol (MIB), or as a result of bacterial decay of surface scums [1–3]. VOCs have been associated with many different types of phytoplankton, including cyanobacteria, algae and diatoms, when these species periodically reach bloom proportions in the freshwater environment [4–7]. Consequently, the presence of VOCs in recreational or drinking water in Australia often leads to public concern, which has stimulated research investigating the chemical nature of these compounds.

Volatile products of cyanobacteria can be classified as *nor*-carotenoids (degradation products of carotenoids or ketones and alcohols with irregular terpene structures), fatty acid degradation products, such as aldehydes, that may be liberated during lipoxygenase action or lipid peroxidation, metabolites released during growth, terpenoids, sulfur compounds, aromatic compounds and amines [8–11]. The terpenoids are secondary metabolites of higher plants and microorganisms and, like the sterols and carotenoids, are synthesised from isoprenoid units by the mevalonate pathway [12]. The function of terpenoids varies widely from their use in plants as an insect attractant during pollination, a natural insect repellent and as an inhibitor of seed germination and plant growth [13]. There is also evidence indicating that the production of volatile and terpenoid compounds, such as geranyl acetone, provide a competitive advantage of some phytoplankton species as allelopathic agents, by inhibiting the growth of surrounding phytoplankton [13, 14]. Additionally, volatile compounds have been implicated as chemical messengers in the aquatic environment, particularly as attractants during fertilisation of marine *Phaeophyceae* ova [4, 15]. These products include octadiene, ectocarpene (6-(*cis*-1-butenyl)cyclohepta-1,4-diene), dictyopterene A (*trans*-1-(*trans*-1-hexenyl)-2-vinylcyclopropane) and dictyopterene C′ (6-butylcyclohepta-1,4-diene), and these have also been isolated from algal phytoplankton samples [4, 15].

The most abundant VOCs detected in *Microcystis* species taken from the field are β-cyclocitral, β-ionone, some terpenoid-derived compounds and aliphatic

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hydrocarbons [1, 3, 11]. However, there is little laboratory-based research investigating the effects of parameters, such as irradiance and nutrient availability, on the production and/or content of VOCs from cultured strains. Presumably, since *nor*-carotenoids and fatty acid derivatives are degradation products of membrane components, exposure of cyanobacteria to prolonged irradiance could lead to their increased production. In addition, carotene-oxygenase activity, responsible for specific carotenoid cleavage, is stimulated in the presence of ferric ions [10, 16]. Therefore, the presence of pro-oxidant forms of iron (both Fe^{2+} and Fe^{3+}) [17] may also be responsible for alterations in the production of VOCs. Iron concentrations vary widely within natural surface waters of Australian waterways [18]. Therefore, the aims of the present study were to investigate the production of VOCs from a laboratory strain of *M. aeruginosa* exposed to prolonged periods of sunlight, in the presence of low and high concentrations of iron.

RESULTS

Composition of volatiles

The lipophilic volatile fraction from *M. aeruginosa* consisted mainly of either cyclic components (such as β-cyclocitral), terpenoids or hydrocarbon-based compounds (Fig. 1). A series of long-chain aliphatic hydrocarbons were detected ranging from pentadecane to henicosane. Heptadecane (Peak 22; Fig. 1) was the most abundant aliphatic hydrocarbon from all irradiance and iron treatments, ranging from 14 to 70% of the total volatile concentration (Tables 1 and 2).

There were nine compounds designated as degradation products of carotenoids or fatty acids (Tables 1 and 2). These included β-cyclocitral and β-ionone, and several short-chain compounds, such as 3-penten-2-one, 5-hexadecyne and 2,4-nonedienal. The terpenoids, cedrol (3*R*-octahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-6-ol) (Peak 17; mass spectrum Fig. 2A) and thujopsene (1,1a,4,4a,5,6,7,8-octahydro-2,4a,8,8-tetramethylcyclopropa[d]naphthalene) (Peak 11; mass spectrum Fig. 2B), were detected in all low and high iron cells exposed to the sunlight regime, ranging from 1.4–3.9% and 5.8–12.9%, respectively. Five naphthalene derivatives (Figs 2D, E and F) were detected in all the low iron cells and the high iron cells exposed to 3 and 6 h standard and sunlight irradiance. Several volatile compounds produced were not identified but had similar mass spectra to cedrol (Fig. 2A) and, hence, are referred to numerically as unknown terpenoids 1–4. Diethyl phthalate and *bis*(2-methylpropyl)phthalate (1) and (2) were detected in all the iron and irradiance treatments.

Sunlight exposure with low iron

The concentrations of volatile components from *M. aeruginosa* grown in the low iron medium in response to the sunlight or standard irradiance are summarised in Table 1. The *nor*-carotenoid, β-cyclocitral (Peak 3), was significantly lower in the low iron cells after exposure to 3, 6 and 9 h sunlight, compared with the corresponding cells exposed to the standard irradiance (Fig. 3A). β-Ionone (Peak 8) was also lower in the cells exposed to sunlight for the 3 and 6 h periods, compared with the equivalent standard irradiance cells (Fig. 3B). β-Cyclocitral and β-ionone from the standard irradiance cells increased by a factor of 5 between the 6 and 9 h exposure periods. However, there were no similar alterations observed in β-cyclocitral and β-ionone concentrations in the corresponding cells exposed to the sunlight regime.

The two naphthalene-derived compounds, 1,2,3,4,5,6,7,8-octahydro-1,4,9,9-tetramethyl-4,7-methanoazulene (Peak 9; mass spectrum Fig. 2C), 1,2,3-trimethyl-4-propenylnaphthalene (1) (Peak 20), and an unknown terpenoid (4) (Peak 16) were significantly lower in *M. aeruginosa* exposed to sunlight after 3 h, compared with the standard irradiance cells. The concentration of pentadecane was significantly lower in the low iron cells after 6 h (but not 9 h) sunlight exposure, compared with the standard irradiance cells.

Many of the naphthalene-derived compounds were not significantly different between the sunlight and standard treatments. However, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl) naphthalene (Peak 14) was negatively correlated with time during the standard irradiance exposure ($P < 0.05$). 1,2,3-trimethyl-4-propenyl naphthalene (Peak 20) was positively correlated during the sunlight exposure in a time-dependent manner ($P < 0.05$) (Table 1). Production of heptadecane (Fig. 3C) and octadecane increased linearly with time ($P < 0.05$) in both the standard and sunlight regimes, whereas nonadecane and eicosane increased with time only during the standard irradiance regime (Table 1). Pentadecane decreased linearly with time in the low iron cells from both the standard and sunlight irradiance treatments ($P < 0.05$). The unknown terpenoids 1–4 were all negatively correlated with exposure time in the standard irradiance cells but were not correlated with exposure time in the sunlight treated cells (Table 1).

Although the amounts of VOCs produced by cells in the sunlight irradiance were always lower than those produced in the standard irradiance cells, the total concentrations of volatile components did not differ significantly between the sunlight and standard irradiance regimes after the 3, 6 and 9 h exposure periods. The production of the total volatile components in the cells exposed to the standard and sunlight irradiance treatments was positively correlated with time ($P < 0.05$) (Table 1, Fig. 3D).

Sunlight exposure with high iron

The changes in the volatile components of *M. aeruginosa* grown in the high iron medium in response to

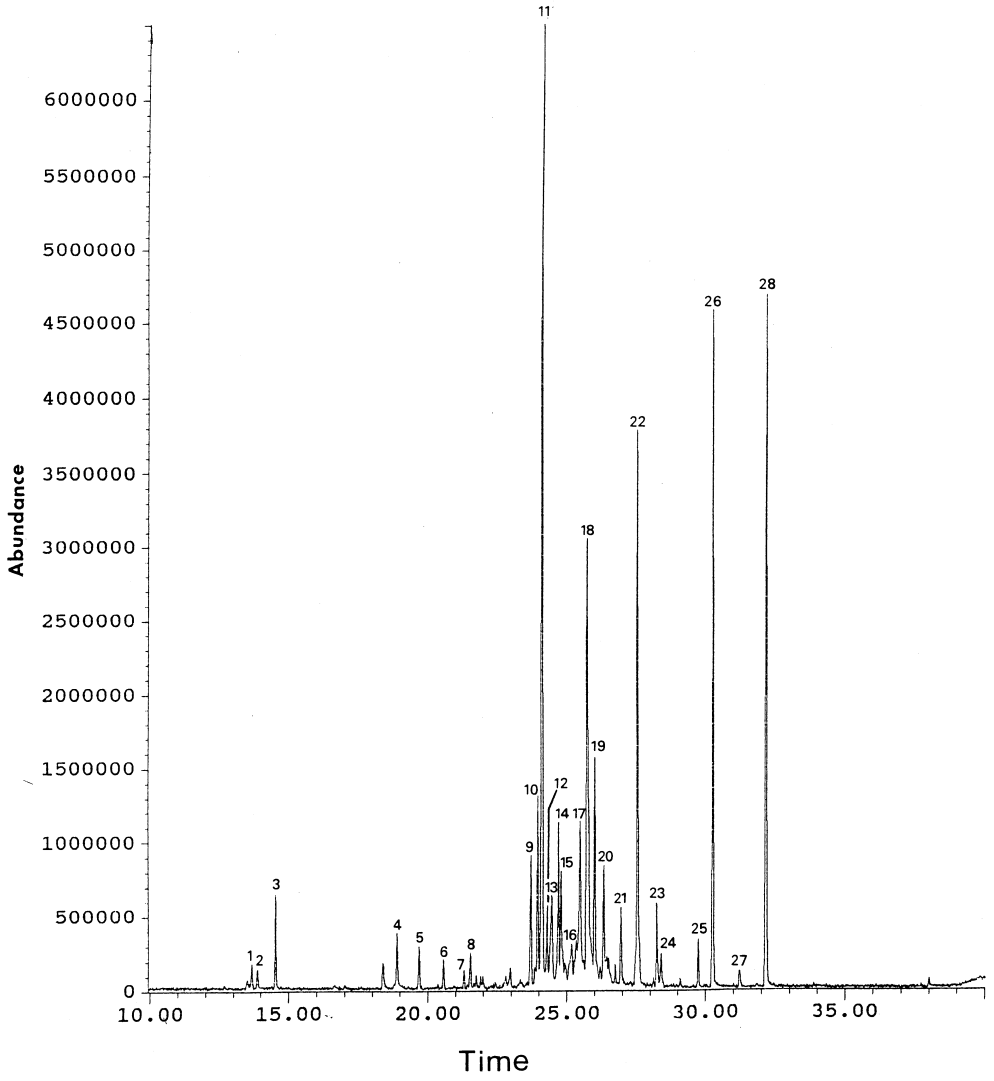

Fig. 1. Chromatogram of volatile components extracted after 6 h standard irradiance exposure from high iron *M. aeruginosa*. All volatile compounds that were assessed are listed below. Compounds that occurred at low abundances and are not presented on the chromatogram are numerically represented in elution order by $T_{1–10}$ = trace.

1 2,3-Dimethyl-1,4-hexadiene
2 1-Methanolcyclohexene
3 *β*-Cyclocitral
4 Propanoic acid, 2-methyl-3-hydroxy-2,4,4-trimethyl-pentyl ester
5 d^3-Geosmin (internal standard)
6 5-Hexadecyne
7 2,6-*bis*(1,1-dimethylethyl)-4-methylphenol
8 *β*-Ionone
9 1,2,3,4,5,6,7,8-Octahydro-1,4,9,9-tetramethyl-4,7-methanoazulene
10 Diethyl phthalate
11 Thujopsene
12 Unknown terpenoid 1
13 Unknown terpenoid 2
14 1,2,3,5,6,8a-Hexahydro-4,7-dimethyl-1-(1-methylethyl)naphthalene
15 Unknown terpenoid 3
16 Unknown terpenoid 4
17 Cedrol
18 1,2,3,4,4a,5,6,8a-Octahydro-7-methyl-4-methylene-1-(1-methylethyl)naphthalene$_{(1)}$
19 1,2,3,4,4a,5,6,8a-Octahydro-7-methyl-4-methylene-1-(1-methylethyl)naphthalene$_{(2)}$
20 1,2,3-Trimethyl-4-propenylnaphthalene$_{(1)}$
21 1,2,3-Trimethyl-4-propenylnaphthalene$_{(2)}$
22 Heptadecane
23 3-Penten-2-one
24 Cyclohexen-1-one relative
25 Octadecane
26 *bis*(2-Methylpropyl)phthalate$_{(1)}$
27 Nonadecane
28 *bis*(2-Methlpropyl)phthalate$_{(2)}$
*T*1 Naphthalene
*T*2 2,4-Nonedienal
*T*3 1,4-Diene-2,6-*bis*(1,1-dimethylethyl)-2,5-cyclohexadiene
*T*4 Pentadecane
*T*5 2-Methyl-, 1-(1,1-dimethylethyl)propanoic acid
*T*6 Hexadecane
*T*7 Benzoic acid, pentyl ester
*T*8 Eicosane
*T*9 Henicosane
*T*10 Propanetricarboxylic acid.

Table 1. Concentration of volatile components ($\mu g\ g^{-1}$ geosmin equivalents/dry wt) from the low iron *M. aeruginosa* exposed to the sunlight and standard irradiance

Peak no.	Volatile odour compounds	3 h Sunlight $n=4$ Mean	3 h Sunlight $n=4$ S.E.	3 h Standard $n=4$ Mean	3 h Standard $n=4$ S.E.	6 h Sunlight $n=4$ Mean	6 h Sunlight $n=4$ S.E.	6 h Standard $n=4$ Mean	6 h Standard $n=4$ S.E.	9 h Sunlight $n=5$ Mean	9 h Sunlight $n=5$ S.E.	9 h Standard $n=5$ Mean	9 h Standard $n=5$ S.E.	Correlations $P<0.05$ Sun	Correlations $P<0.05$ Standard
Breakdown products															
1	2,3-Dimethyl-1,4-Hexadiene	—	—	—	—	—	—	—	—	0.57	*0.35*	0.10	*0.10*		
3	*β*-Cyclocitral	**0.07	*0.07*	2.08	*0.35*	***0.02	*0.02*	2.00	*0.27*	**0.43	*0.28*	11.20	*2.90*		0.68
6	5-Hexadecyne	—	—	—	—	—	—	—	—	0.21	*0.21*	0.21	*0.21*		
8	*β*-Ionone	**0.08	*0.08*	0.88	*0.20*	***0.05	*0.03*	0.88	*0.02*	0.54	*0.34*	5.16	*2.20*		
23	3-Penten-2-one	0.54	*0.05*	0.63	*0.35*	0.02	*0.02*	0.03	*0.03*	1.83	*1.47*	2.17	1.68		0.65
24	Cyclohexen-1-one relative	—	—	0.37	*0.16*	—	—	—	—	0.73	*0.73*	—	—		
*T*2	2,4-Nonedienal	—	—	0.07	*0.07*	—	—	—	—	—	—	0.18	*0.18*		
*T*3	1,4-Diene-2,6-*bis*(1,1-dimethylethyl)-2,5-cyclohexadiene	—	—	0.06	*0.06*	0.09	*0.06*	0.17	*0.09*	—	—	0.25	*0.25*		
*T*10	Propanetricarboxylic acid	—	—	—	—	—	—	—	—	1.22	*1.22*	—	—		
Naphthalene/cyclic compounds															
2	1-Methanolcyclohexene	—	—	—	—	—	—	—	—	0.24	0.24	—	—		
4	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	0.58	*0.16*	0.68	*0.30*	0.24	*0.07*	0.42	*0.12*	0.96	*0.70*	0.68	*0.57*		
7	2,6-*bis*(1,1-Dimethylethyl)-4-methylphenol	1.45	*0.49*	0.98	*0.32*	0.06	*0.04*	0.13	*0.02*	0.31	*0.20*	1.13	*0.83*	−0.57	
9	1,2,3,4,5,6,7,8-Octahydro-1,4,9,9-tetramethyl-4,7-methanoazulene	*0.27	*0.11*	0.73	*0.15*	0.05	*0.03*	0.08	*0.06*	1.32	*1.01*	0.49	*0.37*		
11	Thujopsene	2.95	*0.41*	5.69	*1.41*	0.43	*0.15*	0.80	*0.34*	11.71	*6.17*	6.92	*3.45*		
12	Unknown terpenoid$_{(1)}$	0.22	*0.13*	0.73	*0.29*	0.02	*0.02*	0.04	*0.02*	0.90	*0.64*	—	—		−0.68
13	Unknown terpenoid$_{(2)}$	0.33	*0.21*	0.91	*0.23*	0.07	*0.04*	0.15	*0.07*	1.56	*1.01*	—	—		−0.80
14	1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)naphthalene	1.59	*0.67*	1.77	*0.55*	0.19	*0.08*	0.14	*0.04*	2.48	*1.01*	0.35	*0.35*		−0.57
15	Unknown terpenoid$_{(3)}$	0.21	*0.12*	0.77	*0.23*	0.04	*0.03*	0.23	*0.12*	0.97	*0.97*	—	—		−0.77
16	Unknown terpenoid$_{(4)}$	***—	—	0.37	*0.05*	0.03	*0.03*	0.05	*0.03*	0.89	*0.88*	—	—		−0.87
17	Cedrol	0.80	*0.17*	1.64	*0.37*	0.15	*0.05*	0.22	*0.11*	2.20	*2.20*	—	—		
18	1,2,3,4,4a,5,6,8a-Octahydro-7-methyl-4-methylene-1-(1-methylethyl)naphthalene$_{(1)}$	2.32	*0.39*	3.98	*0.79*	0.34	*0.15*	0.63	*0.30*	9.32	*4.28*	3.00	*1.14*		

19	1,2,3,4,4a,5,6,8a-Octahydro-7-methyl-4-methylene-1-(1-methylethyl)naphthalene$_{(2)}$	0.77	*0.11*	1.53	*0.36*	0.13	*0.05*	0.17	*0.09*	3.49	*1.64*	3.18	*2.36*		
20	1,2,3-Trimethyl-4-propenyl naphthalene$_{(1)}$	0.37	*0.15*	0.74	*0.15*	0.12	*0.05*	0.23	*0.09*	2.32	0.76	2.46	*1.14*	0.60	
21	1,2,3-Trimethyl-4-propenyl naphthalene$_{(2)}$	*0.28	*0.11*	0.84	*0.17*	0.10	*0.04*	0.22	*0.06*	1.32	0.69	1.26	*0.47*		
*T*1	Naphthalene	—	—	—	—	—	—	—	—	0.53	*0.32*	0.07	*0.07*		
*T*7	Benzoic acid, pentyl ester	—	—	—	—	—	—	—	—	—	—	0.87	*0.78*		
*T*5	2-Methyl-, 1-(1,1-dimethylethyl) propanoic acid	—	—	—	—	—	—	—	—	—	—	4.74	*3.35*		
Phthalates															
10	Diethyl phthalate	0.74	*0.24*	1.50	*0.74*	0.09	*0.06*	0.10	*0.06*	4.67	*3.66*	7.90	*5.29*		
26	*bis*(2-Methylpropyl)phthalate$_{(1)}$	2.82	*0.51*	4.50	*1.08*	0.54	*0.26*	0.94	*0.34*	8.72	*3.34*	11.00	*6.33*		
28	*bis*(2-Methylpropyl)phthalate$_{(2)}$	3.22	*0.66*	5.11	*1.13*	0.66	*0.34*	1.22	*0.41*	8.40	*3.54*	7.40	*1.23*		
Aliphatic hydrocarbons															
*T*4	Pentadecane	2.59	*0.98*	3.41	*1.06*	*0.54	*0.14*	2.66	*0.61*	—	—	—	—	−0.72	−0.76
*T*6	Hexadecane	0.70	*0.15*	0.92	*0.38*	*0.11	*0.04*	0.46	*0.09*	0.82	*0.53*	2.01	*0.70*		
22	Heptadecane	9.42	*0.93*	12.95	*4.70*	3.18	*0.56*	9.36	*2.29*	56.31	*11.40*	111.10	*29.81*	0.73	0.73
25	Octadecane	0.95	*0.31*	1.08	*0.36*	0.13	*0.02*	0.56	*0.21*	13.48	*3.01*	23.17	*5.32*	0.74	0.74
27	Nonadecane	0.33	*0.15*	0.43	*0.15*	0.04	*0.02*	0.04	*0.02*	0.70	*0.44*	3.27	*1.05*		0.62
*T*8	Eicosane	—	—	—	—	—	—	—	—	0.19	*0.19*	1.92	*0.91*		0.56
*T*9	Henicosane	—	—	—	—	—	—	—	—	—	—	1.49	*1.11*		
	Total volatile components	33.60	*7.50*	55.36	*16.14*	7.44	*2.47*	21.91	*6.36*	139.32	*53.45*	213.62	*74.24*	0.61	0.70

Significant differences between the standard and sunlight treatments for each time period are represented by $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$. Significant correlations (r values, $P < 0.05$) between VOCs and time are shown for the sunlight and standard irradiance treatments. Peak numbers correspond to those in Fig. 1.

Table 2. Concentration of volatile components ($\mu g\ g^{-1}$ geosmin equivalents/dry wt) from the high iron *M. aeruginosa* exposed to the sunlight and standard irradiance.

Peak no.	Volatile odour compounds	3 h Sunlight $n = 2$ Mean	3 h Standard $n = 3$ Mean	3 h Standard $n = 3$ S.E.	6 h Sunlight $n = 5$ Mean	6 h Sunlight $n = 5$ S.E.	6 h Standard $n = 5$ Mean	6 h Standard $n = 5$ S.E.	9 h Sunlight $n = 3$ Mean	9 h Sunlight $n = 3$ S.E.	9 h Standard $n = 3$ Mean	9 h Standard $n = 3$ S.E.
Breakdown products												
1	2,3-Dimethyl-1,4-Hexadiene	—	—	—	—	—	0.36	*0.36*	—	—	0.39	*0.39*
3	*β*-Cyclocitral	0.77	5.18	*2.73*	—	—	1.92	*1.23*	—	—	9.96	*1.61*
6	5-Hexadecyne	—	—	—	0.35	*0.35*	0.69	*0.43*	—	—	—	—
8	*β*-Ionone	—	2.37	*2.37*	0.45	*0.45*	0.84	*0.50*	—	—	—	—
23	3-Penten-2-one	—	—	—	3.38	*2.07*	2.17	*1.19*	3.60	*3.60*	3.80	*3.80*
24	Cyclohexen-1-one relative	1.89	—	—	1.29	*1.10*	0.89	*0.63*	—	—	1.46	*1.09*
*T*2	2,4-Nonedienal	—	—	—	—	—	—	—	—	—	—	—
*T*3	1,4-Diene-2,6-*bis*(1,1-dimethylethyl)-2,5-cyclohexadiene	3.62	1.54	*1.54*	—	—	—	—	—	—	—	—
*T*10	Propanetricarboxylic acid	1.41	3.19	*3.19*	3.41	*2.47*	5.47	*3.57*	1.49	*1.49*	—	—
Naphthalene/cyclic compounds												
2	1-Methanolcyclohexene	—	—	—	—	—	0.66	*0.45*	—	—	0.46	*0.46*
4	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	—	—	—	—	—	0.69	*0.69*	—	—	—	—
7	2,6-*bis*(1,1-Dimethylethyl)-4-methylphenol	2.39	5.91	*1.67*	*1.30	*0.71*	0.45	*0.37*	—	—	—	—
9	1,2,3,4,5,6,7,8-Octahydro-1,4,9,9-tetramethyl-4,7-methanoazulene	4.94	1.21	*1.21*	4.06	*1.90*	3.62	*2.10*	2.61	*2.61*	1.74	*1.44*
11	Thujopsene	38.64	12.19	*6.58*	39.18	*18.31*	26.42	*13.87*	39.81	*35.67*	20.13	*14.18*
12	Unknown terpenoid$_{(1)}$	3.45	0.69	*0.69*	3.24	*1.54*	2.62	*1.43*	3.23	*3.23*	1.25	*1.25*
13	Unknown terpenoid$_{(2)}$	7.14	1.29	*1.29*	5.66	*2.80*	4.36	*2.26*	7.24	*5.83*	2.00	*2.00*
14	1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)naphthalene	7.73	0.69	*0.69*	6.92	*3.83*	5.37	*2.57*	—	—	4.93	*3.39*
15	Unknown terpenoid$_{(3)}$	3.08	—	—	4.16	*2.14*	3.72	*2.08*	—	—	—	—

16	Unknown terpenoid$_{(4)}$	—	—	—	—	—	2.19	*1.51*	—	—	1.79	*0.65*
17	Cedrol	9.49	1.83	*1.83*	11.86	*6.11*	7.71	*5.49*	7.87	*7.87*	5.59	*4.31*
18	1,2,3,4,4a,5,6,8a-Octahydro-7-methyl-4-methylene-1-(1-methylethyl)naphthalene$_{(1)}$	31.24	8.91	*5.73*	33.15	*13.50*	17.84	*9.96*	23.58	*20.47*	11.64	*8.14*
19	1,2,3,4,4a,5,6,8a-Octahydro-7-methyl-4-methylene-1-(1-methylethyl)naphthalene$_{(2)}$	8.78	2.46	*1.60*	11.24	*4.98*	7.59	*3.92*	4.67	*4.67*	3.02	*2.44*
20	1,2,3-Trimethyl-4-propenyl naphthalene$_{(1)}$	10.44	2.00	*1.10*	8.09	*4.50*	4.78	*1.97*	4.45	*2.98*	1.97	*1.15*
21	1,2,3-Trimethyl-4-propenyl naphthalene$_{(2)}$	4.70	1.94	*1.07*	3.96	*2.12*	3.13	*1.35*	—	—	1.21	*0.81*
*T*1	Naphthalene	—	—	—	—	—	—	—	—	—	—	—
*T*7	Benzoic acid, pentyl ester	—	—	—	—	—	—	—	—	—	—	—
*T*5	2-Methyl-,1-(1,1-dimethylethyl)propanoic acid	1.21	2.62	*1.59*	3.10	*1.95*	—	—	—	—	—	—
Phthalates												
10	Diethyl phthalate	10.15	1.94	*1.94*	10.74	*3.63*	4.89	*2.87*	6.87	*6.87*	5.29	*4.08*
26	*bis*(2-Methylpropyl)phthalate$_{(1)}$	25.37	8.65	*5.13*	47.60	*22.01*	30.45	*8.49*	14.73	*12.70*	8.13	*5.44*
28	*bis*(2-Methylpropyl)phthalate$_{(2)}$	24.00	9.85	*5.19*	52.57	*28.74*	43.88	*17.14*	11.36	*9.67*	7.17	*4.85*
Aliphatic hydrocarbons												
*T*4	Pentadecane	—	—	—	—	—	—	—	—	—	—	—
*T*6	Hexadecane	*5.72	2.00	*1.01*	—	—	—	—	—	—	—	—
22	Heptadecane	124.67	68.29	*21.90*	42.09	*17.32*	79.28	*43.35*	*389.42	*164.91*	72.51	*15.08*
25	Octadecane	11.42	6.15	*2.52*	5.05	*2.15*	9.80	*6.02*	*33.12	*11.70*	4.54	*0.70*
27	Nonadecane	—	—	—	1.46	*1.46*	0.71	*0.44*	—	—	—	—
*T*8	Eicosane	—	—	—	—	—	—	—	—	—	—	—
*T*9	Henicosane	—	—	—	—	—	—	—	—	—	—	—
	Total volatile components	342.24	150.86	*72.59*	304.30	*146.19*	272.51	*136.22*	554.05	*294.28*	168.97	*77.26*

Significant differences between the standard and sunlight treatments for each time period are represented by *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. Peak numbers correspond to those in Fig. 1. There were no significant correlations between VOCs and time for the sunlight and standard irradiance treatments.

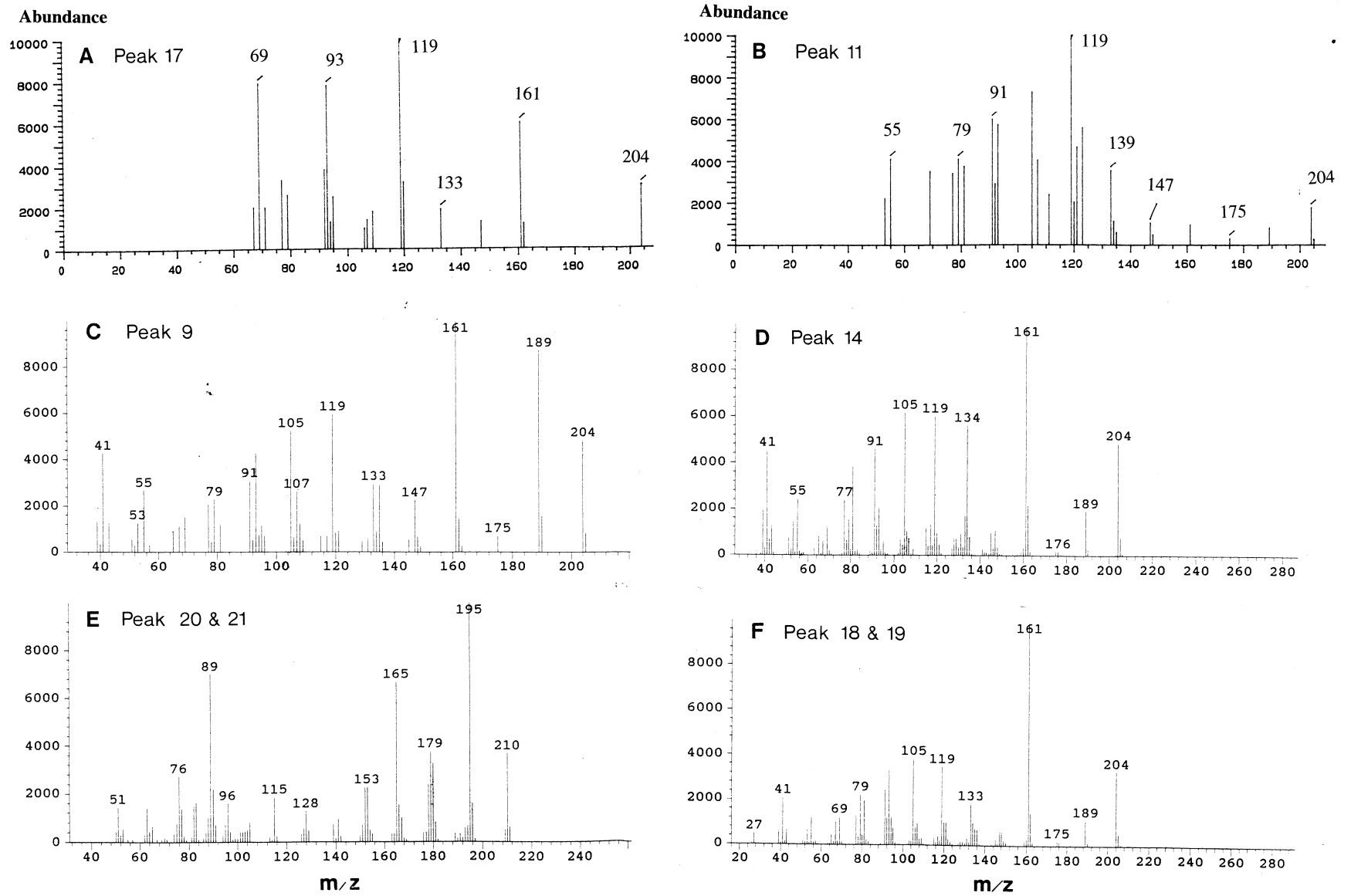

Fig. 2. Mass spectra of terpenoids and naphthalene derivatives detected in the volatile fraction of *M. aeruginosa*, A. Cedrol (Peak 17), B. Thujopsene (Peak 11), C. 1,2,3,4,5,6,7,8-Octahydro-1,4,9,9-tetramethyl-4,7-methanoazulene (Peak 9), D. 1,2,3,5,6,8a-Hexahydro-4,7-dimethyl-1-(1-methylethyl)naphthalene (Peak 14), E. 1,2,3-Trimethyl-4-propenylnaphthalene (Peak 20 and 21), F. 1,2,3,4,4a,5,6,8a-Octahydro-7-methyl-4-methylene-1-(1-methylethyl)naphthalene (Peak 18 and 19).

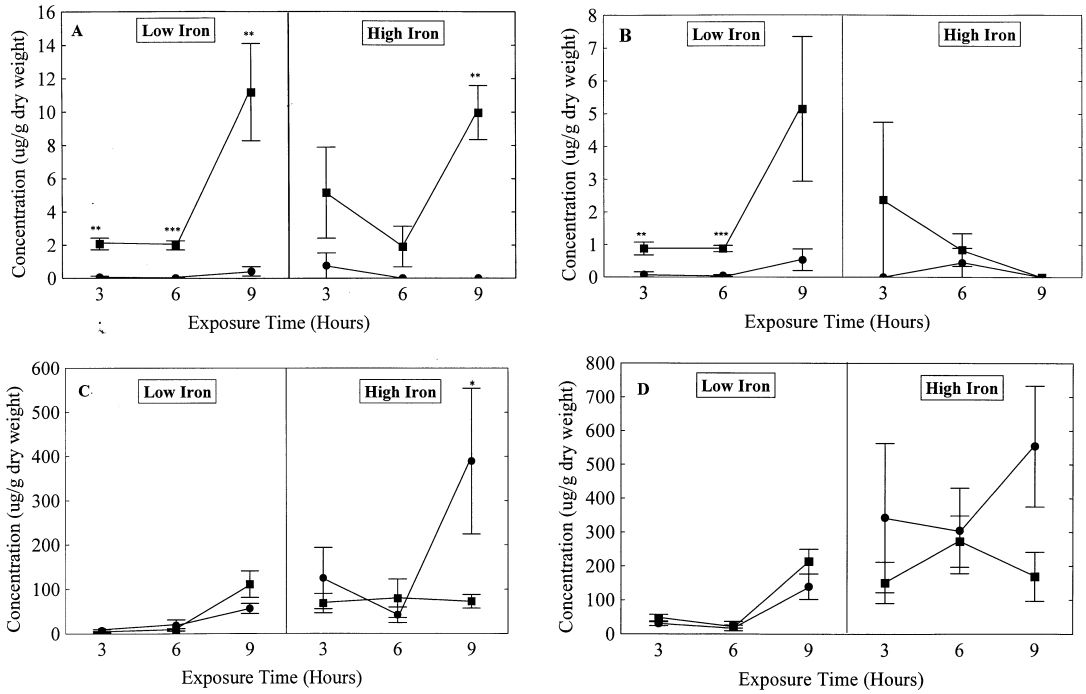

Fig. 3. Concentrations of (A) β-cyclocitral (Peak 3), (B) β-ionone (Peak 8), (C) heptadecane (Peak 22) and (D) the total volatile components extracted from *M. aeruginosa* grown in low and high iron media and exposed to either standard (■) or sunlight (●) conditions.

the sunlight and standard irradiance are summarised in Table 2. Six of the volatile compounds from the low iron cells were not detected in either the standard or sunlight irradiance cells after incubation in the high iron medium. However, those volatile components that were present in the high iron cells, generally occurred at greater abundances than the equivalent low iron cells. The high iron cells had a different VOC profile compared with the low iron cells (Tables 1 and 2). The total volatile concentration was greater in the high iron cells exposed to 9 h sunlight than the low iron cells exposed to 3 and 6 h standard and sunlight irradiance ($P < 0.05$) (Fig. 3D). Furthermore, the total volatile concentration from all the high iron cultures was significantly higher than all the low iron cultures ($P < 0.0001$, $n = 47$).

β-Cyclocitral decreased in cells exposed to both the sunlight and standard irradiance after the 6 h irradiance periods (Fig. 3A). However, after 9 h exposure to the standard irradiance, the production of this carotenoid degradation product increased significantly, compared with those cells from the sunlight regime. β-Ionone was detected in the 3 and 6 h standard exposures and the 6 h sunlight exposure, but was not detected in the other high iron samples (Fig. 3B).

Heptadecane and octadecane were the only aliphatic hydrocarbons detected in the high iron cells after 9 h standard or sunlight irradiance exposure (Table 2). Both of these compounds occurred in significantly greater concentrations after 9 h sunlight irradiance, compared with the cells exposed to the standard irradiance. The high iron cells exposed to 9 h sunlight had a significantly greater concentration of heptadecane than all the other irradiance treatments in both the low and high iron cells ($P < 0.05$) (Fig. 3C). By comparison, pentadecane, eicosane and henicosane were not detected in any of the high iron cells, whereas hexadecane and nonadecane were only detected at low concentrations in the 3 h and 6 h irradiance treatments, respectively.

2,6-*bis*(1,1-Dimethylethyl)-4-methylphenol (Peak 7) decreased with time in the high iron cells exposed to the sunlight irradiance ($P = 0.006$, $r = -0.77$, $n = 10$). Additionally, after 6 h sunlight irradiance, the concentration of 2,6-*bis*(1,1-dimethylethyl)-4-methylphenol (Peak 7) was significantly greater, compared with the equivalent standard irradiance, high iron cells (Table 2).

DISCUSSION

The production of a large range of volatile components from phytoplankton species has been well documented [7, 19–23]. Several VOCs detected in *M. aeruginosa* in the present study, such as β-cyclocitral, β-ionone, 3-pentene-2-one and the aliphatic hydrocarbons (Tables 1 and 2), were consistent with volatile components previously reported for *Microcystis* species [3, 11]. However, 6-methylhept-5-en-2-one and geranyl acetone were not detected in samples from this study. It may be possible that the production of these VOCs were reduced after successive generations in

culture. The production of the numerous naphthalene-derived or terpenoid compounds detected in our study has only been previously reported in field samples of *M. aeruginosa* [20, 21] and not in laboratory cultures. Comparison of the mass spectra of these naphthalene and terpenoid compounds, and their common fragmentation ions (such as $m/z = 119$, 161 and 204, Fig. 2), illustrate the structural similarity of these VOCs in *M. aeruginosa*. Furthermore, VOC composition and total volatile concentration altered with both high iron and high irradiance conditions, suggesting that photooxidative conditions may promote the production of nuisance VOCs from cyanobacterial sources within the natural environment.

Although phthalate derivatives can be indicative of sample contamination with plastics [24], diisoocytl phthalate, diethyl phthalate, diisobutyl phthalate and dibutyl phthalate have been detected in cyanobacterial extracts from a freshwater lake in Canada [20] and dimethyl phthalate is a metabolite of the fungus, *Gibberella fujikuroi* [25]. All care was taken to avoid the use of plastics during sample preparation. Additionally, the abundances of the phthalate components were observed to fluctuate in a similar manner to many of the remaining VOCs (with respect to the internal standard) during the irradiance and iron treatments. Consequently, the diethyl and *bis*(2-methylpropyl) phthalates detected in the cyanobacterial extracts were considered to be metabolic products of *M. aeruginosa*.

Terpenoids, such as cedrol (Peak 17), 1,2,3,4,5,6,7,8-octahydro-1,4,9,9-tetramethyl-4,7-methanoazulene (Peak 9) and thujopsene (Peak 11), were observed in the volatile fraction of *M. aeruginosa* from our study (Fig. 1). These terpenoids have previously been characterised from plant volatile extracts including cedar, cypress and the Japanese Hiba tree, respectively [25]. Cedrol, a C_{15} sesquiterpenoid, is also referred to as cypress or cedar camphor [25–27]. Some terpenoids are thought to be allelopathic agents. Exposure to volatile terpenes can lead to the accumulation of lipid globules in the cytoplasm, reduction in organelles, including mitochondria, and disruption of membranes surrounding mitochondria and nuclei [13]. The terpenoids observed in *M. aeruginosa* may be phytotoxic to other phytoplankton and/or zooplankton grazer species or may be released as a result of cellular stress initiated by photooxidation, but the exact function of these compounds still remains unclear.

Heptadecane was the major volatile component detected within the cells after exposure to the iron and irradiance treatments. The prominent appearance of heptadecane is a characteristic marker of most cyanobacteria [21] and may be an important constituent of their membrane. Production of long-chain alkanes occurs in higher plants, fungi, bacteria, algae, insects and animals, often as components of waxes (for a barrier against water-exchange) or as constituents in the myelin sheaths of peripheral nerves [28–30]. The formation of long-chain alkanes were originally thought to occur via decarboxylation of fatty acids [28]. However, although the reaction mechanism has not been fully elucidated, there is evidence that the production of long, odd-chain hydrocarbons, such as heptadecane, are derived during the enzymic decarbonylation of aldehydes generated from an even-chain, precursor fatty acid [29]. In addition, metal chelators, such as EDTA, were found to strongly inhibit the production of these alkanes, suggesting that the decarbonylase reaction is catalysed by a metalloenzyme [29, 30]. Consequently, the observed increase in the production and/or release of heptadecane after prolonged sunlight exposure in the high iron cells from our study, may be due to the stimulation of a metalloenzymic decarbonylase within the cyanobacteria.

The production of heptadecane from fatty acids via lipid peroxidation processes is unlikely to occur in *M. aeruginosa*. This is because during decarboxylation, the parent unsaturated fatty acid would be required to have a chain length equal to C_{20}–C_{22}, with a double bond close to the carboxyl group, to yield a hydrocarbon with a chain length of C_{17}. Since these types of fatty acids were not detected in abundance in the extracts from *M. aeruginosa* [31], the formation of the high concentrations of heptadecane observed in the present work are unlikely to have originated directly from photooxidation and lipid peroxidation. Further consideration as to the biochemical role and the mechanism of production of heptadecane in *M. aeruginosa* is therefore warranted.

Release of heptadecane into the surrounding water may act as an allelopathic agent as the general bioaccumulation of aliphatic hydrocarbons are well known to be toxic [24, 32, 33]. For example, unsaturated hydrocarbons, such as myrcene, limonene and α-phellandrene, have been reported to cause a 50% reduction in seed germination at concentrations of 2 mM [13]. α-Phellandrene has a structure resembling that of 1,4-diene-2,6-*bis*(1,1-dimethylethyl)-2,5-cyclohexadiene (T_3, Table 1 and 2) and may therefore have an allelopathic function in cyanobacteria against competing phytoplankton species. The volatile compound, 3-penten-2-one (Peak 23) may have a similar function as 4-methyl-3-penten-2-one (mesityloxide), which is produced and excreted in large quantities by the cyanobacterium, *Anabaena cylindrica* [11]. Mesityloxide is thought to be a metabolite originating from fatty acid biosynthesis in cyanobacteria [11]. Growth rates of cyanobacteria (*Synechococcus*) are not affected by concentrations up to 100 $\mu g\ l^{-1}$ of mesityloxide [11]. Consequently, this volatile compound may be either a degradation product of fatty acids and/or be used as a defence against other species.

Alterations in the production of volatile components were observed during exposure to the sunlight irradiance in the high iron cells. The increase in the concentration of the total volatile components after exposure to both the sunlight and standard irradiance

in *M. aeruginosa* was predominantly due to the increase in heptadecane (Peak 22), 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl) naphthalene (1) and (2) (Peaks 18 and 19), and thujopsene (Peak 11). The increase in the total volatile components with time in the low iron cells during the sunlight exposure and the high iron cells during both the irradiance regimes, suggests that the length of irradiance exposure is important in the production and release of VOCs (Tables 1 and 2). Furthermore, alterations in the concentration of numerous individual volatile components were observed to be time-dependent, particularly in the low iron cells. These time-dependent alterations in the volatile composition of *M. aeruginosa* were concluded to occur primarily in response to sunlight irradiance in the low iron cells. In addition, several VOCs were not detected in the high iron cells after either the standard or sunlight irradiance, demonstrating that incubation in the high iron media promoted a different VOCs profile, compared with the low iron cells.

Cleavage of β-carotene by β-carotene 7,8 (7′,8′)-oxygenase stoichiometrically yields two molecules of β-cyclocitral and one molecule of crocetindial [10, 16]. However, the oxidative cleavage of β-carotene during increased oxygen concentrations may also yield *nor*-carotenoid products including the β-*apo*-carotenal series [34] and β-ionone [14]. *Nor*-carotenoids have a demonstrated antimicrobial, growth modifying activity against photoautotropic organisms, bacteria and fungi [14]. If these *nor*-carotenoids play an active allelopathic role for *M. aeruginosa*, then their production would presumably be under tight regulation and not rely entirely on photooxidation of carotenoids. β-Cyclocitral and β-ionone may be formed by a carotene-oxygenase or by photooxidative degradation of β-carotene, in addition to other cleavage products of the β-*apo*-carotenal series. It was not possible from the data obtained to determine the mechanism of production of these *nor*-carotenoids but they did indicate that the concentrations of β-cyclocitral and β-ionone were related to the concentration of β-carotene in the low iron cells (Table 1 and [35]).

The concentrations of β-cyclocitral and β-ionone (Figs 3A and B) from the low and high iron cells exposed to the sunlight irradiance were lower than the respective standard treatments for 3, 6 and 9 h exposure time. In contrast, β-cyclocitral and β-ionone have been detected in natural Australian waters in association with specific blooms of cyanobacteria [3]. The presence of β-ionone has been shown to activate the biosynthesis of β-carotene [14]. Alterations in the concentrations of *nor*-carotenoids from *M. aeruginosa* were concluded to result from time-dependent exposure to sunlight irradiance, which was further enhanced in the presence of high iron concentrations.

The detection of degradation products, such as alkenes, alkynes, ketones and alkadienals (Tables 1 and 2) is evidence for the peroxidation of unsaturated fatty acids in *M. aeruginosa* exposed to photooxidative conditions [36, 37]. Saturated and unsaturated, short-chained alkanes, alkenes, ketones and reactive aldehydes (such as alkenals, 2-alkenals and 2,4-alkadienals), are recurrent oxidation products of fatty acid chain cleavage [9, 37, 38]. The increase in the alkyne and alkadiene components, 2,3-trimethyl-1,4-hexadiene (Peak 1); 5-hexadecyne (Peak 22) and 1,4-diene-2,6-*bis*(1,1-dimethylethyl)-2,5-cyclohexadiene (T_3), as well as the ketone, 3-penten-2-one (Peak 23), with time, in the low iron cells may be due to prolonged irradiance exposure. However, lipid peroxidation radical products are known to be short-lived ($\geqslant 10^{-9}$ s) [17]. Many of the degradation products detected were absent from the high iron cells exposed to 9 h irradiance, suggesting that under these conditions, formation and consequently breakdown of these degradation compounds may have occurred more rapidly. In addition, the formation of more stable peroxidation by-products, such as malondialdehyde, traditionally detected using non-volatile extracts, can reach a maximum concentration after only 1 h (in rat liver microsomes) [39, 17], compared with the 9 h exposure periods in our study. Consequently, the detection of lipid degradation products was proposed to be reliant on the length of irradiance exposure and the concentration of pro-oxidant cations, such as iron.

In conclusion, alterations in the production of VOCs were observed in the cells exposed to sunlight and/or incubated in the high iron media compared to those cells from the standard conditions. Furthermore, the concentration of many volatile components increased with sunlight exposure over time in the low and high iron cells. *Microcystis aeruginosa* exposed to the high iron concentrations produced significantly higher concentrations of the total VOCs than the equivalent low iron cells after 9 h exposure time. Therefore, exposure to high solar irradiance and high iron concentrations altered the volatile composition of the cyanobacteria.

An increase in the concentration of VOCs from cyanobacterial sources as a result of natural photooxidative conditions prevalent during the Australian summer months, could lead to problematic taste and odour problems in water supplies. Furthermore, there is potential to use VOCs as sensitive biomarkers for some species of cyanobacteria [3]. Consequently, further examination of the environmental and biological factors which influence the production of volatile compounds from cyanobacteria may serve to further enhance water-treatment facilities.

EXPERIMENTAL

Culturing and irradiance exposure

Stock cultures of *M. aeruginosa* (strain MASH01-CSIRO Culture Collection, Hobart, Australia) were grown and maintained in 500 ml conical flasks con-

taining 300 ml of either standard ASM medium [40] modified by substitution of metal stocks with MBL trace metals (1 μM $FeCl_3$) [41] or iron-enriched ASM medium containing an additional 30 μM iron as ferric citrate. Cultures were grown at 25° under 12:12 cyclic illumination using cool white fluorescent lamps and an incident photon flux density of 35–45 μmol m^{-2} s^{-1}. Cultures grown in low and high iron media were directly transferred from conical flasks to open Pyrex Petri dishes (50×200 mm) immediately prior to irradiance exposure at the same iron concn. Cells in the original low and high iron media were exposed to either standard artificial irradiance (as for stock cultures) or sunlight regimes for either 3, 6 or 9 h exposure time during the summer months of October to mid-March (1994–1995). Temperature and irradiance were recorded regularly throughout the sunlight exposure periods. Attempts to minimise temp. fluctuations during sunlight exposure were carried out by placing each Petri dish inside a larger, water-filled Petri dish lid. All cyanobacterial samples were maintained at −20° after irradiance exposure in sealed glass Schott bottles (50 ml) containing 2 drops of 1 mM $HgCl_2$ to prevent bacterial growth prior to extraction. Cultures exposed under the standard culture conditions (standard irradiance and low iron), served as a ref. for comparison with the high irradiance (sunlight) and high iron treatments. The terms "high" and "low" concn are used only in a relative sense. The low iron concn corresponds to that present in ASM culture medium (1 μM $FeCl_3$), whereas the high iron treatment contained an additional 30 μM ferric citrate in ASM medium (final iron concn of 31 μM). Consequently the iron concns employed were within a range comparable to concns measured in Australian inland waterways [18]. Sunlight exposure in the low iron expt had incident radiation ranging from 1100–1800 μmol m^{-2} s^{-1}, with an average irradiance of 1430 μmol m^{-2} s^{-1}. During the 9 h exposure in low iron, the temp. of the cultures increased from 25° to 44°, with an average temp. of 32°. Sunlight exposures in the high iron expt had incident radiation ranging from 800–1900 μmol m^{-2} s^{-1}, with an average irradiance of 1510 μmol m^{-2} s^{-1}. The temp. of the high iron cultures increased from 25° to a maximum of 42°, with an average temp. of 39°. Whilst these temp. changes were not ideal from a controlled experiment perspective, they do reflect conditions experienced by a surface scum on a hot Australian summers' day.

Extraction of volatile odour compounds

VOCs from *M. aeruginosa* exposed to sunlight or standard irradiance and grown in either the low or high iron media were extracted within 48 h after harvest via closed loop stripping and analysed by GC-MS [42]. This involved the release of VOCs during sample aeration and concn onto an activated carbon filter. Briefly, ca 50 ml sample was dild to 900 ml with Milli Q H_2O with subsequent addition of dry NaCl (90 g) and the int. standard, deuterated geosmin (9 μl, 41.6 ng μg^{-1}). Prior to extraction, contaminating organic components present in the head space of the apparatus were removed onto an axillary carbon filter by commencing stripping for 10 s. VOCs were collected during stripping via sparging with air onto a second, clean, activated carbon filter maintained at ca 40°. Cyanobacterial samples were maintained at 25° during the closed loop stripping process (90 min). After closed loop stripping, the activated carbon filter was washed $\times 3$ with CS_2 to concentrate the VOCs and the sample was then stored prior to analysis (−40°). The sample was manually injected (1 μl) without derivatisation.

GC-MS

VOCs were separated according to the method of ref. [42]. The GC was fitted with a fused silica capillary column (25 m, 0.20 mm int. diameter, coated with 0.30 μm cross-linked Me silicone) and split/splitless injector. The GC-MS was run with the following settings; inj. temp. 260° and a 1.5 min hold at 25° with the splitter off for 1 min. The splitter was then turned on and the column heated from 25–80° at 10° min^{-1}, and then 5° min^{-1} to 250°, with a 5 min hold at 250°.

Volatile components were identified by comparison of their MS with ref. library spectra and the RIs of available standards (β-cyclocitral, β-ionone and the aliphatic hydrocarbons). Concentrations of volatile compounds were calculated with respect to the concn of the int. standard (d^3-geosmin).

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