Studies on Arsenic Absorption, Adsorption, Accumulation and Analysis of Biofuel Compounds in Microalgae for the Use of Arsenic Detoxification and Biofuel Productions

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ABSTRACT - Microalgae are autotrophic microorganisms that can grow slowly or rapidly and live in various climatic conditions due to their cellular structure. Microalgae can be used to produce energy in different processes. One of the best effective methods is to convert the algal oil derivatives in to biodiesel. The present investigation was done to study the arsenic absorption, adsorption and accumulation potential of three microalgal species isolated from highly arsenic contaminated areas for the use of arsenic detoxification. The present investigation was also done to analyze the biofuel compounds in microalgae for biofuel productions. The pure microalgal species such as *Chlorella vulgaris, Scenedesmus acutus* and *Oscillatoria acuminata* were collected and isolated from various districts of West Bengal, India. The three potential microalgal species grown in BBM growth medium with various arsenic concentrations were harvested and subjected to fatty acids methyl esters extraction and transesterification to determine the biofuel compounds by GCMS for the production of biodiesel. High content of saturated fatty acids (SFA) were observed in all the arsenic treated microalgal samples over control samples. EDAX is an X-ray spectroscopic method used for determining arsenic elemental compositions in microalgal species. The EDAX analysis system worked as an integrated feature of a scanning electron microscope (SEM). SEM combined with EDAX analysis helps to confirm the arsenic adsorption, uptake, accumulation and detoxification in microalgal species.

Key words: ***Microalgae, Arsenic Adsorption, SEM, EDAX, GCMS and Biofuel Compounds.***

I. INTRODUCTION

Arsenic Pollution

Arsenic is a highly toxic and carcinogenic chemical element with the symbol *As*. Groundwater contamination of arsenic is the serious environmental problem that affects more than 100 millions of people all over the world. Most of the people in West Bengal are drinking arsenic contaminated water above the permissible limit (0.01 mg / L). Millions of people in Western Bengal and Bangladesh have been drinking groundwater from wells that contain 100-2,000 µg / L As, and many of these people have succumbed to diseases that are caused by the arsenic contaminated ground water (Mandal *et al*., 1996[[5]]). According to a latest report, in West Bengal alone there are 1.04 crore persons are affected by arsenic contamination (The Hindu, 19th March, 2017[[7]]).

Use Microalgae for Arsenic Detoxification

Microalgae are autotrophic microorganisms that can grow slowly or rapidly and live in various climatic conditions due to their cellular structure. Microalgae and Cyanobacteria are among the fastest-growing phototrophs on the earth. Between 3,00,000 and 10 million species of algae are may be found in the universe. Microalgae are play an important role in arsenic cycling and continuous oxidation reduction and methylation of arsenic is the main natural detoxification process of microalgae (Duncan *et al*., 2015[[1]]; Maeda *et al*., 1990[[4]], 1993[[3]]). Toxicity of *As* (V) and *As* (III) in *Chlorella* sp. and *Monoraphidium arcuatum*, was determined using 72-h growth rate inhibition bioassays by Xi-Xiang Yin *et al*., 2011[[8]]. Both organisms were tolerant to *As* (III) and *As* (V).

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Scanning Electron Microscopy and EDAX Analysis

Scanning Electron Microscopy (SEM), produces images of a sample by scanning the surface with a focused beam of electrons. For SEM, a specimen is normally required to be completely dry, since the living cells and tissues and whole, soft-bodied organisms require chemical fixation to preserve and stabilize their structure. EDAX (or EDX) is an X-ray spectroscopic method for determining elemental compositions such as arsenic and so on. The EDX analysis system works as an integrated feature of a scanning electron microscope (SEM).

Biodiesel Compounds Produced from the Microalgae

The biodiesel is produced from the algae, vegetable and animal fat or alcohol through the transesterification process. This reaction converts the esters into the mixture of esters of fatty acids that makes the oil. The biodiesel is obtained from the purification of the mixture of fatty acid methyl ester fatty acid methyl esters (FAME). A catalyst is used to accelerate the reaction. According to the catalyst used, transesterification can be basic, acidic or enzymatic. Stages or different steps involved for biodiesel production is treatment of raw materials, alcohol catalyst mixing, chemical reaction (transesterification), and separation of the reaction product and purification of the reaction product. Gas chromatography–mass spectrometry (GC-MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample.

In this study, the biological methods such as phycoremediation have been used to remove or detoxify heavy metal arsenic in aquatic environments are studied with specific emphasis on absorption. Adsorption is the adhesion of atoms, ions or molecules from a gas, liquid or dissolved solid to a surface. This process creates a film of the adsorbate on the surface of the adsorbent. The potential of prokaryotic and eukaryotic microalgae living cells or their dead cell biomass in comparison to currently available physicochemical processes aimed at removing toxic heavy metal arsenic is studied and discussed. The present research work had been done to study the Arsenic Absorption, Adsorption, Accumulation and detoxification by microalgal species and Analysis of Biofuel Compounds in Microalgae for the Use of Biofuel Productions.

II. MATERIALS AND METHODS

Sample Collection

The sample collection was made from various places in different districts of West Bengal, India at four different seasons (Winter, Summer, Monsoon and Autumn) between January 2014- August 2016. West Bengal is located in strategic position of Eastern India, lies between 21° 31´ and 27° 14´ N latitude and between 85°91´ and 89° 53´ E longitude.

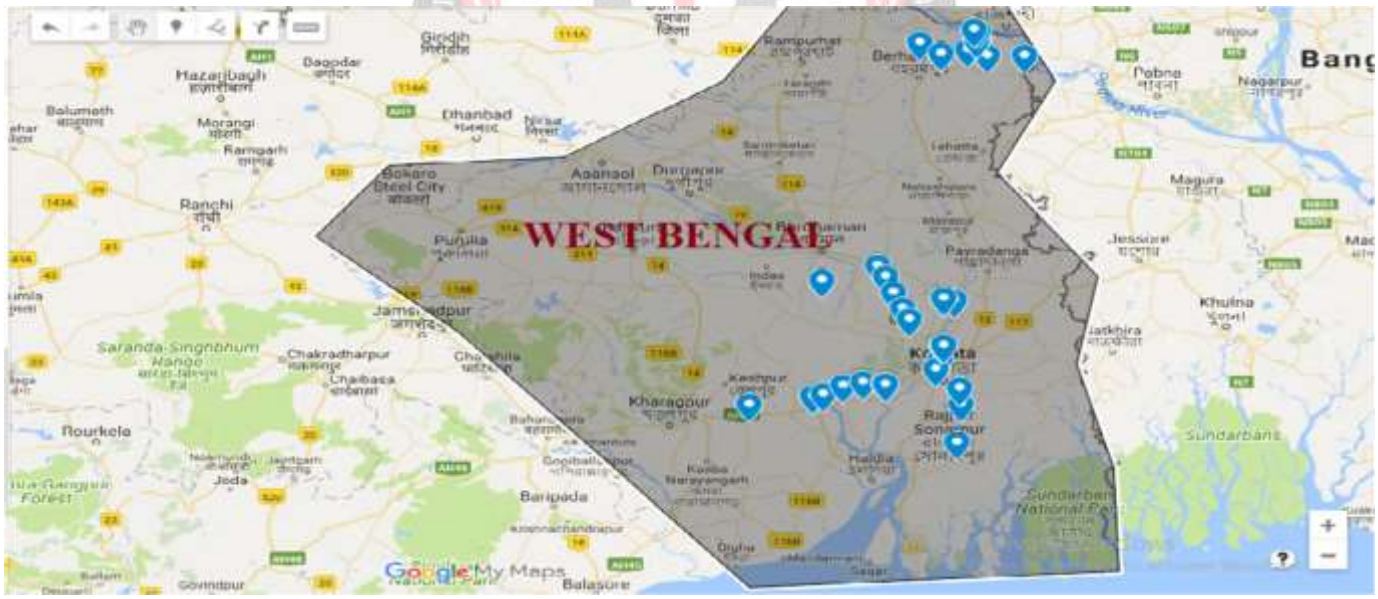

Fig. 1: Map of West Bengal, highlighting the sampling sites of arsenic contaminated areas.

Morphological Identification and Isolation of Microalgal Species

The collected samples were transferred to Erlenmeyer flasks containing BBM medium and Algal culture maintenance in Algal biotechnology laboratory, Presidency College. The microalgal samples were microscopically observed by using Olympus CH20i microscope and photographed by Sony digital still camera model DSC-W320 and identified by comparison with monographs. The micro algal samples were isolated by using serial dilution, spread plate and streak plate method. These isolated Cyanobacterial species were cultured in BBM medium under 3000 lux light intensity with static condition, for 12 h under illumination and 12 h under darkness.

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Arsenic absorption and Biodiesel compounds studies in Microalgae

The stock solutions of arsenic (100ppm) were used to prepare different concentrations of arsenic solutions. The working solutions were prepared in the sterilized BBM medium under aseptic conditions for repeated use at predetermined concentrations. Sample of 10 mL of the exponentially grown culture (Optical Density 0.2) was inoculated into the flasks containing 100 mL of fresh media at a concentration 5ppm, 15ppm, 25ppm and 50ppm of arsenic and incubated in the culture room for 21 days. Cyanobacterial cultures were harvested after a production period of 21days used for arsenic absorption and GCMS studies.

Arsenic adsorption by Microalgal species

The three microalgal biomass was sun-dried and then dried in oven at 50°C for 24 h. The dried algal biomass was shredded, ground in a mortar and an average size of 500-600 µm was used for biosorption experiments at a concentration of 0.5 g/l. For arsenic adsorption studies the dried microalgal samples were incubated in 100 ppm arsenic solution for 48 hours after that filtered and filtrate was used for arsenic analysis. The flow injection hydride generation atomic absorption spectrometry (FI-HG-AAS) method was used for arsenic analysis. A Perkin Elmer system of Flow injection hydride generation atomic absorption spectrometry (FI-HG-AAS), Model AAnalyst 700 and FIAS 400, was used for total *As* in water. Arsenic reduction by microalgal dry biomass was expressed as percentage growth reduction with respect to control. Percentage of arsenic reduction by both wet and dry microalgal biomass was calculated using the below given formula.

$$\text{Percentage of arsenic reduction} = \frac{\text{Initial arsenic Concentration} - \text{Final arsenic Concentration}}{\text{Initial arsenic Concentration}} \times 100$$

SEM and EDAX Analysis

The microalgae species used for arsenic absorption and adsorption studies were analyzed for the confirmation of arsenic uptake and accumulation by using scanning electron microscope and EDAX analysis. SEM and EDAX analysis was done by following the method of Jagna Karcz Katowice, 2009[[2]]. Specimen preparation includes the following steps: Fixation using glutaraldehyde, washing by phosphate buffer, post-fixation - osmium tetroxide, dehydration by using ethanol or acetone solutions, Critical Point Drying (CPD), Coating & viewing specimens in SEM and image analysis.

Extraction of Lipids from Wet Microalgal Biomass and Transesterification

These selected potential microalgal species were subjected to fatty acids extraction and transesterification to determine the fatty acid compounds for the production of biodiesel. The cells were obtained from 40ml medium containing culture flask, centrifuged at 8000X g for 5 min, at 15°C and washed once with distilled water. The fresh culture cells were well homogenized using a mortar and pestle for 20 min at room temperature. Extraction of lipids from wet biomass was performed according to the procedure of Sharif Hossain *et al*., 2008[[6]]. The algal cells were spread over a clean glass plate for air drying. The dried biomass was mixed with citric acid for making algal beads. Allow it for oven drying at 120c for 1 min. Fatty acid presenting in the algal cell contents were extract using petroleum ether, catalyst such as NaOH and methanol. The dried biomass was soaked with petroleum ether (1:1 by vol) solvent in a beaker overnight. The yellow colored oil extracts were collected on the top of the solution and mixed with catalyst (0.30g NaOH and 2ml of methanol). Then allow that the solution for 16h to settle their sediments clearly.

GC-MS studies

Gas chromatography is used to identify the biochemical (biofuel) components from three (3) different microalgal samples (Both treated with 50 ppm arsenic and control samples grown for 21 days) such as *Chlorella vulgaris*, *Scenedesmus acutus* and *Oscillatoria acuminata*. The collected samples were processed with Agilent 6890 gas chromatograph coupled to a 5975 MSD mass spectrometer (GC-MS with Data system is a high resolution, double focusing instrument. Maximum resolution: 6000 Maximum calibrated mass: 1500 Daltons. Source options: Electron impact (EI); Chemical ionization (CI)). The sample was evaporated in a split less injector at 300°c. The fatty acids were quantified by a gas chromatography. The column (HP5) were fused silica 50m x 0.25 mm I.D. Analysis in 20 minutes at 100°C the 3°/ min to 235°C for column temperature, 240°C for injector temperature, helium was the carrier gas. The weight percentages of extracted sample containing compound were approximated by the area of the detector response. The compound was identified by the mass spectrum data base software (NIST/EPA/NIH Mass Spectral Library with Search Program installed in GC-MS) to identify the fatty acid and biofuel compounds present in the samples and the data were recorded and results were compared.

III. RESULTS AND DISCUSSION

Totally eighty (80) microalgal samples were collected from sixty five (65)different places of Murshidabad, Kolkata, Howrah, South 24 Parganas, Hoogly, Barddhaman, East Medinipur and West Medinipur districts of West Bengal. Three microalgal species such as *Chlorella vulgaris*, *Scenedesmus acutus* and *Oscillatoria acuminata* were screened and isolated for further studies as they are dominant species over all the arsenic contaminated sites (Figure-2).

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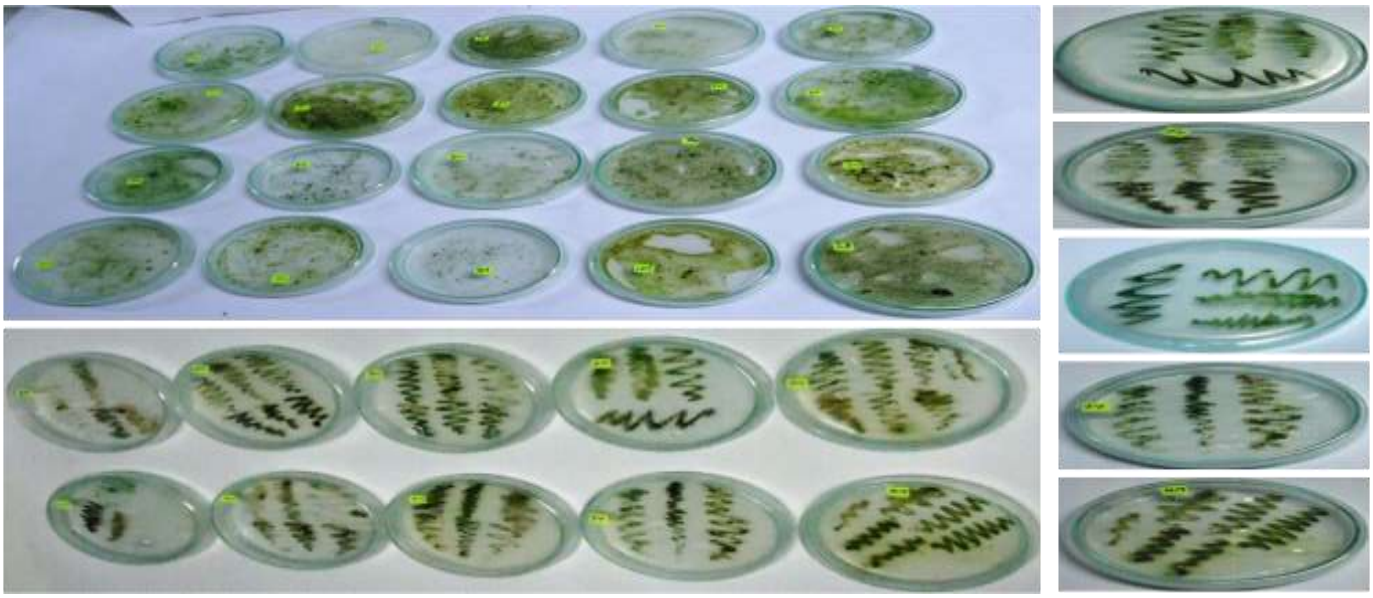

Figure 2: Isolation of microalgal species in BBM medium by spread plate and streak method from the samples collected from arsenic polluted areas of West Bengal, India. The microalgae samples were observed under microscope, photographed and morphologically identified as *Chlorella vulgaris*, *Scenedesmus acutus* and *Oscillatoria acuminata* (Figure-3).

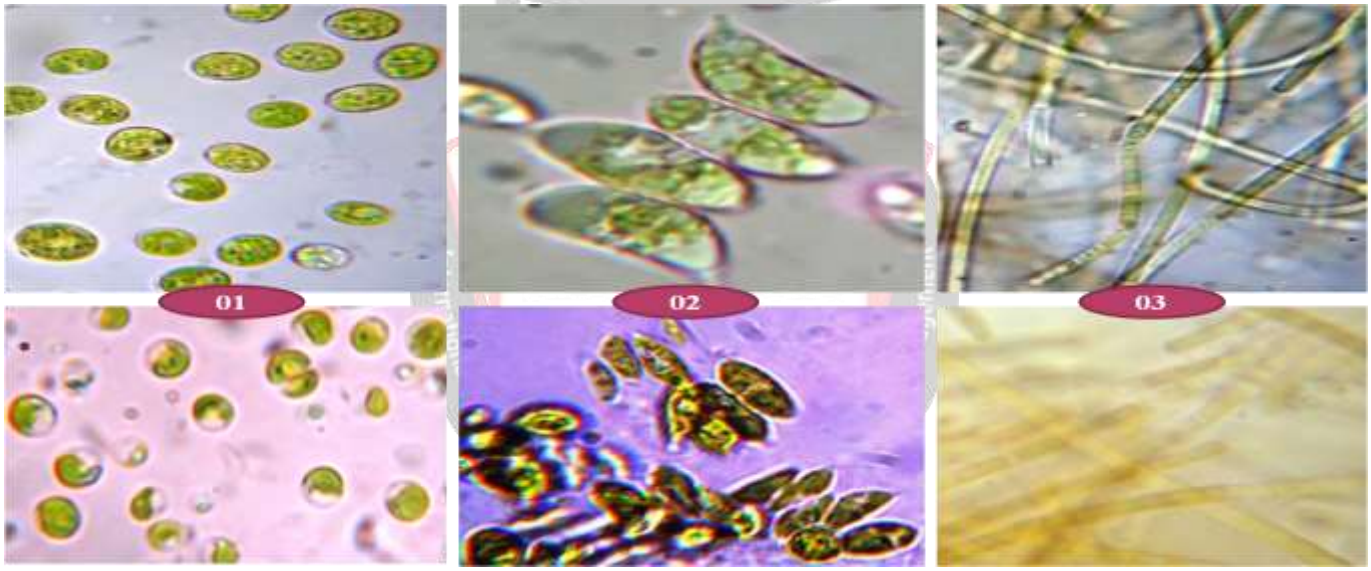

Fig. 2: Morphologically identified microalgae strains isolated from arsenic contaminated sites.

Systematic-position: *Chlorella vulgaris*

Empire : Eukaryota, Kingdom : Plantae, Phylum : Chlorophyta, Class : Trebouxiophyceae, Order : Chlorellales, Family : Chlorellaceae, Genus : *Chlorella*, Species: *vulgaris*.

Systematic-position: *Scenedesmus acutus*

Empire : Prokaryota, Kingdom : Viridiplantae, Phylum : Chlorophyta, Class : Chlorophyceae, Order : Sphaeropleales, Family : Scenedesmaceae, Genus : *Scenedesmus*, Species: *acutus*.

Systematic-position: *Oscillatoria acuminata*

Empire : Prokaryota, Kingdom : Eubacteria, Phylum : Cyanobacteria, Class : Cyanophyceae, Order : Oscillatoriales, Family : *Oscillatoriaceae*, Genus : *Oscillatoria*, Species: *acuminata*.

Arsenic Absorption by Microalgal Species

The three different microalgal species such as *Chlorella vulgaris*, *Scenedesmus acutus* and *Oscillatoria acuminata* were grown for 21 days (Figure-4) in BBM growth media with different arsenic concentrations (5, 15, 25, 50ppm and control). After 21 days of growth with different arsenic concentrations the percentages of arsenic reduction by three microalgal species in growth media were calculated and the results were tabulated (Table-1) and represented as graph (Figure-5 & 6).

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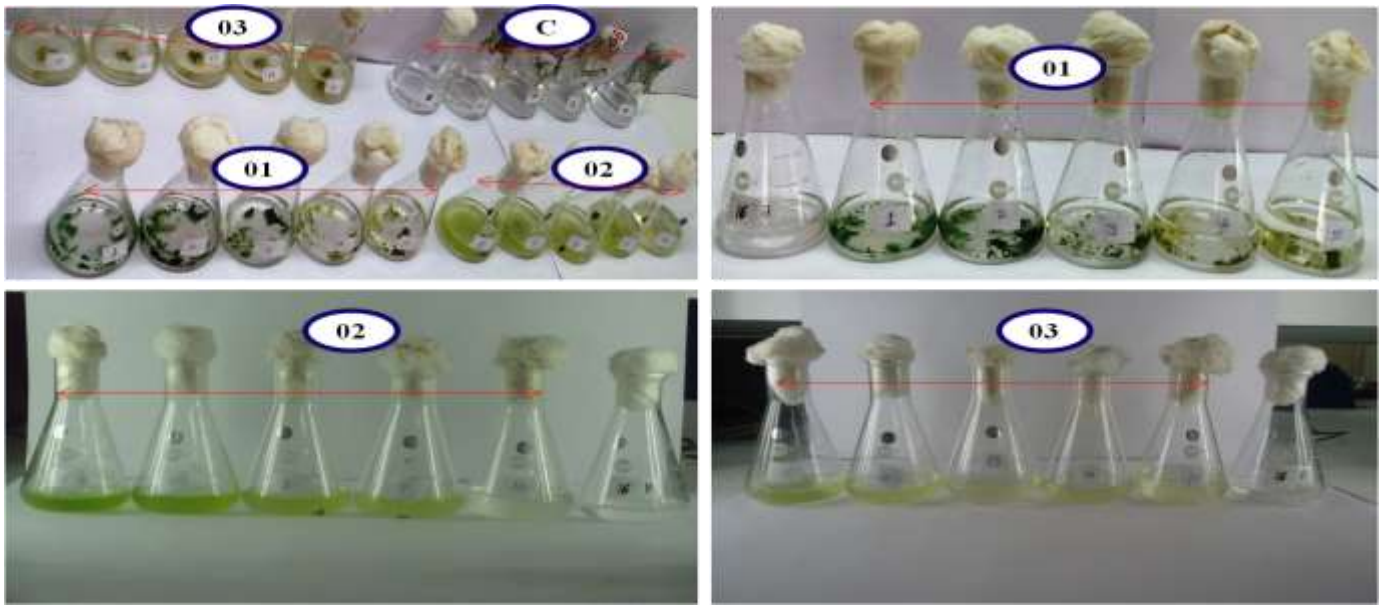

Figure 4: Microalgal samples after growth of 21 days with four different concentrations of arsenic and control samples (without arsenic and microalgae). Note: 01 - *Oscillatoria acuminata* Gom., 02 - *Chlorella vulgaris* Beyerinck, 03 - *Scenedesmus acutus* var. Obliquus Rabenh, C – Control.

Highest arsenic removal (41.98 % at 50 ppm concentration) from liquid Bold Basal Media (BBM) after growth of 21 days when compared to *Chlorella vulgaris* Beyerinck (40.14 % at 50 ppm concentration) and *Scenedesmus acutus* var. Obliquus Rabenh (32.06 % at 50 ppm concentration) was achieved by *Oscillatoria acuminata* Gom. Lowest arsenic removal (4.2 % at 5 ppm concentration) from liquid Bold Basal Media (BBM) after growth of 21 days when compared to *Oscillatoria acuminata* Gom. (25 % at 15 ppm concentration) and *Chlorella vulgaris* Beyerinck (9.4 % at 15 ppm concentration) was achieved by *Scenedesmus acutus* var. Obliquus Rabenh.

Experiments	Initial Arsenic Concentration	Final As Concentration (PPM)	Percentage (%) of As reduction
***Oscillatoria acuminata* Gom. + BBM + Arsenic**	5 PPM	**2.92**	**41.6**
	15 PPM	**11.25**	**25**
	25 PPM	**17.55**	**29.8**
	50 PPM	**29.01**	**41.98**
***Chlorella vulgaris* Beyerinck + BBM + Arsenic**	5 PPM	3.84	23.2
	15 PPM	13.6	9.4
	25 PPM	18.44	26.24
	50 PPM	29.93	40.14
***Scenedesmus acutus* var. Obliquus Rabenh + BBM + Arsenic**	5 PPM	**4.79**	**4.2**
	15 PPM	**13.84**	**7.74**
	25 PPM	**20.32**	**18.72**
	50 PPM	**33.97**	**32.06**
BBM + Arsenic	5 PPM	4.86	2.8
	15 PPM	14.81	1.27
	25 PPM	24.93	0.28
	50 PPM	49.98	0.04
1. Positive Control - 1: BBM + *Oscillatoria acuminata* Gom.	0 PPM	0 PPM	0 PPM
2. Positive Control - 2: BBM+ *Chlorella vulgaris* Beyerinck	0 PPM	0 PPM	0 PPM

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3. Positive Control - 3: BBM+*Scenedesmus acutus* var. Obliquus Rabenh	0 PPM	0 PPM	0 PPM
4. Negative Control - 1: BBM	0 PPM	0 PPM	0 PPM

Table 1: Arsenic reduction by microalgal species in BBM media after 21 days of growth (Maximum and minimum percentages (%) of arsenic reductions are marked as green and red in colour respectively).

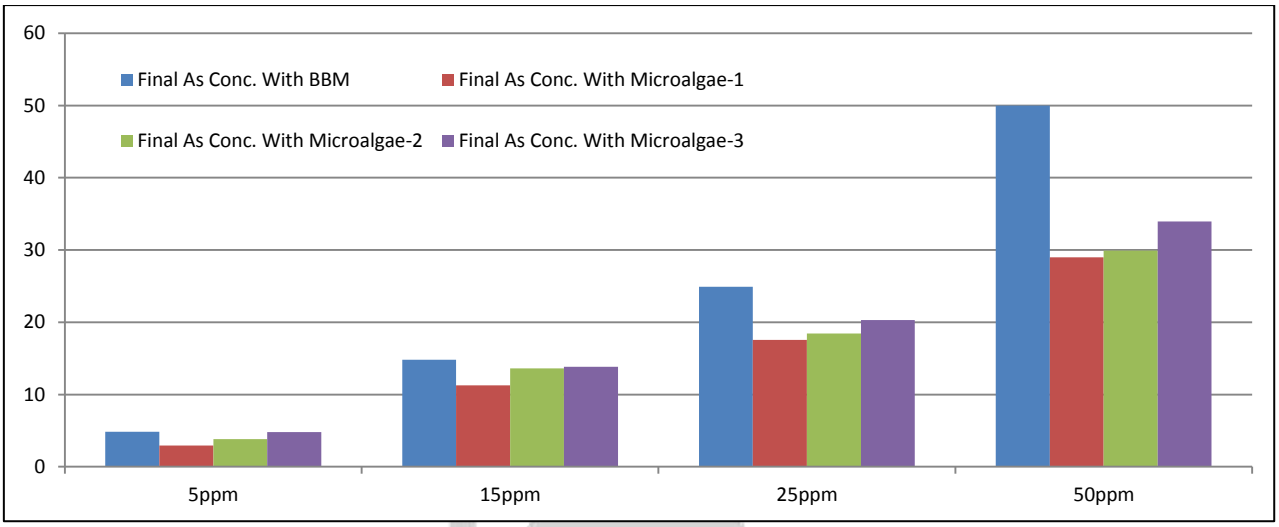

Figure 5: Initial arsenic concentrations vs final arsenic concentrations after growth of microalgal samples for 21 days with four different concentrations of arsenic and control samples (BBM media without microalgae). Note: Microalgae - 1: *Oscillatoria acuminata* Gom., Microalgae - 2: *Chlorella vulgaris* Beyerinck, Microalgae - 3: *Scenedesmus acutus* var. Obliquus Rabenh.

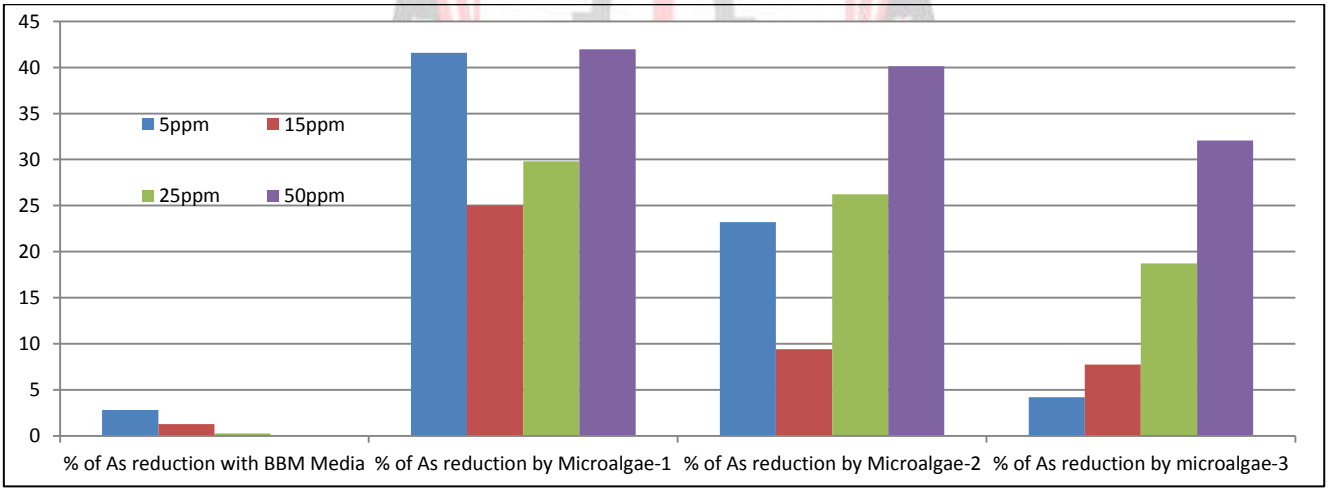

Figure 6: Percentage of arsenic reduction from initial arsenic concentrations after growth of microalgal samples for 21 days with four different concentrations of arsenic and control samples (BBM media without microalgae).Note: Microalgae - 1: *Oscillatoria acuminata* Gom., Microalgae - 2: *Chlorella vulgaris* Beyerinck, Microalgae - 3: *Scenedesmus acutus* var. Obliquus Rabenh.

Arsenic adsorption studies of three microalgal samples:

The dried microalgal samples such as *Oscillatoria acuminata* Gom. and *Chlorella vulgaris* Beyerinck were treated with 100 ppm arsenic for 48 hours to study the arsenic adsorption potential (Figure- 7). Adsorption of arsenic from water after 48 hours of incubation and percentage of reduction of arsenic by two different microalgal species was calculated by using initial and final arsenic concentration in water. The results were tabulated at table- 2. *Chlorella vulgaris* Beyerinck has adsorbed 35.03 % of arsenic and *Oscillatoria acuminata* Gom. has adsorbed 32.77 % of arsenic from drinking water after 48 hours of incubation.

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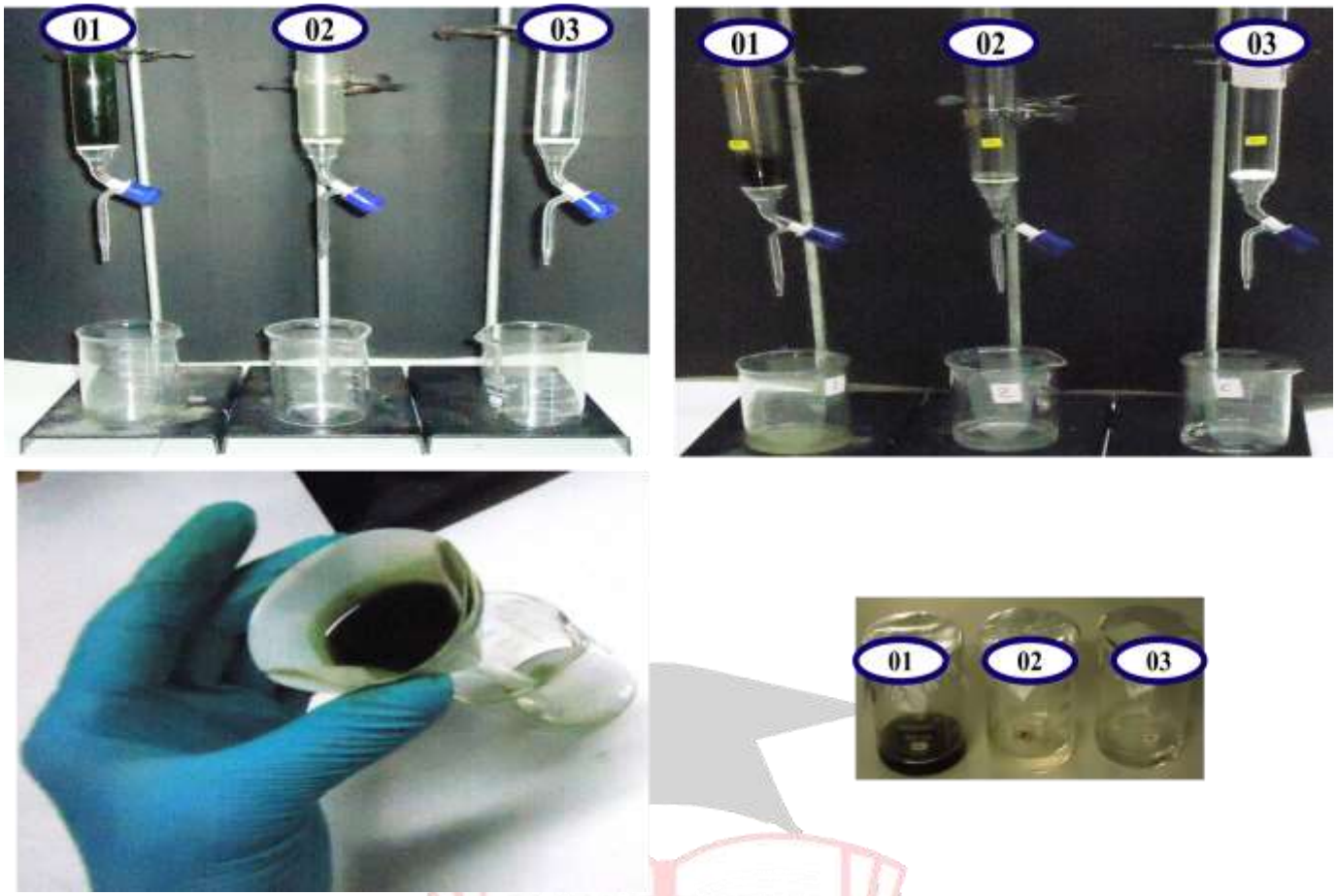

Figure 7: Dried microalgal samples were treated with arsenic for the study of arsenic adsorption potential. Note: Algae-1 = *Oscillatoria acuminata* Gom., Algae-2 = *Chlorella vulgaris* Beyerinck, 3 = Control (Drinking Water).

Sl. No.	Sample Name	Initial Arsenic Conc. (mg/l)	Final Arsenic Concentration (mg/l)	Total Amount of Arsenic Reduced (mg)	Percentage of Arsenic Reduction (%)	Percentage of Arsenic Adsorption (%)
1.	Control	100	99.98	0.02	0.02	0.02
2.	Filtrate-1	100	67.23	32.77	32.77	0
3.	Filtrate-2	100	64.97	35.03	35.03	0
4.	Algae-1	0	32.77	0	0	32.77
5.	Algae-2	0	35.03	0	0	35.03

Table 2: Adsorption of arsenic from water and percentage of arsenic reduction by microalgae after 48 hours of incubation.

Analysis of three different microalgal samples (both treated with arsenic and without arsenic treatment) by Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Microanalysis (EDAX)

The microalgal species such as *Chlorella vulgaris*, *Scenedesmus acutus* and *Oscillatoria acuminata*, which were treated with 50 ppm arsenic concentrations and control samples were viewed under scanning electron microscope and photographed. The arsenic concentrations present in three microalgal samples such as *Chlorella vulgaris*, *Scenedesmus acutus* and *Oscillatoria acuminata* (Control and treated with 50 ppm arsenic) were also studied by EDAX analysis and confirmed the arsenic uptake by microalgal species. Both SEM and EDAX results were confirmed the absorption of arsenic by *Chlorella vulgaris*, *Scenedesmus acutus* and *Oscillatoria acuminata* from liquid BBM media after growth of 21 days. In all the arsenic treated microalgal samples the arsenic accumulation was observed and detected while in all control samples no arsenic accumulation has observed and detected.

a) *Oscillatoria acuminata* Gom.:

The figure-8 shows the control sample of *Oscillatoria acuminata* Gom. (not treated with arsenic and grown for 21 days) has no arsenic absorption in its ultra structural view of Scanning Electron Microscopy (SEM) and also there was no peak on arsenic has observed by Energy Dispersive X-Ray Microanalysis (EDAX) (Table-3) indicates no arsenic absorption and accumulation by control sample of *Oscillatoria acuminata* Gom.

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a) *Oscillatoria acuminata* Gom.:

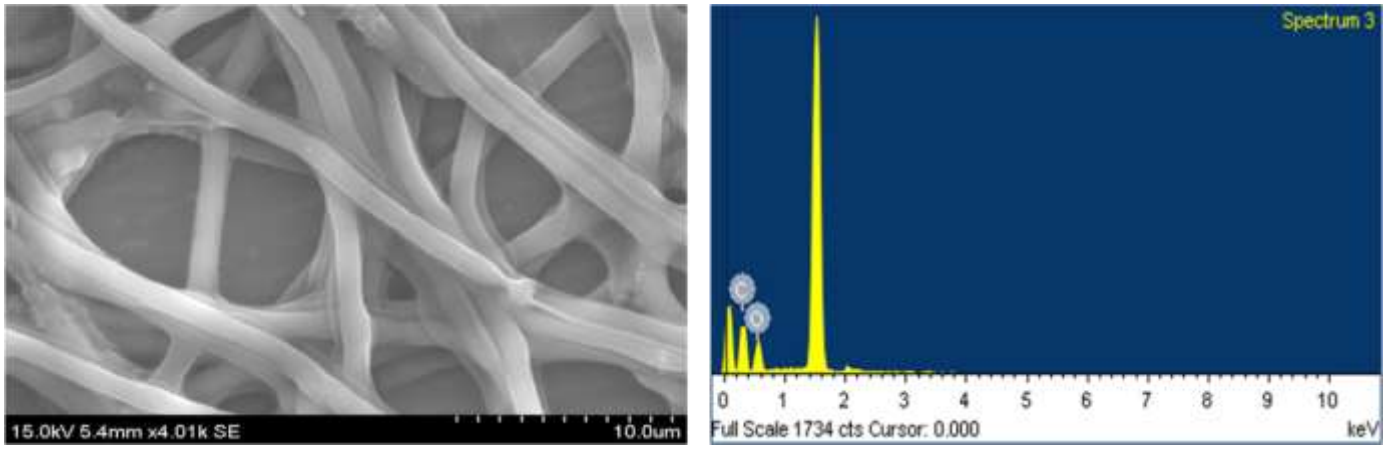

Figure 8: Analysis of microalgal samples of *Oscillatoria acuminata* Gom. not treated with arsenic by Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Microanalysis (EDAX).

Element	Weight%	Atomic%
C K	49.12	56.26
O K	50.88	43.74
Totals	100.00	

Table 3: Analysis of microalgal samples of *Oscillatoria acuminata* Gom. not treated with arsenic (15 ppm) by Energy Dispersive X-Ray Microanalysis (EDAX).

The figure- 9 shows the treated sample of *Oscillatoria acuminata* Gom. (treated with 50 ppm arsenic and grown for 21 days) has absorbed arsenic in its ultra structural view of Scanning Electron Microscopy (SEM) and also there was a peak on arsenic has observed by Energy Dispersive X-Ray Microanalysis (EDAX) (Table- 4) indicates arsenic absorption and accumulation by 50 ppm arsenic treated sample of *Oscillatoria acuminata* Gom.

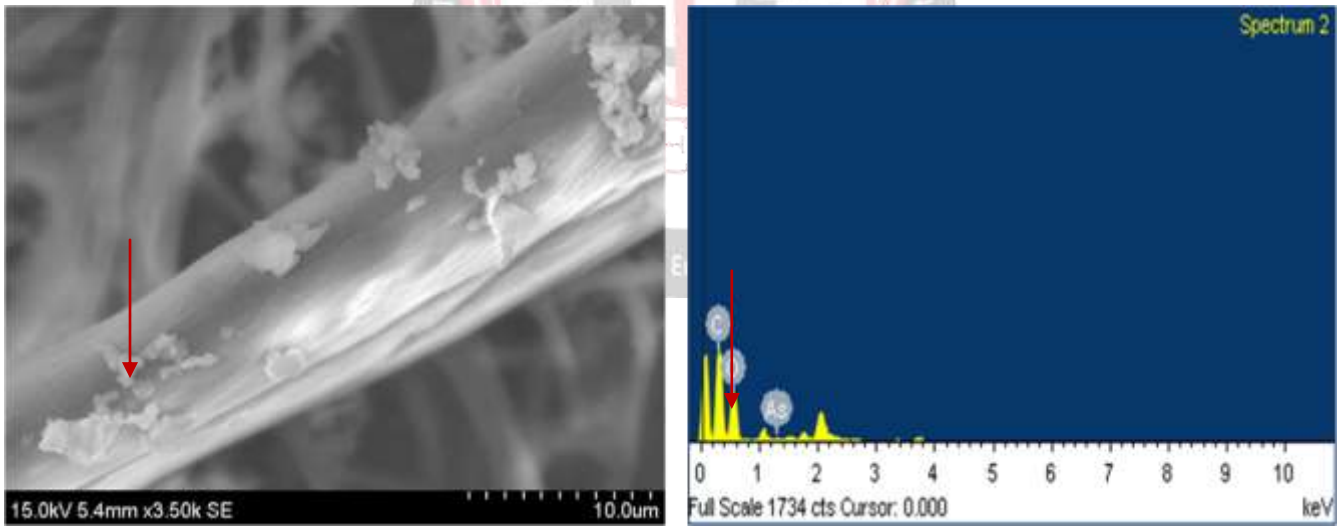

Figure 9: Analysis of Microalgal Samples of *Oscillatoria acuminata* Gom. treated with Arsenic (15 ppm) by Scanning Electron Microscopy (SEM) – Energy –Dispersive X-Ray Microanalysis (EDAX).

Element	Weight%	Atomic%
C K	50.16	57.48
O K	49.32	42.43
As	0.52	0.10
Totals	100.00	

Table 4: Analysis of Microalgal Samples of *Oscillatoria acuminata* Gom. treated with Arsenic (15 ppm) by Energy Dispersive X-Ray Microanalysis (EDAX).

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b) *Scenedesmus acutus* var. Obliquus Rabenh

The figure- 10 shows the control sample of *Scenedesmus acutus* var. Obliquus Rabenh (not treated with arsenic and grown for 21 days) has no arsenic absorption in its ultra structural view of Scanning Electron Microscopy (SEM) and also there was no peak on arsenic has observed by Energy Dispersive X-Ray Microanalysis (EDAX) (Table- 5) indicates no arsenic absorption and accumulation by control sample of *Scenedesmus acutus* var. Obliquus Rabenh.

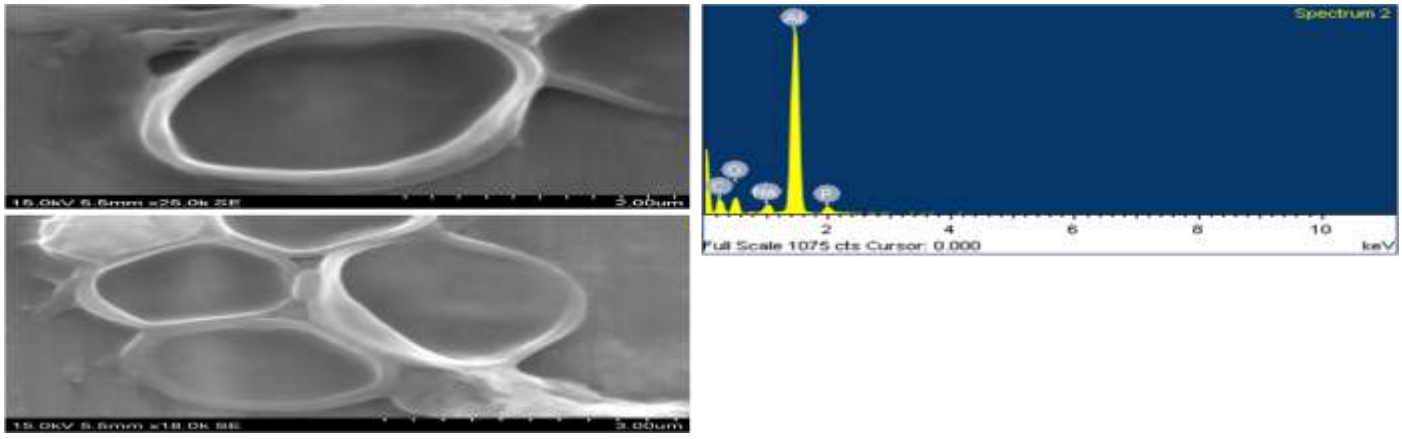

Figure 10: Analysis of Microalgal Samples of *Scenedesmus acutus* var. Obliquus Rabenh not Treated with Arsenic by Scanning Electron Microscopy (SEM) – Energy –Dispersive X-Ray Microanalysis (EDAX).

Element	Weight%	Atomic%
C K	38.91	53.07
O K	23.35	23.91
Na K	2.51	1.79
Al K	33.26	20.19
P K	1.96	1.04
Totals	100.00	

Table- 5: Analysis of Microalgal Samples of *Scenedesmus acutus* var. Obliquus Rabenh not treated with Arsenic by Energy Dispersive X-Ray Microanalysis (EDAX).

The figure-11 shows the treated sample of *Scenedesmus acutus* var. Obliquus Rabenh (treated with 50 ppm arsenic and grown for 21 days) has absorbed arsenic in its ultra structural view of Scanning Electron Microscopy (SEM) and also there was a peak on arsenic has observed by Energy Dispersive X-Ray Microanalysis (EDAX) (Table- 6) indicates arsenic absorption and accumulation by 50 ppm arsenic treated sample of *Scenedesmus acutus* var. Obliquus Rabenh.

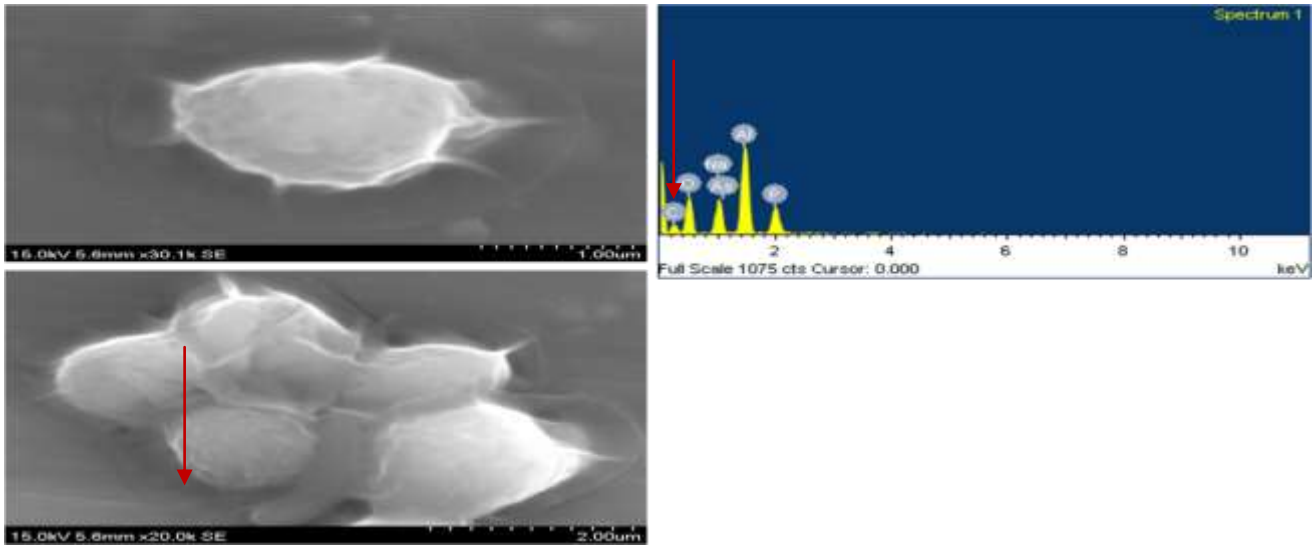

Figure 11: Analysis of Microalgal Samples of *Scenedesmus acutus* var. Obliquus Rabenh treated with Arsenic (15 ppm) by Scanning Electron Microscopy (SEM) – Energy –Dispersive X-Ray Microanalysis (EDAX).

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Element	Weight%	Atomic%
C K	22.49	32.63
O K	38.39	41.81
Na K	10.73	8.13
Al K	20.32	13.12
P K	7.37	4.15
As	0.70	0.16
Totals	100.00	

Table- 6: Analysis of microalgal samples of *Scenedesmus acutus* var. Obliquus Rabenh treated with arsenic (15 ppm) by Energy Dispersive X-Ray Microanalysis (EDAX) .

c) *Chlorella vulgaris* Beyerinck:

The figure-12 shows the control sample of *Chlorella vulgaris* Beyerinck (not treated with arsenic and grown for 21 days) has no arsenic absorption in its ultra structural view of Scanning Electron Microscopy (SEM) and also there was no peak on arsenic has observed by Energy Dispersive X-Ray Microanalysis (EDAX) (Table -7) indicates no arsenic absorption and accumulation by control sample of *Chlorella vulgaris* Beyerinck.

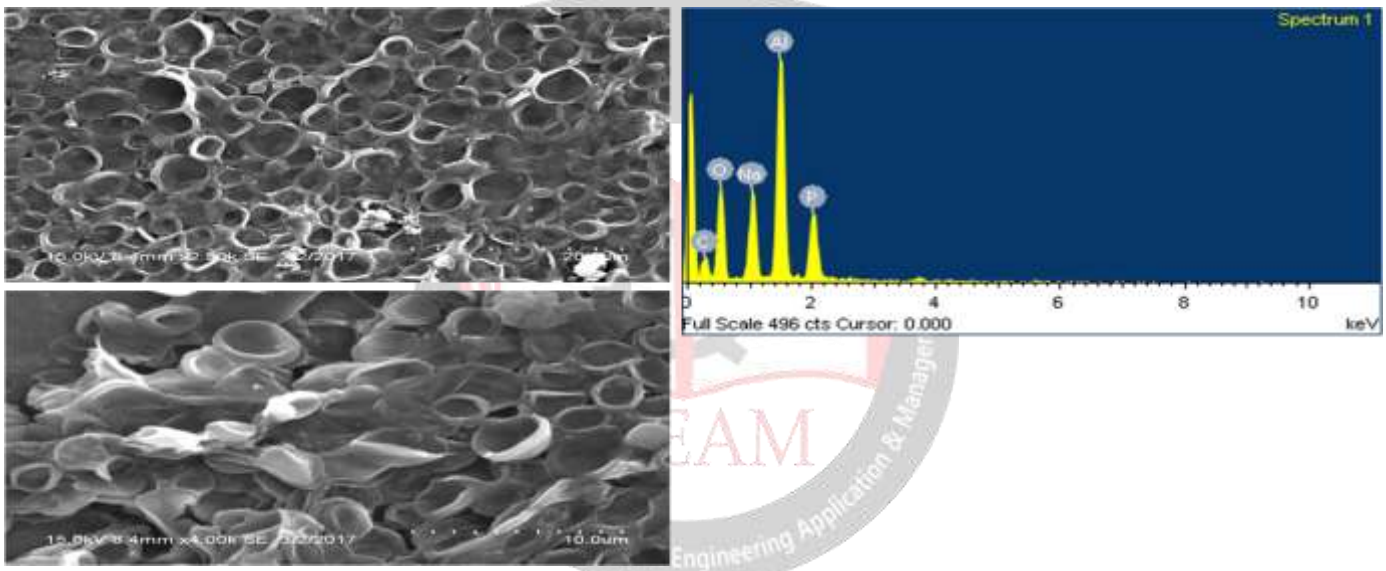

Figure 12: Analysis of microalgal samples of *Chlorella vulgaris* Beyerinck not treated with arsenic by Scanning Electron Microscopy (SEM) – Energy Dispersive X-Ray Microanalysis (EDAX).

Element	Weight%	Atomic%
C K	22.59	32.59
O K	38.71	41.93
Na K	11.02	8.31
Al K	20.26	13.02
P K	7.42	4.15
Totals	100.00	

Table- 7: Analysis of microalgal samples of *Chlorella vulgaris* Beyerinck not treated with arsenic by Energy Dispersive X-Ray Microanalysis (EDAX).

The figure- 13 shows the treated sample of *Chlorella vulgaris* Beyerinck (treated with 50 ppm arsenic and grown for 21 days) has absorbed arsenic in its ultra structural view of Scanning Electron Microscopy (SEM) and also there was a peak on arsenic has observed by Energy Dispersive X-Ray Microanalysis (EDAX) (Table 8) indicates arsenic absorption and accumulation by 50 ppm arsenic treated sample of *Chlorella vulgaris* Beyerinck.

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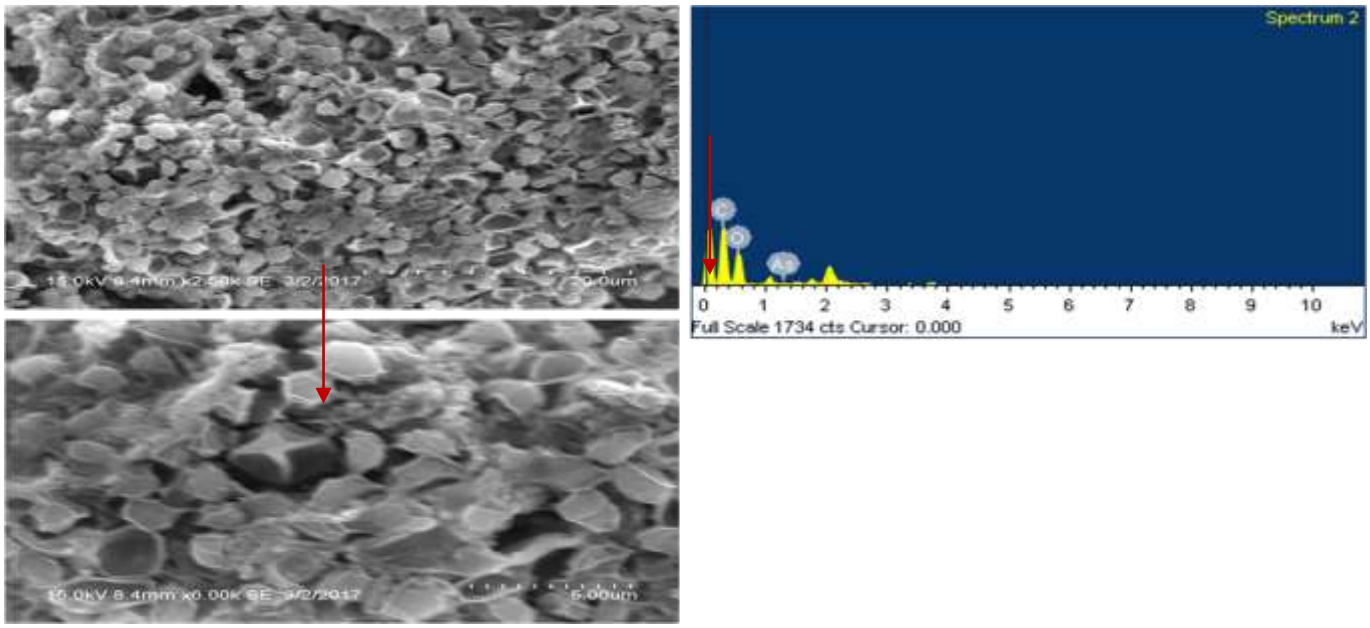

Figure 13: Analysis of microalgal samples of *Chlorella vulgaris* Beyerinck treated with arsenic (15 ppm) by Scanning Electron Microscopy (SEM) – Energy Dispersive X-Ray Microanalysis (EDAX).

Element	Weight%	Atomic%
C K	50.16	57.48
O K	49.32	42.43
As	0.73	0.14
Totals	100.00	

Table- 8: Analysis of microalgal samples of *Chlorella vulgaris* Beyerinck treated with arsenic (15 ppm) by Energy Dispersive X-Ray Microanalysis (EDAX).

Gas Chromatography Mass Spectrometry (GCMS) Analysis:

The selected potential microalgal species were subjected to fatty acids extraction and transesterification to determine the fatty acid compounds by GC-MS analysis for the production of biodiesel. Gas chromatography and Mass Spectrometry is used to identify the biochemical (biofuel) components from three (3) different microalgal samples (Both treated with 50 ppm arsenic and control samples grown for 21 days) such as *Chlorella vulgaris*, *Scenedesmus acutus* and *Oscillatoria acuminata*. High content of saturated fatty acids (SFA) were observed in all the arsenic treated microalgal samples over control samples.

a.) *Scenedesmus acutus* var. Obliquus Rabenh (Control)

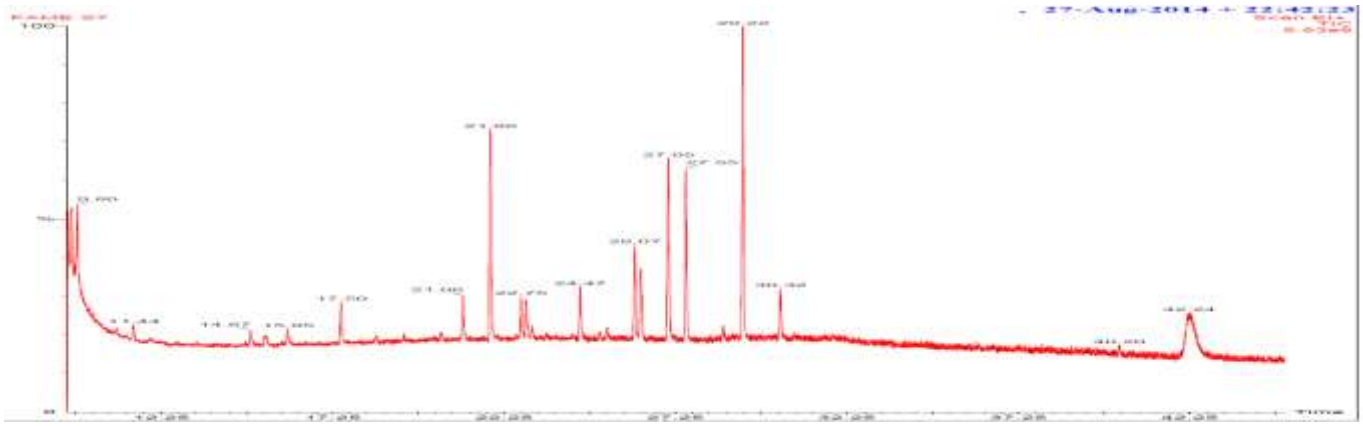

Figure 14: The GC spectrum of *Scenedesmus acutus* var. Obliquus Rabenh (Control).

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Retention time	Compounds	Chemical formula	Molecular weight (g/mol)
9.87	Decanoate	$C_{10}H_{20}O_2$	172.26
11.44	Undecanoate	$C_{30}H_{48}O_3$	456.7003
14.87	Penta decanoate	$C_{15}H_{30}O_2$	242.3975
15.95	Hexa decanoate, 2,2 D-hexadecanoate, 3,3D-hexadecanoate, 4,4D- hexadecanoate, 2-^{13}C-hexa decanoate	$C_{16}H_{31}O_2^-$	255.422
17.50	hepta decanoate	$C_{17}H_{34}O_2$	270.45
21.06	Heneicosanoate	$C_{23}H_{46}O_2$	354.619
21.86	Docosanoate	$C_{22}H_{43}O_2$	339.584
22.75	Tricosanoate	$C_{23}H_{46}O_2$	354.6124
24.47	Tetracosanoate	$C_{24}H_{48}O_2$	368.63
26.07	Hexacosanoate	$C_{27}H_{54}O_2$	410.727
27.05	Octacosanoate	$C_{29}H_{58}O_2$	438.781
27.55	Octacosanoate	$C_{29}H_{58}O_2$	438.781
29.22	Nonacosanoate	$C_{30}H_{60}O_2$	452.808

Table 9: Molecular weight and retention time of fatty acid obtain from *Scenedesmus acutus* var. Obliquus Rabenh (Control).

The fatty acids and biofuel compounds observed in *Scenedesmus acutus* var. Obliquus Rabenh (Control) were (Decanoate - 9.87 Retention time, Undecanoate -11.44 Retention time, Pentadecanoate – 14.87 Retention time, Hexadecanoate, 2, 2 D-Hexadecanoate, 3, 3D- Hexadecanoate, 4, 4D- Hexadecanoate, 2-^{13}C-Hexadecanoate – 15.95 Retention time, Heptadecanoate – 17.50 Retention time, Heneicosanoate – 21.06 Retention time, Docosanoate – 21.86 Retention time, Tricosanoate – 22.75 Retention time, Tetracosanoate – 24.47 Retention time, Hexacosanoate – 26.07 Retention time, Octacosanoate – 27.05 Retention time, Octacosanoate – 27.55 Retention time and Nonacosanoate – 29.22 Retention time. The high content of Hexadecanoate and Octacosanoate were observed in *Scenedesmus acutus* var. Obliquus Rabenh (Control).

b.) *Scenedesmus acutus* var. Obliquus Rabenh (Treated)

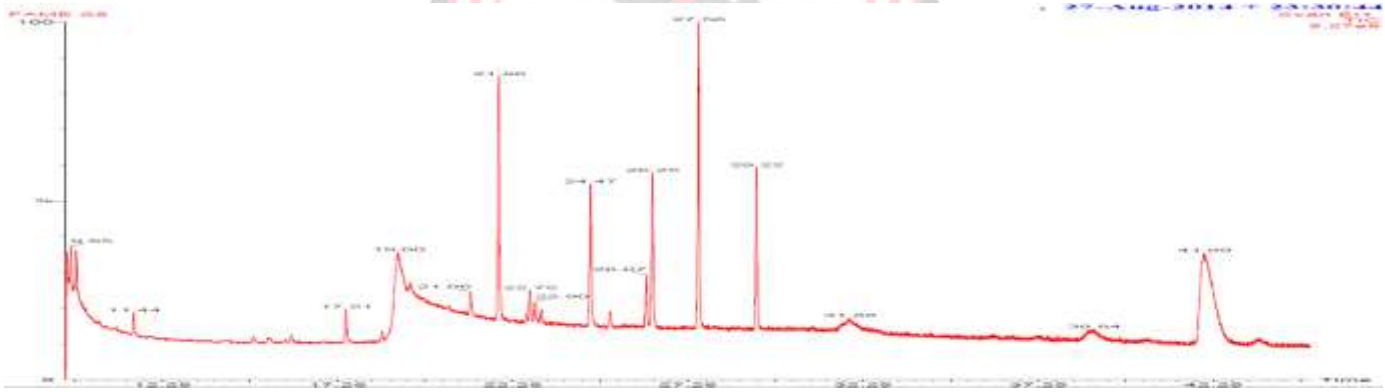

Figure 15: The GC spectrum of *Scenedesmus acutus* var. Obliquus Rabenh (Treated).

Retention time	Compounds	Chemical formula	Molecular weight (g/mol)
9.65	Decanoate	$C_{10}H_{20}O_2$	172.26
11.44	Undecanoate	$C_{30}H_{48}O_3$	456.7003
17.51	Octadecanoate	$C_{17}H_{35}CO_2H$	284.48
19.0	Nonadecanoate	$C_{19}H_{38}O_2$	298.5038
21.06	Heneicosanoate	$C_{23}H_{46}O_2$	354.619
21.86	Docosanoate	$C_{22}H_{43}O_2$	339.584
22.75	Tricosanoate	$C_{23}H_{46}O_2$	354.6124
22.90	Tricosanoate	$C_{23}H_{46}O_2$	354.6124
24.47	Tetracosanoate	$C_{24}H_{48}O_2$	368.63
26.07	Hexacosanoate	$C_{27}H_{54}O_2$	410.727
26.25	Hexacosanoate	$C_{27}H_{54}O_2$	410.727
27.56	Octacosanoate	$C_{29}H_{58}O_2$	438.781
29.22	Nonacosanoate	$C_{30}H_{60}O_2$	452.808

Table 10: Molecular weight and retention time of fatty acid obtain from *Scenedesmus acutus* var. Obliquus Rabenh (Treated).

The fatty acids and biofuel compounds observed in *Scenedesmus acutus* var. Obliquus Rabenh (Treated) were (decanoate – 9.65 Retention time, Undecanoate – 11.44 Retention time, octadecanoate – 17.51 Retention time, Nonadecanoate – 19.0

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Retention time, Heneicosanoate – 21.06 Retention time, Docosanoate – 21.86 Retention time, Tricosanoate – 22.75 Retention time, Tricosanoate – 22.90 Retention time, Tetracosanoate – 24.47 Retention time, Hexacosanoate – 26.07 Retention time, Hexacosanoate – 26.25 Retention time, Octacosanoate – 27.56 Retention time and Nonacosanoate – 29.22 Retention time. The high content of Tricosanoate and Hexacosanoate were observed in *Scenedesmus acutus* var. Obliquus Rabenh (Treated).

c.) *Oscillatoria acuminata* Gom. (Control)

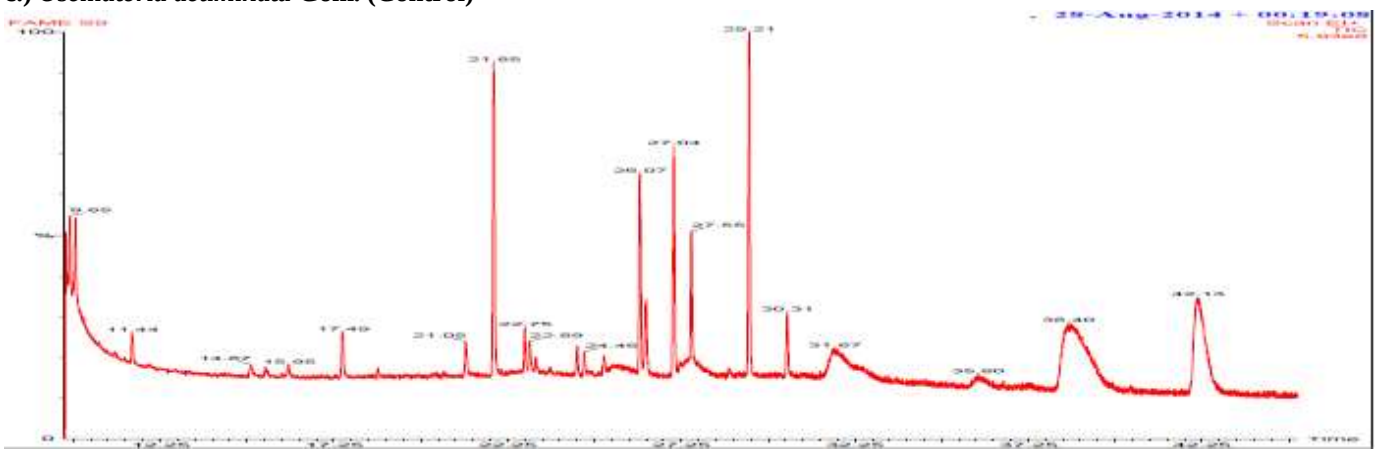

Figure 16: The GC spectrum of *Oscillatoria acuminata* Gom. (Control).

Retention time	Compounds	Chemical formula	Molecular weight (g/mol)
9.65	Decanoate	$C_{10}H_{20}O_2$	172.26
11.44	Undecanoate	$C_{30}H_{48}O_3$	456.7003
14.87	Pentadecanoate	$C_{15}H_30O2$	242.3975
15.95	Hexadecanoate	$C_{16}H_{31}O2^-$	255.422
17.49	Octadecanoate	$C_{17}H_{35}CO_2H$	284.48
21.05	Heneicosanoate	$C_{23}H_{46}O_2$	354.619
21.85	Docosanoate	$C_{22}H_{43}O_2$	339.584
22.75	Tricosanoate	$C_{23}H_{46}O_2$	354.6124
22.89	Tricosanoate	$C_{23}H_{46}O_2$	354.6124
24.46	Pentacosanoate	$C_{25}H_{49}O_2$	382.67
26.07	Hexacosanoate	$C_{27}H_{54}O_2$	410.727
27.04	Heptacosanoate	$C_{28}H_{56}O_2$	424.754
27.55	Nonacosanoate	$C_{30}H_{60}O_2$	452.808
29.21	Triacontanoate	$C_{30}H_{59}O_{22}$	451.8
30.31	Hentriacontanoate	$C_{30}H_{60}O_2$	452.808

Table 11: Molecular weight and retention time of fatty acid obtain from *Oscillatoria acuminata* Gom. (Control).

The fatty acids and biofuel compounds observed in *Oscillatoria acuminata* Gom. (Control) were (Decanoate – 9.65 Retention time, Undecanoate – 11.44 Retention time, Pentadecanoate – 14.87 Retention time, Octadecanoate – 17.49 Retention time, Heneicosanoate – 21.05 Retention time, Docosanoate – 21.85 Retention time, Tricosanoate – 22.75 Retention time, Tricosanoate – 22.89 Retention time, Pentacosanoate – 24.46 Retention time, Hexacosanoate – 26.07 Retention time, Hectacosanoate – 27.04 Retention time, Nonacosanoate – 29.55 Retention time, Triacontanoate – 29.21 Retention time and Hentriacontanoate – 30.31 Retention time. The high content of Tricosanoate was observed in *Oscillatoria acuminata* Gom. (Control).

d.) *Oscillatoria acuminata* Gom. (Treated)

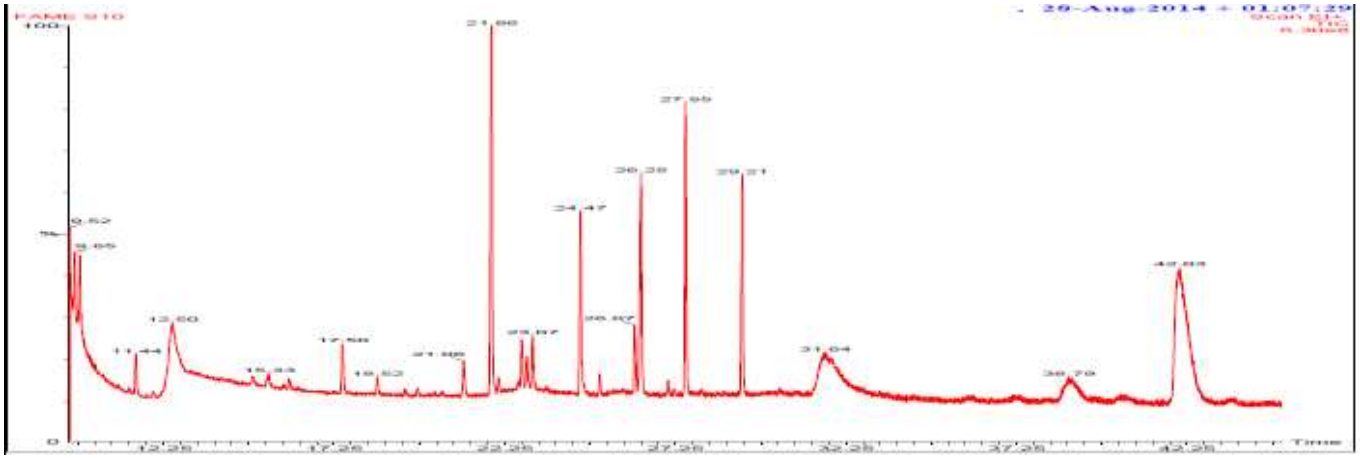

Figure 17: The GC spectrum of *Oscillatoria acuminata* Gom. (Treated).

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Retention time	Compounds	Chemical formula	Molecular weight (g/mol)
9.52	Decanoate	$C_{10}H_{20}O_2$	172.26
9.65	Decanoate	$C_{10}H_{20}O_2$	172.26
11.44	Undecanoate	$C_{30}H_{48}O_3$	456.7003
12.50	Tridecanoate	$C_{14}H_{28}O_2$	214.348
15.33	Pentadecanoate	$C_{15}H_{30}O_2$	242.3975
17.50	Octa decanoate	$C_{17}H_{35}CO_2H$	284.48
18.52	Nonadecanoate	$C_{30}H_{60}O_2$	452.808
21.06	Heneicosanoate	$C_{23}H_{46}O_2$	354.619
21.86	Docosanoate	$C_{22}H_{43}O_2$	339.584
23.07	Tricosanoate	$C_{23}H_{46}O_2$	354.6124
24.47	Pentacosanoate	$C_{25}H_{49}O_2$	382.67
26.07	Hecxacosanoate	$C_{27}H_{54}O_2$	410.727
26.25	Heptacosanoate	$C_{28}H_{56}O_2$	424.754
27.55	Octacosanoate	$C_{29}H_{58}O_2$	438.781
29.21	Triacontanoate	$C_{30}H_{59}O_2$	451.8

Table 12: Molecular weight and retention time of fatty acid obtain from *Oscillatoria acuminata* Gom. (Treated).

The fatty acids and biofuel compounds observed in *Oscillatoria acuminata* Gom. (Treated) were (Decanoate – 9.52 and 9.65 Retention times, Undecanoate – 11.44 Retention time, Tridecanoate – 12.50 Retention time, Pentadecanoate – 15.33 Retention time, Octadecanoate – 17.50 Retention time, Nonadecanoate – 18.52 Retention time, Heneicosanoate – 21.06 Retention time, Docosanoate – 21.86 Retention time, Tricosanoate – 23.07 Retention time, Pentacosanoate – 24.47 Retention time, Hexacosanoate – 26.07 Retention time, Heptacosanoate – 26.25 Retention time, Octacosanoate – 27.55 Retention time and Triacontanoate – 29.21 Retention time. The high content of Decanoate was observed in *Oscillatoria acuminata* Gom. (Treated).

e.) *Chlorella vulgaris* Beyerinck (Control)

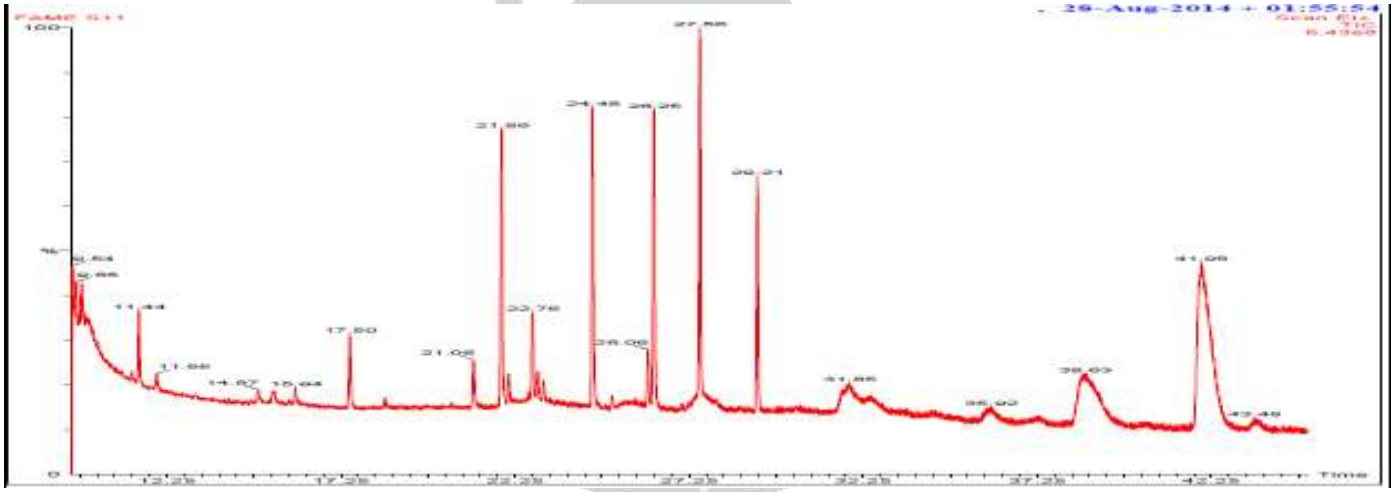

Figure 18: The GC spectrum of *Chlorella vulgaris* Beyerinck (Control).

Retention time	Compounds	Chemical formula	Molecular weight (g/mol)
9.54	Decanoate	$C_{10}H_{20}O_2$	172.26
9.65	Decanoate	$C_{10}H_{20}O_2$	172.26
11.44	Undecanoate	$C_{30}H_{48}O_3$	456.7003
11.95	Dodecanoate	$C_{12}H_{24}O_2$	200.3178
14.87	Pentadecanoate	$C_{15}H_{30}O_2$	242.3975
15.94	Hexa decanoate	$C_{16}H_{31}O2^-$	255.422
17.50	Octadecanoate	$C_{17}H_{35}CO_2H$	284.48
21.06	Heneicosanoate	$C_{23}H_{46}O_2$	354.619
21.86	Docosanoate	$C_{22}H_{43}O_2$	339.584
22.76	Tricosanoate	$C_{23}H_{46}O_2$	354.6124
24.48	Pentacosanoate	$C_{25}H_{49}O_2$	382.67
26.06	Hecxacosanoate	$C_{27}H_{54}O_2$	410.727
26.25	Heptacosanoate	$C_{28}H_{56}O_2$	424.754
27.56	Octacosanoate	$C_{29}H_{58}O_2$	438.781
29.21	Nonocosanoate	$C_{30}H_{60}O_2$	452.808

Table 13: Molecular weight and retention time of fatty acid obtain from *Chlorella vulgaris* Beyerinck (Control).

The fatty acids and biofuel compounds observed in *Chlorella vulgaris* Beyerinck (Control) were (Decanoate – 9.54 and 9.65 Retention times, Undecanoate – 11.44 Retention time, Dodecanoate – 11.95 Retention time, Pentadecanoate – 14.87 Retention time, Hexadecanoate – 15.94 Retention time, Octadecanoate – 17.50 Retention time, Heneicosanoate – 21.06 Retention time,

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Docosanoate – 21.86 Retention time, Tricosanoate – 22.76 Retention time, Pentacosanoate – 24.48 Retention time, Hecxacosanoate – 26.06 Retention time, Heptacosanoate – 26.25 Retention time, Octacosanoate – 27.56 Retention time and Nonacosanoate – 29.21 Retention time. The high content of Decanoate was observed in *Chlorella vulgaris* Beyerinck (Control).

f.) *Chlorella vulgaris* Beyerinck (Treated)

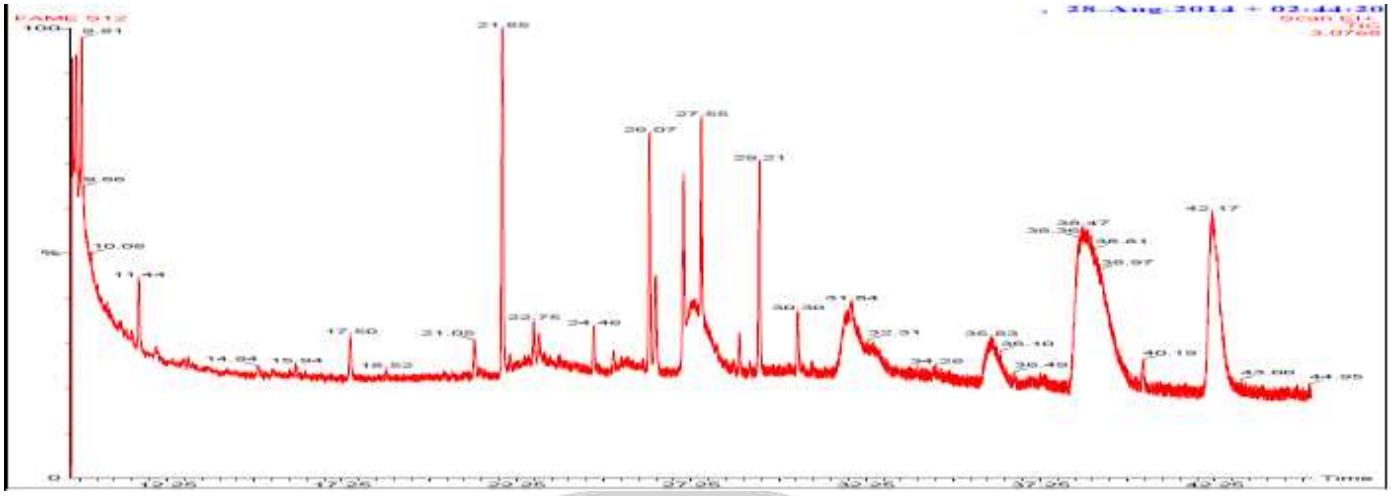

Figure 19: The GC spectrum of *Chlorella vulgaris* Beyerinck (Treated).

Retention time	Compounds	Chemical formula	Molecular weight (g/mol)
9.81	Decanoate	$C_{10}H_{20}O_2$	172.26
9.86	Decanoate	$C_{10}H_{20}O_2$	172.26
10.08	Decanoate	$C_{10}H_{20}O_2$	172.26
11.44	Undecanoate	$C_{30}H_{48}O_3$	456.7003
14.84	Pentadecanoate	$C_{15}H_30O2$	242.3975
15.94	hexa decanoate	$C_{16}H_{31}O2^-$	255.422
17.50	Octadecanoate	$C_{17}H_{35}CO_2H$	284.48
18.52	Nonadecanoate	$C_{30}H_{60}O_2$	452.808
21.05	Heneicosanoate	$C_{23}H_{46}O_2$	354.619
21.85	Docosanoate	$C_{22}H_{43}O_2$	339.584
22.75	Tricosanoate	$C_{23}H_{46}O_2$	354.6124
24.46	Pentacosanoate	$C_{25}H_{49}O_2$	382.67
26.07	hexacosanoate	$C_{27}H_{54}O_2$	410.727
27.56	Octacosanoate	$C_{29}H_{58}O_2$	438.781
29.21	Nonacosanoate	$C_{30}H_{60}O_2$	452.808
30.30	Triacontanoate	$C_{30}H_{59}O_{22}$	451.8

Table 14: Molecular weight and retention time of fatty acid obtain from *Chlorella vulgaris* Beyerinck (Treated).

The fatty acids and biofuel compounds observed in *Chlorella vulgaris* Beyerinck (Treated) were (Decanoate – 9.81, 9.86 and 10.08 Retention times, Undecanoate – 11.44 Retention time, Pentadecanoate – 14.84 Retention time, Hexadecanoate – 15.94 Retention time, Octadecanoate – 17.50 Retention time, Nonadecanoate – 18.52 Retention time Heneicosanoate – 21.05 Retention time, Docosanoate – 21.85 Retention time, Tricosanoate – 22.75 Retention time, Pentacosanoate –24.46 Retention time, Pentacosanoate – 24.46 Retention time, Hecxacosanoate – 26.07 Retention time, Octacosanoate – 27.56 Retention time, Nonacosanoate – 29.21 Retention time and triacontanoate – 30.30 Retention time . The high content of Decanoate was observed in *Chlorella vulgaris* Beyerinck (Treated).

Oscillatoria acuminata Gom 41.98%, *Scenedesmus acutus* var. Obliquus Rabenh 40.14 % and *Chlorella vulgaris* Beyerinck 32.06 % are reduced maximum arsenic concentration in liquid growth media added with 50 ppm arsenic after 40 days of growth period. Arsenic absorbed microalgal samples (*Oscillatoria acuminata* Gom, *Scenedesmus acutus* var. Obliquus Rabenh and *Chlorella vulgaris* Beyerinck) treated with 25 ppm arsenic and control samples (without arsenic treated) were viewed under scanning electron microscopy and confirmed the presence of arsenic uptake. Highest arsenic removal from liquid Bold Basal Media (BBM) after growth of 21 days when compared to *Chlorella vulgaris* Beyerinck and *Scenedesmus acutus* var. Obliquus Rabenh was achieved by *Oscillatoria acuminata* Gom. Lowest arsenic removal from liquid Bold Basal Media (BBM) after growth of 21 days when compared to *Oscillatoria acuminata* Gom. and *Chlorella vulgaris* Beyerinck was achieved by *Scenedesmus acutus* var. Obliquus Rabenh. *Oscillatoria acuminata* Gom (32 %) and *Chlorella vulgaris* Beyerinck (35 %) adsorbed the arsenic while tested with 100 ppm

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concentration of arsenic trioxide. Arsenic absorption and accumulation of microalgae viewed by Scanning Electron Microscopy (SEM) and also the peak on arsenic has observed by Energy Dispersive X-Ray Microanalysis (EDAX) indicates arsenic absorption high in *Chlorella vulgaris* Beyerinck compared with other microalgae.

Microalgae are playing an important role in production of various fatty acid compound like Decanoate, Undecanoate, Pentadecanoate, Hexadecanoate, Octadecanoate, Nonadecanoate, Heneicosanoate, Docosanoate, Tricosanoate, Pentacosanoate, Pentacosanoate, Hecxacosanoate, Octacosanoate, Nonacosanoate and Triacontanoate. The high content of Decanoate was observed in *Chlorella vulgaris*. High content of saturated fatty acids (SFA) were observed in all the arsenic treated microalgal samples over control samples.

IV. CONCLUSION

The *Chlorella vulgaris*, *Scenedesmus acutus* and *Oscillatoria acuminate* could be a potent microalgal species for arsenic reduction and detoxification from drinking water samples of West Bengal, India. Arsenic removal by phycotechnology (by using microalgae) will be the potential, cost effective and ecofriendly technology. The microalgal system for removal of arsenic from drinking water could be a cost effective, eco friendly and easily accessible method for all the people in West Bengal, India and the biomass could be used for the biofuel productions.

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