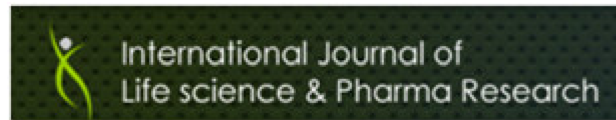

EFFECT OF ZINC STRESS ON BIOCHEMICAL PROFILING IN *DUNALIELLA SALINA* TEOD. AND *SPIRULINA PLATENSIS* GOMO.

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ABSTRACT

All biomolecules like lipids, carbohydrates and fatty acid compositions of a native microalgae *Dunaliella salina* (Teod.) and *Spirulina platensis* (Gomo.) were studied in batch culture at light intensity 100 μ mol photons m^{-2} s^{-1} temperature 25 ± 1°C and 16:8 h light and dark diurnal cycles. It was reported in our previous paper that both lipids and carbohydrate content decreased with increasing concentration of Cu stress. Similar scenario of contents was observed in lipid and carbohydrate on various treatment doses of zinc as heavy metal. GC-MS profiling of lipids showed that various compounds separated according to their retention time in which most of the compounds were Fatty Acid Methyl Ester. Further, purification of carbohydrates was confirmed by HPLC analysis using reference compounds like fructose, glucose and sucrose. The microalgae is rich deposits of essential nutrients so they could be candidate for aqua-culture feeding and/or biofuel production.

KEYWORDS : *Dunaliella salina; Spirulina platensis; lipids; carbohydrates; GC-MS profiling; HPLC; Zn stress.*

INTRODUCTION

Heavy metals at toxic levels hamper normal plant functioning and act as an impediment to metabolic processes in a variety of ways, including disturbance or displacement of building blocks of protein structure, which arises from the formation of bonds between heavy metals and sulfhydryl groups [1] hindering functional groups of important cellular molecules [2] , disrupting functionality of essential metals in biomolecules such as pigments or enzymes [3], and adversely affecting the integrity of the cytoplasmic membrane [4] , resulting in the repression of vital events in plants such as photosynthesis, respiration, and enzymatic activities. Conversely, Zn^{2+} is an essential micronutrient for plants, and it is therefore essential for plant growth, development, and many metabolic processes being one of the major cofactors, together with iron and manganese, in numerous enzymes [5]. More than 1200 proteins are predicted to contain, bind, or transport Zn^{2+} [6]. Furthermore, the zinc-inorganic phosphate (Pi) relationship has been observed in numerous plant species such as tomato, cotton, barley, and *Arabidopsis* [7]. Besides being a micronutrient, zinc is also a heavy metal and can have detrimental effects on many vital processes in plant cells. Microalgae biomass contains products with high commercial importance like proteins, lipids, carbohydrates [8]. Environmental stress condition when nutrients are limited invariably causes a steadily declining cell division rate. Surprisingly, active biosynthesis of fatty acids is maintained in some algae species under such conditions, provided there is enough light and CO_2 available for photosynthesis [9]. Their main photosynthetic pigment is phycocyanin, which is blue in colour. These bacteria also contain chlorophyll a, b and carotenoids. Some contain the pigment phycoerythrin, giving the bacteria a red or pink colour. *Dunaliella* species belong to the phylum Chlorophyta, order Volvocales and family Polyblepharidaceae, and are unicellular, photosynthetic and motile biflagellate microalgae morphologically distinguished by the lack of a rigid cell wall [10] . One who has grown microalgae under

laboratory or outdoor condition is well aware of the fact that to obtain high lipid content, external stress or lipid induction techniques need to be applied. Naturally under ideal growth conditions many microalgae produce saturated and unsaturated fatty acids , which have high nutritional value, but are less ideal for biofuels. However, the synthesis of neutral lipids in the form of Triacylglycerides (TAGs) can be induced in many species under stress conditions. In the present study, two algae *Dunaliella salina* (Teod.) and *Spirulina platensis* (Gomo.) were subjected to different concentrations of Zinc stress and the effect on the carbohydrates and fatty acid profiling was investigated. The aim of present study is to determine if nutritional composition of algae can be refereed as sensitive physiological factors in toxicological studies of effect of heavy metals after treatment on these algae.

MATERIALS AND METHODS

Isolation, purification, Identification and Cultivation of algal strains from different areas of Jaipur.

Algal samples (*Spirulina platensis)* were collected periodically from different sites of Jaipur city especially from Jal Mahal and Tal Katora. The other algae (*Dunaliella salina)* was collected from Sambhar Lake (Rajasthan). (Table 1) From different water bodies cultures of different algal strains were obtained and were purified and maintained in suitable culture medium. For their optimum growth, cultures were maintained in agar slants and cultured in liquid medium. The cultures were cultivated once in every 25 days and their slants were prepared once in three months.

Table 1
Culture Collection of algae

S.No.	*Collection site*	*Month*	*Name of algae collected*
1.	Jal mahal,jaipur	August 2014	Spirullina platensis
2.	Sambhar lake, jaipur	November 2014	Dunaliella salina
3.	Tal katora,jaipur	July 2015	Spirullina platensis

By centrifugation method algal samples with very low cell count and in mixed forms were concentrated, while those with high cell counts were diluted with suitable medium. Enrichment and isolations were carried out using enrichment culture media till unialgal forms of species were obtained. In enrichment method, the inoculum was prepared by mixing the samples with the selected medium and then serial dilutions were made in test tubes containing similar media. Direct isolations were done by picking up single filament or single cell using sterile Pasteur pipettes, which were pulled out into a very thin capillary, using a dissecting microscope. Streaking method was used to contaminated cultures which can be made unialgal. In streaking method, a loop full of algal suspension is taken and drawn into a long zigzag streak on an algal plate. After incubation, isolated colonies appearing at the tail end of the streak are expected to be unialgal.

Maintenance of Stock Cultures

The unialgal cultures were grown on agar slant with suitable medium contained in screw capped culture tubes to reduce frequent sub culturing without losing viability. The inoculated slants in 10-15 ml culture tubes with cotton plugs were incubated in a growth room for 7-10 days. The cotton plugs were replaced by pre sterilized, bakelite screw caps provided with rubber liner under aseptic conditions. After sufficient algal growth appeared on the agar slants, culture tubes were transferred to stock culture room under conditions to keep them in viable state. Low temperature (15-20°C) and light intensity (50-200 lux) maintains the culture in viable state for longer duration.

Cultivation

For the cultivation, 3-5 day old cultures of algae of prepared inoculum of unialgal culture was added to 250 ml conical flasks containing 100 ml culture medium and subjected to controlled culture conditions. The cultures were grown with photoperiod of 12 hours light/dark provided by white lamps at a light intensity of 2,500 lux and temperature of 26 ± 2 °C. The cultures were shaken gently twice a day, as shaken cultures in contrast to the static ones have demonstrated better growth.

Inorganic media for Spirulina platensis and Dunaliella salina

Growth media: The composition of the growth medium in the case of *Spirulina platensis* was in accordance with Zarrouk's medium while for *Dunaliella salina* the artificial sea water media conditions were applied. Zn^{+2}metal at different concentration (0.5ppm, 2ppm, 4ppm and 6 ppm) was chosen for experiments. Five different

solutions were prepared in case of each microelement: one control solution and 2 solutions with diverse metal concentrations [11] Algae suspensions with 10 mgl^{-1} concentration were prepared in Erlenmeyer-flasks (500 ml) by the application of the above described growing media. The experiments were performed in triplicates (n=3) and after the incubation time the whole amount of the gained algae was analyzed. The growing period was 2 weeks; the temperature was 25°C in case of *Dunella* and 32°C in case of *Spirulina.*

Biochemical Profiling

Total Soluble Sugars

The algal material (50 mg each) was homogenized in pestle and mortar with 20 mL of 80% ethanol separately and left overnight. Each sample was centrifuged at 1200 rpm for 15 minutes; the supernatants were collected separately and concentrated on a water bath using the established method [12]. Distilled water was added to make up the volume up to 50 mL and processed further for quantitative analysis.

Starch

The pellet was dissolved in 5 mL of 52% perchloric acid. 5 ml of distilled water was added to raise the volume to 10 mL and was shaken further [13].

Quantitative Estimation

Glucose was used as standard. To 1 mL of sample of both TSS and starch phenol and sulphuric acid was added [14] The optical density was measured at 490 nm using spectrophotometer (Carl Zeiss, Jena DDR, VSU 2 P), after setting for 100% transmission against a blank (distilled water). Standard regression curve was computed between the known concentration of glucose and their respective optical density, which followed Lambert Beer‘s Law. All samples were analyzed in the same way as described above and contents of total soluble sugars and starch were calculated by computing optical density of each of the samples with standard curve.

Lipids

Extraction and Quantification

The test sample was dried, powdered and 100mg was macerated with 10 mL distilled water, transferred to a conical flask containing 30 mL of chloroform and methanol (2/1:v/v;) [15] . The mixture was thoroughly mixed and left overnight at room temperature in dark for complete extraction. Later, to 20 mL of chloroform, 2 mL of water was added and centrifuged. Two layers were separated and the lower layer of chloroform, which contained all the lipids, was carefully collected in preweighed glass vials and the colored aqueous layer of methanol which contained all the water soluble substances and thick interface layer were discarded in each test sample. The chloroform layers were dried in vacuo and weighed. Each treatment was repeated thrice and their mean values were calculated.

GC-MS Profiling

The extract and the standard samples were analyzed by GC-MS of Hewlett-Packard 6890/5973 operating at 1000 eV ionization energy, equipped with using Agilent 7890A/5975C GC HP-5. Capillary column (phenyl methyl siloxane, 25 m×0.25 mm i.d) with Helium (He) was used as the carrier gas with split ratio 1:5. Oven temperature was 100 °C (3 min) to 280°C at 1 to 40 °C/min; detector temperature, 250 to 280°C; carrier gas, He (0.9 mL/min). Retention indices were determined by using retention times of samples that were injected under the same chromatographic conditions. The components of the standard and plant samples were identified by comparison of their mass spectra and retention time with those given in literature and by comparison with the mass spectra of the Wiley library or with the published mass spectra.

HPLC analysis

HPLC conditions were chosen by first evaluating common biomass and food chemistry analytical techniques. Additional chromatography conditions were chosen from carbohydrate application notes provided by Grace by Elico Privated Limited. Single component solutions were injected onto these columns to record individual retention times, followed by injections of the prepared compound solution of various carbohydrates. The conditions include Binary pump with flow rate of 1ml/min having solvent phase of Acetonitrile : Methanol (75:25). 50 µL of samples were injected in C-18 column. Compounds were identified on the basis of retention time. Detectors were set at 190 nm for detection of carbohydrates.

RESULTS AND DISCUSSION

The screening of algal strains was carried out to select the potential algal strains in terms of biomass and bio pigment production especially carotenoids and phycocyanin. Blue green algae is screened as a potential source of biomass and biopigments so

strains were compared for their biomass and biopigments i.e. chlorophyll – a, carotenoids, phycocyanin, allophycocyanin and Phycoerythrin composition of the cyanobacteria. Growth (i.e. optical density and dry weight) and productivity (growth rate) analysis of different algal isolates showed significant difference in growth pattern. On the 25th day of experiment maximum growth i.e .optical density and biomass (as dry weight) respectively was observed in cultures of both the algae. A screening of the blue green algal strains was carried out to select the elite strains in terms of biomass and pigment levels which could be a potential source of biopigments. The first step prior to mass culture of algae in open ponds or bioreactors is to select the strains most suitable for mass culturing in terms of biomass, rate of growth and products of commercial value. Growth is a good indicator to determine the effect of any toxic compound in susceptible microorganism since it reflects the metabolism of the cell. Therefore, carbohydrates and lipids were observed in order to estimate the growth of both the algae It was observed that in Zn stress (0.5 to 6 ppm) *Spirulina platensis* maximum amount of carbohydrates was observed at 0.5 ppm (3.7 mg/gdw) while minimum was at 6 ppm dose level (0.74 mg/gdw) while in *Dunaliella salina* it was observed that maximum carbohydrates content was at 0.5 ppm (3.27mg/gdw) while minimum at 6 ppm dose level (0.36 ppm). Overall *S. platensis* had better carbohydrate content than *D. salina*. In case of lipids it was observed that when Zn stress was applied maximum content was observed at 0.5 ppm (11.08 mg/gdw) and minimum at 6 ppm (3.87mg/gdw) in *S. platensis* and in case of *D. salina* maximum amount was at same dose level (10.81mg/gdw) and least in 6ppm (3.89mg/gdw). In the present investigation both carbohydrates and lipid content decreased with increase in concentration of stress (Tables 2 and 3). These findings are in agreement with earlier reports[17] who studied the biochemical changes in response to metal tolerance in *Anabena doliolum* exposed to Cu and Cd. The growth inhibition and decrease in biomolecules may be due to inhibition of photosynthesis, enzyme system, protein content, pigment degradation and functioning of PSII in both the algae. It has been reported by several workers [16] that addition of heavy metals cause alteration in whole chain and electron transport activities. It is quite possible that the reduction in content may be due to inhibition of normal cell division by binding at sulphydryl group. Further GC- MS profiling of control in *Spirulina* revealed many compounds which were mainly observed at retention time of 9.37, 13.43, 16.59, 19.89, 24.75, 33.17, 26.15 and 28.87 min while in similarly in Zn stressed culture of *Spirulina* major compounds were observed at 11.76, 16.33, 18.82, 20.94, 23.23, 27.97 and 30.62 min. respectively (Fig. 1 and 2 and Tables 4 and 5). Many compounds were observed in GC- MS profiling of *Dunaliella salina* (control). Majority of the compounds were observed at 7.32, 10.86 13.43 15.38, 17.94, 23.31 and 26.41min respectively while in Zn stress most of the compounds were observed at 13.44, 16.34, 20.92, 23.22, 27.96 and 30.56 (Fig. 3 and 4 and Tables 6 and 7). In HPLC profiling different compounds were separated in the synthetic mix. The retention times for the individual monosaccharides using these methods are shown. Example chromatograms of the monosaccharide mix run on HPLC configurations various figures are shown. (Fig.5-9). In both algae effect of various stress induced some new compounds which are newly reported. The HPLC methods tested showed either several missing components or several closely eluting peaks, which limit their usefulness for this algal carbohydrate application. Most of the configurations allowed for the detection of the neutral monosaccharides and disaccharides Some of the problems with the detection of the monosaccharides, , are often related to the low or non-selective and inconsistent responses from the detector. This, in addition to the often-poor separation, makes these methods unsuitable for algal biomass derived monosaccharide quantification.

Table 2
Effects of Zinc stress on carbohydrate and lipids contents (in mg/gdw) on Spirulina platensis

Carbohydrates				
CONTROL	0.5 ppm	2 ppm	4 ppm	6 ppm
3.29±0.02	3.7±0.04	3.54±0.009	2.69±0.007	0.74±0.003
Lipids				
CONTROL	0.5 ppm	2 ppm	4 ppm	6 ppm
10.21±0.83	11.08±0.9	8.64±0.76	6.59±0.66	3.87±0.52

Table 3
Effect of Zinc stress on carbohydrate and lipids contents (in mg/gdw) on Dunaliella salina

Carbohydrates				
CONTROL	0.5 ppm	2 ppm	4 ppm	6 ppm
3.11±0.01	3.27±0.01	3.05±0.009	2.95±0.006	0.36±0.001
Lipids				
CONTROL	0.5 ppm	2 ppm	4 ppm	6 ppm
10.13±0.78	10.81±0.87	8.72±0.62	4.97±0.57	3.89±0.44

Table 4
Various compounds identified by GC –MS in Spirulina platensis (Control)

RT	Compound Name	Area	Area %
6.52	Methyl 4methyl4nitroso2trimethylsiloxypentanoate	16205	2.32
7.45	5[2(1,3Dioxolan2yl)ethyl]2methyl1cyclopentene1carboxaldehyde	11389	1.63
7.63	Malonic acid, 3methylpentyl propyl ester	10373	1.49
9.15	3 [2(4Methylphenylthio) ethyl]4Hsydnone	11358	1.63
9.37	1H1Pyrindine2,7dione, 1[2(1,3benzodioxol5yl) ethyl]3,4,5,6tetrahydro	26027	3.73
9.60	Benzene, (1nitropropyl)	12159	1.74
10.21	1,2Benzendiol, O,O'di(cyclopentanecarbonyl	12289	1.76
11.53	1,3Dioxolane, 2(phenylmethyl)	10203	1.46
12.32	2Methyl1dimethyl(dichloromethyl) silyloxypropane	8488	1.22
12.67	Phosphonic acid, (3methyl3penten1ynyl) diethyl ester, (E)	13708	1.96
12.81	Penicillamine, triTMS	9282	1.33
13.05	3Methylthio6phenyl5oxo4,5dihydro1,2,4triazine	13766	1.97
13.12	3Hepten1ol, acetate	18248	2.61
13.20	1Pentanone, 1(2pyridinyl	16406	2.35
13.31	Carbonic acid, butyl cyclohexyl ester	9136	1.31
13.43	NDifluorophosphoxyOtrimethylsilylhydroxylamine	44080	6.32
13.67	1Hexyl2nitrocyclohexane	14569	2.09
13.90	Butanamide, N(1oxopropyl)	17737	2.54
15.50	áDGlucopyranoside, methyl 2(acetylamino)2deoxy3,4,6triOmethyl	13751	1.97
15.57	Decane, 4methylene	8240	1.18
16.59	4Nonene, 1bromo, (Z)	11663	1.67
17.44	5Decene, 1bromo, (Z)	12119	1.74
17.53	Benzenamine, N[2methoxy1(methoxymethyl) 2methylpropylidene	15832	2.27
18.07	DarabinoHex1enitol, 1,5anhydro2deoxy	6205	0.89
18.12	2Cyclohexen1ol, benzoate	11922	1.71
18.24	Methyl 6anisoyládgalactopyranoside	11119	1.59
18.34	Benzeneacetamide, àethylNformylàhydroxy	14167	2.03
18.77	4Tridecanol	7911	1.13
19.39	Benzenepropanoic acid, ethyl ester	9336	1.34
19.89	Cyclobutanone, 2methyl2oxiranyl	10657	1.53
20.15	Tetradecanoic acid, 13oxo, methyl ester	18666	2.67
20.90	Phosphorochloridous dihydrazide, hexamethyl	15411	2.21
21.67	Propane, 3,3dichloro1,1,1,2,2pentafluoro	19227	2.76
22.13	Trimethyl (3,3difluoro2propenyl) silane	11528	1.65
22.26	Benzeneacetic acid, àmethyl3phenoxy, trimethylsilyl ester	18620	2.67
22.56	Silane, 2cyclohexen1yltrimethyl	9589	1.37
22.89	Propanedioic acid, propyl	16763	2.40
23.21	7Oxo4,7dihydrotriazolo(3,2c) triazine	20475	2.93

23.96	[1,4]Dioxino[2,3b] 1,4dioxin, hexahydro2,3,6,7tetramethyl	27552	3.95
24.74	àDXylofuranoside, methyl	14916	2.14
26.82	NBenzyloxy2isopropoxycarbonylazetidine	6149	0.88
31.95	Methyl 4methyl4nitroso2trimethylsiloxypentanoate	9886	1.42
33.17	Butanoic acid, 3methyl, trimethylsilyl ester	18015	2.58
33.29	N,O bis (trimethylsilyl)5(aminomethyl) isoxazol3ol	13166	1.89
33.51	3Hexene, 1(1ethoxyethoxy) (Z)	10548	1.51
34.05	Cyclohexanol, 2(trimethylsilyl), cis	10591	1.52
34.27	1Trimethylsilyloxynoctene	8803	1.26
35.28	Butanoic acid, 2methylcyclohexyl ester, cis	14408	2.06
35.52	(3Nitrobenzyl) phenethylamine	10482	1.50
35.93	[(Pyridine2carbonyl) amino] Acetic acid, ethyl ester	14679	2.10

Table 5

Various compounds identified by GC -MS in Spirulina platensis (with Zn stress at 4 ppm)

RT	Compound Name	Area	Area %
4.57	1[(2Pyridinylcarbonyl) oxy]2,5pyrrolidinedione	2612359	0.65
5.10	NEthoxy2carbomenthyloxyaziridine	1563710	0.39
15.05	1Hexadecanol	721147	0.18
15.13	Pentadecane, 2,6,10trimethyl	860795	0.21
16.33	1Heptadecyne	3488007	0.87
18.11	10Heneicosene	1355037	0.34
18.18	1Iodo2methylundecane	695370	0.17
18.82	3,7,11,15Tetramethyl2hexadecen1ol	5003223	1.24
19.18	3,7,11,15Tetramethyl2hexadecen1ol	2397366	0.59
19.44	3,7,11,15Tetramethyl2hexadecen1ol	4475506	1.11
20.18	1Dodecanol, 3,7,11trimethyl	2679284	0.66
20.94	nHexadecanoic acid	101529563	25.20
21.06	Oxalic acid, allyl tridecyl ester	14067620	3.49
22.15	Oxalic acid, isohexyl pentyl ester	1462881	0.36
22.24	2Hexyl1octanol	1327248	0.33
22.35	2Hexyl1octanol	1364193	0.34
22.51	1Hexyl2nitrocyclohexane	1894317	0.47
23.23	Oleic Acid	78979596	19.60
23.46	Octadecanoic acid	28771943	7.14
23.89	2Heptadecenal	2625552	0.65
24.32	1Hexyl2nitrocyclohexane	5533037	1.37
24.44	1Hexyl2nitrocyclohexane	4109990	1.02
24.74	2Hexyl1octanol	2462190	0.61
24.81	2Hexyl1octanol	2528763	0.63
25.05	2Hexyl1octanol	1996396	0.50
25.74	1Decanol, 2hexyl	971698	0.24
25.85	Oxalic acid, allyl nonyl ester	8757774	2.17
26.15	Sulfurous acid, butyl heptadecyl ester	859816	0.21
26.61	Cyclohexane, 1(1,5dimethylhexyl) 4(4methylpentyl	8371292	2.08
26.85	2Hexyl1octanol	2297962	0.57
26.94	2Hexyl1octanol	7471865	1.85
27.23	2Hexyl1octanol	2066913	0.51
27.34	2Isopropyl5methylcyclohexyl 3(1(4chlorophenyl)3oxobutyl)coumarin4yl carbonate	6619159	1.64
27.73	1Methylene2bhydroxymethyl3,3dimethyl4b(3methyl but2enyl)	1801495	0.45

	cyclohexane		
27.90	1Hexyl2nitrocyclohexane	4017203	1.00
27.97	1Decanol, 2hexyl	22043766	5.47
28.24	2Hexyl1octanol	4299189	1.07
28.45	8a(2H)Phenanthrenol, 7ethenyldodecahydro1,1,4a, 7tetramethyl, acetate, [4as(4aà,4bá,7á,8aà,10aá)]	1602154	0.40
28.81	8a(2H)Phenanthrenol, 7ethenyldodecahydro1,1,4a, 7tetramethyl, acetate, [4as(4aà,4bá,7á,8aà,10aá)	4312748	1.07
28.89	1Hexyl2nitrocyclohexane	3083383	0.77
29.11	5,10Pentadecadiyne, 1chloro	5128239	1.27
29.24	1Methylene2bhydroxymethyl3,3dimethyl4b(3methyl but2enyl) cyclohexane	17930027	4.45
29.57	16Heptadecenal	2700031	0.67
30.10	9Dodecenoic acid, methyl ester, (E)	4386237	1.09
30.48	4,8,12Tetradecatrien1ol, 5,9,13trimethyl	2521419	0.63
30.82	1Hexyl2nitrocyclohexane	5303119	1.32
31.06	2,6,10,14Hexadecatetraenoic acid, 3,7,11,15tetramethyl9(phenylsulfonyl), methyl ester, (E,E,E)	5648214	1.40
31.30	Cyclopentadecanone, oxime	1284072	0.32

Table 6

Various compounds identified by GC –MS in Dunaliella salina (Control)

RT	Compound Name	Area	Area %
4.40	Tetrakis (methylsulfonyl)hydrazine	3508655	1.99
5.85	N,N'Bis [(5Z) cyclooctenylidene] hydrazine	1140917	0.65
5.92	2Ethyl1hexanol, trifluoroacetate	1329230	0.75
7.32	3Methylheptyl acetate	3593413	2.03
9.14	Decane, 2,5,6trimethyl	598073	0.34
10.86	10Methylnonadecane	785126	0.44
13.43	Sulfurous acid, butyl decyl ester	866103	0.49
15.38	Oxalic acid, hexyl neopentyl ester	690735	0.39
16.54	4Hydroxyàbromoethylphenone	1164150	0.66
17.41	Oxalic acid, hexyl neopentyl ester	1603320	0.91
17.94	Phthalic acid, ethyl pentyl ester	3940032	2.23
20.10	Cyclohexanemethyl propanoate	3602613	2.04
20.44	2RAcetoxymethyl1,3,3trimethyl4t(3methyl2buten1yl) 1tcyclohexanol	1098297	0.62
20.52	2,2,7,7Tetramethyloctane	674794	0.38
20.94	DL4,5Octanediol	1123096	0.64
21.14	1Cyclohexylnonene	2136659	1.21
21.75	Dodecanal	3882763	2.20
22.38	1Hexyl2nitrocyclohexane	1811283	1.03
22.48	1Hexyl2nitrocyclohexane	1743061	0.99
22.75	2Hexyl1octanol	3970883	2.25
22.84	2Hexyl1octanol	2627666	1.49
23.31	Pentadecanoic acid, 14methyl, methyl ester	25890686	14.65
23.52	2Hexyl1octanol	10534030	5.96
23.62	DL4,5Octanediol	2439309	1.38
23.92	1Hexyl2nitrocyclohexane	378959	0.21
24.09	1Octene, 3,3,4,4,7,7,8,8,8nonafluoro	4399269	2.49
24.20	Oxalic acid, allyl octadecyl ester	19736681	11.17
24.30	Oxalic acid, allyl undecyl ester	3508937	1.99
24.46	2Hexyl1octanol	1445097	0.82

24.81	1Hexyl2nitrocyclohexane	1492103	0.84
24.91	2(1Methylcyclopentyloxy) tetrahydropyran	2965771	1.68
24.98	1Hexyl2nitrocyclohexane	1289080	0.73
25.40	2Hexyl1octanol	1628030	0.92
25.61	2Hexyl1octanol	1112487	0.63
25.73	1Hexyl2nitrocyclohexane	869336	0.49
25.83	Decane, 3bromo	1415675	0.80
26.06	9Dodecenoic acid, methyl ester, (E)	10132168	5.73
26.20	1Hexyl2nitrocyclohexane	358514	0.20
26.33	1Hexyl2nitrocyclohexane	604817	0.34
26.41	Hexadecanoic acid, 15methyl, methyl ester	11672218	6.61
26.58	2Methyl2chloro3nitroso4cyclohexyloxybutane	1497000	0.85
26.63	Cyclohexanemethyl propanoate	1687922	0.96
26.68	2(1Methylcyclopentyloxy) tetrahydropyran	3612649	2.04
26.90	Oxalic acid, allyl octadecyl ester	4031501	2.28
26.96	2Hexyl1octanol	7972719	4.51
27.07	Decane, 2,5,6trimethyl	885150	0.50
27.20	Oxalic acid, allyl tridecyl ester	5630634	3.19
27.27	Tetradecane, 1iodo	2009711	1.14
27.40	2Hexyl1octanol	2151921	1.22
27.82	2Hexyl1octanol	3458365	1.96

Table 7
Various compounds identified by GC –MS in Dunaliella salina (with Zn stress at 4 ppm)

RT	Compound Name	Area	Area %
20.92	nHexadecanoic acid	38321515	28.16
22.15	Oxalic acid, allyl decyl ester	1576433	1.16
22.30	Cis1methyl3nnonylcyclohexane	407422	0.30
23.22	Oleic Acid	28287837	20.79
23.45	Octadecanoic acid	11549964	8.49
23.82	1Hexyl2nitrocyclohexane	820713	0.60
25.35	1Hexyl2nitrocyclohexane	142973	0.11
25.81	2Nonadecanol	3902402	2.87
26.60	1Hexyl2nitrocyclohexane	419233	0.31
26.78	1Hexyl2nitrocyclohexane	147898	0.11
26.95	1Hexyl2nitrocyclohexane	347708	0.26
27.04	Dodecanal	97500	0.07
27.46	2Nonadecanol	145263	0.11
27.57	2Nonadecanol	273279	0.20
27.66	Dodecanal	167396	0.12
27.77	10Heneicosene	2959731	2.18
27.96	Oxalic acid, allyl octadecyl ester	10628502	7.81
28.30	1,3Cyclohexadiene1carboxylic acid, 5(acetyloxy)6methoxy, methyl ester, trans	112843	0.08
28.44	1,3,3Trimethyl2hydroxymethyl3,3dimethyl4(3methyl but2enyl) cyclohexene	1203831	0.88
28.56	1Methylene2bhydroxymethyl3,3dimethyl4b(3methyl but2enyl) cyclohexane	6384162	4.69
28.67	Decane, 1,10dibromo	291606	0.21
28.89	2Nonadecanol	1046535	0.77
29.04	Cyclohexanol, 1(4cyanophenyl) 4pentyl	3237923	2.38
29.16	Cyclopentadecanone, oxime	352700	0.26

29.40	meso2,5Dimethyl3,4hexanediol	671205	0.49
29.51	3Dodecanol, 3,7,11trimethyl	3785098	2.78
29.66	meso2,5Dimethyl3,4hexanediol	350297	0.26
29.71	meso2,5Dimethyl3,4hexanediol	350297	0.26
29.76	3Dodecanol, 3,7,11trimethyl	574131	0.42
29.86	3Dodecanol, 3,7,11trimethyl	998463	0.73
30.02	3Dodecanol, 3,7,11trimethyl	998463	0.73
30.14	4Methyl2,4bis(4'trimethylsilyloxyphenyl) pentene1	153273	0.11
30.31	1Methylene2bhydroxymethyl3,3dimethyl4b(3methyl but2enyl) cyclohexane	8399718	6.17
30.56	Azuleno[4,5b] furan2,8(3H,4H)dione, 3a,5,6,6a,7,9bhexahydro6hydroxy3,6,9trimethyl, [3S(3à,3aà,6à,6aà,9bá)]	299694	0.22
31.08	Azuleno[4,5b] furan2,8(3H,4H)dione, 3a,5,6,6a,7,9bhexahydro6hydroxy3,6,9trimethyl, [3S(3à,3aà,6à,6aà,9bá)	569357	0.42
31.29	3,6Dioxa2,7disilaoctane,2,2,7,7tetramethyl3[(2methylphenoxy) methyl	372332	0.27
31.60	3,6Dioxa2,7disilaoctane, 2,2,7,7tetramethyl3[(2methylphenoxy) methyl	336444	0.25

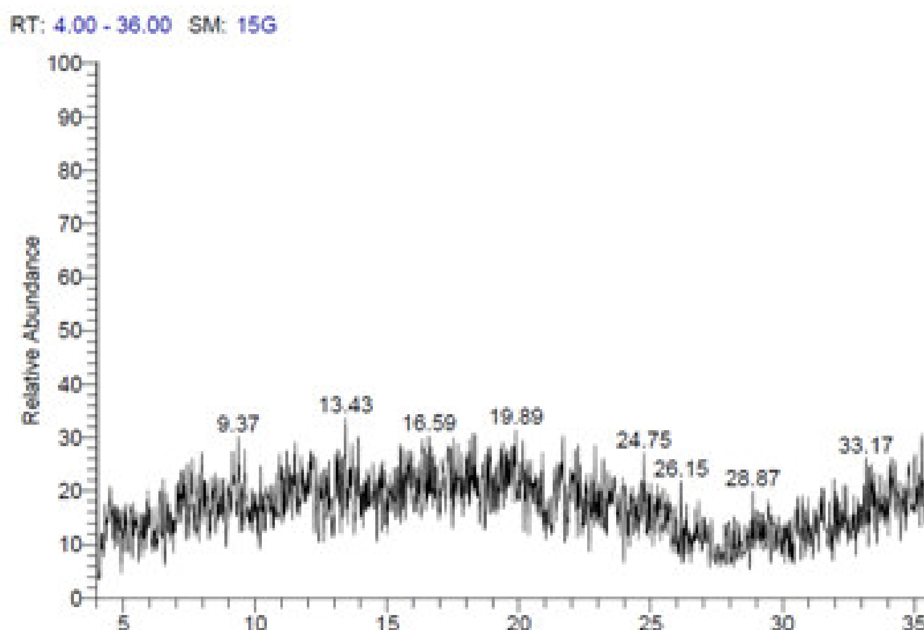

Figure 1
Peak representing retention time of various compounds identified by GC –MS in Spirulina platensis (Control)

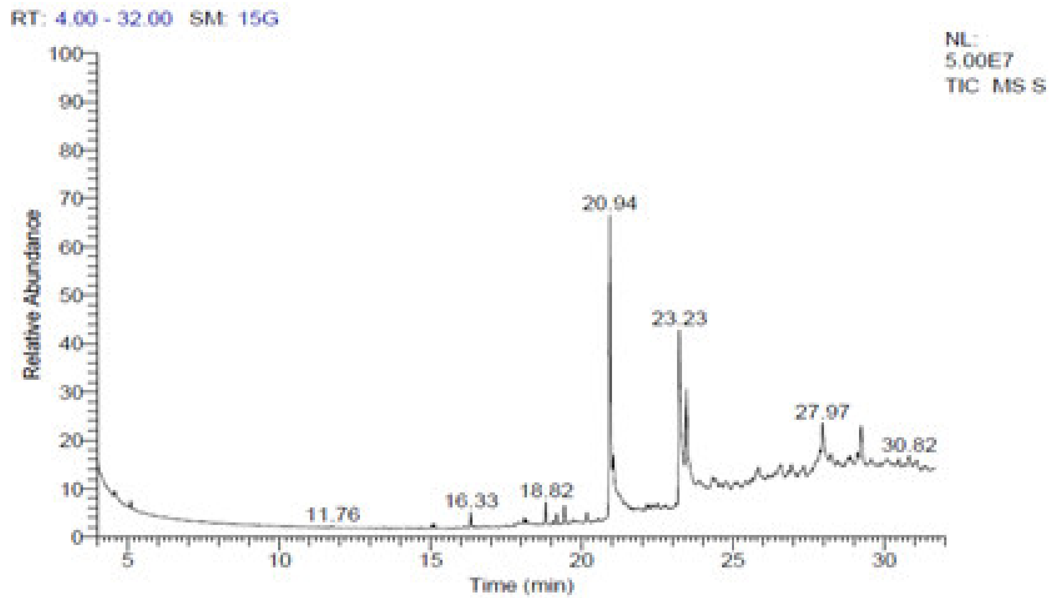

Figure 2
Peak representing retention time of various compounds identified by GC –MS in Spirulina platensis (With Zn stress at 4 ppm)

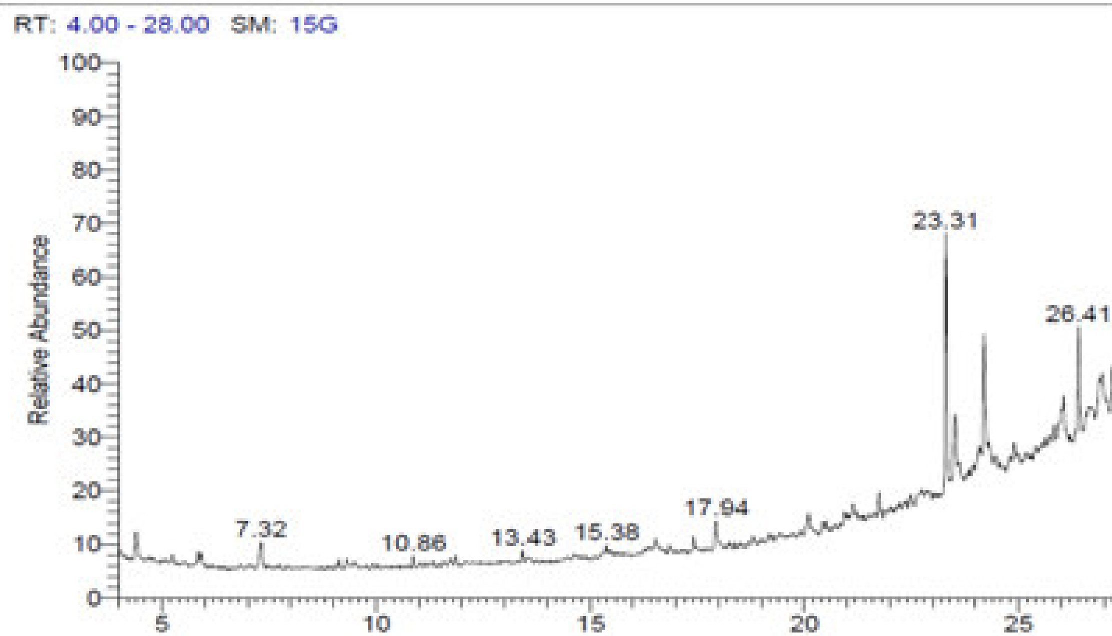

Figure 3
Peak representing retention time of various compounds identified by GC –MS in Dunaliella salina (Control)

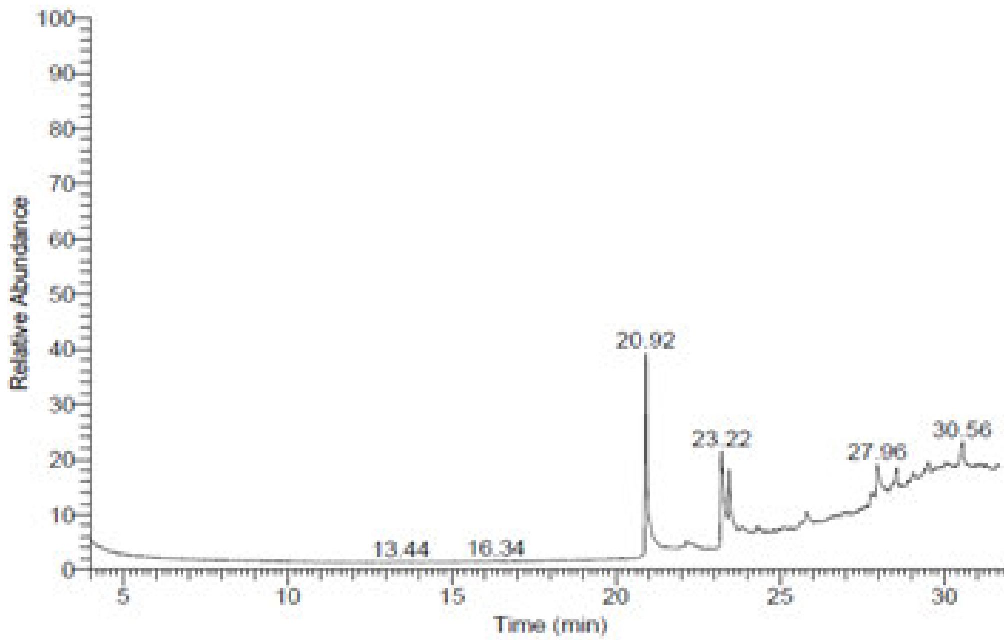

Figure 4
Peak representing retention time of various compounds identified by GC –MS in Dunaliella salina (With Zn stress at 4 ppm)

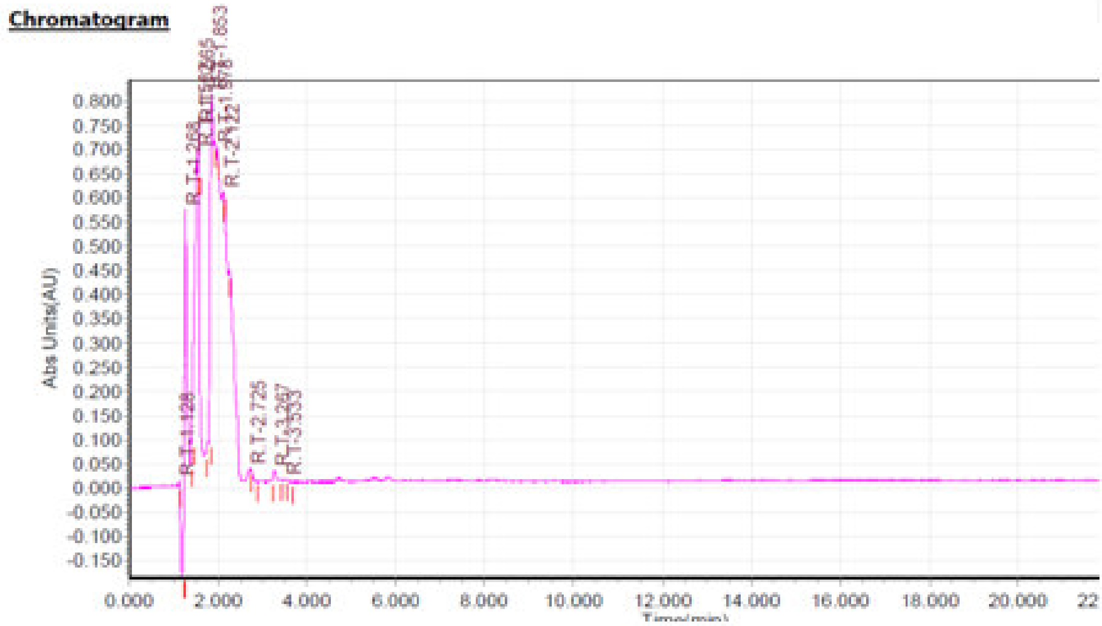

Figure 5
Peak representing retention time of various compounds identified by HPLC in standard carbohydrates

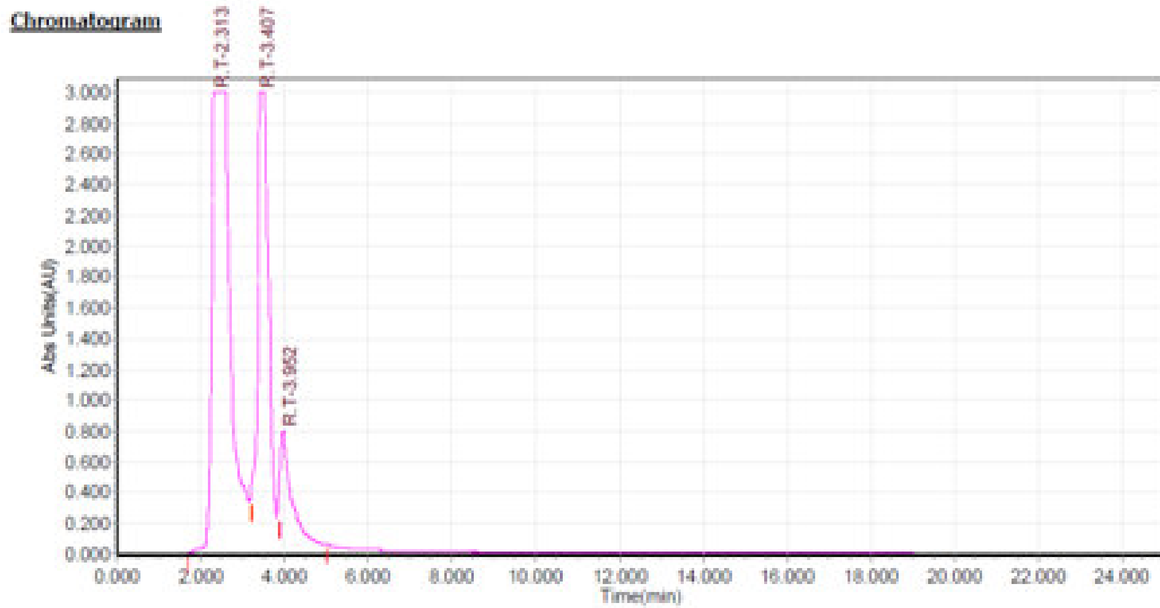

Figure 6
Peak representing retention time of various compounds identified by HPLC in Spirullina carbohydrates (Control)

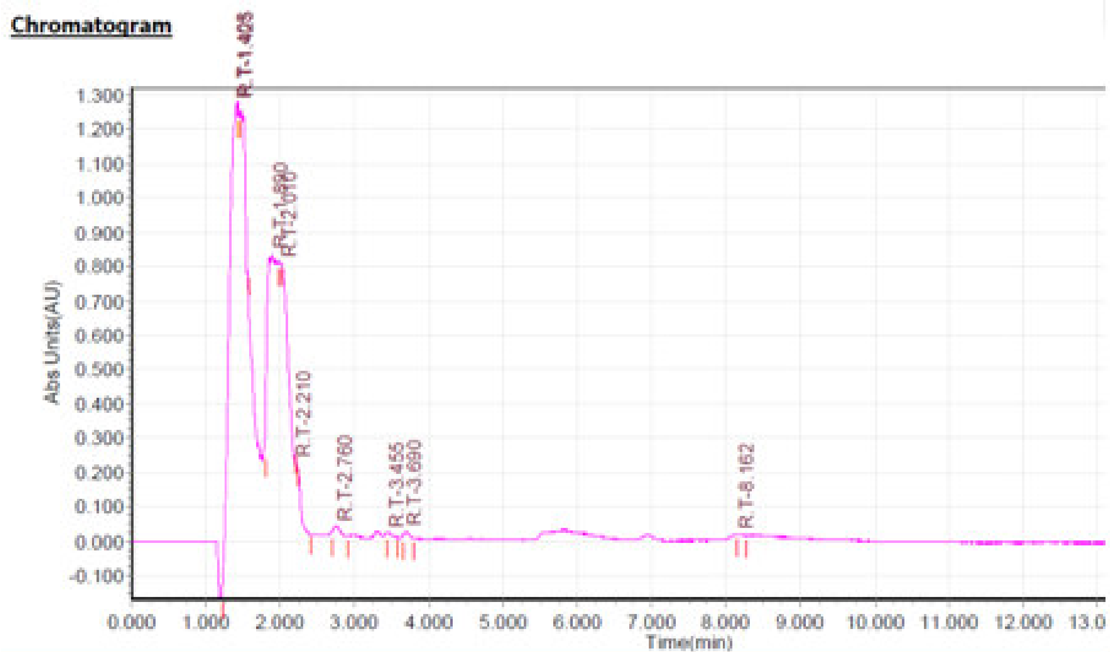

Figure 7
Peak representing retention time of various compounds identified by HPLC in Spirullina carbohydrates (with Zn stress at 4ppm)

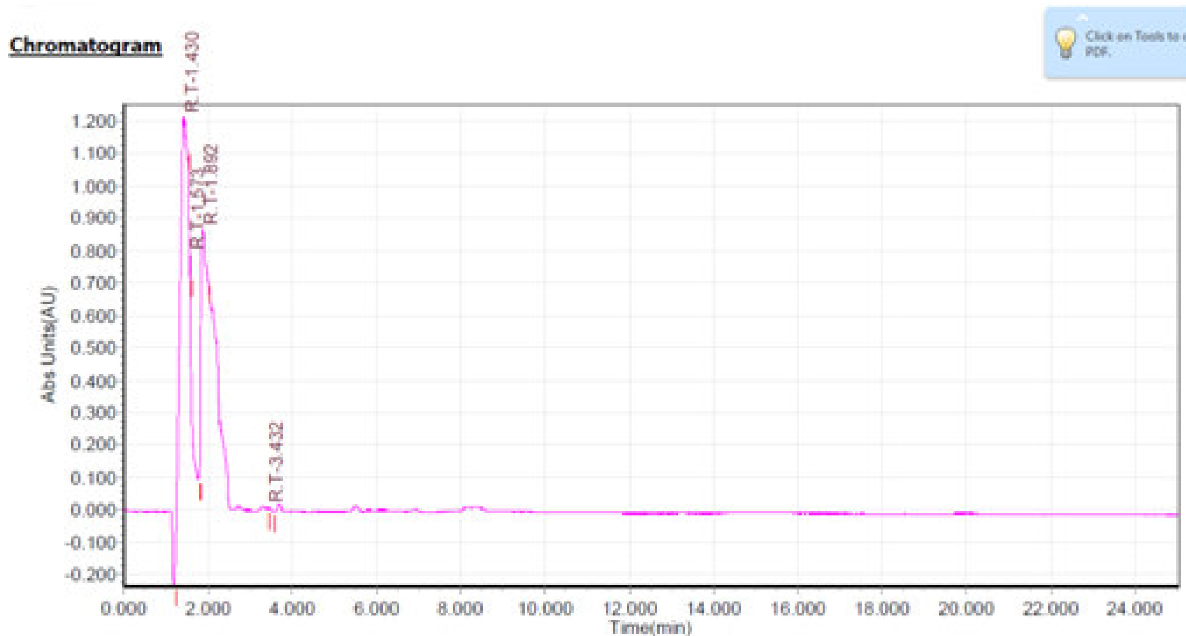

Figure 8
Peak representing retention time of various compounds identified by HPLC in Dunaliella salina carbohydrates (Control)

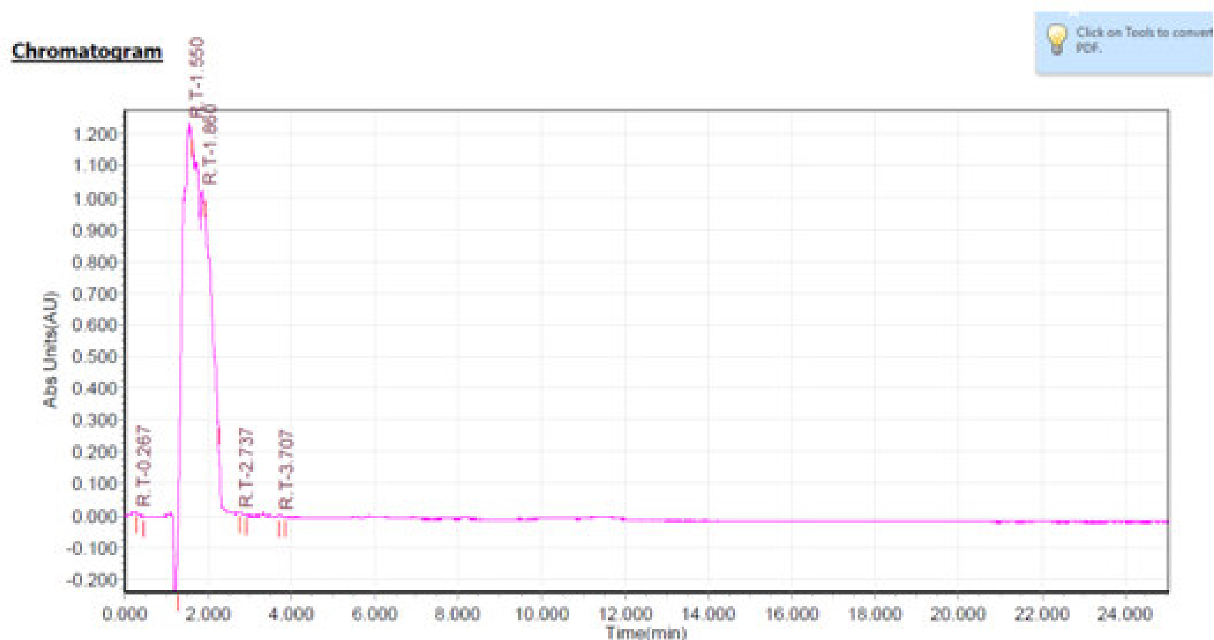

Figure 9
Peak representing retention time of various compounds identified by HPLC in Dunaliella salina carbohydrates (with Zn stress at 4 ppm)

CONCLUSION

The present investigation deals with culture and maintenance of various algae for their mechanism of action against various stresses. Further biochemical estimation of these algae was also done and it was observed that they are good source of proteins and carbohydrates and their content was affected by this heavy metal stress. It was established that significantly less amount of algae has to be consumed to comply with the physiological needs of some relevant microelements, if they are incubated in metal fortified media. The above mentioned results confirm the relevance of development of microelement enriched products based on specifically selected and grown microalga species. The most efficient ways and conditions of metal

bioaccumulation have been established laying the foundations for functional food application in the future.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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