https://doi.org/10.26651/allelo.j/2018-45-1-1176 0971-4693/94 Euro 20.00
Allelopathy Journal 45 (1): 65-76 (September, 2018) *International Allelopathy Foundation* 2018
Table: 2, Figs : 4

Effects of *Litchi chinensis* leaf litter extract on the growth, photosynthesis and metabolic activity in *Microcystis aeruginosa*

X.X. Wang[1], Y.T. Szeto[2]*, C.C. Jiang[1] and X.J. Wang[3]
School of Civil and Environmental Engineering,
Shenzhen Polytechnic, Shenzhen 518055, China
E-Mail: ytszeto@alumni.vtc.edu.hk; ytszeto@hotmail.com.hk

(Received in revised form: July 21, 2018)

ABSTRACT

We investigated the use of *Litchi chinensis* leaf litter extract (LCLLE) on the growth, chlorophyll a (Chl-*a*) fluorescence and physiological properties for *Microcystis aeruginosa*. The algal growth, photosynthesis and metabolic functions were effectively inhibited in a dose dependent manner but the effect was only temporary and the extract functions only as an algicistat. Furthermore, GC-MS analysis showed that several kinds of volatile chemicals, such as n-hexadecanoic acid in LCLLE suppressed the growth of algae.

Key words: Algae, allelochemicals, allelopathy, GCMS, growth, harmful algal blooms (HAB), leaf litter, litchi, *Litchi chinensis*, *Microcystis aeruginosa*

INTRODUCTION

Widespread eutrophication has caused multiple toxic cyanobacterial blooms in all continents (1) and such harmful algal blooms (HABs) have drawn great attention for their environmental, social, and economic impacts (40). The negative influences of HABs includes aesthetic changes in water bodies such as changes in odour, colour, taste, oxygen and suppresses the survival of aquatic animals (20). Cyanobacterial harmful algal blooms (CHABs) are highly toxic because of their secondary metabolites namely microcystins, produced by *Microcystis* sp. (6,14,20,37). Microcystins are hepatotoxic to humans and animals (4). The control of CHABs is therefore an important issue in eutrophic water body management.

Numerous studies [including the chemical, physical and biological treatments) have been done (41)] to control the formation of HABs. The use of allelopathy to control cyanobacterial blooms has also been suggested as a promising method (24). The aquatic macropyte *Potamogeton maackianus* inhibits the growth of *M. aeruginosa* (38) and *Ceratophyllum demersum* inhibits that of nitrogen-fixing cyanobacteria (13)**.** The terrestrial plants effectively controls the algal growth in water bodies through allelopathy (35). Agricultural byproducts (barley and rice straw) are used to control algal growth (32) and inhibits the growth of *M. aeruginosa* (3,23,41). Despite the low cost and apparent safety, the use of crop residues needs considerable management effort and long-term

*Correspondence author, [2]Diagnostix Medical Centre, Room 601, 6/F, China Insurance Group Building, 141 Des Voeux Road Central, Hong Kong SAR, China, [3]College of Environment and Energy, South China University of Technology, Guangzhou 510006, China.

ecological safety monitoring (26). A large amount of straw (8.0-10.0 g/L) is required for effective inhibition but their high dose adds higher quantities of nutrients into the water bodies. Therefore, it has become necessary to explore the use of allelopathy, which can be more efficient and do not adds excess nutrients into the water bodies (26). In our previous study (35) the extract of *Dracontomelon duperreanum* leaf litter strong inhibited the algal growth. However, studies using the leaf litter extracts of terrestrial plants for algal growth inhibition are limited.

Litchi chinensis (fam Sapindaceae), is widely distributed terrestrial plant and its leaf litter extract has strong activity against *M. aeruginosa* (36). However, no precise studies on its anti-algal properties have been done. This study aimed to investigate the specific biological responses of cyanobacterial cells to LCLLE exposure and the composition of litchi leaf litter extract (LCLLE).

MATERIALS AND METHODS

I. LCLLE preparation

L. chinensis leaf litter (fallen mature leaves) was collected from Shenzhen, Guangdong Province, China. Leaves were washed with tap water, dried at 40 ℃ for 4 days in an oven and were cut into 1 cm pieces. To prepare the extract, 50 g sample were immersed in 0.5 L (10%) of reverse osmosis (RO) water and then incubated at 26±1^{o}C for 4 days in dark without preservative.

II. Cyanobacterial strain and culture conditions

The *M. aeruginosa* FACHB-915 strain was obtained from the Freshwater Algae Culture of Hydrobiology, Wuhan, Hubei, China. It was grown in batch cultures in 250-mL Erlenmeyer flasks containing 10 mL *M. aeruginosa* strain suspension and 20 mL BG-11 medium (27) under a 12/12 h light/dark cycle with a light intensity of about 30 μmol photons·m^{-2} s^{-1} at 26 °C for 7-10 days. Then, 1 mL of algal suspension in the exponential growth phase was transferred to 100 mL of BG-11 medium and the LCLLE was added to microalgae cultures at the final concentrations : 0,0.4,0.8,1.2,1.6,2.0 g dry weight of extract/L of leaf extract or 0,0.04,0.08,0.12,0.16,0.2 % concentrations as per earlier studies (35). Three mL (pulse amplitude-modulated fluorescence phytoplankton analyzer measurement) or one ml (flow cytometry measurement) of samples was withdrawn for analysis on 1, 4, 7, 10, 15 days after LCLLE exposure.

III. Growth inhibition

The algal growth was determined by measuring the Chlorophyll a (Chl-*a*) concentrations on 1,4,7,10 and 15 days, using pulse amplitude-modulated fluorescence phytoplankton analyzer (Phyto-PAM, Walz, Germany). The analysis was performed with software of PhytoWin v2.13 and calculated by the equation given below:

$$\text{Percentage inhibition } (\%) = \left(\frac{C_0 - C}{C_0}\right) \times 100$$

Where, C_0 and C are Chl-*a* concentrations in control and extract-treated cultures, respectively.

IV. Photosynthetic activity

The rapid photosynthesis versus irradiance curves technique was used to determine the photosynthetic activity of *M. aeruginosa* treated with LCLLE. The effective quantum yields produced by 10-incremental actinic light intensities (photosynthetically available radiation, PAR) viz., 16, 164, 264, 464, 664, 864, 1064, 1264, 1464 and 1664 μmol photons $m^{-2}\cdot s^{-1}$ were determined The corresponding relative electron transport rates (rETRs) were calculated, and a complete rapid light curve (RLC) was produced. After the samples were adapted for 2.0 min in darkness, the actinic light irradiance step was applied for 30 s, as previously described (17). RLC curves were fitted using the model described (18).

V. *In vivo* Chl-a fluorescence, cell membrane integrity and metabolic activity

The fluorescence signal of Chl-*a* was measured with fluorescent channel (FL3) detectors (> 670 nm) using flow cytometry (BD-Accuri 6, USA) and non-cyanobacterial particles were excluded by setting gate levels as previously described (41). Changes in cell membrane integrity and esterase activity were determined by FL2 (585/40 nm) and FL1 (533/30 nm) detectors respectively. Cells were stained with 65.2 mg/L propidium iodide (PI, Sigma, St Louis, MO) and 10 mg/L of fluorescein diacetate (FDA, Sigma, St Louis, MO) for 15 min. Data were analyzed using BD-Accuri 6 software. Medium flow rate was used and about 12,000 cells were counted by flow cytometry for each sample. Detailed procedures are described elsewhere (41,42).

VI. Identification of antialgal compounds in LCLLE

The constituents of LCLLE were extracted with 10 mL hexane. The mixture was scrolled for 1min and ultrasonic 15 min, the sample was extracted and repeated thrice. The sample was blown with nitrogen to about 4 mL and made up to final volume of 5 mL. The sample was then analyzed by GC-MS (7890B-5977A, Agilent, USA) equipped with DB-5MS quartz capillary column. Temperature of injection port and transmission line was 280 ℃. The temperature programme was as under: initial oven temperature 80 ℃; increased to 180 ℃ at 10 ℃/min and kept for 10 min; and increased to the final temperature of 290 ℃ and kept for 25 min. One μL sample was injected in a splitless mode. Carrier gas was helium at a rate of 1 mL/min. MS conditions: mass spectra were recorded over 50-550 amu range with electron impact ionization energy of 70 eV. Preliminary identification of constituents was done by peak matching against standards in the standard NIST library spectra. Relative proportions of the constituents were calculated from GC-MS peak areas.

VII. Statistical analysis

All experiments were performed in triplicate. Means and standard deviations were calculated. The data were analyzed by SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Origin 8.5 was used in drawing the figures and analyzed by one-way analysis of variance (Origin version 8.5, Microcal Software Inc., Northampton, MA, USA), then followed by Tukey's multiple comparison test. Differences were considered to be significant at $p<0.05$.

RESULTS AND DISCUSSION

Growth and membrane integrity of *M. aeruginosa*

After 15-d incubation (Fig. 1), LCLLE showed significant concentration-dependent inhibition of *M. aeruginosa* growth (p <0.05, ANOVA). The extract began exerting strong inhibitory activity after 4-d. After 10-d, the Chl-*a* content of cyanobacterial biomass in the treated groups declined to the minimal level at the highest concentration (2.0 g/L) of extract with 92 % inhibition. However, the inhibitory effect weakened 15 d after exposure (Fig. 1). It might be due to the short half-lives of the allelochemicals and hence, the activity was maintained for short period of time.

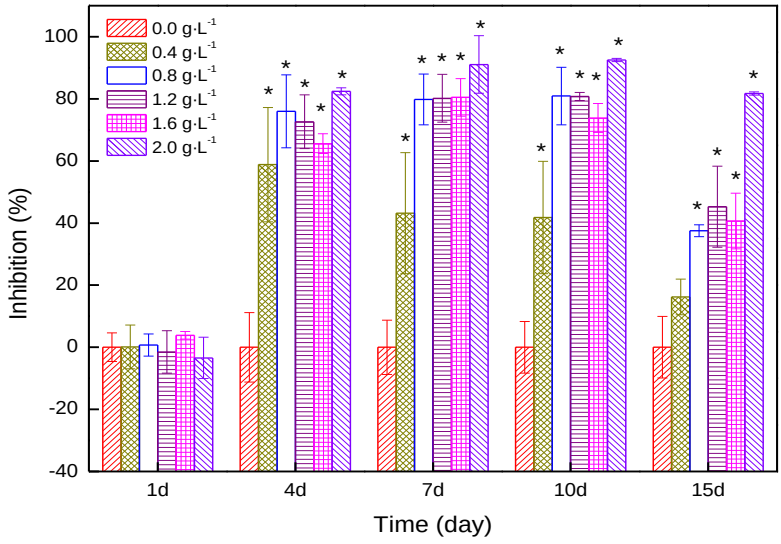

Figure 1. Changes in inhibition activity of *M. aeruginosa* after 1-, 4-, 7-, 10- and 15- day LCLLE exposure

Cellular membrane integrity was determined by using flow cytometry**,** usually with fluorescent PI (8). The cell membrane could be damaged, if the chemical is algicidal but not if the chemical is only algistatic (39). In our experiments, we noticed only a slight change in the membrane integrity at the highest LCLLE concentration. The lowest membrane integrity was 96.2% on day 15 with 2.0 g/L of LCLLE. No statistically significant difference was seen compared to the control (P > 0.05) (Table 1). This indicated that the cell membranes remained intact and the mechanism of LCLLE on algal growth was algistatic in nature. These results are consistent with previous reports (33,35,41). In the present study, leaf extract prepared after 4-d soaking, was sufficient to inhibit the growth of *M. aeruginosa*. In using the LCLLE, not only the extract was effective but also the introduction of unwanted nutrients was also avoided.

Table 1. Cell membrane integrity of *M. aeruginosa* cells after exposure to different concentrations of LCLLE

LCLLE (g/L)	1 d (%)	5 d (%)	15 d (%)
0.00	98.60 (0.04) [a]	98.30 (0.06)	99.10 (0.06)
0.40	98.70 (0.05)	99.00 (0.06)	99.10 (0.06)
0.80	99.20 (0.05)	99.00 (0.06)	96.20 (0.04)
1.20	98.80 (0.05)	99.40 (0.07)	98.70 (0.07)
1.60	97.60 (0.06)	99.10 (0.05)	99.90 (0.07)
2.00	98.60 (0.07)	99.10 (0.06)	100.00 (0.05)

Cell membrane integrity expressed as the percentage of PI-unstained cells (with intact cell membrane) compared to the total amount of cells analyzed by flow cytometry.
[a] The values indicate mean of triplicate (SD in parentheses), and at least 12000 cells were analyzed for each trial.

In vivo Chl-*a* fluorescence measured by flow cytometry

Probing photosynthesis by measuring Chl-*a* fluorescence is widely used method to understand the efficiency of photosynthesis (32). Changes in mean Chl-*a* fluorescence intensity (MFI) of *M. aeruginosa* cells by flow cytometry were analyzed on day 1, 5 and 15 (n >12 000). Results showed (Fig. 2) that LCLLE at all concentrations caused concentration- dependent decrease in Chl-a fluorescence of the cyanobacterial cells. The change of MFI was 8 % at 2.0 g/L LCLLE on day 1. After 5 and 15 days, the concentration above 1.2 g/L LCLLE decreased the MFI in a dose-dependent manner. When exposed to 2.0 g/L extract dose, after 15 days the MFI was 86.2 % of the control. The Results of growth and MFI analysis demonstrated similar trend with regard to the concentration of LCLLE and the exposure time (Fig.1, 2).

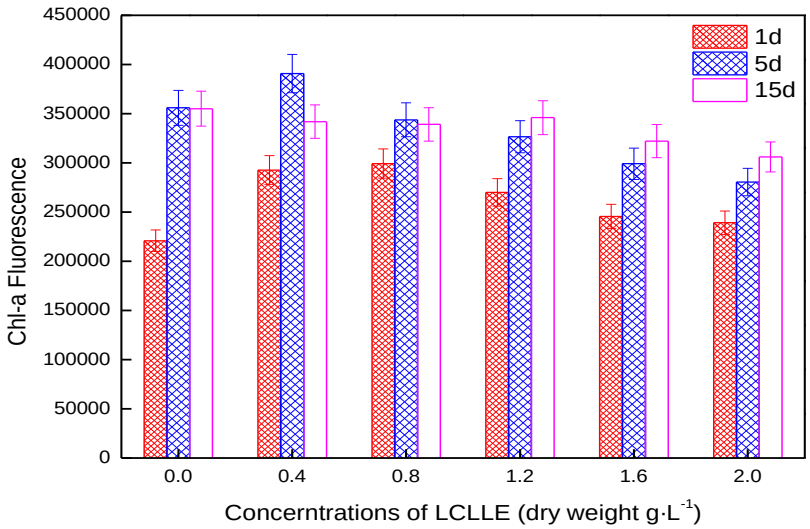

Figure 2. Changes in Chl-*a* fluorescence of *M. aeruginosa* cells Chl-*a* measured by flow cytometry after 1-, 5- and 15-day LCLLE exposure

Chl-*a* fluorescence provides information about the photosynthetic process, including energy absorption, distribution and utilization (44). Pigment inhibition suggests that the extract might introduce critical effect on the photosynthetic system of *M. aeruginosa* (19). One of the possibilities is the inhibition to the PSII system through decreasing Chl-*a* fluorescence (5). In the present experiments, cyanobacteria exposed to LCLLE were critically harmed in the photosynthetic system by decreasing the MFI in a concentration-dependent manner. At low levels of LCLLE, the effect on the photosynthetic capacity (*in vivo* Chl-*a* fluorescence) was limited. However, at higher concentrations, LCLLE efficiently suppressed the photosynthetic capacity, which was reflected by the decreasing MFI. Later, the MFI level increased slightly implying the need of continuous supply of LCLLE to inhibit the algae growth.

Previous studies (34) using agricultural residues to control the algal growth have shown that factors affecting the fluorescence of Chl-*a* include the nutrients in the agricultural residues (2,33). However, flavonolignans isolated from barley straw extract, did not affect the MFI in 10-day incubation (43). The rice straw extract significantly reduced the *in-vivo* Chl-*a* fluorescence of *M. aeruginosa* after 7 days exposure, but the fluorescence was not changed after 15 days (33).

Inhibition of cyanobacterial photosynthetic activity

Measuring the PS II activity in algae by phytoplankton analyzer pulse-amplitude-modulation (Phyto-PAM) fluorometry allows a better understanding of the physiological mechanisms involved in electron transport rate and photochemical efficiency (2,3,19). The photosynthesis versus irradiance (rETR/E) curves of *M. aeruginosa* treated with LCLLE compared to Phyto-PAM fluorometry are shown in Fig. 3. The LCLLE treatment showed a similar pattern of rETR in response to Photosynthetically Available Radiation (PAR) which was generated by PAM. Fig. 3a showed no obvious difference in the rETR in response to PAR between the treatment and control on day 1. However, the rETR values significantly decreased ($P<0.01$), especially at high extract concentrations (1.2, 1.6, and 2.0 g/L) on day 4 (Fig. 3b). On day 7 and 15, the values of rETR increased gradually corresponding to PAR and LCLLE concentrations (Fig. 3c, 3d) which suggested the presence of algistatic effect.

In this study, the photosynthetic parameters obtained from the pulse-amplitude-modulation, reflect the state of algal cells at different times. The rETR/E remained at high levels in control and at low doses of LCLLE but decreased at higher doses (1.2, 1.6, and 2.0 g/L) and earlier (on day 4), which agrees with the stable mass of *M. aeruginosa* after LCLLE exposure. These findings suggested that the MFI and rETR/E parameters are useful parameters to reflect the state of *M. aeruginosa* photosynthesis process. Through suppression of both MFI and rETR/E parameters, the growth of algae was temporarily inhibited by LCLLE.

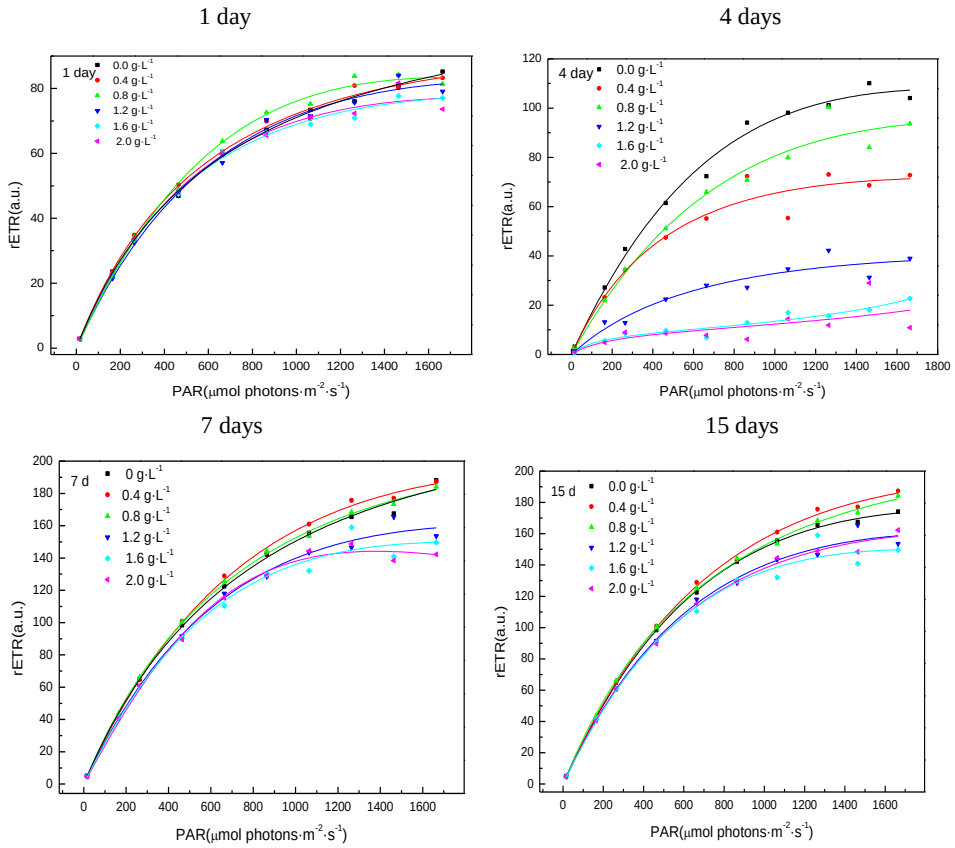

Figure 3. Rapid photosynthesis-irradiance response (rETR/E) curves of *M. aeruginosa* treated with different dosages of LCLLE. Each point represents the mean of three replicates. The solid line represents model results. Comparisons were performed using the Ratkowsky method for non-linear models (23).

Esterase activity of *M. aeruginosa*

Healthy cells take up FDA and convert to fluorescein. This conversion could be used to estimate the hydrolysis rate of esterase (41). Inhibition of esterase activity was detected as the shift in the FL1 histogram to the left compared to the control region. S2 indicated esterase activity in the control cells while S1 and S3 represented a decrease or increase in esterase activity respectively. The effect of LCLLE on the esterase activity in *M. aeruginosa* is shown in Fig. 4a. After 1 day exposure of esterase activity, approximately 0.5-1.6 % cells was decreased (1.6 % in control) and increased in 6.8-9.0 % cells (9.0% in control) (Fig. 4a). However, on day 5, more diverse results were seen. Cellular esterase activity was reduced (5.5-11.7 %) in all concentrations of extract, 11.7

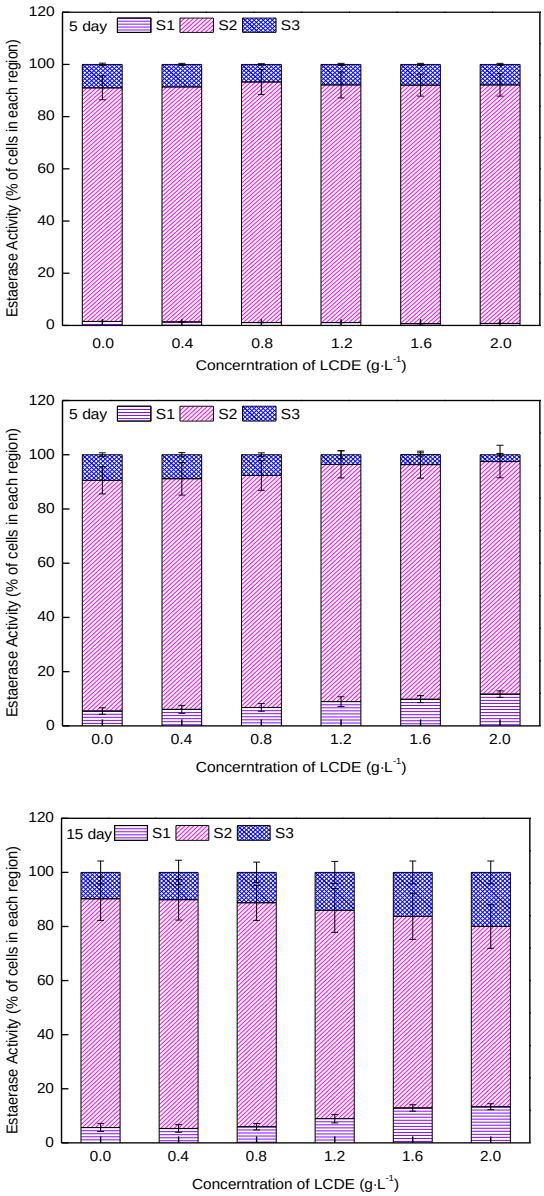

Figure 4. Distribution of cells in different esterase activities of *M. aeruginosa* after 1, 5 and 15 days LCLLE exposure. S1 represents a decrease in esterase activity;
S2 indicates esterase activity in the control cells; S3 represents an increase in esterase activity.
Data are expressed as the percentage of the fluorescence of control cells.

% of cells shifted to the lower activity state at 2.0 g/L dosage (5.5 % in control), while the higher esterase activity state of cells decreased to 2.5-9.5% (9.5% in control) (Fig. 4b). After 15 days exposure, the higher esterase activity state of cells increased, when exposed to each concentration of LCLLE including the control, reaching approximately 20.0 % and litter change for the lower esterase activity of cells (Fig. 4c). The esterase enzyme activities might be affected by LCLLE only at early time and this aligned with the temporary lowered electron transport capacity. The short half-lives of allelochemicals in LCLLE might contribute to the observation and hence multiple introduction of the extarct was the logical application of LCLLE.

Potential allelochemicals in LCLLE

GC-MS is important tool to determine the chemical components in plant extracts (15,25). To determine the nature and quantity of allelochemicals in LCLLE, it was subjected to GC-MS analysis. In LCLLE, the major organic compunds were: (Z)-9-octadecenamide (40.6%), 1, 3-bis (1, 1-dimethylethyl) -benzene (15.6%) and n-hexadecanoic acid (8.6%) (Table 2).

Table 2. Major Chemicals in LCLLE detected by GC-MS analysis

Chemical	Chemical formula	Relative amount (%)
1,3-bis(1,1-dimethylethyl)-Benzene	$C_{14}H_{22}$	15.59
Dodecane	$C_{12}H_{26}$	2.54
1-butyl-2-propyl-Cyclopentane	$C_{12}H_{24}$	2.91
Hexacosyl heptafluorobutyrate	$C_{30}H_{53}F_7O_2$	3.44
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	8.15
5-Methyl-2-(1-methylethenyl)-Cyclohexanol	$C_{10}H_{18}O$	2.66
Dotriacontyl heptafluorobutyrate	$C_{36}H_{65}F_7O_2$	2.10
(Z)-9-Octadecenamide	$C_{18}H_{35}NO$	3.45
2,2'-methylenebis[6-(1,1-dimethylethyl) -4-methyl-Phenol	$C_{23}H_{32}O_2$	3.12
Tetracosane	$C_{24}H_{50}$	2.21
(Z)-13-Docosenamide	$C_{22}H_{43}NO$	40.60

Many plant organic chemicals (such as lipids, ketones, alkaloids, etc.) suppresses the growth of algae (10). Previous studies have shown that some fatty acids viz., cis-6-octadecenoic and cis-9-octadecenoic acids significantly inhibited the growth of *M. aeruginosa* (17). In the present study, n-hexadecanoic acid was identified in LCLLE and this might act as potential allelochemical to inhibit the growth of algae. This finding is consistent with previous report (21) of hexadecanoic acid being effective against *M. aeruginosa*. However, to the best of our knowledge, (Z)-9-octadecenamide and 1, 3-bis (1, 1-dimethylethyl)-benzene found in LCLLE are not active against *M. aeruginosa*. Further experimental verification on the effect of these phytochemicals on the growth of *M. aeruginosa* mentioned is required.

CONCLUSIONS

In our research, LCLLE was tested for its ability to inhibit the growth of *M. aeruginosa*; *In vivo* Chl-*a* fluorescence, RCL of photosynthetic center PSII, PI and FDA staining assays suggested that the growth of *M. aeruginosa* was suppressed in concentration dependent manner. However, growth suppression 15 days after incubation, suggested that LCLLE was algistatic. GCMS analysis of the extract showed the presence of chemicals which may inhibit the growth of the algae.

ACKNOWLEDGEMENTS

The research was supported by funds from the National Natural Science Foundation of China (51378316). We also thank the Science, Industry, Trade and Information Technology Commission of Shenzhen Municipality (201504301657307).

REFERENCES

1. Barrington, D.J. and Ghadouani, A. (2008). Application of hydrogen peroxide for the removal of toxic cyanobacteria and other phytoplankton from wastewater. *Environmental Science and Technology* **42**: 8916-8921.
2. Bailey, S. and Grossman, A. (2008). Photoprotection in cyanobacteria: Regulation of light harvesting. *Photochemistry and Photobiology* **84**: 1410-1420.
3. Choe, S. and Jung, I. (2002). Growth inhibition of freshwater algae by ester compounds released from rotted plants. *Journal of Industrial and Engineering Chemistry* **8**: 297-304.
4. Codd, G.A., Morrison, L.F. and Metcalf, J.S. (2005). Cyanobacterial toxins: Risk management for health protection. *Toxicology and Applied Pharmacology* **203**: 264-272.
5. Falkowski, P. and Kiefer, D.A. (1985). Chlorophyll a fluorescence in phytoplankton: Relationship to photosynthesis and biomass. *Journal of Plant Research* **7**: 715-731.
6. Figueiredo, D.R.D., Azeiteiro, U.M., Esteves, S.M., Goncalves, F.J.M. and Pereira, M.J. (2004). Microcystin-producing blooms-a serious global public health issue. *Ecotoxicology and Environmental Safety* **59**: 151-163.
7. Franklin, N.M., Stauber, J.L. and Lim, R.P. (2004). Development of multispecies algal bioassays using flow cytometry. *Environmental Toxicology and Chemistry* **23**: 1452-1462.
8. Gao, L., Yuan, T., Zhou, C.Q., Cheng, P., Bai, Q.F., Ao, J.J., Wang, W.H. and Zhang, H.M. (2013). Effects of four commonly used UV filters on the growth, cell viability and oxidative stress responses of the *Tetrahymena thermophile*. *Chemosphere* **93**: 2507-2513.
9. Hagström, J.A., Sengco, M.R. and Villareal, T.A. (2010). Potential methods for managing *Prymnesium parvum* blooms and toxicity, with emphasis on clay and barley straw: A review. *Journal of the American Water Resources Association* **46**: 187-198.
10. Hong, Y., Hu, H.Y., Sakoda, A. and Sagehashi, M. (2011). Straw preservation effects of *Arundo donax* L. on its allelopathic activity to toxic and bloom-forming *Microcystis aeruginosa*. *Water Science and Technology* **63**: 1566-73.
11. Huang, H.M., Xiao, X., Ghadouani, A., Wu, J.P., Nie, Z.Y., Peng, C., Xu, X.H. and Shi, J.Y. (2015). Effects of natural flavonoids on photosynthetic activity and cell integrity in *Microcystis aeruginosa*. *Toxins* **7**: 66-80.

12. Kalaji, H.M., Schansker, G., Ladle, R.J., Goltsev, V., Bosa, K., Allakhverdiev, S.I., Brestic, M., Bussotti, F., Calatayud, A., Dabrowski, P., Elsheery, N.I., Ferroni, L., Guidi, L., Hogewoning, S.W., Jajoo, A., Misra, A.N., Nebauer, S.G., Pancaldi, S., Penella, C., Poli, D.B., Pollastrini, M., Romanowska-Duda, Z.B., Rutkowska, B., Serǒdio, J., Suresh, K., Szulc, W., Tambussi, E., Yanniccari, M. and Zivcak, M. (2014). Frequently asked questions about *in-vivo* chlorophyll fluorescence: Practical issues. *Photosynthesis Research* **122**: 121-158.
13. Kogan, S.I. and Chinnova, G.A. (1972). Relations between *Ceratophyllum demersum* (L.) and some blue-green algae. *Hydrobiologia* **8**: 14-19.
14. Kong, Y., Xu, X.Y., Zhu, L. and Miao, L.H. (2013). Control of the harmful alga *Microcystis aeruginosa* and absorption of nitrogen and phosphorus by *Candida utilis*. *Applied Biochemistry and Biotechnology* **169**: 88-99.
15. Li, F.M. and Hu, H.Y. (2005). Isolation and characterization of a novel anti-algal allelochemical from *Phragmites communis*. *Applied and Environmental Microbiology* **71**: 6545-6553.
16. Murray, D., Jefferson, B., Jarvis, P. and Parsons, S.A. (2010). Inhibition of three algae species using chemicals released from barley straw. *Environmental Technology* **31**: 455-466.
17. Nakai, S., Yamad, S. and Hosomi, M. (2005). Anti-cyanobacterial fatty acids released from *Myriophyllum spicatum*. *Hydrobiologia* **543**: 71-78.
18. Nelder, J.A. and Mead, R.A. (1965). A simple method for function minimization. *Computer Journal* **7**: 308-313.
19. Ou, H., Gao, N.Y., Deng, Y., Qiao, J.L. and Wang, H. (2012). Immediate and long-term impacts of UV-C irradiation on photosynthetic capacity, survival and microcystin-LR release risk of *Microcystis aeruginosa. Water Research* **46**: 1241-1250.
20. Paerl, H.W. and Huisman, J. (2009). Climate change: A catalyst for global expansion of harmful cyanobacterial blooms. *Environmental Microbiology Reports* **1**: 27-37.
21. Pan, Q., Zou, G.Y., Song, X.F., Nakai, S.S. and Fu, Z.S. (2014). Inhibitory effects of the roots of floating bed plants of *Canna indica* on *Microcystis aeruginosa*. *Research in Environmental Science* **27**: 193-1198. (Chinese)
22. Papageorgiou, G.C. and Govindjee (2004). *Chl-a Fluorescence: A Signature of Photosynthesis.* Springer Science and Business Media, Dordrecht.
23. Ratkowsky, D.A. (1983). *Nonlinear Regression Modeling: A Unified Practical Approach*. Marcel Dekker, New York.
24. Rice, E.L. (1984). *Allelopathy*, II Ed. Academic Press, Orlando, USA Pp. 424.
25. Ridge, I. and Pillinger, J.M. (1996). Towards understanding the nature of algal inhibitors from barley straw. *Hydrobiologia* **120**: 301-305.
26. Ridge, I. Walters, J. and Street, M. (1999). Algal growth control by terrestrial leaf litter: Arealistic tool? *Hydrobiologia* 3**95**-**396**: 173-180.
27. Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M. and Stanier, R.Y. (1979). Genetic assignments, stain histories and properties of pure culture of cyanobacteria. *Journal of General and Applied Microbiology* **111**: 1-61.
28. Rioboo, C., O'Connor, J.E., Prado, R., Herrero, C. and Cid, A. (2009). Cell proliferation alterations in Chlorella cells under stress conditions. *Aquatic Toxicology* **94**: 229-237.
29. Samuilov, V.D., Bezryadnov, D.B., Gusev, M.V., Kitashov, A.V. and Fedorenko, T.A. (2001). Hydrogen peroxide inhibits photosynthetic electron transport in cells of cyanobacteria. *Biochemistry* **66**: 640-645.
30. Samuilov, V.D., Timofeev, K.N., Sinitsyn S.V. and Bezryadnov, D.V. (2004). H_2O_2-induced inhibition of photosynthetic O_2 evolution by *Anabaena variabilis* cells. *Biochemistry* (Moscow) **69**: 926-933.
31. Schrader, K.K., de Regt, M.Q., Tidwell, P.R., Turker C.S. and Duke, S.O. (1998). Selective growth inhibition of the mushy-odour producing cyanobacterium *Oscillatria cf chalybea* by natural compounds. *Bulletin of Environmental Contamination and Toxicology* **60**: 651-658.
32. Street, M. (1978). Research on the improvement of gravel pits for waterfowl by adding straw. *The Game Conservancy Annual Review* **10**: 56-61.
33. Su, W., Johannes, A.H., Jia, Y.H., Lu, Y.P. and Kong, F.X. (2014). Effects of rice straw on the cell viability, photosynthesis and growth of *Microcystis aeruginosa. Chinese Journal of Oceanology and Limnology* **32**: 120-129.

34. Veldhuis, M.J.W. and Kraay, G.W. (2000). Application of flow cytometry in marine phytoplankton research: Current applications and future perspectives. *Scientia Marina* **64**: 121-134.
35. Wang, X.X., Jiang, C.C., Szeto, Y.T., Li, H.K., Yam, K.L. and Wang, X.J. (2016). Effects of *Dracontomelon duperreanum* defoliation extract on *Microcystis aeruginosa*: Physiological and morphological aspects. *Environmental Science and Pollution Research* **23**: 8731-8740.
36. Wang, X.X., Jiang, C.C., Li, J.W. and Wang, X.J. (2015). Effects of *Litchi chinensis* defoliation on growth and photosynthesis of *Microcystis aeruginosa. Journal Environmental Science* (China) **36**: 1648-1954. (Chinese)
37. Wicks, R.J. and Thiel, P.G. (2002). Environmental factors affecting the production of peptide toxins in floating scums of the cyanobacterium *Microcystis aeruginosa* in a hypertrophic African reservoir. *Environmental Science and Technology* **24**: 1413-1418.
38. Wu, Z., Deng, P., Wu, X., Luo, S. and Gao, Y. (2007). Allelopathic effects of the submerged macrophyte *Potamogeton malaianus* on *Scenedesmus obliquus*. *Hydrobiologia* **592**: 465-474.
39. Wu, Y., Ge, H.H. and Zhou, Z.Z. (2014). Effects of *Fructusligustri lucidi* on the growth, cell integrity and metabolic activity of the *Microcystis aeruginosa*. *Environmental Science and Technology* **22**: 8471-8479.
40. Wu, Z.X., Shi, J.Q. and Yang, S.Q. (2013). The effects of pyrogallic acid on growth, oxidative stress, and gene expression in *Cylindrospermopsis raciborskii* (Cyanobacteria). *Ecotoxicology* **22**: 271-278.
41. Xiao, X., Chen, Y.X., Liang, X.Q., Lou, L.P. and Tang, X.J. (2010). Effects of Tibetan hulless barley on bloom-forming cyanobacterium (*Microcystis aeruginosa*) measured by different physiological and morphologic parameters. *Chemosphere* **81**: 1118-1123.
42. Xiao, X., Han, Z.Y., Chen, Y.X., Liang, X.Q., Li, H. and Qian, Y.C. (2011). Optimization of FDA-PI method using flow cytometry to measure metabolic activity of the cyanobacteria, *Microcystis aeruginosa*. *Physics and Chemistry of the Earth* **36**: 424-429.
43. Xiao, X., Huang, H., Ge, Z., Rounge, T.B., Shi, J., Xu, X., Li, R. and Chen, Y.X. (2014). A pair of chiral flavonolignans as novel anti-Cyanobacterial allelochemicals derived from barley straw (*Hordeum vulgare*): Characterization and comparison of their anti-Cyanobacterial activities. *Environmental Microbiology* **16**: 1238-1251.
44. Zhang, M., Kong, F.X., Xing, P. and Tan, X. (2007). Effects of interspecific interactions between *Microcystis aeruginosa* and *Chlorella pyrenoidosa* on their growth and physiology. *International Review of Hydrobiology* **92**: 281-290.