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RESEARCH ARTICLE

SCREENING OF SOME FRESH WATER PHYTOPLANKTON FOR THE REQUIREMENT OF IRON, A MICRONUTRIENT IN TERMS OF GROWTH AND PRODUCTIVITY

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ABSTRACT

The current project analyzes the requirement of iron for the growth, chlorophyll *a* content and productivity of three fresh water phytoplankton *viz*. *Chlorococcum humicola, Chlorella ellipsoidea and Scenedesmus bijuga.* The test organisms were treated with different concentrations of iron like 0.0075ppm, 0.015ppm, 0.03ppm, 0.06ppm and 0.12ppm supplied in the form of $FeCl_3$. The culture devoid of iron was kept as control. Results obtained revealed the indispensable role of iron for the growth of these organisms as they showed decreased values for all the parameters in iron deficient medium. Optimum concentration of iron for the growth and productivity of *Chlorococcum humicola* was found to be in the range 0.0075ppm to 0.015ppm and it was 0.015ppm for *Chlorella ellipsoidea* and *Scenedesmus bijuga*. Iron scarcity exerted more retarding effect on the growth of *Chlorella ellipsoidea. Chlorococcum humicola* appeared as more susceptible to the toxic effect of higher concentrations of iron than other two test algae. The above observations suggest that algal species vary in their tolerance to iron.

INTRODUCTION

Microalgae play an important role in the biosynthesis of organic matter (primary production) in aquatic systems which directly or indirectly serve all the living organisms of a water body as food. Extensive researches on the various aspects of algal physiology established the potentiality of these organisms as food, feed, fodder and manure and their role in public health problems of fast developing civilization. Unlike conventional food crops, these organisms can be cultivated throughout the year and have the capacity to trap maximum solar energy, thus indicating the relevance of these organisms in the present era of food and energy shortage. The large scale cultivation of any algal species for various purposes demands a thorough understanding of its requirements for both macro and micro nutrients. Not much work has been done so far in determining the importance and species specific requirement of micro nutrients on algal growth and metabolism (Lin *et al.*, 1994; Moreau *et al*., 1994; Chow *et al.,* 1998 and Katiyar and Katiyar, 2000). In this context, the present study investigates the species specific requirement of Fe, a micronutrient for the optimum growth and productivity of three economically important fresh water phytoplankton *viz*. *Chlorella ellipsoidea, Chlorococcum humicola, and Scenedesmus bijuga.*

MATERIALS AND METHODS

Isolation and culturing of test organisms

The test organisms were isolated from water samples collected from different fresh water ponds, in and around Ernakulam district, Kerala, India. Serial dilution method was adopted for isolation. Stock cultures and experimental cultures of isolated algae were raised in Ward and Parish medium (Ward and Parish, 1982) that contained 0.03ppm Fe in the form of $FeCl_3$. Reculturing of mother culture was done after every 14 days to keep the cells in the exponential phase. The mother cultures and experimental cultures were exposed to a photoperiod of 10 hrs light and 14 hrs darkness. Illumination was provided by day light fluorescent tubes with the intensity of 2000 lux. The ambient temperature ranged from 27˚C to 32˚C. Cultures were not bacteria free.

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Test Procedure

In order to evaluate the requirement of Fe, the experiments were carried out using culture media containing various concentrations of iron as 0.0075ppm, 0.015ppm, 0.03ppm, 0.06ppm and 0.12ppm, supplied in the form of $FeCl_3$. Medium devoid of iron was used as control. Modified Ward and Parish medium containing lesser number of micronutrients was used as the culture media for the experimental purpose. The inoculums for all the experiments were taken from the mother cultures during the exponential phase. The cells to be inoculated were starved in the medium devoid of Fe for a week, prior to the inoculation. The initial concentration of test organisms in all the experiments was $10x10^4$ cells/ml. The experiment was conducted in triplicate for a period of 21 days. The cultures were analyzed at regular intervals for the following parameters:

a) Population growth

About 1ml of culture from each conical flask was fixed in Lugol's iodine in every alternate days and its cell number was counted using calibrated haemocytometer and expressed as cells x 10^4 /ml.

b) Chlorophyll *a* content

It was estimated using the method described by Parsons *et al*. (1984) by determining the absorbance of pigment extract at various wavelengths using a spectrophotometer. Chlorophyll *a* content was calculated using the formula:

Chlorophyll *a* (mg/m^3) = 11.85 E_{664}-1.54 E_{647}-0.08 E_{630}

c) Gross primary productivity (GPP)

GPP was determined by using light and dark bottle method (Gaarder and Gran, 1927).

RESULTS

Chlorococcum humicola revealed significant higher yield in 0.0075ppm and 0.015ppm compared to control, the maximum cell count being observed in 0.0075ppm Fe on 21st day of the experiment. Beyond 0.015ppm retarding effect of Fe led to a decline in the cell number (Table 1.1). Higher chlorophyll *a* content was also noticed in 0.0075ppm throughout the sampling period (Table 1.2). GPP recorded higher values in 0.0075ppm up to 13th day. On accompanying days 0.015ppm revealed higher values for GPP. On 21st day of the experiment, primary production in 0.015ppm Fe was 20.4% higher than that of Fe deficient medium (Table 1.3)

Table 1.1. Effect of iron on the growth (no.of cells/mlx10^4) of *Chlorococcum humicola*

Days	control	0.0075ppm	0.015ppm	0.03ppm	0.06ppm	0.12ppm
1	10	10	10	10	10	10
3	15	19	19	18	18	14
5	29	51	42	34	28	14
7	47	72	64	49	32	18
9	73	90	77	59	36	22
11	153	206	198	143	47	30
13	174	257	232	189	68	38
15	206	299	296	220	81	49
17	366	493	481	381	173	91
19	522	603	568	514	292	186
21	744	846	770	727	567	252

Between days F=134.5328, P-value=1.49E-32
Between concentrations F=34.74836, P-value= 3.78E-15

Table 1. 2. Effect of iron on the chlorophyll *a* content (mg/m^3) of *Chlorococcum humicola*

Days	control	0.0075 ppm	0.015 ppm	0.03 ppm	0.06 ppm	0.12 ppm
5	55.16	56.82	49.355	45.01	40.585	39.085
9	127.19	191.97	184.5	180.12	122.49	102.11
13	504.49	686.07	681.37	563.66	468.86	382
17	647.28	957.59	865.87	640.29	479.41	390.27
21	791.02	1249.51	1249.49	1234.12	1061.69	719.92

Between days F=74.53199, P-value= 1E-11
Between concentrations F=5.161239, P value= 0.00335

Table 1.3. Effect of iron on the gross primary production (mg C l^{-1}hr^{-1}) of *Chlorococcum humicola*

Days	control	0.0075 ppm	0.015 ppm	0.03 ppm	0.06 ppm	0.12 ppm
5	0.0845	0.109	0.109	0.0604	0.0604	0.036
9	0.207	0.242	0.155	0.138	0.121	0.052
13	0.191	0.262	0.262	0.202	0.171	0.091
17	0.250	0.285	0.302	0.276	0.224	0.173
21	0.443	0.494	0.534	0.524	0.464	0.332

Between days F= 85.31148, P-value=2.83E-12
Between concentrations F=12.27191, P value= 1.56E-05

Chlorella ellipsoidea offered maximum cell number in the lowest concentration of Fe applied, *i.*e. in 0.0075ppm on 5th day. On succeeding days, the alga flourished well in 0.015ppm, the tendency being prolonged to the 21st day of the experiment (Table 2.1). Chlorophyll *a* and gross primary production also revealed higher values in 0.015ppm Fe supplied medium (Tables 2.2 & 2.3). All the parameters unveiled the least values in the control, provided with no Fe supply.

Scenedesmus bijuga delivered maximum growth rate in 0.03ppm Fe up to 5th day. From 7th day onwards rapid rate of cell division was noted in 0.015ppm and the maximum population size (488x10^4 cells /ml) about 84% more than that of control was noted in 0.015ppm on 21st day. Up to 13th day, the highest concentration tried exerted more detrimental effect on cell multiplication. Thereafter Fe deficient cultures imparted greater degree of obstruction to algal

Table 2.1. Effect of iron on the growth (no.of cells/mlx10^4) of *Chlorella ellipsoidea*

Days	control	0.0075 ppm	0.015 ppm	0.03 ppm	0.06 ppm	0.12 ppm
1	10	10	10	10	10	10
3	27	73	72	68	49	43
5	50	77	73	70	54	52
7	58	80	89	77	64	65
9	92	124	137	134	125	107
11	184	227	297	240	196	191
13	262	366	398	323	302	298
15	392	396	448	446	445	412
17	396	457	609	512	480	457
19	416	486	622	550	497	465
21	436	515	635	587	511	482

Between days F= 134.5328P-value=1.49E-32
Between concentrations F=34.74836, P value= 3.78E-15

Table 2.2. Effect of iron on chlorophyll *a* content (mg/m^3) of *Chlorella ellipsoidea*

Days	control	0.0075 ppm	0.015 ppm	0.03 ppm	0.06 ppm	0.12 ppm
5	106.95	223.39	178.38	168.88	140.915	140.875
9	150.375	258.13	438.09	344.99	312.76	243.28
13	217.29	361.46	460.17	368.69	356.84	265.2
17	324.53	562.12	697.84	662.37	451.56	387.78
21	470.56	820.17	1075.14	986.82	787.78	596.05

Between days F=53.48189, P-value= 2.12E-10
Between concentrations F=10.10212, P value= 6.12E-05

Table 2.3. Effect of iron on the gross primary production (mg C l^{-1}hr^{-1}) of *Chlorella ellipsoidea*

Days	control	0.0075 ppm	0.015 ppm	0.03 ppm	0.06 ppm	0.12 ppm
5	0.101	0.121	0.131	0.131	0.111	0.101
9	0.171	0.282	0.363	0.292	0.222	0.141
13	0.292	0.302	0.373	0.353	0.343	0.292
17	0.411	0.411	0.447	0.447	0.423	0.399
21	0.413	0.423	0.484	0.443	0.423	0.413

Between days F= 186.99, P-value= 1.54E-15
Between concentrations F=6.905, P value= 0.000681

Table 3.1. Effect of iron on the growth (no.of cells/mlx10^4) of *Scenedesmus bijuga*

Days	control	0.0075ppm	0.015ppm	0.03ppm	0.06ppm	0.12ppm
1	10	10	10	10	10	10
3	13	17	18	23	15	12
5	20	21	21	28	17	17
7	31	56	60	44	23	20
9	64	90	99	93	60	38
11	136	170	214	97	82	76
13	217	247	338	104	92	86
15	106	172	186	169	120	113
17	172	307	394	356	191	172
19	219	343	441	360	251	243
21	265	379	488	364	329	295

Between days F=182.181, P-value=9.96E-36
Between concentrations F=14.96877, P-value= 5.67E-09

Table 3.2. Effect of iron on chlorophyll *a* content (mg/m^3) of *Scenedesmus bijuga*

Days	Control	0.0075 ppm	0.015 ppm	0.03 ppm	0.06 ppm	0.12 ppm
5	81	103.1	133.95	121.31	66.55	22.58
9	100.25	130.56	183.87	140.915	127.19	45.78
13	147.94	168.27	206.98	156.34	156.26	124.03
17	267.3	727.15	750.85	817.01	743.62	739
21	166.57	290.6	761.08	896.55	832.18	796.55

Between days F=22.92989, P-value= 3.11E-07
Between concentrations F=2.3062, P value= 0.082838

Table 3.3. Effect of iron on the gross primary production (mg C $l^{-1}hr^{-1}$) of *Scenedesmus bijuga*

Days	Control	0.0075 ppm	0.015 ppm	0.03 ppm	0.06 ppm	0.12 ppm
5	0.060	0.081	0.133	0.133	0.071	0.030
9	0.072	0.085	0.218	0.171	0.084	0.036
13	0.254	0.532	0.556	0.532	0.484	0.387
17	0.278	0.399	0.483	0.592	0.580	0.447
21	0.112	0.328	0.354	0.362	0.397	0.138

Between days F= 53.307, P-value= 2.18E-10
Between concentrations F=8.275, P value= 0.000226

growth (Table 3.1). The algae possessed more chlorophyll *a* and GPP in 0.015ppm Fe till 13th day, while on 17th day onwards 0.03ppm showed higher values for these parameters (Tables 3.2 & 3.3). A harmful effect was noted in all the three algal species when the amount of Fe exceeded the optimum levels.

DISCUSSION

Iron has long been considered as an essential element to algae growth. Most of the algal species require this nutrient only in micro quantities; hence it is included under micronutrients or trace elements. Iron is a constituent of many enzymes like catalase and peroxidase and of ferredoxin, cytochromes and certain other porphyrins, hence its deficiency will obviously lead to retarded growth of organisms. Iron has also been found to be essential for the hydrogenase development (Yanagi and Sasa, 1966). A major fraction of iron in the cell is found to be associated with chloroplast. Decreased synthesis of protein in the chloroplast and reduced incorporation of magnesium into the porphyrin molecule (Granick 1951, 1955) were suggested as the probable reasons for the reduced chlorophyll synthesis in the chloroplast due to iron scarcity. Reduced rate of photosynthesis due to decreased chlorophyll may account for the retarded algal growth in Fe deficient medium. A close correlation between the concentration of dissolvable iron and chlorophyll *a* content was observed in red tide diatom especially *Skeletonema costatum* (Lin *et al.*,1994) and red tide organism *Prorocentrum micans* (Wang *et al.*, 1995) and *Chlorella ellipsoidea* (Chow *et al.*, 1998). Researches by Banse (1991), Bizsel *et al*. (1997) and Paerl (1997) have shown that the reactive iron is an important factor for the phytoplankton bloom and its distribution in coastal waters. According to Street and Paytan (2005) and *Hassler et al*. (2012) iron plays the pivotal role in determining the phytoplankton growth, productivity and community structure in a variety of open ocean environments. The current investigation establishes the necessity of iron for the proper growth of test organisms under controlled culture conditions.

Conclusion

The current study attempts to investigate the influence of iron, a micronutrient on the growth, chlorophyll *a* content and productivity of three algal species *viz. Chlorococcum humicola, Chlorella ellipsoidea* and *Scenedesmus bijuga* and to determine the optimum requirement of this nutrient for each algal species. Results of the study reveal the indispensable role of iron for the proper growth and productivity of the test organisms in the controlled culture conditions as all parameters studied unveiled higher values in the presence of iron than its absence. Scarcity of iron exerted more growth inhibition in *Chlorella ellipsoidea* compared to other two test algae, while *Chlorococcum humicola* appeared to be more susceptible to higher concentrations of iron than *Chlorella ellipsoidea* and *Scenedesmus bijuga*. Optimum concentration of iron for the growth and productivity of *Chlorococcum humicola* was found to be in the range 0.0075ppm to 0.015ppm and for *Chlorella ellipsoidea* and *Scenedesmus bijuga* it was 0.015ppm. The above observations suggest that algal species vary in their tolerance to iron.

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