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Nutritional studies of *Chara corallina*

Uday Bhan Singh[1*], R.K. Thakur[2], Ravish Verma[3] and A.S. Ahluwalia[1]

[1]Laboratory of Algal Biology and Diversity, Department of Botany, Panjab University, Chandigarh-160 014, India
[2]Department of Zoology, Panjab University, Chandigarh-160 014, India
[3]Department of Botany, University of Rajasthan, Jaipur-302 004, India

Abstract
Physico-chemical analysis of the pond water supporting growth of *Chara corallina* and of those grown in different culture media in the laboratory conditions have been carried out. The physico-chemical parameters of the water of Napli forest and P.N. Mehra Botanical Garden show that *Chara corallina* requires less amount of nutrients and the level of pollution is quite low as is evident from the data and standards. The water is clear enough to allow penetration of visibility to its bottom. Napli forest and P.N. Mehra Botanical Garden lack rich diversity which may be attributed to some chemicals released by *Chara corallina*. In laboratory culture, it is found that *Chara corallina* can be grown in all three culture media, *viz.,* Allen and Arnon's medium , Chu-10 medium and BG-11 medium but growth is seen to be the best in Allen and Arnon's culture medium in comparison with the other two culture media.

Keywords: *Chara,* Physicochemical parameters, Algae, Water pollution.

INTRODUCTION

Characean algae are called stoneworts or brittleworts due to extensive extracellular calcification. The presence of Charophyceae (stoneworts) is often associated with clean and rather hard water with a low phosphorous (P) concentration (Fig. 1).

Fig 1. Showing the growth of *Chara corallina* in Batanical Garden

Forsberg reported that soluble reactive phosphorus (SRP) concentration as low as 15 µg P L^{-1} inhibited the plant growth and the sensitivity to P explained the absence of charophytes in very eutrophic waters and their disappearance from polluted localities [1].

Contrary to earlier findings, later studies have shown that the plant can grow well in water with a high P concentration, for example 1.0 mg P L^{-1} [2] and 0.8 mg P L^{-1} [3] and the disappearance of the plant is caused primarily by shading through phytoplankton and other algae [4]. Dense *Chara* beds in hard water may act as nutrient sinks in several ways, such as nutrient incorporation in plant biomass, nutrient withdrawal from decomposing detritus, release of allelophatic compounds, reduced sediment resuspension, slow decomposition rate on plant senescence, and the co-precipitation of P with calcite [5]. Charophytes thrive and may dominate the vegetation in unpolluted fresh and brackish waters [6]. Charophytes may also indirectly affect nutrient cycling in lakes. Utilization of bicarbonate is accompanied by precipitation of calcite during periods of intensive photosynthesis, favoring immobilization of P by binding in the crystal structure or sorption on sedimenting mineral particles. Charophytes are able to deliver oxygen to the sediment, thus potentially enhancing nitrification/denitrification processes and preventing iron-bound sediment phosphorus from being released to the overlying water. Furthermore, dense *Chara* meadows restrict sediment resuspension, consequently blocking an important internal source of nutrients to planktonic algae. Moreover, the plant can be heavily calcified-more than 50% (as $CaCO_3$) of the total plant biomass dry weight has been reported [7,8].

Keeping this in view, the present investigation has been undertaken to assess the water quality of freshwater ponds supporting vegetation of *Chara corallina*, and also suitability of best growth medium to grow *Chara corallina,* for its exploitation as a source for carbon sequestration programs.

MATERIALS AND METHODS

Collection sites

In the present study, two sample collection sites were selected in (76°46′45.96″E, 30°44′01.19″N) Chandigarh. Both sites showed profuse growth of *Chara corallina*. (1) Botanical garden pond: Inside Panjab University botanical garden, water recharged every week

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*Corresponding Author
Uday Bhan Singh
Laboratory of Algal Biology and Diversity, Department of Botany, Panjab University, Chandigarh-160 014, India

Email: ubsday@gmail.com

with ground water. Almost crystal clear water maintained and presence of *Chara corallina.* No addition of wastewater. (2) Nepli forest pond: Natural and conserved pond, surrounded by forest cover, no anthropogenic activities allowed. Growth of *Chara corallina* has been observed. No addition of wastewater.

Physico-chemical analysis

Physico-chemical parameters of the water were analyzed using standard methods APHA [9]. Water samples in triplicates were procured from the P.N. Mehra Botanical Garden Chandigarh, and Napli forest, Chandigarh. Parameters like temperature, pH, fixation of dissolved oxygen, secchi-transparency, and free carbon dioxide were determined on the spot. For rest of the parameters, 2.5 L of pond water was brought to the laboratory. It was filtered and analyzed for total alkalinity, total hardness, chlorides, sulphate, nitrates, and phosphates. Total dissolved solids (TDS) by using E-Merck's TDS meter; Water temperature by using centigrade thermometer and pH was recorded with BDH paper on the spot and later by pH meter in the laboratory. The data was statistically analyzed using software SPSS version 18, to calculate average and standard deviation.

Estimation of growth rate

Fresh and dry weight was estimated according to the method given by International Atomic Energy Agency [10]. The media selected for the culture of *Chara corallina* are Chu-10 medium [11], Allen and Arnon's medium [12], and BG-11 medium [13]. The apical growing tips of the plant material were cut at approximately 1 cm each and inoculated in the medium with utmost care under aseptic conditions. Observations were recorded for growth in terms of length, fresh weight and dry weight in order to estimate their growth rates after every four days, upto 12 days.

RESULTS AND DISCUSSION

Physico-chemical characteristics of water in P.N. Mehra Botanical Garden and Napli forest were investigated (Table 1).

Table 1. Physico-chemical parameters of water supporting growth of *Chara corallina* in P.N. Mehra Botanical Garden and Napli Forest, Chandigarh.

S.No.	Parameter	Botanical Garden	Napli Forest
1	Secchi-transparency (cm)	71.3±0.9	124.3±2.3
2	Total Dissolved Solid(mg/L)	216.7±3.3	201.7±6.0
3	pH	7.5±0.03	7.7±0.06
4	Temperature (air)(^{0}C)	25.3±0.17	25.8±0.08
5	Temperature (water)(^{0}C)	26.1±0.03	26.5±0.08
6	Free CO_2 (mg/L)	21.7±0.08	38.0±1.15
7	Carbonate - CO_3^{2+} (mg/L)	Absent	Absent
8	Alkalinity (mg/L)	91.7±0.03	103.6±2.96
9	Total Hardness (mg/L)	348.3±6.0	366.7±2.4
10	Ca^{2+} (mg/L)	101.7±0.45	110.9±0.31
11	Mg^{2+} (mg/L)	246.6±5.89	255.8±2.6
12	Cl^- (mg/L)	64.0±3.5	65.0±2.6
13	Dissolved O_2 (mg/L)	20.8±1.1	20.7±2.4
14	Total Phosphate PO_4^{2-} (mg/L)	0.025±0.002	0.036±0.003
15	Nitrate - NO_3^- (mg/L)	0.123±0.004	0.213±0.006

Observations of secchi-transparency of two sites indicate that water quality of Napli forest water body is cleaner as visibility of secchi disc is more in this (Table 1).Total dissolved solids (mg/l) and pH was found to be broadly in an identical range. Free CO_2 was recorded to be higher in Napli forest water body. Most of the parameters (alkalinity,hardness and calcium) were found to be higher in P.N. Mehra Botanical Garden water. Few of the parameters were significant in having uniform values in both the habitats (Table 1).Comparing the observed values with the standard values of potable water, the water in the P.N. Mehra Botanical Garden and Napli forest is non-polluted. However, it supported the growth of *Chara corallina*. This also indicates that *Chara* would prefer clean water for its establishment and further growth. These water bodies lack rich biodiversity, may be due to non-polluted and poor levels of nitrogen and phosphorus.

Comparing various physicochemical parameters of studied water samples with National and International Standards, Table 2 [14,15,16], reveals that all the parameters analyzed were with the permissible limits of drinking water standards.

Table 2. Standards for drinking water

Parameters	WHO	ISI	ICMR
pH	6.5-8.5	6.5-8.5	7-8.5
Total Alkalinity	120	-	-
Total hardness	500	300	300
Chloride	250	250-1000	250-1000
Nitrate	50-100	50-100	20-100
Phosphate	0.1-1	0.5-1	-
BOD	5.0	-	-
DO	5.0	3.0	-
TDS	500	-	500

All parameters are expressed in mgL^{-1} except pH

Based on certain parameters, Central Pollution Control Board (Table 3) has classified fresh water into five classes (A, B, C, D and E) suitable for various purposes. On the basis of this classification, the water at ponds could be categorized as “A”. The values of water quality clearly revealed that the water can be used for drinking purposes after disinfection.

Table 3. Water quality criteria for various uses
(Central Pollution Control Board – 1978)*

S. No.	Designated-Best-Use	Class of water	Criteria
1.	Drinking Water Source without conventional treatment but after disinfection	A	Total Coliforms Organism MPN/100ml shall be 50 or less pH between 6.5 and 8.5 Dissolved Oxygen 6mg/L or more Biochemical Oxygen Demand 5 days 20°C 2mg/L or less 5.0
2.	Outdoor bathing (Organized)	B	Total Coliforms Organism MPN/100ml shall be 500 or less pH between 6.5 and 8.5 Dissolved Oxygen 5mg/L or more Biochemical Oxygen Demand 5 days 20°C 3mg/L or less
3.	Drinking water source after conventional treatment and disinfection	C	Total Coliforms Organism MPN/100ml shall be 5000 or less pH between 6 to 9 Dissolved Oxygen 4mg/L or more Biochemical Oxygen Demand 5 days 20°C 3mg/L or less
4.	Propagation of Wild life and Fisheries	D	pH between 6.5 to 8.5 Dissolved Oxygen 4mg/L or moreFree Ammonia (as N) 1.2 mg/L or less
5.	Irrigation, Industrial Cooling, Controlled Waste disposal	E	pH betwwn 6.0 to 8.5 Electrical Conductivity at 25°C micro mhos/cm Max.2250 Sodium absorption Ratio Max. 26 Boron Max. 2mg/

**www.cpcb.nic.in/classi.htm*

As stated earlier, 1 cm long apical portions of *Chara corallina* were grown in 3 different media, each medium being inoculated with ten samples each (Fig. 2).

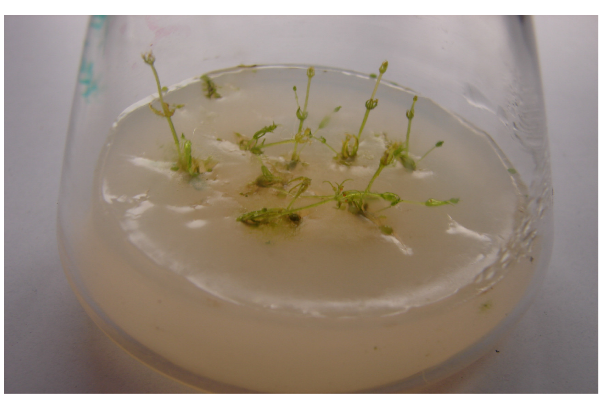

Figure 2. Showing the growth of *Chara corallina* in solid agar medium

3 replicates of each medium was maintained to minimize the error. In these samples some profoundly visible observations could be made. After 4 days of culture, the older basal plant portion of the samples in Chu-10 medium became pale in coloration (lost green color) while those grown in Allen and Arnon’s and BG-11 media retained their green coloration (Table 4). As the culture proceeded to the 8th day after culture (Table 5), most of the samples in Chu-10 medium were pale yellow in color, became soft and had rotten base; survival was low in Chu-10 medium but those which survived, attained proper length. In BG-11 medium, samples became greenish-brown. Few of them were seen to have deteriorated with rotten basal portion. But in Allen and Arnon’s medium, all samples survived and were lush green in color. Rhizoids of about 0.3-0.5 cm were also seen to have developed on all the samples.

Table 4. Comparison of the growth rates of *Chara corallina* after 4 days of culture in different culture media through fresh and dry weight and differences in length.

Parameter / Medium	Fresh weight (g)		Dry weight (g)		Length(cm)		Survival out of 10
	Replicates	Mean	Replicates	Mean	Replicates	Mean	
Chu 10 medium	0.059	0.059	0.010	0.011	12.1	12.63	10
	0.055		0.010		13.2		10
	0.062		0.012		12.6		10
BG 11 medium	0.049		0.012		13.2		10
	0.034	0.045	0.011	0.012	13.7	13.30	10
	0.052		0.012		13.0		10
Allen and Arnon's medium	0.040		0.013		14.0		10
	0.054	0.044	0.013	0.014	13.4	13.27	10
	0.037		0.016		12.8		10

Table 5. Comparison of the growth rates of *Chara corallina* after 8 days of culture in different culture media through fresh and dry weight and differences in length.

Parameter / Medium	Fresh weight (g)		Dry weight (g)		Length(cm)		Survival out of 10
	Replicates	Mean	Replicates	Mean	Replicates	Mean	
Chu 10 medium	0.045	0.093	0.007	0.011	8.3	13.00	6
	0.090		0.010		14.3		9
	0.145		0.015		16.3		9
BG 11 medium	0.180		0.015		15.8		10
	0.084	0.147	0.008	0.014	10.5	13.23	8
	0.177		0.018		13.4		10
Allen and Arnon's medium	0.179		0.017		16.7		10
	0.201	0.176	0.018	0.017	14.6	15.60	10
	0.149		0.016		15.4		10

After the 12th day of culture (Table 6), survival of sample was low in Chu-10 medium. In Allen and Arnon's medium, basal portion became yellowish-green like old stem and initiation of new apex from nodes was seen. Further, all samples became fertile and rhizoids of about 1.5-2.5 cm were seen to arise from all samples. However, in BG-11 medium, the old basal body of plant got deteriorated but the upper portions still retained their green coloration.

Table 6. Comparison of the growth rates of *Chara corallina* after 12 days of culture in different culture media through fresh and dry weight and differences in length.

Parameter / Medium	Fresh weight (g)		Dry weight (g)		Length(cm)		Survival out of 10
	Replicates	Mean	Replicates	Mean	Replicates	Mean	
Chu 10 medium	0.041	0.069	0.008	0.009	11.9	13.33	8
	0.090		0.010		14.6		9
	0.075		0.010		13.2		9
BG 11 medium	0.074		0.008		11.3		10
	0.095	0.094	0.010	0.011	11.2	11.7	10
	0.113		0.014		12.6		10
Allen and Arnon's medium	0.123		0.016		13.6		10
	0.068	0.083	0.015	0.015	11.6	12.83	10
	0.058		0.015		13.3		10

CONCLUSIONS

Conclusions can be made from these observations that the samples grown in Allen and Arnon's medium had the maximum survival and growth rate (Tables 4-6). Further, rhizoids were seen to arise from the plant body only in the Allen and Arnon's medium. Maximum deterioration was seen in plant grown in Chu-10 medium while BG-11 medium could support the growth of the algae moderately. As stated earlier, after the 8th day of culture, no further increase in growth could be seen in all media (Tables 5-6).This may be due to the reason that *Chara corallina* requires hard surface to anchor the plant body through rhizoids, so after some time the deterioration of plant body starts. Due to low nutritional requirements and fast growth rate, Chara chorallina can be exploit as a possible candidate for carbon sequestration programs.

Physico-chemical parameters of water supporting growth of *Chara corallina* also indicates that *Chara* would prefer clean water for its establishment and further growth. These water bodies lack rich biodiversity, may be due to non-polluted and poor levels of nitrogen and phosphorus. *Chara corallina* with the low pollution clearly indicates that these macrophytes prefer water with significantly different nutrient concentrations for their growth, and can be used as indicators of pollution status quality of water.

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