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# Use of Some Phytoplankton from Shatt al-Arab Estuary in the Production of Hydrocarbons

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**Abstract:** ***The idea of the hydrocarbons extraction from algae as biofuel is not new but it has recently been seriously thought of as an alternative to petroleum fuel because it is less polluting the environment, it is eco-friend as well as the escalating price of petroleum. So, the current study included six species of algae related to three classes were Nostoc, Microcystis and Spirulina related to Cyanbacteria, Chlorella and Cladophora related to Chlorophyta and Compsopgon related to Rhodophyta, to estimate its total content of hydrocarbons. The Cyanobacteria were more producing to the hydrocarbonic compounds represented by Nostoc, Microcystis and Spirulina (108.292, 85.369, 37.687) µg/g respectively followed by Cladophora and Chlorella (17.831, 10.160) µg/g respectively then Compsopgon (2.438) µg/g, then they were separated to identify Aliphatic compound by using GC (Gas Chromatography), Chlorella was more product to these compound which was (68181.32) ng/g then Spirulina, Microcystis and Nostoc (52559.57, 37262.72, 25236.42) ng/g respectively. Aromatic compounds were identified by using HPLC, the total of these compounds in Microcystis was (6091.92) ng/g where was in Spirulina and Nostoc (3307.09, 1722.81) ng/g respectively then Chlorella and Cladophora (1687.17, 1162.98) ng/g respectively.***

**Keywords:** hydrocarbon production, biofuel, algae

## 1. Introduction

In recent years, biodiesel has been received a great attention as it is biodegradable, renewable and non-toxic, and it does not contribute to the emission of carbon dioxide or sulfur in the atmosphere and less polluting the environment than traditional diesel fuel [1], [2], [3]. The use of algae as a source of biofuels and biogas for energy applications has been emphasized [4] because of higher photosynthetic efficiency, higher biomass production, a faster growth rate than higher plants, highest $CO_2$ fixation and $O_2$ production, growing in liquid medium which can be handled easily make the algae to stand high in front of other oil seed crops [5], [6]. They include a wide variety of simple, autotrophic organisms that ranging from unicellular to multi-cellular form which has exceptional potential for cultivation as an energy product [4]. They convert carbon dioxide to potential biofuels, valuable bioactive compounds such as carbohydrates, proteins, lipids and pigments [7], [8]. In addition to other co-products such as residual biomass after oil extraction which can be used as feed or fertilizer [7], or fermented to produce bioethanol or biomethane [9]. Among these are blue-green algae, which are an important source of a wide range of fats, oils, hydrocarbons and sterols not only as a source of renewable energy but also for the production of a range of important pharmaceutical and industrial products [10]. An important factor in the manufacture of hydrocarbons in microalgae is the presence of the decarbonylase enzyme, which was recently discovered to produce hydrocarbons of fatty acids in cyanobacteria [11]. Hydrocarbons can be produced by four main pathways: fermentation, gasification, pyrolysis, and algal conversion [12].

This study aims to determind the ability of some algal species to produce hydrocarbon compounds by assessing total concentration of these compounds and separating them to Aliphatic and Aromatic compounds and identified them.

## 2. Materials and Methods

Algal species were collected from different station of Shatt Al-Arab River. Three types of cyanobacteia which were *Nostoc sp*. related to Nostocales order, *Microcystis sp.* related to Chroococcales and *Spirulina platensis* related to Oscillatoriales, and one species of chlorophyta which was *Chlorella vulgaris* belong to chlorococcales, have been isolated, identified, purified and inoculated in BG11 Medium (pH 7.5) and Incubated at 27± 2̊c in 16:8 hours light and dark cycle and harvested in stationary phase for Laboratory experiments. Other species blooming in Shatt Al- Arab were collected, observed under microscope and depending on their morphological features were classified as: one species of chlorophyta which was *Cladophora sp.* belong to siphonocladales and *Compsopogon sp*. related to compsopogonales (Rhodophyta), washed with distilled water and freez dried.

### 2.1 Hydrocarbons Estimation

As described in [13], hydrocarbons extracted with Methanol: Benzene mixture (1:1) with suxolite for 24 hours, after that saponification was done for two hours, the samples was separated by separating funnels and purified by glass chromatography column. Total hydrocarbons were measured by spectrofluorometer and Aliphatic compounds were measured by (GC) Agilent 7890 Gas Chromatography (autosamplar, column Hp-5 30m, 0.32mm, flam ionization detector (FID) with 1ml/mnt Helium flow rate) in South Oil Company (S.O.C.) laboratories; while Aromatic compounds measured by Shimadzu HPLC (High performance liquid chromatography) in marine science center laboratories (Exitation 310nm, Emission 360nm).

### 2.2 Statistical analysis

Statistical Package for Social Science (SPSS Ver. 20) was adopted in the analysis of the results under significant level (P<0.05) where the T- test was used for logarithm of total hydrocarbons and less significant difference test (LSD) for logarithm Aliphatic and Aromatic compounds concentration.

## 3. Results and Discussion

### 3.1 Total hydrocarbons

Results showed algal susceptibility to hydrocarbons production in different concentrations, cyanobacteria and eukaryotic algae are expected to become a promising feedstock in future [14], and there is significant differences at probability < 0.05 between studied algae (T = 5.406), Fig. (1) shows that blue-green algae were more productive for hydrocarbons. As the production of *Nostoc* (108.292) µg which was the highest, followed by *Microcystis* (85.369) µg, then *Spirulina* (37.687) µg. cyanobacteria are one of only a few types of organisms that are known to directly produce hydrocarbons [15] and hydrocarbons could be produced in a variety of cyanobacterial strains (e.g., *Nostoc sp ,.Anacystis sp., Anacystis nidulans, Trichodesmium erythaeum, Microcoleus chthonoplastes, Plectonema terebrans, Oscillatoria williamsii ,Lyngbya lagerhaimii*, etc.) [16, 17].

### 3.2. Aliphatic compounds

From Table (1) we find that the aliphatic compounds in studied algae were between nC-12 and nC-38. Bacteria, microalgae and land plants produce odd-chain hydrocarbons [18, 19] and there were significant differences between the Aliphatic compounds on the one hand and the studied algae on the other (P< 0.05). The highest total of these compounds was in the *chlorella*, reaching 68181.32ng/g where the best algae for biodiesel would be microalgae [20] and *Chlorella* sp. could be more appropriate microalgae for lipid production [21] which can be used as a feed stock for liquid fuels, such as biodiesel [4], followed by *Spirulina*, *Microcystis***,** and *Nostoc* with the total aliphatic compounds produced (52559.57, 37262.72 and 25236.42) ng/g respectively microalgae have a large capacity for producing lipids for biodiesel and carbohydrates for bioethanol. [22]. Cyanobacteria synthesize long chain alk (a/e)nes using two different pathways, one of which involves a deformylation of fatty aldehydes and the other decarboxylation of fatty acids [17, 23, 24].

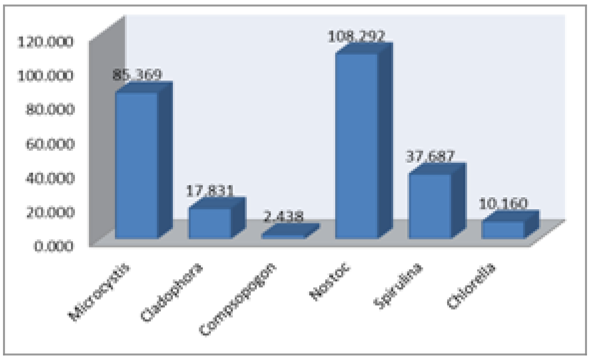


**Figure 1:** Total hydrocarbons concentrations (µg) in six species

**Table 1:** Aliphatic compounds (ng/g) in studied algae

| compound | Microcystis | Nostoc | Compsopogon | Cladophora | Spirulina | Chlorella | S.D |
|---|---|---|---|---|---|---|---|
| n-c12 | 0.00 | 0.00 | 6.80 | 0.00 | 0.00 | 0.00 | 0.34 |
| n-c13 | 0.00 | 241.52 | 4.00 | 0.00 | 0.00 | 0.00 | 0.95 |
| n-c14 | 124.80 | 101.78 | 10.78 | 9.82 | 0.00 | 0.00 | 0.92 |
| n-c15 | 247.34 | 227.56 | 24.60 | 14.32 | 107.52 | 0.00 | 0.91 |
| n-c16 | 543.98 | 725.77 | 149.97 | 20.70 | 649.61 | 407.07 | 0.59 |
| n-c17 | 12066.06 | 7376.10 | 5710.91 | 55.65 | 40652.64 | 4210.79 | 0.98 |
| n-c18 | 2357.32 | 946.99 | 204.65 | 99.80 | 768.51 | 2720.78 | 0.57 |
| n-c19 | 1163.56 | 578.95 | 132.65 | 55.01 | 278.70 | 1539.23 | 0.56 |
| n-c20 | 5915.15 | 4506.29 | 540.06 | 436.89 | 895.75 | 2054.27 | 0.48 |
| n-c21 | 7710.28 | 6055.63 | 473.90 | 753.42 | 417.20 | 5682.50 | 0.60 |
| n-c22 | 2883.10 | 1939.48 | 220.62 | 215.11 | 814.21 | 3744.32 | 0.55 |
| n-c23 | 236.27 | 330.79 | 351.04 | 51.27 | 470.12 | 2754.40 | 0.56 |
| n-c24 | 813.18 | 565.24 | 386.54 | 70.95 | 467.88 | 2664.68 | 0.51 |
| n-c25 | 504.88 | 180.50 | 71.97 | 44.64 | 508.30 | 2905.15 | 0.66 |
| n-c26 | 510.14 | 147.59 | 98.18 | 46.08 | 408.24 | 2408.33 | 0.61 |
| n-c27 | 353.35 | 127.91 | 76.68 | 28.70 | 305.76 | 1979.99 | 0.63 |
| n-c28 | 294.12 | 95.34 | 139.46 | 18.67 | 406.89 | 2303.62 | 0.70 |
| n-c29 | 266.65 | 89.20 | 120.22 | 55.56 | 324.43 | 2237.24 | 0.57 |
| n-c30 | 180.96 | 62.60 | 129.81 | 16.23 | 243.83 | 962.04 | 0.59 |
| n-c31 | 174.27 | 279.19 | 240.81 | 8.97 | 795.94 | 4296.87 | 0.88 |
| n-c32 | 105.74 | 293.15 | 173.13 | 6.49 | 292.02 | 527.28 | 0.68 |
| n-c33 | 66.30 | 42.68 | 268.80 | 11.79 | 109.63 | 4545.24 | 0.88 |
| n-c34 | 158.44 | 137.02 | 88.05 | 0.00 | 0.00 | 10574.06 | 1.53 |
| n-c35 | 0.00 | 0.00 | 52.62 | 0.00 | 295.11 | 845.56 | 1.36 |
| n-c36 | 586.85 | 185.12 | 64.93 | 55.39 | 1153.33 | 7047.74 | 0.81 |
| n-c37 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1770.14 | 2.30 |
| n-c38 | 0.00 | 0.00 | 0.00 | 0.00 | 2193.98 | 0.00 | 2.36 |
| total | 37262.72 | 25236.42 | 9741.19 | 2075.44 | 52559.57 | 68181.32 | 0.34 |
| S.D | 1.08 | 0.92 | 0.65 | 0.78 | 1.18 | 1.37 | |
| LSD (P<0.05) compounds | | 0.825 | | LSD (P<0.05) algae | | 0.413 | |

The figures (2, 3, 4, 5 and 6) generally showed that the predominance of n-C17 and n-C21 compounds in algae. [13] stated that ordinary alkanes with fewer than n-21 individual carbon atoms were commonly found in algae. In addition, the bio-processing of the aliphatic compounds was recorded in several types of cyanobacteria [25] and n-C17 hydrocarbon is widely distributed in most cyanobacterial strains [26]. In general, the n-C17 family of heptadecane, heptadecene and 8-methylheptadecane comprise the main hydrocarbons found in most cyanobacteria studied to date [27].

Early research in the 1960s revealed that cyanobacteria mainly produced hydrocarbons with carbon chain lengths varying from n-C15 to n-C18 with a predominance of n-C17 compounds [15, 17].

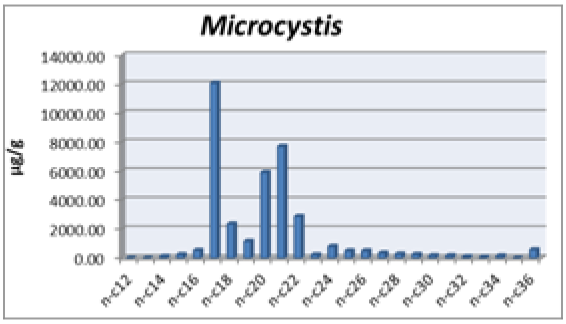


**Figure 2:** Aliphatic compound in *Microcystis sp.*

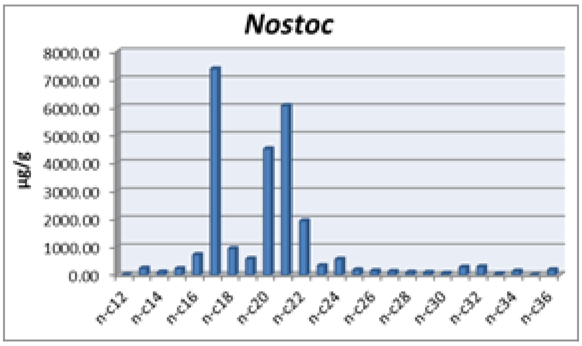


**Figure 3:** Aliphatic compound in *Nostoc sp.*

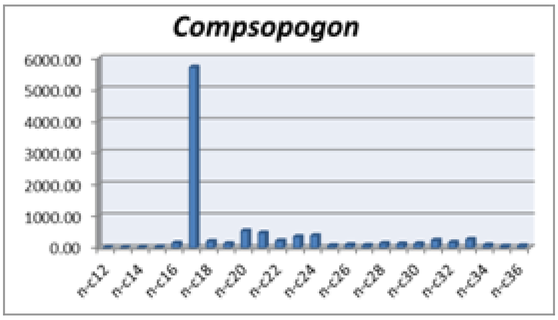


**Figure 4:** Aliphatic compound in *Compsopogon sp*

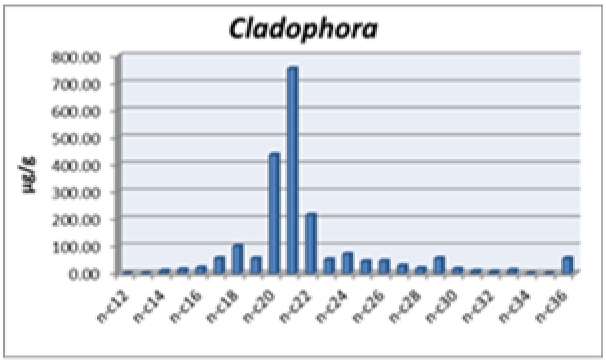


**Figure 5:** Aliphatic compound in *Cladophora sp.*

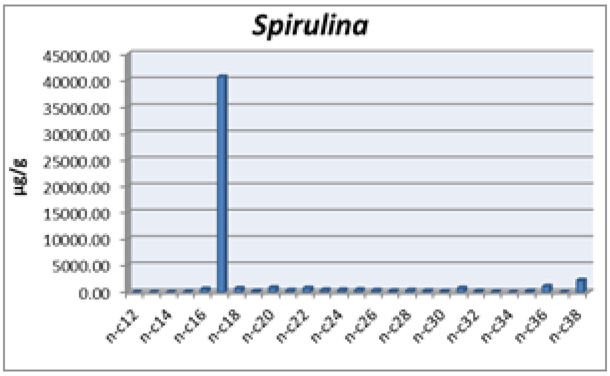


**Figure 6:** Aliphatic compound in *Spirulina sp.*

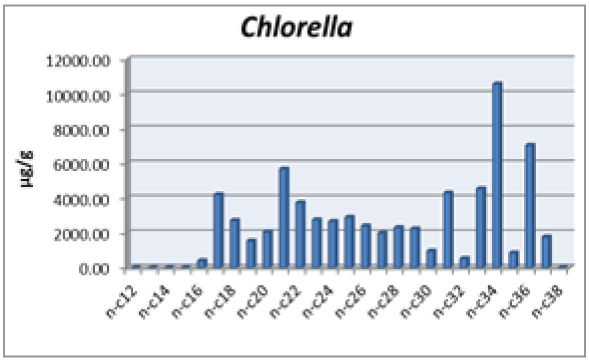


**Figure 7:** Aliphatic compound in *Chlorella sp.*

### 3.3 Aromatic compounds

Table (2) shows the concentrations of aromatic compounds in the studied algae, [28] have demonstrated their presence in some micro-algae species, including *chlorella vulgaris* in freshwater, for their ability to produce polycyclic aromatic compounds. The concentrations of these compounds differed between the studied algae, and there were significant differences between the studied algae and between Aromatic compounds ($P< 0.05$), *Microcystis* recorded the highest total of aromatic compounds (6091.92) ng/g followed by *Spirullina* and *Nostoc* about (3307.09, 1722.81) ng/g respectively. Cyanobacteria, which are capable of the photosynthetic production of hydrocarbons and exhibit multiple adaptive morphological, biochemical and metabolic properties, have garnered particular attention because of their huge potential for renewable energies [23, 29, 30] followed by green algae *chlorella* and *Cladophora* (1687.17, 1162.98) ng/g respectively, and finally, red alga *Compsopogon* about (280.45) ng/g, where Many aromatic compounds were found in all algal species and most of these compounds are ester derivatives of benzenedicarboxylic acid, benzoic acid, dimethylphthalate, tetraphthalic, methylvinyl ester and phenanthrene carboxylic acid ester derivatives [31].

**Table 2:** Aromatic compounds (ng/g) in studied algae

| Compounds | Microcystis | Nostoc | Compsopogon | Cladophora | Spirulina | Chlorella | S.D. |
|---|---|---|---|---|---|---|---|
| Naphtalene | 231.54 | 70.36 | 0 | 7.75 | 14.31 | 0.00 | 0.67 |
| Acenaphtylene | 0 | 1209.56 | 258.32 | 0 | 482.15 | 0.00 | 0.34 |
| Acenaphthene | 1133.65 | 73.70 | 0 | 193.83 | 436.04 | 176.99 | 0.45 |
| Fluorene | 0 | 61.74 | 0 | 0 | 992.86 | 454.17 | 0.62 |
| Phenanthrene | 0 | 156.28 | 0 | 24.07 | 0.00 | 0.00 | 0.57 |
| Anthracene | 4247.18 | 0 | 0 | 758.37 | 700.40 | 141.28 | 0.60 |
| Fluoranthene | 0 | 0 | 14.62 | 0 | 0.00 | 0.00 | |
| Pyrene | 0 | 0 | 0 | 0 | 5.65 | 0.00 | |
| Benzo[a]anthracene | 77.25 | 0 | 6.90 | 20.20 | 30.96 | 0.00 | 0.44 |
| Chrysene | 107.19 | 0 | 0 | 79.33 | 35.29 | 8.42 | 0.49 |
| Benzo[b]fluoranthene | 0 | 24.84 | 0.61 | 0 | 23.51 | 0.00 | 0.45 |
| Benzo[k]fluoranthene | 295.11 | 126.33 | 0 | 79.41 | 276.32 | 261.45 | 0.25 |
| Benzo[a]pyrene | 0 | 0 | 0 | 0 | 19.73 | 2.05 | 0.69 |
| Dibenzo[a, h]anthracene | 0 | 0 | 0 | 0 | 289.86 | 642.82 | 0.24 |
| Benzo[g, h, i]perylene | 0 | 0 | 0 | 0 | 0.00 | 0.00 | |
| Indeno[1, 2, 3-c, d]pyrene | 0 | 0 | 0 | 0 | 0.00 | 0.00 | |
| Total | 6091.92 | 1722.81 | 280.45 | 1162.98 | 3307.09 | 1687.17 | |
| S.D. | 0.66 | 0.53 | 0.80 | 0.67 | 0.78 | 0.94 | |
| LSD< 0.05 compounds | 0.582 | | | LSD< 0.05 algae | 0.455 | | |

The figures (8, 9, 10, 11, 12 and 13) showed the diagnosis of aromatic compounds for all studied algae using HPLC, which showed the difference of aromatic compounds among these species. This may be due to the difference between different species [32, 33]. In the *Microcystis*, there is a clear presence of anthracene, as well as in the *Cladophora*. In the *Spirulina*, the salient compounds are fluorene and anthracene. The acenaphytylene compound was the most widely produced in the *Nostoc* and *Compsopogon* while in the *Chlorella*, the compounds were dibenzo[a, h] anthracene and fluorene were most present while some studies [34, 35] indicated that benzothiazole derivatives are pyrolytic products of cyanobacteria.

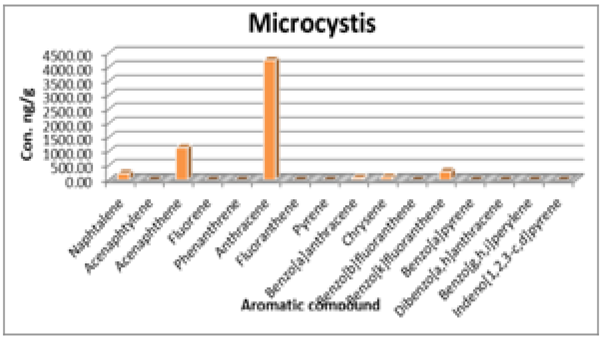


**Figure 8:** Aromatic compound in *Microcystis sp.*

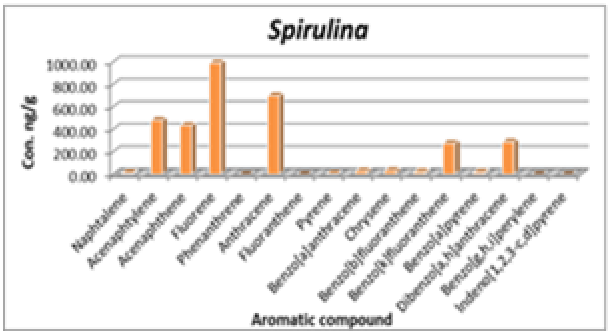


**Figure 9:** Aromatic compound in *Spiulina sp.*

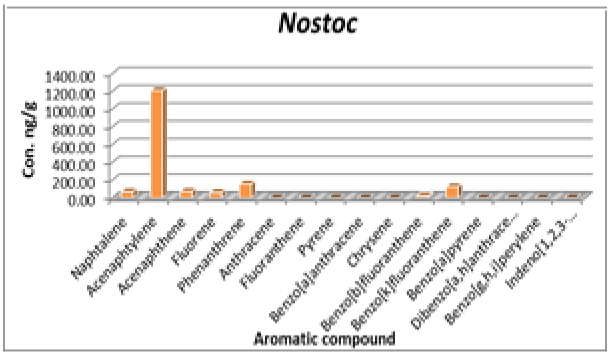


**Figure 10:** Aromatic compound in *Nostoc sp.*

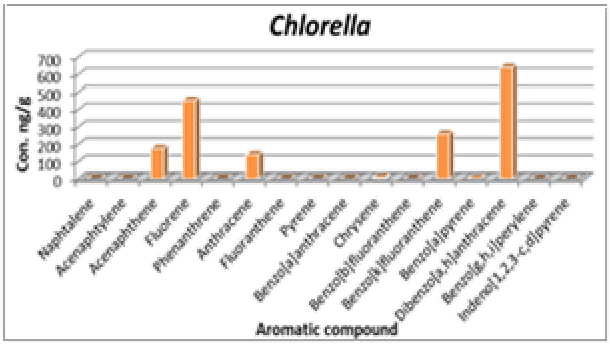


**Figure 11:** Aromatic compound in *Chlorella sp.*

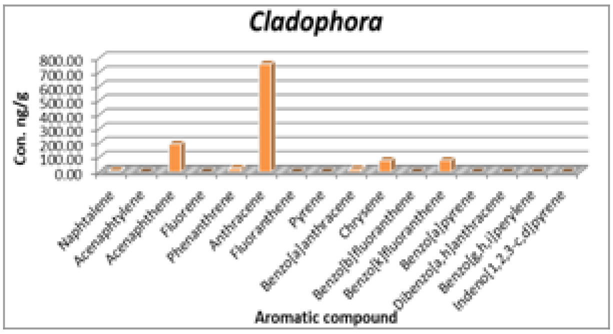


**Figure 12:** Aromatic compound in *Cladophora sp.*

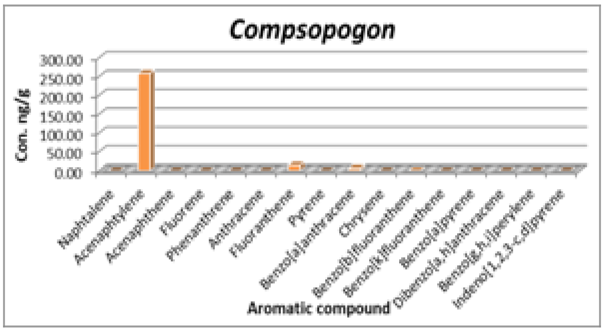


**Figure 13:** Aromatic compound in *Compsopogon sp.*

## 4. Conclusions

1) Cyanobacteria are more productive for total hydrocarbons.
2) In general, there is a clear dominance of n-C17 and n-C21 compounds in most studied algae.
3) Algae differed in their production of aromatic compounds and there is no clear dominance for anyone of them.
4) The production of algae is better for aliphatic compounds than for aromatics

## 5. Acknowledgements

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