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Measurement of some chemical and Biochemical parameters in *Cladophora glomerata* L. from Farahabad Region of Iran

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Algae has many different applications such as food, agricultural (fertilizer making), pharmaceutical, and medical industries. *Cladophora glomerata* is one the abundant filamentous green algae in the southern coast of Caspian. Algae samples obtained from 4 sampling points in Farahabad region of Iran (late 2016-early 2017). Selection of stations was for touristic importance, discharge of pollutions, and oil residues, hence study of some chemical and biochemical indexes of *Cladophora glomerata* as indicator algae can be important in this region. In this paper, some chemical and biochemical characterizations such as, dry weight, chlorophyll a, b, total Chlorophyll, Carotenoids, Total protein, Proline content, and Antioxidant activity) measured based on standard methods. Results showed that station 3 had lowest amount of dry weight, chlorophyll a, b, total Chlorophyll, Carotenoids, and Total protein ($p < 0.05$) and there were significant different between stations but proline content and Antioxidant activity were high amount in station 3 ($p < 0.05$). Results showed and proved high stress conditions in this point for higher content of heavy metals based on before studies.

Keywords: *Cladophora glomerata*, chemical and biochemical parameters, Farhabad region, Iran

INTRODUCTION

In recent years, algae from marine ecology viewpoints, considered as first producers and supply chain for receiving energy from the sun that can guarantee the energy for aquatic life. In the global division of plants, algae have about 1,800 genera and 21,000 species. Due to the extensive presence of algae in the air and groundwater, occupied wider area in comparison with other plants. An alga has many applications and uses such as food industries, making different fertilizers, pharmaceutical and medical industries [1-3].

Cladophora glomerata L. is green filamentous alga that has almost highly distribution in the southern coast of Caspian Sea. It has mostly grown on big stones, wood residues and also beside walls in southern coast part of Caspian Sea as long filaments such as Farahabad region. This region is the pleasant location for local and international tourists in Mazandaran Province, for enjoy from seaside and monitoring of this place is very important for human health. Some researchers showed, Marine organisms such as *Cladophora glomerata*, can be a useful biomarker or bio indicator for highly absorbable heavy metals. Hence, it is very more reliable than chemical analysis of water and sediment for environmental researchers [4].

In former research by Ebadi and Hisoriev [4], content of three heavy metals chromium (Cr), lead (Pb), and cadmium (Cd) in four sampling sites, measured for one year (2016) in *Cladophora glomerata* samples. Results of this study showed that the range of Cr, Pb and Cd in various algal samples was 29–55, 2 to 8, and 1.5 to 8.2 ppm/g dry weight respectively. Although amount of metals are not very serious but can be environmental alarm for human health and ecological risks. With increasing of pollutions in the Caspian Sea that mostly related to petroleum products, pesticides, agricultural fertilizers, heavy metals and domestic sewage, can effect on growth and physiology of this organism. This finally leads to environmental problems such as reducing oxygen to marine plants and animals and death of them [1,4-5]. The goal of this study was to examine some chemical and biochemical characterizations mainly in *Cladophora glomerata* for show effects of measured pollutions in different studies on factors such as, Dry weight, Chlorophyll a, b, Total Chlorophyll, Carotenoids, Total protein, Proline content, and Antioxidant activity. This study also shows the response of this alga to environmental pollutions as an ecological stress.

EXPERIMENTAL

Algae sampling and preparation

Samples obtained from 4 sampling points of Farahabad region (Mazandaran Province of Iran). Sampling, Preparation and drying procedures performed based on standard methods. All samples collected in same conditions from weather in the late of 2016 and early 2017 (Table 1 & Figure 1).

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Fig. 1. *Cladophora glomerata* obtained from Farahabad region of Caspian Sea (2016-2017).

Table1. Coordinates of sampling points in Farahabad region (2016-2017).

Farahabad Region sampling points	Geographical characteristics
Point 1	36°48'41.59" N ; 53°06'24.38" E
Point 2	36°48'48.40" N ; 53°06'55.33" E
Point 3	36°48'46.94" N ; 53°06'39.84" E
Point 4	36°48'37.70" N ; 53°06'09.86" E

Dry weight detection, Chlorophyll a, b, Total Chlorophyll and Carotenoids

Dry weight measured based on standard method [5]. Chlorophyll a, b, Total Chlorophyll and Carotenoids measured based on below formula and shown based on mg/g wet weight [6].

Chlorophyll a = (19.3*A663 - 0.86*A645) V/100W (1)

Chlorophyll b = (19.3*A645 - 3.6*A663) V/100W (2)

Total Chlorophyll = Chlorophyll a + Chlorophyll b (3)

Carotenoids = 100(A470) - 3.27(mg chl. a) - 104(mg chl. b)/227 (4)

Where, V: volume of supernatant liquid; A: light absorbance in different wavelengths (663, 645, and 470 nm); W: sample wet weight based on gram.

Total protein, Proline content and Antioxidant activity

Total protein, Proline content and antioxidant activity measured based on standard methods [7-9]. Total protein shown based on mg/g wet weight. Proline amount shown based on µmol/ g wet weight based on below formula and in wavelength of 520 nm:

µmol in each gram = µgram Proline in each ml * amount of consumed Toluene (ml) / 115.17 µgram in mol (5)

Antioxidant activity measured in wavelength of 517 nm and based on capacity of damage of active radicals [5] based on below formula:

% of damage of active radicals = [absorb in control sample – absorb in test sample / absorb in control sample] * 100 (6)

Statistical analysis

Statistical analysis performed by SPSS program version 9. Mean comparison done based on Duncan test in $p < 0.05$ level. All diagrams prepared with excel program.

RESULTS AND DISCUSSION

Chlorophyll a content

Amount of Chlorophyll a in four sampling points showed in Figure 2. Based on this figure, order of Chl a was S1> S4> S2> S3. Highest amount was for point 1 (8.65 mg/g wet weight) and lowest for point 3 with 6.5 mg/g wet weight.

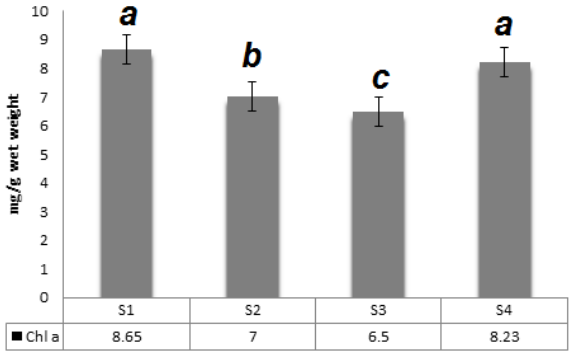

Fig. 2. Measurement of Chlorophyll a content in *Cladophora glomerata* samples from stations in 2016-2017 (Mean ± SE, $p < 0.05$).

Duncan test showed that treatment divided in three groups and also significant different between sampling points ($p < 0.05$).

Chlorophyll b content

Amount of Chlorophyll b in four sampling points showed in Figure 3. Based on this figure, order of Chl b was as S1> S4> S2> S3. Highest amount was for point 1 (9.53 mg/g wet weight) and lowest for point 3 with 6.63 mg/g wet weight.

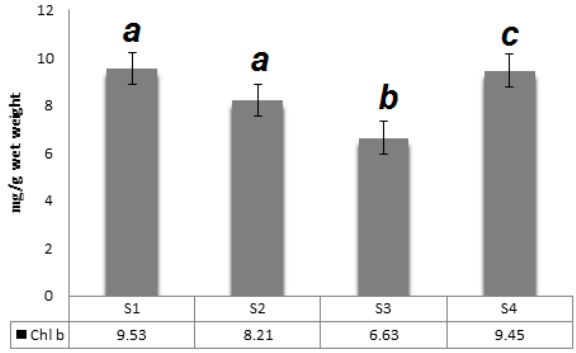

Fig. 3. Measurement of Chlorophyll b content in *Cladophora glomerata* samples from stations in 2016-2017 (Mean ± SE, $p < 0.05$).

Duncan test showed that treatment divided in three groups and also different between points was significant ($p < 0.05$).

Total Chlorophyll

Total Chlorophyll amount (a+b) in four sampling points showed in Figure 4. Based on this figure, order of total Chl is S1> S4> S2> S3. Highest amount was for point 1 (9 mg/g wet weight) and lowest for point 3 with 7.35 mg/g wet weight. Duncan test also showed that treatment divided in three groups and significant different between points ($p < 0.05$).

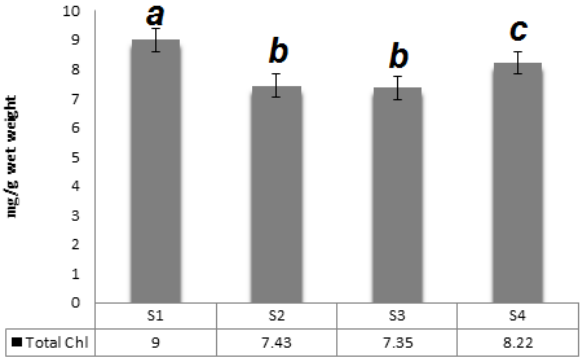

Fig. 4. Measurement of Total Chlorophyll (a+b) content in *Cladophora glomerata* samples from different stations in 2016-2017 (Mean ± SE, $p < 0.05$).

Carotenoids content

Carotenoids content in four sampling points showed in Figure 5. Based on this figure, order of Carotenoids content was S1> S4> S2> S3. Highest amount was for point 1 (1.41 mg/g wet weight) and lowest for point 3 with 1 mg/g wet weight. Duncan test also showed that treatment divided in three groups and also significant different between points ($p < 0.05$).

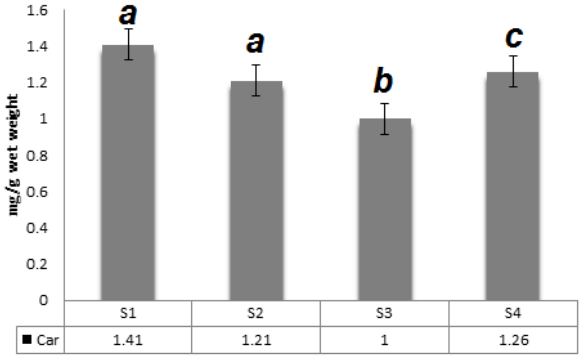

Fig. 5. Measurement of Carotenoids content in *Cladophora glomerata* samples from different stations in 2016-2017 (Mean ± SE, $p < 0.05$).

Total Proteins

Total protein content in four sampling points showed in Figure 6. Based on this figure, order of total protein is S1> S4> S2> S3. Highest amount was for point 1 (3.7 mg/g wet weight) and lowest for point 3 with 2 mg/g wet weight. Duncan test also showed that treatment divided in two groups and significant different between points ($p < 0.05$).

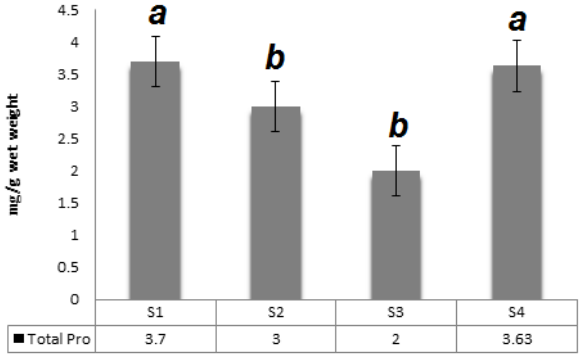

Fig. 6. Measurement of Total Proteins in *Cladophora glomerata* samples from different stations in 2016-2017 (Mean ± SE, $p < 0.05$).

Proline content

Proline content in four sampling points showed in Figure 7. Based on this figure, order of proline content is S3> S2> S4> S1. Highest amount was for point 3 (0.65 mg/g wet weight) and lowest for point 1 with 0.25 mg/g wet weight. Duncan test also showed that treatment divided in three groups and significant different between points ($p < 0.05$).

Antioxidant activity

Antioxidant activity in four sampling points showed in Figure 8. Based on this figure, order of activity is S3> S2> S4> S1. Highest amount was for point 3 (67 µmol/g wet weight) and lowest for point

1 with 25 µmol/g wet weight. Duncan test also showed that treatment divided in three groups and significant different between points ($p < 0.05$).

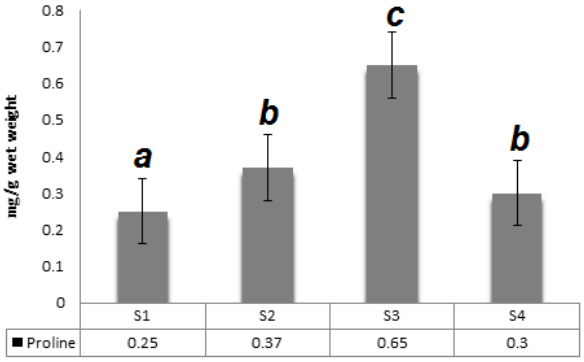

Fig. 7. Measurement of Proline content in *Cladophora glomerata* samples from different stations in 2016-2017 (Mean ± SE, $p < 0.05$).

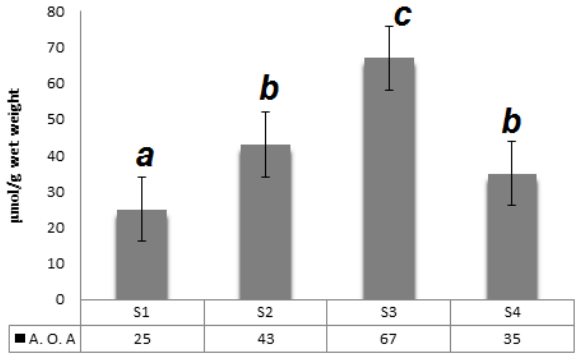

Fig. 8. Measurement of Antioxidant activity in *Cladophora glomerata* samples from different stations in 2016-2017 (Mean ± SE, $p < 0.05$).

Dry weight

Dry weight amount in four sampling points showed in Figure 9. Based on this figure, order of dry weight is S1> S4> S2> S3. Highest amount was for point 1 (16%) and lowest for point 3 with 8.45%. Duncan test also showed that treatment divided in three groups and significant different between points ($p < 0.05$).

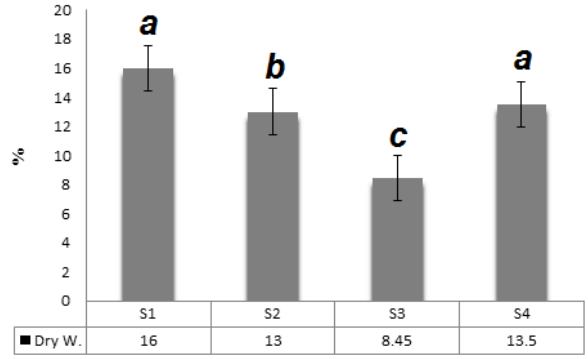

Fig. 9. Measurement of Dry weight in *Cladophora glomerata* samples from different stations in 2016-2017 (Mean ± SE, $p < 0.05$).

Based on results and former researches of current authors, accumulation of heavy metals and also agricultural and industrial pollutants can make high stress conditions specially in sampling point of 3. Content of Chl a, b, total Chl and also Carotenoids presented lower synthesis in stress point (point 3) and also higher content was for points with lower stress effects. Many studies proved this reality means in stress condition, amount of Chl can be as a physiologic assessment in *Cladophora glomerata*. Chlorophyll content has direct relation with stress condition for example, salty, drought, and also heavy metal pollution. Some researchers proved that decrease in Chl content under stress can increase amount of Chlorophyllase activity [9-10].

Statistical analysis about proline content showed that created stress due to heavy metal accumulation based on former researches in this region and on this algae [4], was significant in sampling point of 3. Increase of proline can have many reasons for increase of resistance against of stress such as prevent of proline oxidation and prevent of its contribution in protein synthesis [11].

Increase of Antioxidant activity based on results, showed also direct relationship with increase of stress. Entrance, absorption, and accumulation of heavy metals in plants and algae can increase free radicals in the cells hence antioxidant system activates for decrease of destruction effects. Results of this study proved that in sampling point of 3, increase in antioxidant activity can exit oxygen free radicals and more protection of cellular lipids, proteins and even nucleic acids and finally increase of algae tolerance [11]. Finally many studies proved that stress can decrease dry weight and protein synthesis. This relates to decrease in Chlorophyll making for increase of plant economy for more tolerance in stress condition. There is also balanced division of photosynthetic materials in Algae [12].

CONCLUSION

The southern shores of the Caspian Sea, especially Farahabad region, host many tourists from inside and outside of Iran every year. Unfortunately, due to poor management about control on agricultural-industrial and human pollutants, it has become a potential environment for the growth of harmful algae. Since *Cladophora glomerata* is the dominant algae in this region, this study showed a relative cognition of chemical and biochemical properties. Antioxidant activity and also proline content showed higher amount in Station of 3. This resulted in higher stress conditions in this point. This increase can be for heavy metal accumulation and also oil residues in this region. The results of this

study are crucial for solving some environmental and economic problems, and finally introduction of an industrial method for the use of these algae and its application for remediation researches are highly necessary.

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ИЗМЕРВАНЕ НА НЯКОИ ХИМИЧНИ И БИОХИМИЧНИ ПАРАМЕТРИ В *Cladophora glomerata* L. ОТ РЕГИОНА НА ФАРАХАБАД В ИРАН

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(Резюме)

Водораслите имат много различни приложения като храни, в селскостопанската (торове), фармацевтичната и медицинската промишленост. *Cladophora glomerata* е от изобилните филаментозни зелени водорасли в южния бряг на Каспийско море. Проби от водорасли, бяха получени от 4 пункта за вземане на проби в района на Фарахабад в Иран (края на 2016 г. - началото на 2017 г.). Изборът на станции е от значение за туризма, изхвърлянето на замърсявания и остатъчни масла, поради което проучването на някои химични и биохимични показатели на *Cladophora glomerata* като индикаторни водорасли може да бъде важно за този регион. В тази статия са определени някои химични и биохимични характеристики като сухо тегло, хлорофил *a* и *b*, общ хлорофил, каротеноиди, общ протеин, съдържание на пролин и антиоксидантна активност, измерени на базата на стандартни методи. Резултатите показват, че станция 3 има най-малко количество сухо тегло, хлорофил *a* и *b*, общ хлорофил, каротеноиди и общ белтък ($p < 0,05$) и има значителни разлики между станциите, но съдържанието на пролин и антиоксидантна активност са високи в станция 3 ($p < 0.05$). Резултатите показаха и доказаха високи стресови условия от гледна точка на тежки метали в тази точка (основани на предишни проучвания).