

Removal efficiency of marine filamentous Cyanobacteria for Pyrethroids and their effects on the biochemical parameters and growth

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ABSTRACT

Pesticides, widely used in agricultural fields, eventually washed into surface waters, affecting a wide range of non-target organisms, including marine biota. In order to evaluate the response of two filamentous cyanobacteria isolated from marine environment, *Pseudanabaena* sp. (MB.1007) and *Leptolyngbya* sp. (MB.1010) were exposed to different concentration of technical grades (5–100 µg.mL⁻¹) and formulated commercial products (5–500 µg.mL⁻¹) of cypermethrin and deltamethrin. Morphological parameters, growth rates, photosynthetic pigments, carbohydrates, lipids, ash, moisture, and protein contents were estimated. Cypermethrin at concentrations up to 20 µg.mL⁻¹ stimulated growth rates, photosynthetic pigments, and biochemical parameters while deltamethrin showed inhibitory effects. Cypermethrin treated cells exhibited significant survivability as compared to those treated with deltamethrin. Analysis of recovered pesticides from the treated media showed the efficiency of both strains in pyrethroid removal. *Pseudanabaena* sp. (MB.1007) was found to be more efficient with up to 98.0% for cypermethrin and 99.0% for deltamethrin removal as compare to *Leptolyngbya* sp. (MB.1010) which showed removal efficiency of 82.0% for cypermethrin and 97.8% for deltamethrin. After removal study, a total of 35 chemical constituents have been identified from four different pooled culture media including 13 hydrocarbons (6, 10, 11, 12, 14, 15, 16, 18, 19, 31–33 and 35), 8 fatty alcohols and their derivatives (1, 2, 4, 7, 17, 20, 30, and 34), 8 aromatics including 2 phthalates (13 and 24), a fatty ketone (8), a furan (9), a fatty acid ester (23) and a fulvene (26). 3-phenoxy-benzaldehyde (3) and 1-(2-methyl-cyclopropyl)-ethanone (29), the degradation products of cypermethrin and deltamethrin, were also identified from the media exploiting GCMS.

1. Introduction

As a result of anthropogenic activities, the levels of organic pollutants found in surface water have increased in recent decades of which pesticides are the most common in aquatic environments [1,2]. These are sprayed in agricultural fields and enter the aquatic system through runoff and can potentially reach groundwater and eventually in sea water flowing through streams and rivers [3]. Large quantities of pesticides are applied annually, but only a smaller amount reaches the target pests efficiently. The rest is deposited in the soil, leached into water and hence contaminated to non-target organisms and the environment [4,5].

Exposure to pesticides has caused toxicity to different macroalgae and microalgae, resulting in reduced biodiversity and harmful algal blooms. This interaction is responsible for shifts in food webs of typical ecosystem, which in turn increases the heterotrophic activity [6,7]. Microorganisms, including algae, bacteria, protozoa, and fungi are essential biota of aquatic networks, which provide vital services such as primary food production, decomposition and recycling of different nutrients and anthropogenic chemicals. Pesticides alter the microbial population resulting in remarkable changes and damages water inhabitants as well as poses threats to human health [8]. Several studies have been conducted to evaluate the role of both naturally occurring and lab-derived microorganisms in the removal and degradation of

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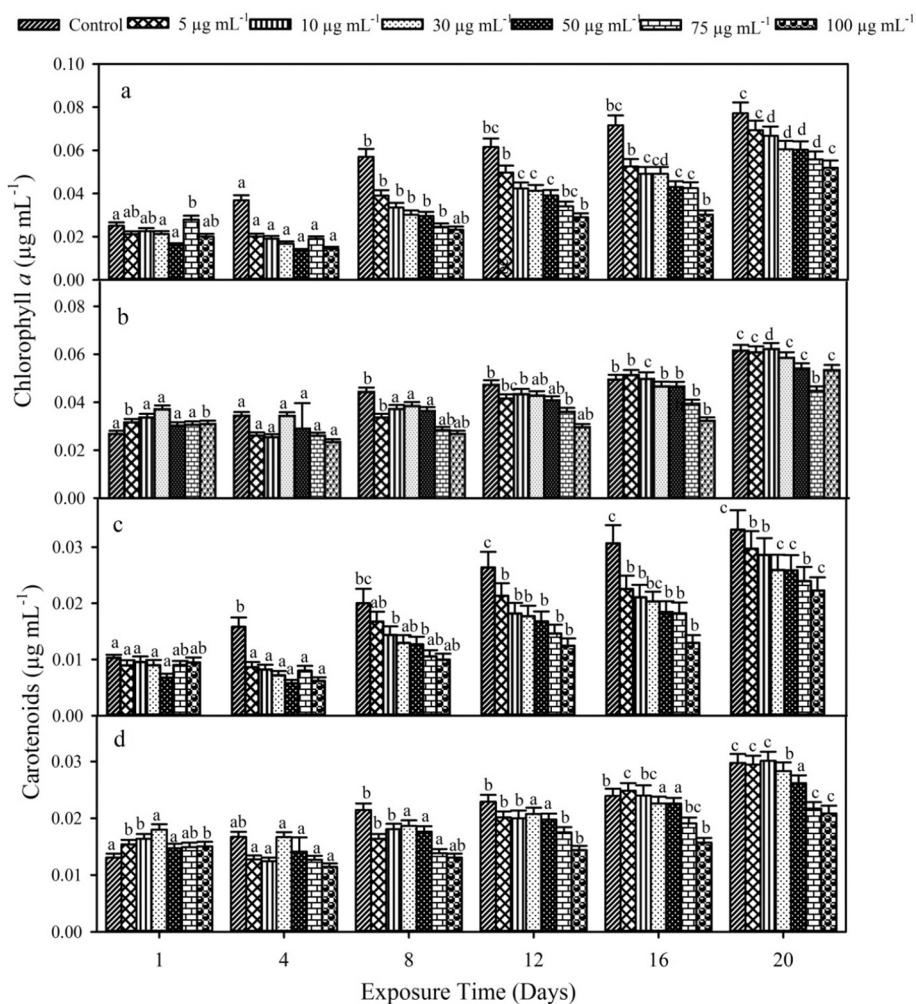


Fig. 1. Effect of technical grade cypermethrin on chlorophyll *a* of (a) *Pseudanabaena* sp. (MB.1007) (b) *Leptolyngbya* sp. (MB.1010) and total carotenoids of (c) *Pseudanabaena* sp. (MB.1007) and (d) *Leptolyngbya* sp. (MB.1010). Values are given as mean \pm SE of triplicates. Bars with different lower case letters denote statistically significant differences ($P < 0.05$) in chlorophyll *a* and total carotenoids with increasing exposure time.

Table 1

Variance analysis which shows the effects of technical grade pyrethroids on the pigments of cyanobacterial strains.

Pesticide concentration ($\mu\text{g mL}^{-1}$)	Cypermethrin				Deltamethrin			
	<i>Pseudanabaena</i> sp. (MB.1007)		<i>Leptolyngbya</i> sp. (MB.1010)		<i>Pseudanabaena</i> sp. (MB.1007)		<i>Leptolyngbya</i> sp. (MB.1010)	
	Chlorophyll <i>a</i>	Carotenoids	Chlorophyll <i>a</i>	Carotenoids	Chlorophyll <i>a</i>	Carotenoids	Chlorophyll <i>a</i>	Carotenoids
Control	68.754***	66.13***	13.11***	11.85***	8.05**	7.95**	10.75***	12.85***
5	36.45***	34.94***	4.99**	2.35ns	2.79*	2.39ns	121.13***	14.72***
20	37.70***	38.19***	6.11**	3.20ns	0.12ns	0.11ns	29.32***	14.73***
50	22.98***	23.50***	0.64ns	0.67ns	35.05***	14.04***	15.75***	5.58**
100	24.93***	24.71***	15.80***	14.12***	15.96***	12.85***	29.48***	22.81***
500	12.69***	12.33***	39.78***	21.47***	18.41***	13.44***	34.58***	19.50***

*** $P < 0.001$ = highly significant.

** $P < 0.01$ = significant.

* $P < 0.05$ = less significant.

ns $P > 0.05$ = non-significant correlations.

Pesticides [9,10,11].

Pyrethroids have been extensively used in agriculture, veterinary, and household formulations for more than 40 years and accounts for approximately 1/4th of the global pesticide market [12]. In Pakistan, pesticides including the class II pyrethroids cypermethrin and delta-methrin are used extensively. Pyrethroids are preferred over other pesticides because of their biodegradation into nontoxic or less toxic metabolites by different organisms including bacteria and cyanobacteria

[13]. Pyrethroids demonstrate low toxicity to mammals and birds, however, fishes are very vulnerable to exposure [14].

Abundance and role of cyanobacteria as the base of the many aquatic food chains are well studied [15,16]. Cyanobacteria are constantly consumed by larger planktonic microbes, filter feeders and grazers [17]. Cyanobacteria have gained attention for being used as an alternative source of food and food supplements due to their lipids and proteins [6,7,17,18]. Cyanobacteria and microalgae are considered as useful

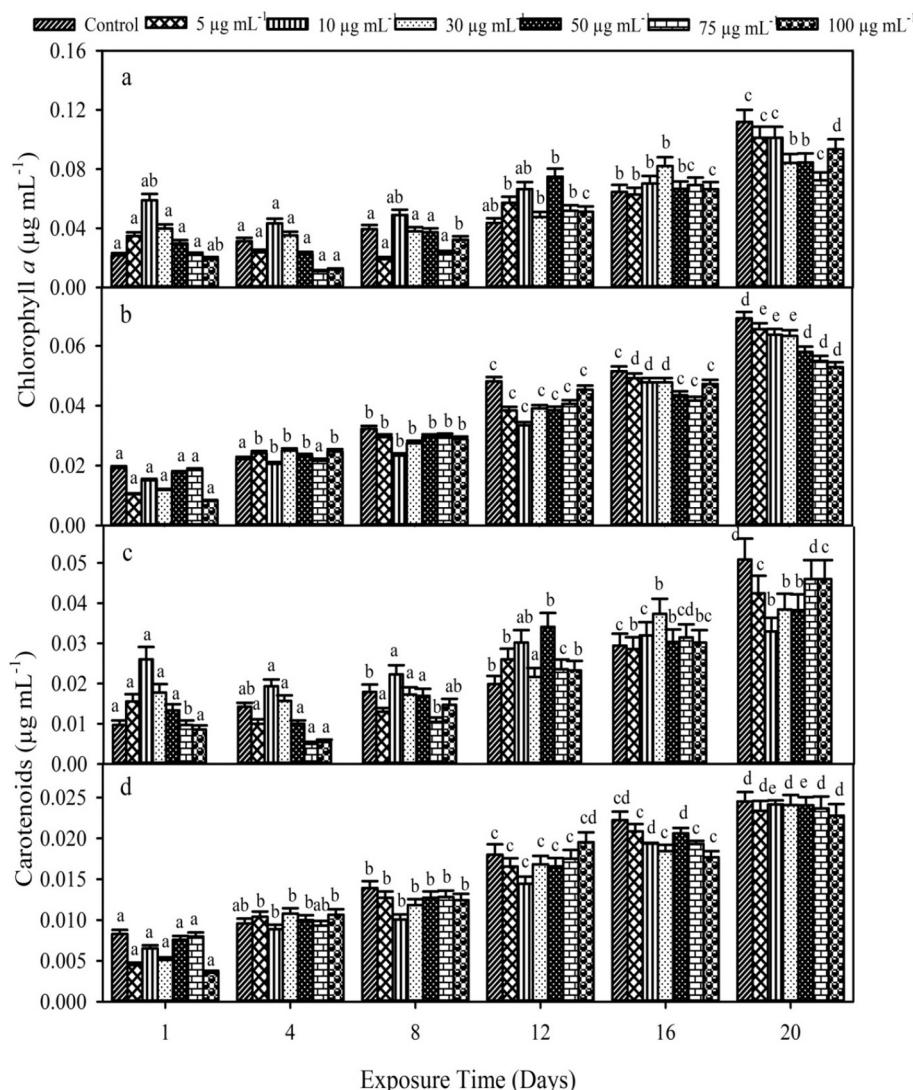


Fig. 2. Effect of technical grade deltamethrin on chlorophyll *a* of (a) *Pseudanabaena* sp. (MB.1007) (b) *Leptolyngbya* sp. (MB.1010) and total carotenoids of (c) *Pseudanabaena* sp. (MB.1007) and (d) *Leptolyngbya* sp. (MB.1010). Values are given as mean \pm SE of triplicates. Bars with different lowercase letters denote statistically significant differences ($P < 0.05$) in chlorophyll *a* and carotenoids with increasing exposure time.

organisms in the production of various secondary metabolites and biofuels or in waste water treatment [19].

Pesticides are proved to be effective against a wide range of pests that affect crop plants. Several reports have been published on the comparative toxicity of Chloryrifos and Methyl parathion against various test organisms such as cyanobacteria [20,21]. Kumar and co-workers [22] have conducted a study which shows effects of various organophosphates on nucleic acids of *Anabaena* sp. Photosynthetic, enzymatic, and biochemical investigations of *Anabaena fertilissima* in response to hexachloro-hexahydromethano-benzodioxathiepine-oxide was also performed [23]. A considerable amount of work relating to the inhibitory effects on growth, photosynthetic pigments, and nitrogen fixation in blue green algae induced by other pesticides have been done. A little has been reported on the interaction of pyrethroids with cyanobacteria [24].

Cyanobacteria are photoautotrophs and can fix the atmospheric nitrogen, which make them more resistant in contaminated sites. Therefore, cyanobacteria are favoured for bioremediation of environmental pollutants, either by biodegradation or bioaccumulation. Various studies have also been conducted to evaluate their efficiency to remove moderate amount of petroleum products and pesticides, [21,25]. The study, in hand, was conducted to evaluate the survival of cyanobacterial

strains in this polluted environment. Pyrethroids (cypermethrin $>$ deltamethrin) are one of the major pesticides used in Pakistan and hence selected for study [26].

The present study is aimed to explicate the effect of exposure of cypermethrin and deltamethrin on the two filamentous cyanobacterial strains, *Pseudanabaena* sp. (MB.1007) and *Leptolyngbya* sp. (MB.1010). These strains isolated from Sandspit backwaters were treated with varying concentrations of the pesticides. The changes in the amount of pigments and other bio-chemicals such as carbohydrates, proteins, lipids, ash, water and moisture content were observed. Analysis for recovery of pyrethroid pesticides from the media, their degradation products, and other metabolites were identified using gas chromatography - mass spectroscopy (GCMS) [15].

2. Materials and methods

2.1. Sample collection

Microbial mats were collected from Sandspit backwaters ($24^{\circ}49'N$ and $66^{\circ}56'E$), Karachi Pakistan, a highly polluted mangrove stand [27,28,29], using sterile glass slides. Samples transferred to sterilized plastic bottles were kept chilled in an ice box and transported back to the

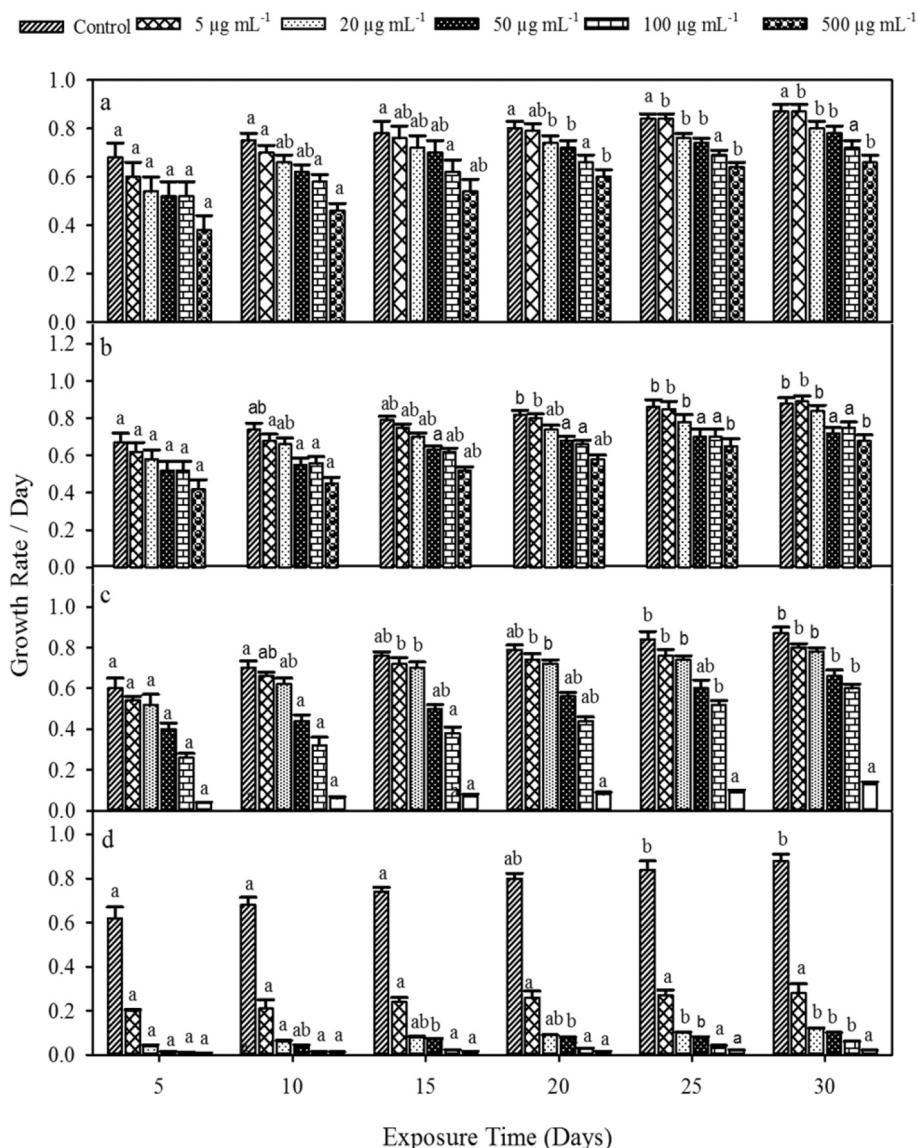


Fig. 3. Effect of cypermethrin on on growth rate of cyanobacteria (a) *Pseudanabaena* sp. (MB.1007) (b) *Leptolyngbya* sp. (MB.1010) and deltamethrin on (c) *Pseudanabaena* sp. (MB.1007) (d) *Leptolyngbya* sp. (MB.1010). Values are given as mean \pm SE of triplicates. Bars with different lowercase letters denote statistically significant differences ($P < 0.05$) in growth rates with increasing exposure time.

Table 2

Variance analysis which shows the effects of different concentrations of formulated pyrethroids on the growth rate of cyanobacterial strains.

Pesticide concentration ($\mu\text{g.mL}^{-1}$)	Cypermethrin		Deltamethrin	
	<i>Pseudanabaena</i> sp. (MB.1007)	<i>Leptolyngbya</i> sp. (MB.1010)	<i>Pseudanabaena</i> sp. (MB.1007)	<i>Leptolyngbya</i> sp. (MB.1010)
Control	2.60 ^{n.s.}	15.29***	2.845 ^{n.s.}	26.30***
5	5.92**	27.93***	2.920 ^{n.s.}	28.02***
20	6.08**	25.43***	3.26*	175.43***
50	6.81**	26.34***	5.82**	343.71***
100	4.82*	28.65***	14.45***	557.27***
500	13.81***	56.31***	23.21 ***	198.39***

*** $P < 0.001$ = highly significant.

** $P < 0.01$ = significant.

* $P < 0.05$ = less significant.

^{n.s.} $P > 0.05$ = non-significant correlations.

laboratory. These microbial consortia and sea water were inoculated in ASN III media and kept under light (2000 lx) at $28 \pm 2^\circ\text{C}$ [20]. ASN III medium was prepared with slight modification using 33 g/L of NaCl instead of 25 g/L mentioned in original recipe, to match with ambient

average salinity prevailing in the coastal waters of Sandspit, Karachi [29]. The pH of ASN III medium was 7.3 ± 0.2 at room temperature.

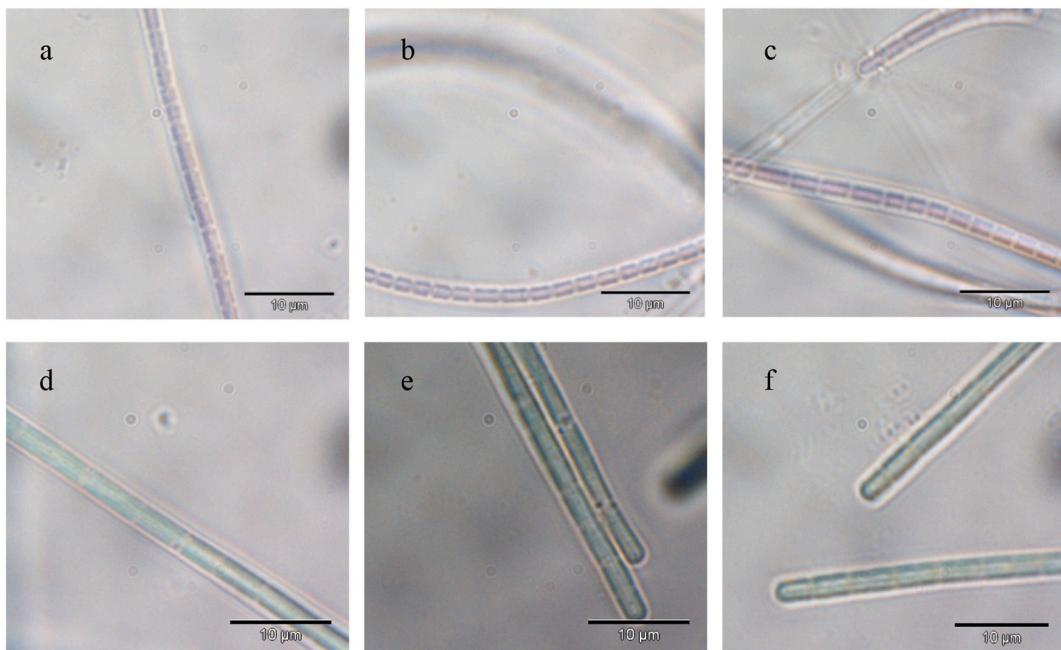


Fig. 4. Morphological variations; (a) Untreated *Pseudanabaena* sp. (b) Cypermethrin treated *Pseudanabaena* sp. (c) Deltamethrin treated *Pseudanabaena* sp. (d) Untreated *Leptolyngbya* sp. (e) Cypermethrin treated *Leptolyngbya* sp. (f) Deltamethrin treated *Leptolyngbya* sp.

Table 3

Effect of cypermethrin and deltamethrin on morphology of *Pseudanabaena* sp. (MB.1007) and *Leptolyngbya* sp. (MB.1010).

Species	Experiment	Length μm	Width μm	Sheath thickness μm	Thallus colour	Mat formation
<i>Pseudanabaena</i> sp. (MB.1007)	Control	2.9 ± 0.2	1.4 ± 0.2	0.54 ± 0.02	Purple	Normal
	Cypermethrin*	3.4 ± 0.2	1.8 ± 0.3	0.58 ± 0.03	Dark purple	Thick
	Deltamethrin*	2.8 ± 0.3	1.7 ± 0.3	0.62 ± 0.02	Light purple	Dispersed
<i>Leptolyngbya</i> sp. (MB.1010)	Control	4.4 ± 0.2	2.4 ± 0.3	0.42 ± 0.02	green	Normal
	Cypermethrin*	5.0 ± 0.4	2.6 ± 0.4	0.44 ± 0.02	Dark green	Thick
	Deltamethrin*	4.6 ± 0.2	2.9 ± 0.4	0.5 ± 0.04	Dull green	Dispersed

* Data were taken from micrographs captured strains grown in 5 $\mu\text{g.mL}^{-1}$ of pesticide at 30th day. Values are mean ± SD observed in 10 to 20 cells from 10 filaments of each strain.

2.2. Isolation and purification and identification

Isolation and purification of organisms were performed by serial dilution and plating method [30]. Identifications were taken to the lowest taxonomic level possible using all available information [31,32,33,34].

2.3. Preparation of media containing pyrethroid

2.3.1. Preparation of media containing technical grade pyrethroid

Technical grade cypermethrin (95%) and deltamethrin (98%) used, were cordially provided by the Aziz Groups Pesticides, Multan, Pakistan. Stock solutions of cypermethrin and deltamethrin ($100 \mu\text{g.L}^{-1}$) were prepared by dissolving pyrethroids in 0.4 mL acetone (Sigma Aldrich, Germany). Further dilutions (5, 10, 30 50 and 75 $\mu\text{g.L}^{-1}$) were prepared in 250 mL Erlenmeyer flasks with 100 mL ASN III media from the stock solution. The quantity of acetone used to mix the insecticide in a culture solution was within the admissible level of 0.5 mL.L^{-1} [35].

2.3.2. Preparation of media containing formulated pyrethroid

Formulated cypermethrin (10% EC, United Phosphorus, Gujarat, India) and deltamethrin (Deltashine® 2.5% EC, Isagro Agrochemicals, Mumbai, India) were used. Stock solutions (500 mg.L^{-1}) were prepared from formulated cypermethrin and deltamethrin, in ASN III media. Stock solution was used to prepare further dilutions containing 5, 20, 50,

and 100 mg.L^{-1} of pyrethroids as final concentrations in test solutions. $500 \text{ mg.L}^{-1}.\text{acre}^{-1}$ was the maximum concentration to the end user as per label claim and hence was used in the experiment as maximum. All concentrations were prepared in 250 mL Erlenmeyer flasks with 100 mL ASN III media from the stock solution.

2.4. Inoculation of cyanobacteria in media containing pyrethroid and incubation

2.4.1. For pigments analyses, growth rate measurements and morphometry

Exponentially growing stock cultures were inoculated in the media containing pesticides to get an initial absorbance of 0.1 A at 720 nm [36]. Pyrethroids were introduced once in the media and aliquots of 10 mL were withdrawn from the culturing flask till 30 d for the growth measurements after an equal interval of 5 d. No fresh media was added.

The experiments were extended to 30 d, based on the initial trials on growth curves. Cultures were always homogenized before inoculation in order to obtain an equal number of cells at the start of each experiments but it led to damages to the filaments of cyanobacteria [37]. Cyanobacteria took 5 d to acclimatize the pesticide stress, therefore, an equal interval of 5 d was selected to note the measureable change in cultures' parameters.

The stock and experimental cultures were maintained in a sterile fuming hood (Pharma Technology, Karachi, Pakistan) at $32 \pm 2^\circ\text{C}$ with continuous shaking (Digital orbital shaker, SHO-2D, Witeg

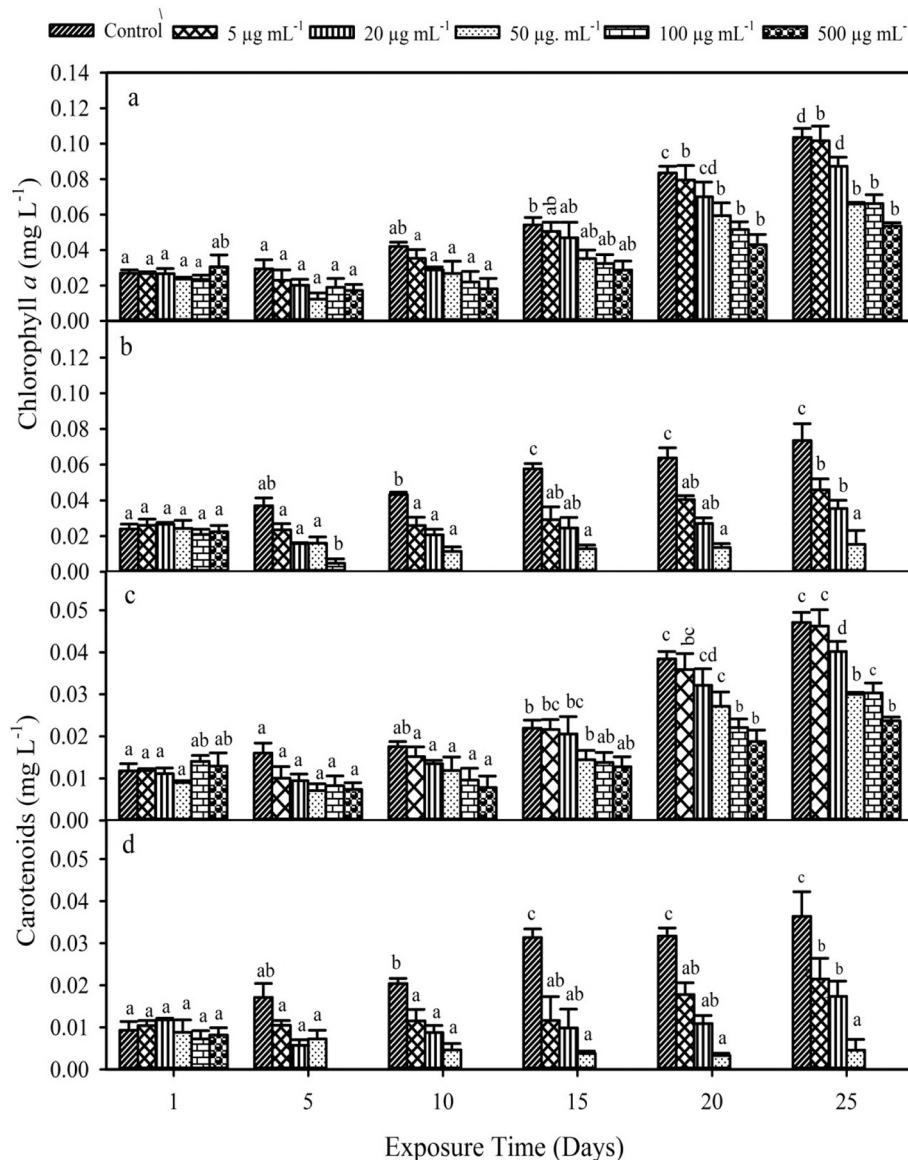


Fig. 5. Effect of formulated cypermethrin on chlorophyll *a* of (a) *Pseudanabaena* sp. (MB.1007) (b) *Leptolyngbya* sp. (MB.1010) and total carotenoids of (c) *Pseudanabaena* sp. (MB.1007) (d) *Leptolyngbya* sp. (MB.1010). Values are given as mean \pm SE of triplicates. Bars with different lowercase letters denote statistically significant differences ($P < 0.05$) in chlorophyll *a* and total carotenoids with increasing exposure time.

Laboratechnik, GmbH, Korea) at 80 rpm, on optimized conditions obtained after several preliminary trials. Culture vessels were illuminated with fluorescent tubes adjusted to a photon flux of $25 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ with light/dark cycle of 14/10 h in the fuming hood. Controls have been treated and analysed in similar way.

2.4.2. For biochemical analyses

A separate set of culture media having final concentration of $5 \mu\text{g} \cdot \text{mL}^{-1}$ pesticides was incubated for biochemical (carbohydrates, lipids, proteins, ash, and moisture) analyses. These tests were conducted after filtering and drying cyanobacterial cells in sterile conditions, on the 30th day from the replicates of mass cultured flasks (5 L). Controls have been treated and analysed in similar way.

2.4.3. For pesticide removal analyses

A separate set of culture media having final concentration of 100 and $500 \mu\text{g} \cdot \text{mL}^{-1}$ pesticides in 100 mL was arranged for pesticide removal studies. A (3×30) mL aliquot was drawn on the 30th day from the respective flasks for analyses of residual pyrethroids. Remaining

volumes from each replicate were pooled to analyse the metabolites and degraded products using GCMS.

2.5. Morphometry

Observations were made using a light microscope (Olympus, Japan) at $100\times$ magnification. Morphometry was done randomly on 10 filaments for each strain. These included vegetative cell width and length and sheath thickness.

2.6. Estimation of growth rate

The growth of the pyrethroid treated cyanobacteria was estimated as dried biomass. Stress was applied by maintaining 5, 20, 50, 100 and $500 \mu\text{g} \cdot \text{mL}^{-1}$ of deltamethrin and cypermethrin separately as final concentrations. The biomass was oven dried at 60°C for overnight and expressed as $\mu\text{g} \cdot \text{mL}^{-1}$ [38].

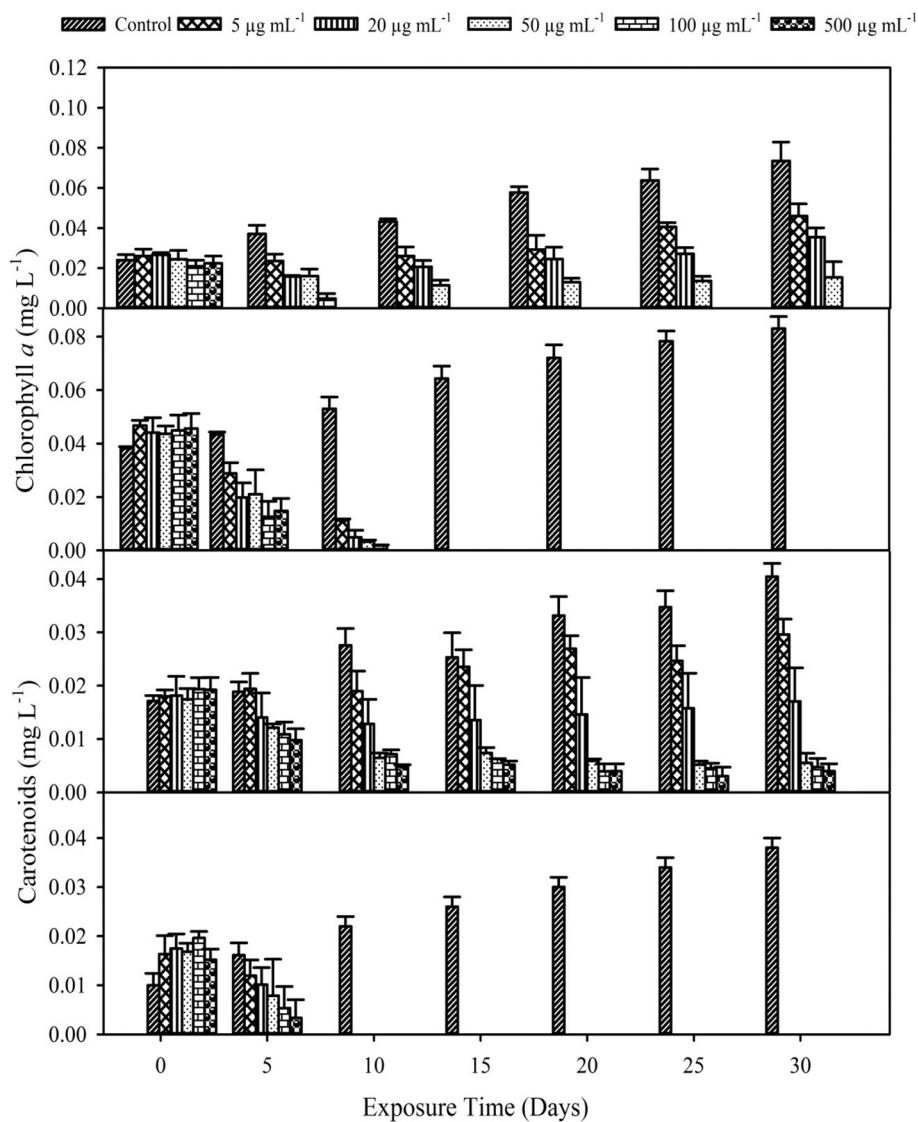


Fig. 6. Effect of formulated deltamethrin on chlorophyll *a* of (a) *Pseudanabaena* sp. (MB.1007) (b) *Leptolyngbya* sp. (MB.1010) and total carotenoids of (c) *Pseudanabaena* sp. (MB.1007) (d) *Leptolyngbya* sp. (MB.1010). Values are given as mean \pm SE of triplicates. Bars with different lowercase letters denote statistically significant differences ($P < 0.05$) in chlorophyll *a* and total carotenoids with increasing exposure time.

Table 4

Variance analysis which shows the effects of formulated pyrethroids on the pigments of cyanobacterial strains.

Pesticide concentration (µg.mL⁻¹)	Cypermethrin				Deltamethrin			
	<i>Pseudanabaena</i> sp. (MB.1007)		<i>Leptolyngbya</i> sp. (MB.1010)		<i>Pseudanabaena</i> sp. (MB.1007)		<i>Leptolyngbya</i> sp. (MB.1010)	
	Chlorophyll <i>a</i>	Carotenoids	Chlorophyll <i>a</i>	Carotenoids	Chlorophyll <i>a</i>	Carotenoids	Chlorophyll <i>a</i>	Carotenoids
Control	29.28***	11.59***	56.75***	24.99***	55.66***	28.16***	148.46***	58.73***
5	42.75***	16.14***	628.31***	276.69***	54.23***	24.05***	225.90***	85.80***
10	42.75***	16.15***	99.55***	43.83***	17.37***	8.88**	211.22***	77.55***
30	43.54***	17.75***	32.06***	14.12***	28.31***	14.57***	251.37***	97.47***
50	54.26***	20.45***	539.79***	237.70***	38.24***	19.57***	244.91***	116.11***
75	33.26***	13.78***	33.10***	14.57***	57.03***	28.76***	162.11***	54.70***
100	43.74***	15.83***	76.11***	33.52***	61.96***	31.34***	201.27***	64.19***

*** $P < 0.001$ = highly significant.

** $P < 0.01$ = significant.

2.7. Estimation of growth using chlorophyll *a* and carotenoids content

Chlorophyll *a* and carotenoids were estimated on spectrophotometer following Strickland and Parson [39]. Aliquot of 10 mL was taken from

each media flask and filtered over glass fibre filters (GF/F 0.7 µm; Whatman®). Cells collected were washed with distilled water and filter paper containing cells was placed in a 20 mL amber coloured test tube. 10 mL acetone was added, followed by incubation at 4 °C in dark for 48

Table 5

biochemical composition (%) of cyanobacteria when treated with cypermethrin and deltamethrin.

Biochemical	Species	Control cultures	Cypermethrin treated cultures	Deltamethrin treated cultures
Ash content*	<i>Pseudanabaena</i> sp. (MB.1007)	8.68 ± 0.34	5.35 ± 0.50	5.68 ± 0.33
	<i>Leptolyngbya</i> sp. (MB.1010)	9.18 ± 0.72	5.80 ± 0.35	6.98 ± 0.53
Moisture*	<i>Pseudanabaena</i> sp. (MB.1007)	15.23 ± 0.21	12.24 ± 0.33	16.60 ± 0.67
	<i>Leptolyngbya</i> sp. (MB.1010)	13.22 ± 0.19	11.02 ± 0.53	13.78 ± 0.78
Water*	<i>Pseudanabaena</i> sp. (MB.1007)	94.1 ± 0.08	93.32 ± 0.06	93.74 ± 0.08
	<i>Leptolyngbya</i> sp. (MB.1010)	94.0 ± 0.05	93.2 ± 0.14	95.31 ± 0.12
Carbohydrates**	<i>Pseudanabaena</i> sp. (MB.1007)	6.73 ± 0.05	10.3 ± 0.12	2.45 ± 0.05
	<i>Leptolyngbya</i> sp. (MB.1010)	12.13 ± 0.08	15.12 ± 0.24	4.43 ± 0.01
Lipids**	<i>Pseudanabaena</i> sp. (MB.1007)	9.16 ± 0.23	12.77 ± 0.27	4.96 ± 0.08
	<i>Leptolyngbya</i> sp. (MB.1010)	12.23 ± 0.24	15.02 ± 0.09	4.63 ± 0.13
Proteins**	<i>Pseudanabaena</i> sp. (MB.1007)	7.23 ± 0.12	9.53 ± 0.16	3.15 ± 0.39
	<i>Leptolyngbya</i> sp. (MB.1010)	4.23 ± 0.24	6.33 ± 0.10	1.23 ± 0.226

All results are reported as mean ± SD, taken from replicates (*n = 3 and **n = 9) and has p < 0.001.

h. Content was centrifuged at 3000 ×g for 20 min, filtered and analysed at 630, 647, and 664 nm for chlorophyll a and 480 and 750 nm for carotenoids.

2.8. Estimation of biochemical parameters

2.8.1. Carbohydrates

For determination of total carbohydrates, 100 mg of dried cyanobacterial samples were shaken with 2.5 mol.L⁻¹ hydrochloric acid at 100 °C for an hour to prepare the hydrolysed extract. Total carbohydrate was determined by phenol-sulphuric acid method [40]. In short, 0.2 mL extract was transferred to a test tube to which 1.0 mL of phenol (5%) and 5.0 mL concentrated sulphuric acid (95–97%) were added. Test tubes were placed in a water bath maintained at 100 °C for 30 min. Samples were analysed on UV-visible spectrophotometer (480 nm) using the standard glucose solution (concentration ranging from 10 to 100 mg.L⁻¹).

2.8.2. Proteins

Proteins were estimated with Folin-phenol reagent [41]. In short, 100 mg of algal biomass was taken in a test tube with 1 mL 1 N NaOH. The test tubes were subjected to centrifugation using SA International Clinical Centrifuge, CL, set at 3000 ×g. Filtrate collected was placed in a boiling water bath for 10 min. Reagents were added, mixed properly and again incubated at room temperature for 30 min. The absorbance of the supernatant was read at 660, 700 and 750 nm against blank, using bovine serum albumin (BSA) as standard in the concentration ranging from 10 to 100 mg.L⁻¹.

2.8.3. Lipids

Total lipid content was evaluated using Folch's method [42]. About 100 mg of the dried samples was extracted with chloroform:methanol (2:1 v/v) mixture and the filtrate was transferred to a pre-weighed vial for drying. Total lipid content was estimated gravimetrically.

2.8.4. Moisture content

For moisture content 1 g sample was dried in pre weighed China dish in oven at 105 °C for 3 h. Dishes were transferred and cooled in desiccator and reweighed to calculate % moisture content [43].

2.8.5. Ash content

For ash content 1 g oven dried samples, taken in pre weighed crucibles were placed in furnace, ignited and then ashed at 550 °C for 8 h. % ash content was calculated as per described method [43].

2.9. Recovery of pesticides and intermediate metabolites

After 30 d of incubation, biomass was harvested from each flask using centrifugation at 3000 ×g for 20 min. Supernatant was saved for analyses. Cell pellet was washed with distilled water to remove any insecticide residues adhering to the cell surface. Aliquots of 30 mL were withdrawn from supernatant and extracted thrice with 10 mL hexane: ethyl acetate (7:3 v/v) in 50 mL glass separating funnel. Hexane and ethyl acetate were purchased from VWR chemicals (France) and Dae Jung (South Korea), respectively. Column was prepared by adding 2 g activated alumina in chromatographic tube. Initial elution was done with 10 mL 15% diethyl ether (Merck, US) in hexane, followed by 20 mL hexane to remove alumina impurities. 30 mL of extracted sample was loaded on to a glass column (50 mL, 1 cm i.d.), packed with 2 g Silica 60, (Merck, Germany) and was eluted with 100 mL of 15% diethyl ether in hexane. Eluate was collected and evaporated on a rotary evaporator (NE-1, Eyela, Japan) to near dryness.

Collected 30 mL of organic phase was dried over anhydrous sodium sulfate and filtered in a 50 mL volumetric flask. Phenanthrene (98%, Sigma Aldrich, Germany) used as internal standard, was prepared as stock solution of 300 µg.mL⁻¹ in hexane:ethyl acetate (7:3 v/v). 10 mL of stock solution of internal standard was added in each flask and the volume was made up to the 50 mL. Thus the final concentration of internal standard in ready to analyse samples for GC was 60 µg.mL⁻¹.

2.10. Gas chromatography - mass spectroscopy (GC-MS) analyses

Extracts containing pyrethroids were analysed on Perkin Elmer Clarus® - 500 Plus gas chromatograph (USA) equipped with a 30 m × 0.32 mm × 0.25 µdf, HP-5MS® capillary column (Agilent, USA). Helium used as carrier gas was pressurized to give a column head pressure of 21.4 kPa and an average flux of 1 mL.min⁻¹. Initial temperature of oven was 170 °C for 2 min and ramped to 300 °C with a rate of 20 °C.min⁻¹. Temperatures of injector and EI source were fixed at 180 and 250 °C, respectively. 5 µL sample was injected with a split ratio of 1:10.

2.11. Statistical analysis

Analysis of variance (one-way ANOVA) was applied to determine whether treatments were significantly different. SPSS® 16.0 software was used. A Bonferroni post-hoc (P < 0.05) was employed to indicate significant differences among replicates mean values. All experiments were repeated minimum for three replicates. Mean values and standard errors of replicates were calculated and used for graphical representation (Sigma Plot®).

3. Result and discussion

The maximum range of concentrations used in the study was kept at higher sides, which are not ecologically or environmentally relevant. However, these ranges have been chosen on the basis of direct applications of pesticides in the field as per label claims (instructions) and as has also been reported in literature [44,45]. These selected concentrations have been chosen after initial survival screening studies. Attempts were initiated using technical grade cypermethrin (99%) and deltamethrin (95%). Acetone was used as carrier to dissolve the pyrethroids

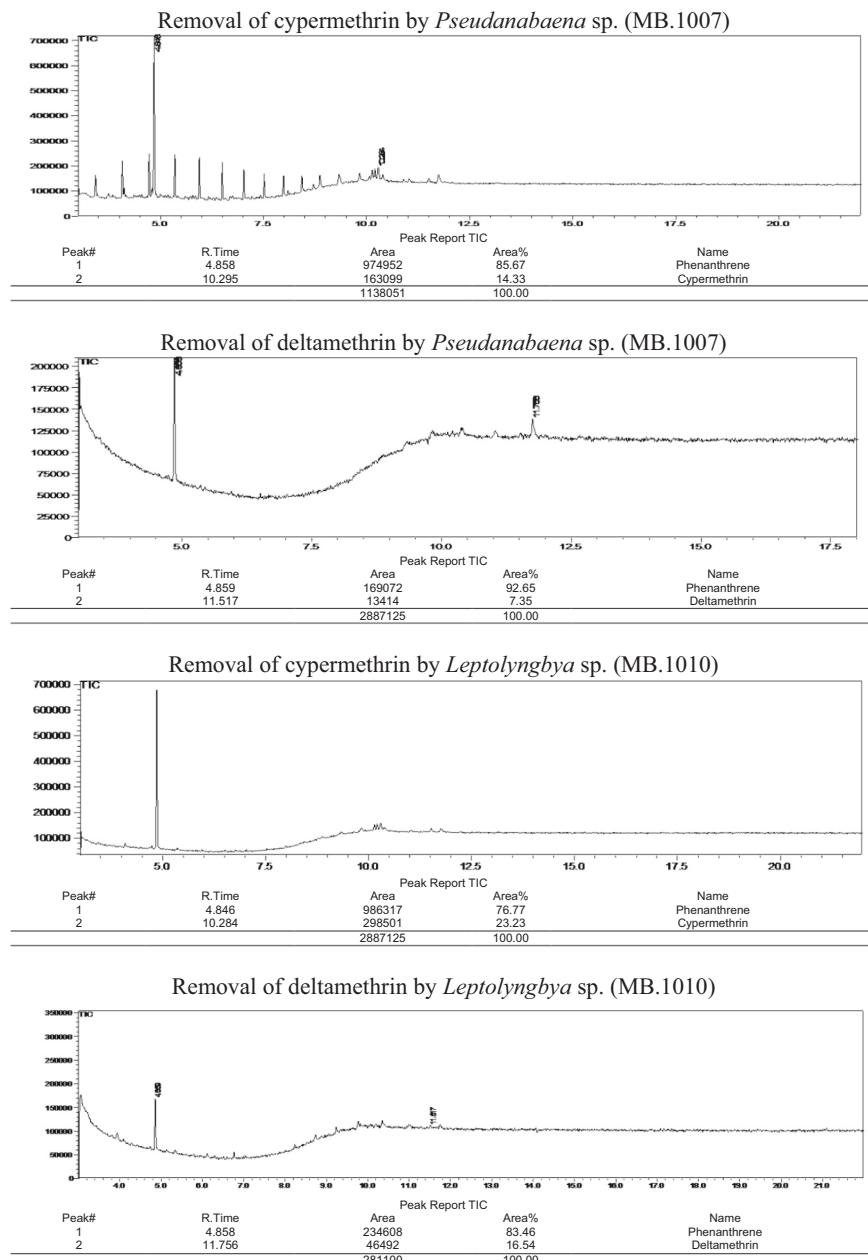


Fig. 7. Gas chromatograms of removal efficiency studies.

Table 6
Recovery of pyrethroids from media treated with cyanobacteria.

Pesticides	Cyanobacteria	Recovery concentration ($\mu\text{g.mL}^{-1}$)	Removed concentration ($\mu\text{g.mL}^{-1}$)	Removal efficiency (%)
Cypermethrin (500 $\mu\text{g.mL}^{-1}$)	<i>Pseudanabaena</i> sp. (MB.1007)	6.0 \pm 0.7	294.0 \pm 2.0	98.0
	<i>Leptolyngbya</i> sp. (MB.1010)	54.5 \pm 3.0	245.5 \pm 3.0	82.0
Deltamethrin (100 $\mu\text{g.mL}^{-1}$)	<i>Pseudanabaena</i> sp. (MB.1007)	0.53 \pm 0.1	59.5 \pm 2.0	99.0
	<i>Leptolyngbya</i> sp. (MB.1010)	1.3 \pm 0.2	58.7 \pm 2.0	97.8

All results are reported as mean \pm SD, taken from triplicates.

in media to facilitate its solubility in water [46]. This procedure probably renders the pyrethroid insoluble in media as solvation cage of pyrethroid-acetone in water breaks. With the passage of time (on standing) acetone may also have evaporated letting pyrethroids no more available for the interaction. More growth was observed in samples compared to blanks as reported earlier.

After preliminary experimentation it was observed that technical grade was rather showing stimulatory effects and were less soluble in water. The results led to use the formulated pyrethroids instead of technical grade against the pollution tolerant test cyanobacterial isolates.

To investigate the survival of cyanobacterial strains in pesticide stress the experiments were extended to 30 d. Cultures were homogenized before inoculation to obtain an equal number of cells at the start of each experiment. Homogenization process always result in some apparent damage to the filaments of cyanobacteria [37]. Starting 5 d were taken by cyanobacteria to acclimatize, therefore, an equal

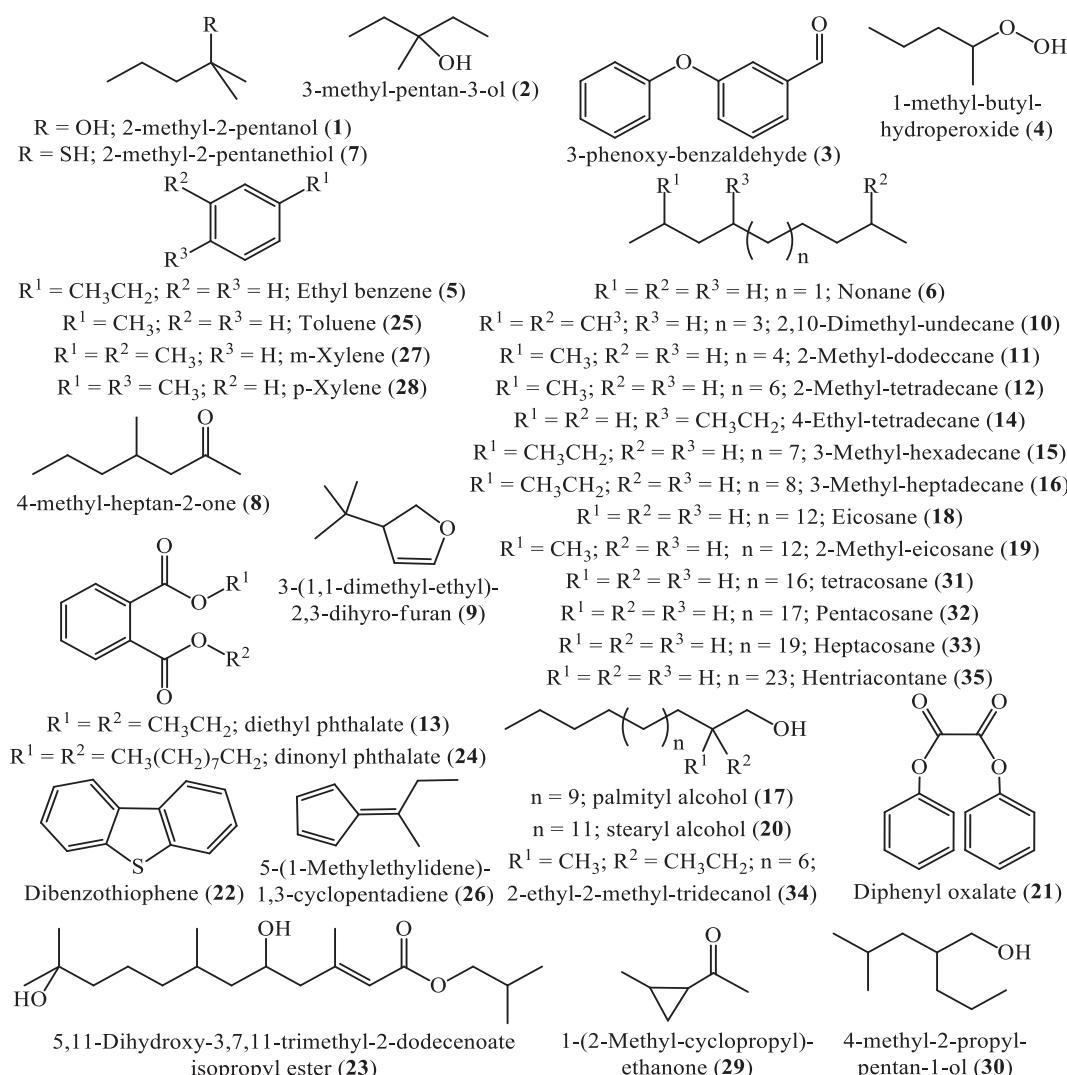


Fig. 8. Degradation products, natural metabolites, and excipients identified in GCMS studies.

interval of 5 d was selected to note the measurable change in cultures' parameters. The experimental design was also reported in an earlier study carried out in this lab on the interaction of unicellular cyanobacteria (*Chroococcidiopsis* sp. (MB.0023) and *Microcystis* sp. (MB.0024)) with cypermethrin and deltamethrin. In lower concentrations cypermethrin was found to be stimulatory or slightly inhibitory in term of chlorophyll *a*, carotenoids and growth rate while deltamethrin showed deleterious effects on both unicellular strains [47].

3.1. Effect of technical grade pyrethroids on chlorophyll *a* and carotenoids

In general, the inferences are made considering the effect of the excipients (xylene as solvent and emulsifiers) present in the formulated pyrethroids on the life cycles of the cyanobacteria as negligible. Initially all cultures treated with technical grades showed growth parallel to control, which demonstrate their indifferent behaviour and cyanobacterial strains developed resistance against cypermethrin. Technical grade cypermethrin was found to be slightly inhibitory in case of *Pseudanabaena* sp. (MB.1007) at all concentrations throughout the 30 days' experiment. Chlorophyll *a* and carotenoids were found to be decreased as compare to control cultures. In case of *Leptolyngbya* sp. (MB.1010) photosynthetic pigments were found to be increased with time and showed parallel growth when compared with control (Fig. 1, Table 1).

Various studies have been conducted to compare the toxicity of technical grade pesticides with their formulations. Active ingredients of pesticides were found to be less toxic as compare to their commercial formulations. They have evaluated the variations of acute toxicity of technical grade (92%) and commercial formulations (10%) of cypermethrin to fresh water organisms [48,49]. Another study revealed that commercial formulations were more toxic than technical grade [50], which is analogous to current study.

Deltamethrin was found to be less inhibitory against both strains of cyanobacteria (Fig. 2, Table 1). After initial inhibition chlorophyll *a* and carotenoids were observed to be increased in all concentrations till 30th day. Formulations of deltamethrin were found to be more toxic than its technical grade against various tested organisms.

The reasons have already been discussed above and were reported in other literatures [38,51,52,53]. The concentrations used in the current experiments were higher than those practically possible in water bodies but are not exceeding from the prescribed doses of applications.

3.2. Effect of formulated pyrethroids on growth rate

Growth rate of cyanobacteria was observed to be increasing in test strains at low concentrations of formulated cypermethrin (5 and 20 µg·mL⁻¹) which showed the tolerance and adaptability of cyanobacteria on pesticides exposure. At higher concentrations, growth rate was found to

Table 7

GC-MS analyses of Metabolites produced by *Pseudanabaena* sp. (MB.1007) (CP) and *Leptolyngbya* (MB.1010) (CL) when treated with cypermethrin.

RT ^a	IUPAC name (trivial name)	Conc. (%)
CP		
3.08	2-Methyl-2-pentanol (1)	1.39
3.44	3-Methyl-3-pentanol (2)	0.28
4.19	3-Phenoxy-benzaldehyde (3)	0.48
6.27	1-Methyl-butyl-hydroperoxide (4)	6.87
6.86	Ethyl benzene (5)	16.09
7.72	n-Nonane (6)	0.43
8.51	2-Methyl-2-pentanethiol (7)	17.61
8.67	4-Methyl-2-heptanone (8)	0.42
9.69	3-(1,1-Dimethylethyl)-2,3-dihydro-furan (9)	0.22
19.48	2,10-Dimethyl-undecane (10)	0.37
19.89	2-Methyl-dodecane (isotridecane) (11)	0.07
25.35	2-Methyl-tetradecane (isopentadecane) (12)	Tr.
28.07	1,2-Benzencarboxylic acid diethyl ester (diethyl phthalate) (13)	1.16
28.13	4-Ethyl-tetradecane (14)	1.16
30.66	3-Methyl-hexadecane (15)	0.36
32.66	3-Methyl heptadecane (16)	6.99
37.02	Hexadecan-1-ol (palmityl alcohol) (17)	1.38
39.55	n-Eicosane (18)	5.19
40.81	2-Methyl-eicosane (isoheneicosane) (19)	0.61
41.21	Octadecan-1-ol (stearyl alcohol) (20)	0.46
Total		61.54
CL		
4.17	Diphenyl oxalate (21)	6.37
4.19	3-Phenoxy-benzaldehyde (3)	0.50
4.75	Dibenzothiophene (22)	7.27
6.68	5,11-Dihydroxy-3,7,11-trimethyl-2-dodecenoate isopropyl ester (23)	2.13
8.50	1,2-benzene dicarboxylic acid dinonyl ester (di-nonyl phthalate) (24)	8.35
Total		24.62

^a RT = Retention time.

Table 8

GC-MS analyses of Metabolites produced by *Pseudanabaena* sp. (MB.1007) (CP) and *Leptolyngbya* (MB.1010) (CL) when treated with deltamethrin.

RT ^a	IUPAC name (trivial name)	Conc. (%)
DP		
3.69	Dimethyl benzene (toluene) (25)	63.16
4.19	3-Phenoxy-benzaldehyde (3)	0.38
6.51	5-(1-Methylethylidene)-1,3-cyclopentadiene (dimethyl fulvene) (26)	3.00
6.90	1,3-Dimethyl benzene (<i>m</i> -xylene) (27)	12.66
7.83	1,4-Dimethyl benzene (<i>p</i> -xylene) (28)	4.41
10.99	1-(2-Methylcyclopropyl)-ethanone (29)	0.57
49.21	4-Methyl-2-propyl-1-pentanol (30)	13.23
Total		97.41
DL		
7.92	Tetracosane (31)	8.56
8.50	Pentacosane (32)	0.08
9.17	Heptacosane (33)	28.10
9.98	2-ethyl-2-methyl-tridecanol (34)	31.20
10.96	Hentriacontane (35)	22.12
Total		90.06

^a RT = Retention time.

be lower as compared to control culture. Exposure time showed no significant effects on growth rate.

The inhibition of growth was observed from initial exposure up to 30 d and found relating directly to the exposure concentrations. This may be due to high permeability or denatured cellular membrane at higher concentration of the cypermethrin in both strains [54].

Increased growth rate at lower concentrations showed tolerance of cyanobacteria to cypermethrin and probably they may have started to

utilize it as carbon source as shown by the increased growth rate at 5 and 20 $\mu\text{g.mL}^{-1}$. The growth rate at 5 $\mu\text{g.mL}^{-1}$ was found exceeding from control on 30th day (Fig. 3 Table 2).

On the other hand, in case of *Pseudanabaena* sp. (MB.1007) deltamethrin was found to be stimulatory but increment in growth rate was less as compared to control. Stunted growth was observed at highest concentration (500 $\mu\text{g.mL}^{-1}$). In case of *Leptolyngbya* (MB.1010) growth rate was found to be inhibited at all concentrations. No significant change in growth rate was observed with time till 30th day. Growth inhibition was also reflected by reduction in photosynthetic pigments and protein content of tested cyanobacterial strains in analogous to previous report, which also demonstrated the greater toxicity of deltamethrin over cypermethrin [55]. The inverse relationship observed between deltamethrin concentrations and growth of cyanobacterial strains is shown in Fig. 3. Similar dose-dependent reduction in growth was also reported using chlorpyrifos [56].

3.3. Effect of formulated pyrethroids on morphometric characters

Microscopic observations of both the *Pseudanabaena* sp. (MB.1007) and *Leptolyngbya* sp. (MB.1010) revealed distinct morphological changes at viable concentrations of both pyrethroids. From microscopy it was observed that sheath size (consisting of polysaccharides) was found thicker in cypermethrin treated cultures than its control, (Fig. 4; Table 3). Cyanobacteria without sheath have been found more susceptible to pesticide stress in comparison to sheathed members [57]. Development of dense mat of filamentous cyanobacteria and thickening of sheath as compared to controlled culture could be a defensive physiological strategy to avoid pesticide and to survive in stressed conditions.

In deltamethrin treated strains sheath thickness was observed but with loss of pigmentation (Fig. 4; Table 3). In addition, the ability of strains to form mat was also found disrupted. Biochemical variations (vide infra) also supported the observation as carbohydrates were found to be decreased in deltamethrin treated cultures of both strains. Reduced carbohydrates may have resulted in their inability to form mats. Loss of pigmentations can also be justified by the reduction of chlorophyll *a* and carotenoids (vide supra) content in both strains.

3.4. Effect of formulated pyrethroids on chlorophyll *a* and carotenoids

The stimulatory effect of cypermethrin observed in *Pseudanabaena* sp. (MB.1007) at lower concentrations, 5 and 20 $\mu\text{g.mL}^{-1}$, was almost equal to the control while at other treatments (50, 100 and 500 $\mu\text{g.mL}^{-1}$) pigments were found to be decreased as compared to the control. Increased proportion of photosynthetic pigments (Fig. 5, Table 3) as has also been reported earlier [58], could not be justified without studying the mode of pesticide action on cyanobacteria. The results of current study with *Pseudanabaena* sp. (MB.1007) were in agreement with the adverse effect of herbicide Anilofos on *Synechocystis* sp., which has caused the cell lysis and decolorization of cultures [38].

Exposure of *Leptolyngbya* sp. (MB.1010) to different concentrations of cypermethrin showed inhibitory effect till 30th day. However, an increase in chlorophyll *a* was observed in media with low concentrations (5 and 20 $\mu\text{g.mL}^{-1}$). Complete inhibition in photosynthetic pigments in case of *Leptolyngbya* sp. (MB.1010) supported the observations of decolorisation of mat and inhibition in growth at higher concentrations (100 and 500 $\mu\text{g.mL}^{-1}$). In case of carotenoids no significant increment was observed with increasing exposure time.

Light harvesting pigments may increase due to utilization of pesticide or mutagenesis [58,59]. In addition to their role in photosynthesis, carotenoids also serve as sensitive biomarkers for screening of aquatic pollutants. Cyanobacteria has been reported to increase their carotenoids as a protective strategy under stress conditions [60]. Carotenoids reduced the stress-induced damages to the photosynthetic machinery by deactivating the chlorophyll molecules. The damages are triggered by

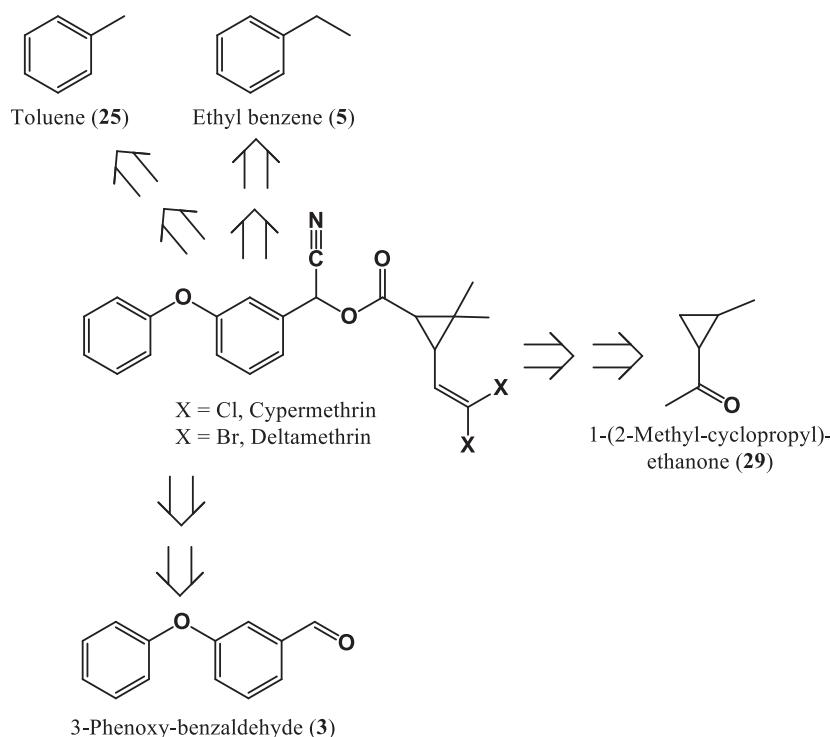


Fig. 9. Degraded products from pyrethroids.

the formation of reactive oxygen species (ROS) upon exposure to toxicants [61].

Deltamethrin further showed deleterious effects on photosynthetic pigments of both strains in all concentration. (Fig. 6, Table 4). Both pigments were found decreasing with decrease in growth until complete inhibition of pigments and growth. *Leptolyngbya* sp. (MB.1010) was found to be more susceptible to deltamethrin as compare to *Pseudanabaena* sp. (MB.1007). Literature showed that studies on interaction of deltamethrin with cyanobacteria are lacking but reports proving the adverse effects of deltamethrin on marine phytoplankton are on record [62]. Results in current study are also in agreement with the effects of pesticides on chlorophyll *a*, carotenoids, and phycobiliproteins of cyanobacteria and marine microalgal communities [63,64].

3.5. Effect of formulated pyrethroids on biochemical compositions

Carbohydrates were found increased in cypermethrin treated *Pseudanabaena* sp. (MB.1007) and *Leptolyngbya* sp. (MB.1010) (Table 5). Similar observations were reported for *Leptolyngbya foveolarum* on exposure to different concentrations of cartap hydrochloride [65].

Deltamethrin treatment has shown significant decrease in carbohydrate content as the carbohydrate metabolism has shifted towards the synthesis of sheath thickness (polysaccharides) in both test strains. Literature survey revealed that pesticide exposure decreases the carbohydrate content in algae. Kumar and co-workers have reported the inhibition of carbohydrates in the algae cultured in higher doses of herbicides; biochemical changes in cyanobacteria (*Aulosira fertilissima*, *Anabaena variabilis* and *Nostoc muscorum*) on endosulfan exposure; and reduction in carbohydrate content of *Aulosira fertilissima* and *Westiellopsis prolifica* with increasing concentrations of the tebuconazole [39,66].

Total lipids increased in both strains when treated with cypermethrin. Lipids are reported to play a vital role in response to tolerate several physiological stresses in photosynthetic organisms [67].

In contrast lipid content decreased in the test strains when exposed to deltamethrin. Deng and co-workers found that an herbicide diquat at

high concentration decreased the algal growth and lipid content. Study also revealed that sethoxydim and simazine at low concentrations showed lesser toxic effects to the alga but cause significant decrease in lipid content [68].

Total protein content increased in cypermethrin treated cyanobacteria. Increase in protein content might be due to the synthesis of stress alleviating proteins in cyanobacteria. In contrast syntheses of normal cell proteins are compromised [69].

Total protein content was found to be decreased in the cyanobacteria treated with deltamethrin. Protein content of *Nostoc* sp. was found decreasing when treated with butachlor [70]. There are reports that total protein is found reduced in *Protosiphon botryoides* when treated with a pesticide, which is related to increased level of protease activity [71]. Interruption in protein synthesis including structural proteins, which are essential for the growth of an organism, has also been found related to the inhibition of enzymes [72].

Decrease in ash content is correlated with the increase in the total organic content of the culture. Moisture content in cypermethrin treated strains found to be decreased. Higher moisture content in biomass can also reduce the lipid extraction efficiency and vice versa [73]. In analogy, low moisture content in studied cyanobacteria has resulted in higher lipid content. An increment was observed in the moisture content in case of deltamethrin treatment and thus lipid decreased.

Cyanobacteria are more tolerant to pesticides than other microalgae, particularly under conditions of elevated nutrient supply. This suggests that cyanobacteria are using pesticides as nutrient source [74].

The results of current study are in agreement with metabolic cost hypothesis, which proposed that all types of toxic stresses induce metabolic changes in the organisms, which results in depletion of its energy reserve leading to growth inhibition and biochemical changes. It has also been suggested that during inhibition of photosynthesis, cells accelerate degradation of photosynthetic pigments to maintain the balance between energy absorption and assimilation [75].

3.6. Recovery and quantitative analyses of pyrethroids

In the current study treatment of cyanobacteria with cypermethrin ($500 \mu\text{g.mL}^{-1}$) and deltamethrin ($100 \mu\text{g.mL}^{-1}$) were selected for the recovery and quantitative analyses (Fig. 7). These concentrations were found lethal for all tested strains showing maximised interaction. Experiments for recovery of pesticides were performed on 30 d - the final day of the treatment.

Data presented in Table 6 showed the recovery concentration and total removal efficiency of pyrethroids with corresponding cyanobacterial isolates. When treated with cypermethrin, recovery results showed that all four strains are capable of removing pesticides from the media. In case of cypermethrin results showed that *Pseudanabaena* sp. (MB.1007) was more efficient with recovery concentration of $6.0 \mu\text{g.mL}^{-1}$ accounting for total removal efficiency of 98.0%. In media treated with *Leptolyngbya* sp. (MB.1010) recovered concentration of cypermethrin was $54.5 \mu\text{g.mL}^{-1}$ (removal efficiency 82.0%). On the other hand, in case of deltamethrin *Pseudanabaena* sp. (MB.1007) was found to be more efficient with recovery concentration of $0.53 \mu\text{g.mL}^{-1}$ (removal efficiency 99.0%). In media treated with *Leptolyngbya* sp. (MB.1010) recovered concentrations of deltamethrin was $1.3 \mu\text{g.mL}^{-1}$ (removal efficiency 97.8%).

To date, biodegradation and removal of cypermethrin and deltamethrin by cyanobacteria is not reported in the literature. Studies have been conducted to evaluate the potential of bacterial strains in the removal and biodegradation of these pyrethroids. A bacterium *Bacillus* sp. was found to be capable of removing about 81% of cypermethrin by biodegradation [76]. Another study showed the degradation of cypermethrin by a bacterium strain (*Pseudomonas* sp.) which is capable of degrading 82% pesticide when treated with $40 \mu\text{g.mL}^{-1}$ [77]. A bacterial isolate IK2a was capable of degrading 84.5% cypermethrin [78]. Results revealed that cyanobacteria in current study have removed pyrethroids more efficiently in comparison to reported bacteria.

3.7. Qualitative analyses of metabolites from culture media

Media containing different concentrations of formulated cypermethrin and deltamethrin ($5, 20, 50, 100$ and $500 \mu\text{g.mL}^{-1}$) for each strain were pooled together to get four samples i.e. CP (*Pseudanabaena* sp. (MB.1007) treated with cypermethrin), CL (*Leptolyngbya* sp. (MB.1010) treated with cypermethrin), DP (*Pseudanabaena* sp. (MB.1007) treated with deltamethrin) and DL (*Leptolyngbya* sp. (MB.1010) treated with deltamethrin). The aim was to identify the chemical constituents recovered from the media after interaction of 30 d. Theoretically these included degradation products of pyrethroids, chemical constituents expected and recovered from formulation and media, as well as regular metabolites. A total of 35 such chemical constituents have been recovered and identified from these pooled media (Fig. 8, Tables 7 and 8).

3-Phenoxy-benzaldehyde (3) was identified from all four pooled samples. Several studies reported that 3 is an intermediate product formed during biodegradation of cypermethrin [79,80] and deltamethrin [81]. Ethyl benzene (5) was identified in CP while toluene (25) was identified in DP. Analysing the carbon skeleton of pyrethroids, 1-(2-methyl-cyclopropyl)-ethanone (29) can also be considered as a degraded product. 29 was identified in DP. Fig. 9 postulates an explanation of degraded fragments originating from pyrethroids. 3, 5, 25, and 29 are justified as degraded products.

Initially both pesticides showed negative impacts on growth of test strains, with the passage of time the cyanobacteria strains developed tolerance against cypermethrin and deltamethrin. The possible reasons of pesticide tolerance, which is a matter of further studies, may include capability of isolates for the bioaccumulation of pyrethroids either by biosorption or uptake inside the cell.

Whole set of pesticide exposure experiments were conducted using formulated emulsifiable concentrates (ECs). Both the procured ECs

contained xylol® (xylanes) as solvents. Thus, all enriched samples were expected to contain xylanes as well as its associated known impurities. Ethyl benzene (5), toluene (25), *m*-xylene (27) and *p*-xylene (28) were identified from different pooled enriched samples in the GC-MS analyses. 5 and 25, which are expected degradation products might also be considered as impurities of 27 and 28 used as solvent in commercial EC formulations of cypermethrin and deltamethrin. Dibenzothiophene (22), a petroleum product, although not common but can be a possible impurity of 27 and 28.

GC-MS analyses further showed presence of many hydrocarbons recovered from the media. These included *n*-nonane (C₉; 6), 2,10-dimethyl-undecane (C₁₁; 10), 2-methyl-dodecane (C₁₂; 11), 2-methyl-tetradecane (C₁₄; 12), 4-ethyl-tetradecane (C₁₄; 14), 3-methyl-hexadecane (C₁₆; 15), 3-methyl heptadecane (C₁₇; 16), *n*-eicosane (C₂₀; 18), and 2-methyl-eicosane (C₂₀; 19). Other hydrocarbons identified from DL included *n*-tetracosane (C₂₄; 31), *n*-pentacosane (C₂₅; 32), *n*-heptacosane (C₂₇; 33) and *n*-hentricontane (C₃₁; 35). The parenthesis showed the catenated carbon skeleton and not the total carbons. Various straight chain and branched alkanes have been detected from the natural unstressed culture media of filamentous cyanobacteria. Medium and long chain linear C₁₂ and C₃₂ hydrocarbon and branched C₁₄ to C₂₈ hydrocarbons were reported from green algae [82,83]. Another study revealed that *Anabaena cylindrica* can biosynthesize very long chain *n*-alkanes, ranging from C₂₃ to C₃₁ under salt stress [84]. Berla and co-workers explicated the function of medium chain hydrocarbons in the regulation of electron flow during cyclic phosphorylation in thylakoid membranes to support cyanobacterial growth under cold stress [85]. Cyanobacteria are also reported to accumulate hydrocarbons in their membranes, but their role in the life cycle is not yet cleared. However, current experiments, conducted in controlled environment using synthetic media and artificial seawater, cannot lead to the conclusion of accumulation and thus proving that the identified hydrocarbons, whether linear or branched has been biosynthesized.

Oxidation of short chain alkane, 2-methyl-pentane into 21 is reported from a fungus. It might be possible that desmethylated product of alcohol 1 under oxidative stress, as expected on exposure of pyrethroids, may generate the hydroperoxide 4 [86]. A report described the synthesis of 1-methyl-butyl-hydroperoxide in seedless long jujube under high-oxygen conditions [87]. 2-methyl-2-pentanethiol (7) is the thiol version of alcohol 1. Alcohol 7 has been reported from ethanol extract from *Canthium dicoccum*, showing potent antimicrobial, anti-tumor, Immunomodulatory and antioxidant activity [88]. 2-Ethyl-2-methyl-tridecanol (34) was identified from cypermethrin treated media of *Leptolyngbya* sp. (MB.1010). Branched fatty alcohol 34 was also reported from the extracts obtained from the leaves of *Momordica angustisepala* which showed antibiotic and antioxidant activities [89]. However, palmityl alcohol (17) and stearyl alcohol (20) are very common fatty alcohols reported from various organisms including cyanobacteria. 3-methyl-3-pentanol (2) and 4-methyl-2-propyl-pentan-1-ol (10) are branched alcohols. The cyanobacteria are reported to produce variety of such branch alcohols e.g., 2-methyl-hexan-3-ol and 3-methyl-2-propyl-1-pentanol was detected when *Pseudanabaena* sp. (MB.1007) was exposed to deltamethrin stress in current study. Some branched alcohol from the extracts of cyanobacterial strains and green alga showed the activity against human pathogenic bacteria and yeast [90].

4-methyl-2-heptanone (8) has not been reported from cyanobacteria, instead its positional isomer 6-methyl-2-heptanone has been identified from a bacterium *Bacillus subtilis*, which the bacteria synthesized to repel the nematodal predators [91]. 3-(1,1-dimethyl ethyl)-2,3-dihydro furan (9) has not been previously reported from cyanobacteria. Some metabolites of 2,3-dihydro-furan have been reported from other organisms as well [92,93]. Diphenyl oxalate (21) is a double ester of phenol with oxalic acids. This might be a product of biotransformation, formed by reaction of phenol (biodegradation product of pyrethroids) with oxalic acids. Reports showed the presence of organic acids such as oxalic acid in plants, algae, cyanobacteria and fungi [94,95]. Oxalates

are produced in cells by the oxidation of carbohydrates. Plants are reported to produce oxalates for their protection against predation [96]. 5,11-Dihydroxy-3,7,11-trimethyl-2-dodecenoate isopropyl ester (**23**), an isoprenoid (sesterpenoid) has been identified from media of *Leptolyngbya* sp. (MB.1010). Isoprenoid **23** has not been yet reported from any cyanobacterial strains. It is a common metabolites reported from various plants [97]. 1-(2-Methyl-cyclopropyl)-ethanone (**29**) earlier justified as degradation product (Fig. 9) was detected along with 3-methyl-2-propyl-1-pentanol (**30**) when *Pseudanabaena* sp. (MB.1007) was exposed to deltamethrin stress.

Phthalates have been identified from the media when cyanobacteria were treated with pyrethroids. These include; diethyl phthalate (**13**) from CP and dinonyl phthalate (**24**) from CL. *Microcystis aeruginosa* and *Phormidium* sp. have been reported to biosynthesized phthalate when subjected to stress conditions [98]. Current experiments, conducted in controlled environment confirm that the phthalates have been biosynthesized.

4. Conclusion

The present study suggests that cyanobacterial strains *Pseudanabaena* sp. (MB.1007) and *Leptolyngbya* sp. (MB.1010) can be used for bioremediation. Growth rates of cyanobacterial strains in cultures containing cypermethrin and deltamethrin were found reduced in comparison to controls. Significant changes in biochemicals of both species before and after exposure, removal efficiency and identification of some degraded metabolites from cypermethrin and deltamethrin suggests that cyanobacteria are utilizing pyrethroids as source of energy.

CRediT authorship contribution statement

Saira Bano: Conceptualization, Data curation, Methodology. **Zaib-Un-Nisa Burhan:** Methodology, Writing – original draft. **Muhammad Nadir:** Visualization, Software, Investigation. **Amir Ahmed:** Software, Validation, Methodology. **Sarwat Ghulam Rasool:** Formal analysis, Data curation. **Pirzada Jamal Ahmad Siddiqui:** Conceptualization, Resources. **Munawwer Rasheed:** Supervision, Writing – review & editing.

Declaration of competing interest

The authors have declared no conflict of interest.

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Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study.

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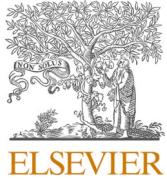
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Corrigendum

Corrigendum to ‘Removal efficiency of marine filamentous Cyanobacteria for Pyrethrins and their effects on the biochemical parameters and growth’ [Algal Res. 60 (2021) 102546]



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The authors apologise for any inconvenience caused to the readers due to an inadvertent error in the references. References for [35–46] were incorrectly listed in the original version of the manuscript and should be listed as:

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