EFFECT OF NATURAL BLUE-GREEN ALGAL CELLS LYSIS ON FRESHWATER QUALITY

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ABSTRACT

Blue-green algae grow in freshwater bodies when they are provided with suitable factors such as nutrients and appropriate weather conditions. Their cell lysis occurs naturally when they encounter unfavourable conditions. This study reports on the types and amounts of fatty acids added into freshwater due to the algal cell lysis. The investigation on the amount of fatty acids released into water due to algal cells lysis was performed by placing samples in two compartments, one with no light allowing the algae cells to die while the other compartment was kept in favourable conditions enough to sustain the life of algae. Fatty acids were then extracted from both dead and living cells as well as their respective water filtrates. Determination of fatty acids was performed using GCxGC-TOF-MS. Identified fatty acids were mainly; tetradecanoic acid, 7-hexadecenoic acid, hexadecanoic acid, 9,12,15-octadecatrienoic acid, 6,9,12,15-octadecatetraenoic acid, 9,12-octadecadienoic, 9-octadecenoic and octadecanoic acids. It was found that water from the dead cells had higher amounts of fatty acids than water samples from the living cells. Unsaturated fatty acids increased from 0.85 mg/L to 2.70 mg/L in filtrate water from the dead algae. The pH of water samples from the dead cells decreased from 6.8 to 6.1.

Keywords; Algae, cells lysis, Fatty acids, gas chromatography time-of-flight mass spectrometry, water quality

INTRODUCTION

Water quality worldwide has remained a problem of concern due to different factors caused by human activities enhanced by the rapid socio-economic growth (Thomas and Durham 2003). Currently, the general environment and freshwater bodies in particular receive massive pollutants from day-to-day activities leading to the limited water resource unfit for both human and animal consumptions (Savenije and Van-der-Zaag 2008, Msagati and Mamba 2011, Kilulya et al. 2012a). The growth of blue-green algae in freshwater is among the major problems that currently affect the water quality. Among others eutrophication (nutrient enrichment) is the major cause of the growth of blue-green algae in freshwater bodies and hence deterioration of its quality. Factors such as municipal and industrial wastewater effluents, agricultural and urban runoff, septic tank leach and any other environmental pollutants which find their destination in water bodies are responsible for the plant nutrient enrichments (Paerl 1997, van Ginkel 2012), hence the growth of algae. Blue-green algae are a diverse group of photosynthetic organisms found mostly in freshwater bodies (Sharathchandra and Rajashekhar 2011). The formation of algal blooms in water bodies cause not only bad smell of water but also bad water taste, release of different toxic metabolites to aquatic organisms, oxygen depletion, change of water pH, change of water colour and disrupting water treatment by making water difficult to treat for human consumption (Sivonen et al. 1990, Paerl 1997). Algal cell

lysis occurs naturally when they encounter unfavourable conditions such as environmental stresses (Singh et al. 2002) resulting in the release of chemical compounds into freshwater bodies. Some of the stresses which cause the death of algae cells include dark stress, light stress (Al-Hasan et al. 1989), temperature stress, water stress and heat stresses (Los and Murata 1999, Singh et al. 2002). When these stresses occur, algae fights to sustain their life by developing different mechanisms in terms of their body physiology (Singh et al. 2002). One of the mechanisms used is the desaturation of fatty acids components to survive the environmental stresses, a process which leads to the increase of unsaturated fatty acids compared to saturated fatty acids. The unsaturation of fatty acids is used to control the fluidity of cell membranes caused by stress and thus are essential for low temperature tolerance (Los and Murata 1999, Singh et al. 2002). This implies that when algal cells enter into winter season from summer season, produce high amount of unsaturated fatty acids which are relatively more toxic to aquatic organisms (Kostamo et al. 2004, Orrego et al. 2010). It has been reported that the toxicity of the blue-green algae produced toxins, microcystins, is enhanced by the presence of polyunsaturated fatty acids. Secondarily, it has been reported that unsaturated long-chain fatty acids are toxic to aquatic organisms and known to inhibit fish gill activities resulting into death of fish and other aquatic microorganisms when they exceed the tolerable levels (Bury et al. 1998, Kostamo et al. 2004, Sharathchandra and Rajashekhar 2011). Generally, both toxins and aquatic toxic lipids such as fatty acids released into water bodies make freshwater unfit for human consumption and failing to sustain the ecosystem. A number of freshwater bodies such as rivers and dams, in South Africa are affected by algal blooms due to eutrophication. This has highly affected the use of water for human consumption and for other organisms (Oberholster et al. 2005). Vaal Dam which is situated in Vaal River is among the Dams in South Africa affected by high levels of blue-green algal blooms. A number of factors can explain the cause of algal blooms in the Vaal River, amongst are high nutrient loading to the river, warmer temperatures, good sunlight penetration and the lack of variation in river flow (Department of Water Affairs 2012). This paper therefore, reports on the types and amounts of fatty acids added into freshwater bodies as a result of blue-green algal cells lysis. The composition of fatty acids both saturated and unsaturated and their seasonal variation in algae biomass and water samples were determined using GCxGC–TOFMS.

MATERIALS AND METHODS

Sample Collection and Pretreatment

Algae biomass samples were collected monthly during summer to winter season in February to May 2013 (in triplicates) in Vaal Dam, South Africa. The collected samples were filtered and washed to remove any contaminants, followed by freeze drying. For the study of the effect of cell lysis on water quality the collected samples were separated in two sets (in duplicate) one set was kept in a compartment with no light allowing the algae cells to die while the second set was kept in favourable conditions for algae cells to remain alive until analysis.

Chemical Reagents and Standards

Hexane, acetone, methanol, chloroform, anhydrous sodium sulphate, hydrochloric acid, methyl tetradecanoate, methyl hexadecanoate, methyl linoleate, methyl oleate, methyl linolenate, methyl octadecanoate were of analytical grade and were all purchased from Sigma Aldrich (St Louis, Mo, USA).

Analytical Procedures and Methods

Extraction of Fatty Acids from Algae Biomass

The extraction of lipids from algae biomass was carried out using ultrasonic solid-liquid

extraction (USLE) using the mixture of acetone and methanol at a ratio of 2:3% v/v (Kilulya et al. 2012b). The extraction was carried out at a temperature of 60 °C for 1 h. The extracts were filtered using PTFE filters of 0.2 µm pore sizes while hot. Solvent was then evaporated to dryness and the extracts re-dissolved in 1 mL of acetone and 0.5 mL of 3M methanolic HCl and heated at 60 °C for 1 h in a thermostated water bath for derivatization. Samples were then cooled and extracted using 1 mL x 3 of hexane. Extracts were mixed and solvent evaporated to dryness. Finally, the extracts; fatty acids methyl esters (FAMEs) were dissolved in 1 mL of HPLC grade hexane, filtered using PTFE disc filters of 0.2 µm pore sizes for GCxGC-TOFMS analysis.

GCxGC-TOFMS Conditions

The analyses of both standards and samples were carried out using GCxGC-TOFMS (Pegasus 4D, LECO Corporation). Helium was used as a carrier gas whereas nitrogen, compressed air and liquid nitrogen were used for the operation of the quad-jet thermal modulator. The sample injector temperature was set at 280 °C, and samples were injected at a volume of 1 µL with splitless mode. The flow of carrier gas was set at a rate of 1 mL/min. The GCxGC column set comprised of a 30 m, RXI-5Sil MS (0.25 mm internal diameter, 0.25 µm stationary film thickness) for the first column while the second column was 1.36 m, RTX-200 (0.18 mm internal diameter, 0.18 µm stationary film thickness). Temperature programme on the first column oven was 80 °C held for 1 minute, then increased to 290 °C at a ramping rate of 10 °C/min and held for 5 minutes. Whereas the second dimension column oven temperature started at 90 °C held for 1 min. then ramped to 300 °C at a ramping rate of 10 °C/min and held for 5 minutes. The modulator interface was set at 15 °C above the secondary oven temperature. The transfer line temperature was set at 250 °C whereas the ion source temperature was set at 240 °C. Electron impact ionization energy was set at 70 eV, while the detector voltage was set at 1600 V. The analysis was carried out at the mass range of 40 – 450 amu.

RESULTS AND DISCUSSION

Qualitative and Quantitative Composition of Fatty Acids

The study investigated the types and amounts of long chain fatty acids added into water due to algal cells lysis and their effects on other water parameters such as pH, odour and colour. The long chain fatty acids identified using GCxGC-TOFMS were dominated by tetradecanoic (C14:0), pentadecanoic (C15:0), 7-hexadecenoic (C16:1), hexadecanoic (C16:0), heptadecanoic (C17:0), 9,12,15-octadecatrienoic (C18:3), γ-linolenic (γ-C18:3), 6,9,12,15-octadecatetraenoic (C18:4), 9,12-octadecadienoic (C18:2), 9-octadecenoic (C18:1) and octadecanoic acids (C18:0). Others were dodecanoic (C12:0), eicosanoic (C20:0) and 11,14-eicosadienoic (C20:2) acids. The qualitative composition of the detected fatty acids was similar to what has been reported previously (Rezanka et al. 2003, Sharathchandra and Rajashekhar 2011). Variations in qualitative and quantitative compositions were observed between the fatty acids determined from living algal cells biomass and those obtained from dead algal cells biomass (Table 1). Generally, it was found that the amount of fatty acid components (saturated and unsaturated) varied between the dead and living algal cells biomass. Saturated long chain fatty acids decreased in dead algal cells whereas the amount of unsaturated fatty acids increased in the same cells

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Table 1: Variations of fatty acids components in living and dead algae biomass (n = 2).

	Living algae cells (mg/g)	Dead algae cells (mg/g)	Percentages increase (↑) or decrease (↓) (%)
Saturated fatty acids	5.65 ± 0.86	2.59 ± 0.51	37.04 ↓
Unsaturated fatty acids	2.39 ± 0.52	2.93 ± 0.39	10.15 ↑

Note: The data shown in the Table are the total values [sum of the averages of individual fatty acids in each component of fatty acids] of each component of fatty acids in the samples.

It can be observed from Table 1 that in the dead algal cells biomass saturated fatty acids decreased from 5.65 mg/g to 2.59 mg/g of dry sample; a decrease of 37.04% while unsaturated fatty acids increased from 2.39 mg/g to 2.93 mg/g of dry sample, an increase of 10.15%. This can be explained by the desaturation process in which algal cells undergo when experience environmental stresses such as cold stress prior to their death. Thus, the desaturation process of fatty acids leads to the decrease of saturated fatty acids and increase of unsaturated ones as it was observed. The decrease of saturated fatty acids and increase of unsaturated fatty acids in the dead cells of algae biomass indicates clearly that desaturation process takes place when algal cells are subjected to environmental stresses to sustain their life as it was previously reported by other researchers (Los and Murata 1999, Singh et al. 2002). Thus, the process of natural algal cell lysis leads to the deterioration of water quality due to the release of secondary metabolites such as microcystins and lipids such as fatty acids. Different studies have reported on the levels of microcystins in South African freshwater bodies as a result of algal blooms. Although different reports identified microcystins as the toxic secondary metabolites from blue-green algae cells of which their production and accumulation is triggered by environmental stresses (El-Shehawy et al. 2012), unsaturated fatty acids have been reported to increase the toxicity of microcystins resulting in exerting combined effects on aquatic organisms.

After the observation of the variations of fatty acid components between living and dead algae cells biomass, the study evaluated the amount of fatty acid components in water filtrates of living and dead algae cells biomass so as to establish the amount of fatty acids released into water due to cells lysis. The observed variation on this aspect revealed that the amount of saturated fatty acids from living algae cells increased slightly in water filtrates whereas the amount of unsaturated fatty acids in water filtrates from dead algae cells increased significantly (Table 2).

From Table 2, it can be clearly observed that the amount of unsaturated fatty acids increased from 0.85 mg/L in water filtrate from living algal cells to 2.70 mg/L in the water filtrates from the dead algal cells, an increase of 52.11%. This gives a clear picture on how much unsaturated fatty acids are added into freshwater bodies when blue-green algae cells die because of environmental stresses. The increase of more than 52% is an alarming situation for water quality since unsaturated fatty acids are known for their toxicity to aquatic organisms and other microorganisms. The observed situation calls for the measures such as collecting and removing the algal blooms from water bodies before their death. For the saturated fatty acids the increase was at the marginal level, it just increased from 1.18 mg/L in water from living cells biomass to 1.35 mg/L in water from dead cells biomass.

Table 2: Variations of fatty acids components in water filtrates from living and dead blue-green algal cells biomass (n = 2).

	Water filtrates from living algal cells (mg/L)	Water filtrates from dead algal cells (mg/L)	Percentage increase (↑) (%)
Saturated fatty acids	1.18 ± 0.08	1.35 ± 0.00	6.72 ↑
Unsaturated fatty acids	0.85 ± 0.26	2.70 ± 0.58	52.11 ↑

Note: The data shown in the Table are the total values [sum of the averages of individual fatty acids in each component of fatty acids] of each component of fatty acids in the samples.

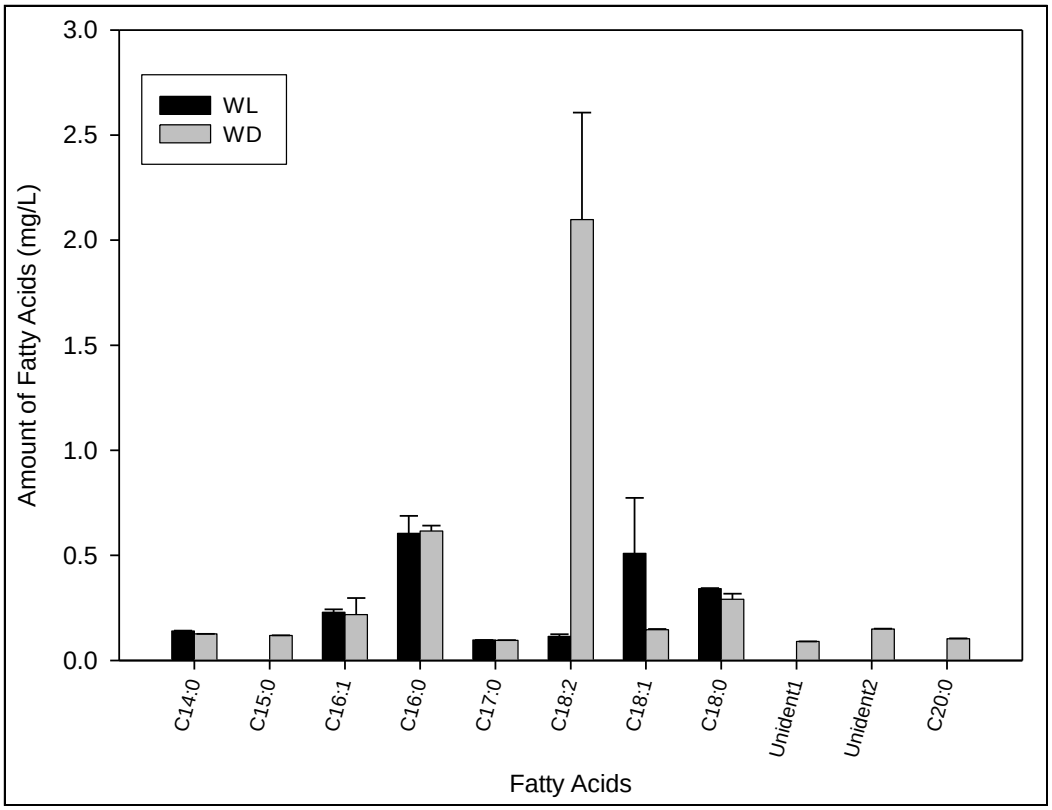

Figure 1: The variation of fatty acids in water samples from living (WL) and dead algal (WD) cells biomass (note; (Unident1 = Unidentified unsaturated fatty acid 1 and Unident2 = Unidentified unsaturated fatty acid 2)

Furthermore, In the water filtrates from both living and dead algal cells biomass it was observed that the total amount of fatty acids was higher in the filtrates from the dead

cells, with the amount of unsaturated fatty acids being significantly higher. Actually the amount of 9,12-octadecedienoic acid (C18:2) (**Fig.1**), dominated the unsaturated fatty acids in water filtrate from dead algal cells. A significant decrease of oleic acid (9-octadecenoic acid) was observed in water filtrates from dead algal cells biomass while increased amount of 9,12-octadecedienoic acid was observed suggesting probably oleic acid desaturated to 9,12-octadecedienoic acid during the desaturation mechanism process in the algal cells.

This observation therefore, is an indication that significant amounts of fatty acids are annually added in freshwater bodies affected by algal blooms. Their combined effects increase the toxicity of the secondary metabolites released in water. Aquatic organisms are the first ones to suffer from this situation followed by water treatment plants, in which the treatment of water becomes difficult and hence expensive. It was also observed that the level of pH decreased from 6.8 to 6.1, a situation which costs the water treatment plant to raise the pH to the suitable one. The water filtrates from dead algal cells biomass were also found to change colour from colourlesss to blue colour with a bad smell.

In the course of the analyses and evaluation of the variations in terms of qualitative composition of fatty acids from both living and dead algal cells biomass and their respective water filtrates, new fatty acids were observed. These newly observed fatty acids were unfortunately not fully identified due to the lack of standards which would allow their fully identification. However, based on the comparison of mass spectra with those in MS Libraly of the GCxGC-TOFMS, it was observed that the two fatty acids were all unsaturated fatty acids (**Fig. 2**).

Just like in the dead algal cells biomass extracts, in the water filtrates from dead algal cells biomass the two fatty acids were also observed indicating that they were released into water. These newly observed fatty acids might be due to the desaturation of unsaturated C18s fatty acids due to the stresses experienced by cells prior to their death. Thus, this observation increases the concern on the types and amounts of fatty acids added into freshwater bodies as a result of blue-green algal cells lysis. **Figure 2** shows the GCxGC-TOFMS overlaid chromatograms in which the additional peaks on the chromatogram of water filtrate from the dead algae cells biomass (DALW) are shown. These peaks were not observed on the chromatogram of the water filtrate from the living algae cells biomass (LALW). The individual fatty acids variations between living and dead algal cells are presented in Table 3.

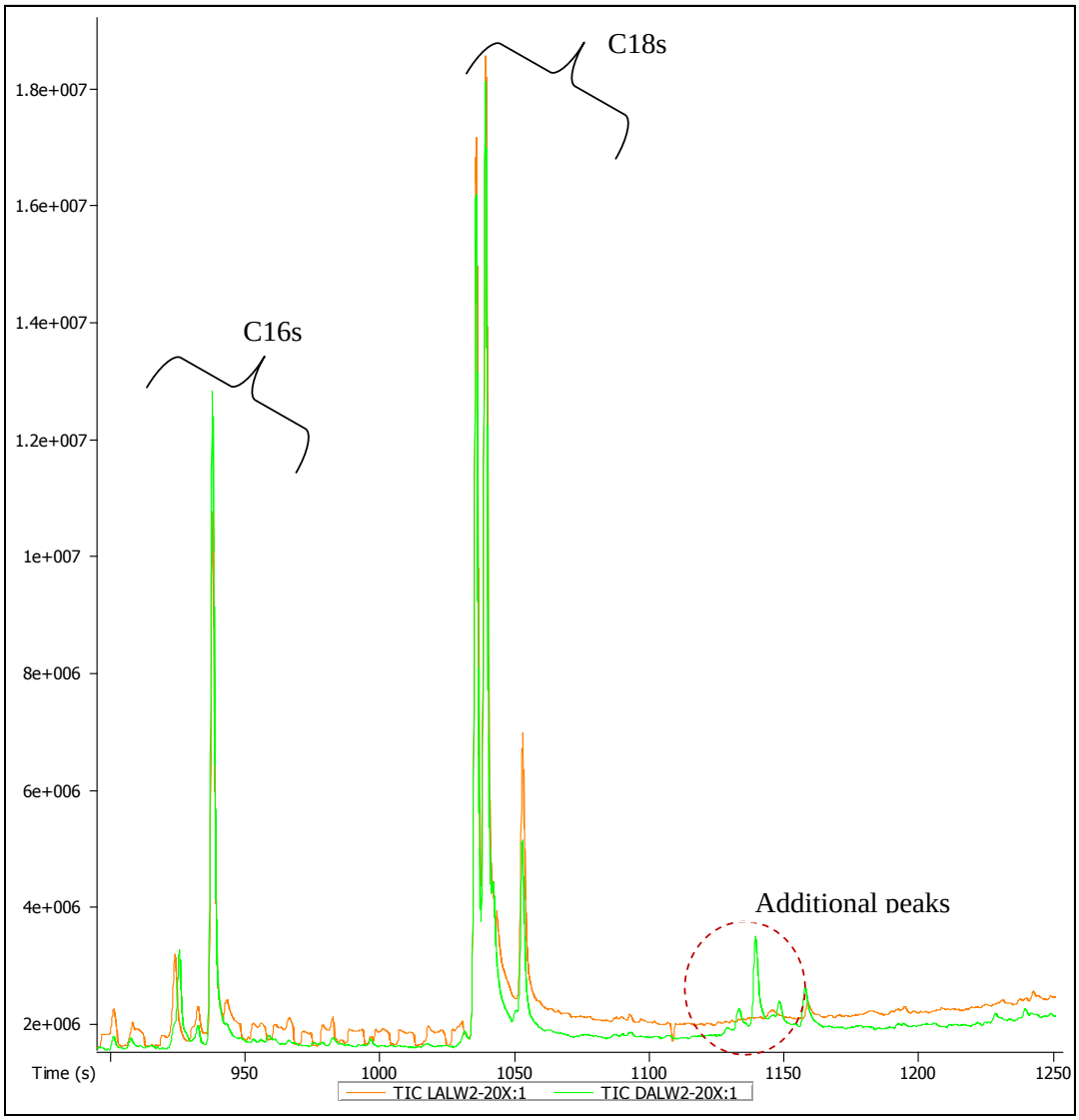

Figure 2: The overlaid GCxGC-TOFMS chromatograms for fatty acids from water filtrates from living and dead algal cells biomass showing the additional peaks of fatty acids on the water filtrate sample from dead algal cells (DALW; green coloured chromatogram) which were not observed on the water filtrate from the living algae cells biomass (LALW; brown coloured chromatogram).

Table 3: Fatty acids composition (mean values) in living and dead algal cells biomass (n = 2).

Saturated Fatty Acids (mg/g)			Unsaturated Fatty Acids (mg/g)		
	LALG	DALG		LALG	DALG
C14:0	0.19 ± 0.02	0.27 ± 0.02	C16:1	0.47 ± 0.06	0.97 ± 0.09
C15:0	0.15 ± 0.00	0.17 ± 0.01	C18:1	1.06 ± 0.21	0.59 ± 0.08
C16:0	4.70 ± 0.63	1.17 ± 0.07	C18:2	0.11 ± 0.00	0.10 ± 0.00
C17:0	0.15 ± 0.01	0.16 ± 0.00	C18:3	ND	0.13 ± 0.00
C18:0	0.46 ± 0.02	0.65 ± 0.21	γ-C18:3	0.23 ± 0.02	0.57 ± 0.09
C20:0	ND	0.17 ± 0.04	C18:4	0.52 ± 0.07	0.12 ± 0.00
			C20:2	ND	0.14 ± 0.01
			Unident1	ND	0.12 ± 0.00
			Unident2	ND	0.19 ± 0.00
Total	5.65	2.59	Total	2.39	2.93

Note; LALG = Living algal cells, DALG = Dead algal cells, Unident1 = Unidentified unsaturated fatty acid 1 and Unident2 = Unidentified unsaturated fatty acid 2.

The most important finding in this variations was the decrease in the amount of hexadecanoic acid (C16:0) which was 4.70 mg/g in the extracts from the living algae cells biomass and 1.17 mg/g in the extracts from the dead algal cells biomass, a decrease of 75.10 %. Hexadecanoic acid is the most abundant saturated fatty acid in blue-green algal cells. Its decrease can be explained by the fact that the large amount of this fatty acid was desaturated to unsaturated fatty acids such as 7-hexadecenoic acid (C16:1) which increased from 0.47 mg/g to 0.97 mg/g in the dead algal cells biomass. The other interesting observation in Table 3 is the decrease in the amount of 9-octadecanoic acid (C18:1) from 1.06 mg/g to 0.59 mg/g which can be explained by its desaturation to polyunsaturated C18s fatty acids such as C18:3 and γ-C18:3 which their amount increased. The amount of C18:3 increased from undetectable level in the extracts from the living algal cells biomass to 0.13 mg/g whereas γ-C18:3 increased from 0.23 mg/g to 0.57 mg/g. The increase of polyunsaturated fatty acids caused by the desaturation of monounsaturated fatty acids increases the toxicity of fatty acids released in water as a result of algal cell lysis, based on the literature reports. On the variation of both qualitative and quantitative composition of fatty acids between the living and dead algal cells biomass, the study evaluated the net effect on the water filtrate. The data presented in Table 4 show the variation of individual fatty acids in water filtrates from the living and dead algal cells biomass. Generally, there was no much difference in saturated fatty acids except for pentadecanoic acid (C15:0) and eicosanoic acid (C20:0) which were not detected in water filtrate from living algal cells but detected in the water filtrate from the dead algal cells biomass.

On the other hand, unsaturated fatty acids such as C18:1 decreased from 0.51 mg/L in water filtrate from the living algal cells to 0.15 mg/L in water filtrate from the dead algal cells biomass whereas the amount of C18:2 increased from 0.11 mg/L in water filtrate from the living algal cells biomass to 2.10 mg/L in the water filtrate from the dead algal cells biomass. These could be explained by the oxidation of C18:1 to C18:2 while in the aquatic environment. Furthermore, the desaturation of monounsaturated fatty acid C18:1 to the polyunsaturated fatty acids such as C18:2, unident1 and unident2 due to the dark and

cold stresses in algae cells could be responsible for the decrease of C18:1 and the increase of C18:2 and the two unidentified fatty acids (unident1 and unident2).

Table 4: Fatty acids composition (mean values) in water filtrates from the living and dead algal cells biomass (n = 2).

Saturated Fatty Acids (mg/g)			Unsaturated Fatty Acids (mg/g)		
	LALW	DALW		LALW	DALW
C14:0	0.14 ± 0.00	0.13 ± 0.00	C16:1	0.23 ± 0.01	0.21 ± 0.08
C15:0	ND	0.12 ± 0.00	C18:1	0.51 ± 0.26	0.15 ± 0.00
C16:0	0.60 ± 0.08	0.61 ± 0.02	C18:2	0.11 ± 0.01	2.10 ± 0.51
C17:0	0.10 ± 0.00	0.10 ± 0.00	Unident1	ND	0.09 ± 0.00
C18:0	0.34 ± 0.00	0.29 ± 0.03	Unident2	ND	0.15 ± 0.00
C20:0	ND	0.10 ± 0.00			
Total	1.18	1.35	Total	0.85	2.70

Note; LALW = Living algae water filtrate, DALW = Dead algae water filtrate, Unident1 = Unidentified unsaturated fatty acid 1 and Unident2 = Unidentified unsaturated fatty acid 2.

Summer-Winter Season Variation of Fatty Acid Composition in Algae Biomass

The results obtained in the analysis of fatty acid composition in the living and dead algal cells biomass conducted at the laboratory scale were compared with the results from the freshly collected samples. Samples collected from Vaal Dam between February and May 2013 (summer to winter season) inclusively were analysed for this evaluation. Generally, the samples collected in May (winter season) had the highest amount of total fatty acids compared to those collected in February and April 2013. This indicates high production of lipids such as fatty acids especially unsaturated fatty acids by algal cells during cold season. These findings are in agreement with what was reported earlier by other researchers, in which it was reported that the production of monounsaturated and polyunsaturated fatty acids in algae cells is influenced by temperature stress (Shyam et al. 2011). Moreover, it was observed that the samples collected in winter season had the higher amount of unsaturated fatty acids compared to saturated fatty acids, which is attributable to the desaturation of saturated fatty acids in the algae cells to survive environmental stress such as cold. The observed variation of the fatty acid composition due to weather changes from summer to winter is in agreement with what was found in the samples treated in the laboratory in this study. **Figure 3** shows the variation of fatty acids composition between February and May 2013. The trend shows that the amount of C16:0, C18:4 and C18:3 were very high in May. However, the amount of C16:0 was observed to increase gradually in every sampling month, ie, February, April and May. The observed composition variation with seasons confirms the addition of high levels of fatty acids especially unsaturated fatty acids in freshwater bodies due to the problem of algal blooms. Thus, algal cells lysis, influenced by environmental stresses, has a significant effect on water quality.

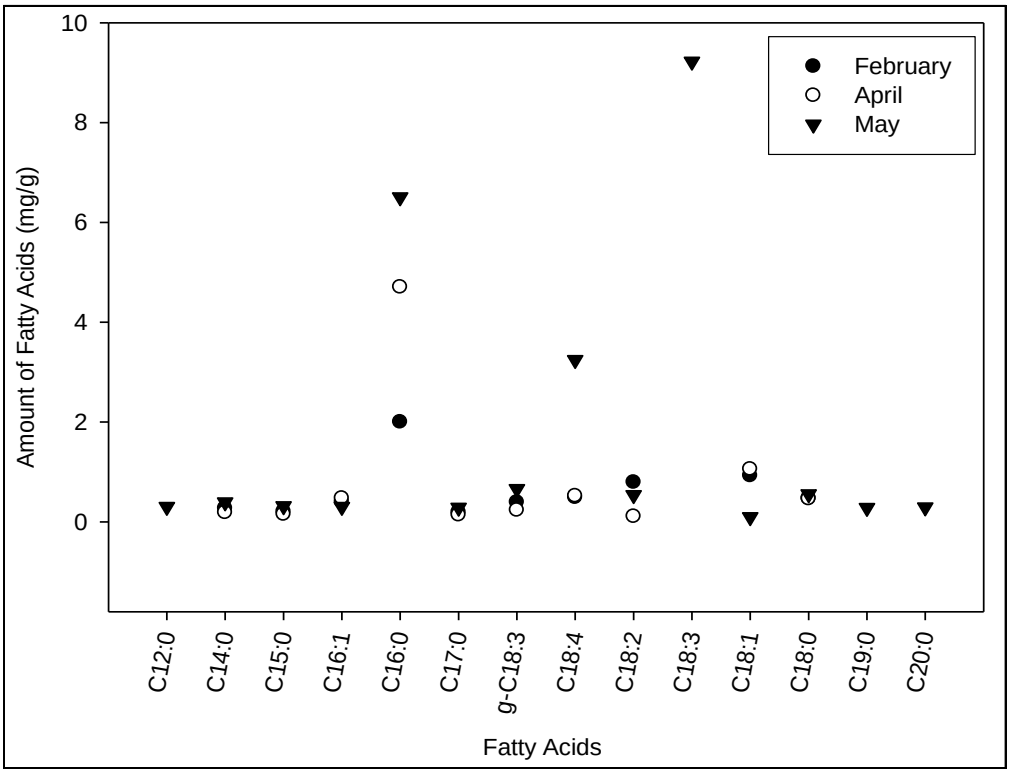

Figure 3: The variation of individual fatty acids in algae biomass samples collected between February and May 2013

The amount of C18:3 were very high in May of which might be highly contributed by the desaturation of monounsaturated C18 to polyunsaturated C18 such as C18:3. As a general trend among the unsaturated fatty acids, polyunsaturated fatty acids (PUFA), which are reported the most toxic in aquatic environment had the highest levels indicating high risk to both aquatic organisms and general ecosystem during winter season.

CONCLUSION

From the data obtained in this study it can be concluded that the total amount of fatty acids production in blue-green algae cells increases with environmental stresses. However, between the two components of fatty acids, saturated and unsaturated, it is unsaturated fatty acids component which increases when algae cells die because of environmental stresses whereas saturated fatty acids component decreases. This study indicated that in the dead algae cells biomass saturated fatty acids decreased from 5.65 mg/g of dry sample in living cells to 2.59 mg/g of dry sample of dead algae cells biomass, a decrease of 37.04% while unsaturated fatty acids incresed from 2.39 mg/g in living algae cells biomass to 2.93 mg/g of dry sample of dead algae cells, an increase of 10.15%. In water filtrates from the living and dead algae cells biomass it was revealed that the amount of unsaturated fatty acids increased from 0.85 mg/L in water filtrate from living algae cells biomass to 2.70 mg/L in the water filtrates from the dead algae cells; an increase of 52.11%.

Thus, the blue-green algal cell lysis increases significantly the amount of unsaturated fatty acids in freshwater bodies which affect the water quality. The algal cell lysis was also found to contribute to the decrease of freshwater pH, causing bad smell and change of water colour making water not suitable for human consumption.

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REFERENCES

Al-Hasan R, Ali A, Radwan S 1989 Effects of light and dark incubation on the lipid and fatty acid composition of marine cyanobacteria. *J. Gen. Microbiol.* **135**:865-872.

Bury N, Codd G, Bonga SW, Flik G 1998 Fatty acids from the cyanobacterium *Microcystis aeruginosa* with potent inhibitory effects on fish gill Na+/K+-ATPase activity. *J. Exp. Biol.* **201**:81-89.

Department of Water Affairs 2012 Department of Water Affairs to address algal bloom eutrophication conditionss in the Vaal River. Media Release by Department of Water Affairs, 08 March 2012, Republic of South Africa.

El-Shehawy R, Gorokhova E, Fernández-Piñas F, del Campo FF 2012 Global warming and hepatotoxin production by cyanobacteria; What can we learn from experiments? *Water Res.* **6** : 1420-1429.

Kilulya KF, Msagati TAM, Mamba BB, Ngila JC, Bush T 2012a Controlling the release of wood extractives into water bodies by selecting suitable eucalyptus species. *Phys. Chem. Earth.* Parts A/B/C. **50**–**52**:217-223.

Kilulya KF, Msagati TAM, Mamba BB, Ngila JC, Bush T 2012b Study of the fate of lipophilic wood extractives during acid sulphite pulping process by ultrasonic solid-liquid extraction and gas chromatography mass spectrometry. *J. Wood Chem. Technol.* **32**:253-267.

Kostamo A, Holmbom B, Kukkonen J 2004 Fate of wood extractives in wastewater treatment plants at kraft pulp mills and mechanical pulp mills. Water Research. **38**:972-982.

Los DA, Murata N 1999 Responses to cold shock in cyanobacteria. *J. Mol Microbiol Biotechnol.* **1**:221-230.

Msagati TAM, Mamba BB 2011 Development of supported liquid membrane techniques for the monitoring of trace levels of organic pollutants in wastewaters and water purification systems. *Phys. Chem. Earth.* **36** 1167-1177.

Oberholster PJ, Botha A-M, Cloete TE 2005 An overview of toxic freshwater cyanobacteria in South Africa with special reference to risk, impact and detection by molecular marker tools. *Biokemistri.* **17**:57-71.

Orrego R, Guchardi J, Krause R, Holdway D 2010 Estrogenic and anti-estrogenic effects of wood extractives present in pulp and paper mill effluents on rainbow trout. *Aquat. Toxicol.* **99**:160-167.

Paerl HW 1997 Coastal eutrophication and harmful algal blooms: Importance of atmospheric deposition and groundwater as" new" nitrogen and other nutrient sources. *Limnol oceanogr.* **42**:1154-1165.

Rezanka T, Dor I, Prell A, Dembitsky VM 2003 Fatty acid composition of six freshwater wild cyanobacterial species. *Folia Microbiol.* **48**:71-75.

Savenije HHG, Van-der-Zaag P 2008 Integrated water resources management: concepts and issues. *Phys. Chem. Earth*. **33**:290-297.

Sharathchandra K, Rajashekhar M 2011 Total lipid and fatty acid composition in some freshwater cyanobacteria. *J. Algal. Biomass Utiln.* **2**:83-97.

Shyam RK, Al-Harbi NA, Thajuddin N 2011 Macromolecular and fatty acid profile studies on symbiotic cyanobacterial isolates of cyanolichens. *J. Med. Plants Res.* **5**:4188-4193.

Singh SC, Sinha RP, Hader DP 2002 Role of lipids and fatty acids in stress tolerance in cyanobacteria. *Acta protozool.* **41**:297-308.

Sivonen K, Niemelä SI, Niemi RM, Lepistö L, Luoma TH, Räsänen LA 1990 Toxic cyanobacteria (blue-green algae) in Finnish fresh and coastal waters. *Hydrobiologia.* **190**:267-275.

Thomas J, Durham B 2003 Integrated water resource management: looking at the whole picture. *Desalination.* **156**:21–28.

van Ginkel C 2012 Algae, phytoplankton and eutrophication research and management in South Africa: past, present and future. *Afr. J. Aquat. Sci.* **37**:17-25.