



Growth of *Chara globularis* under salt stress

1 **Bhoomika N. Patel**

Botany, .M.Sc. Student, Department of Botany, Bioinformatics and Climate Change Impact Management, School Of Science, Gujarat University, Ahmedabad-380009, India.

2. **Juhi Gajjar**

Botany, Research Scholar, Department of Botany, Bioinformatics and Climate Change Impact Management, School Of Science, Gujarat University, Ahmedabad-380009, India.

3. **Dr.Himanshu Pandya**

Vice Chancellor Gujarat University, Gujarat University, Ahmedabad-380009, India.

Received: 10 April Revised: 18 April Accepted: 26 April

Abstract

The effect of Salt Stress on fresh water green algae *Chara globularis*, were investigated in the present study. NaCl salt are main salt causing Salinization and widely exist in aquatic environment, *Chara* may suffer from Salinization stress for high water evaporation. In this study, in order to investigate and compare the toxic effect of different NaCl concentration on *Chara globularis* growth, pH of Water and Chlorophyll content. Under the Salinization (NaCl) stress growth of *Chara* decrease compare to control. pH of water increased and chlorophyll content decreased at high concentration of NaCl.

Key Words: *Chara globularis*, NaCl, Salinity stress, Chlorophyll.

Introduction

Chara are multicellular and superficially resemble land plants because of leaf and stem like structures. *Chara* covered with calcium carbonate deposits and commonly known as stoneworts. Rough to touch because of deposited calcium salts on the cell wall. *Chara* has cosmopolitan distribution. Small aquatic animals and insects use *chara* as a food. *chara* also used as a manure. Algal biomass are potential source of renewable energy converted into energy such as biofuel. [S.Siddiqua., 2015]. *Chara sp.* are strong indicator than water chemistry. Salinity of water or soil is one of the major stress problem to increase production in plant growing areas throughout the world and mainly in arid regions semi arid regions it can severely limit plant production. [Jamil *et al.*, 2006]. Salinity affect nutrient also. Salinization can also affect on cations and anions in water. [Nielsen *et al.*, 2003] Algae are inhabitants of biotops characterized by changing salinities & serve as a model organisms. [Bohnert and Jensen., 1996] Such salinity concentration change in water affect the metabolism, growth and photosynthesis [Moisander *et al.*, 2002; Lartigue *et al.*, 2003]. For aquatic and terrestrial plant salinity stress and unfavourable light both are main limiting factor for growth [Fodorpataki and Bartha., 2004]. Under high salt concentration molecular and photosynthetic apparatus structure affected in *Scenedesmus obliquus* and also reduction of PS-2 quantum yield. [Z.Zuo *et al.*, 2014]. Micro algae differ in tolerance to salinity grouped as halophilic and halotolerant. [Rao *et al.*, 2007] Total chlorophyll contents stimulated at lower concentrations (0.1 and 0.2M) of NaCl compared to control but reduced at high concentration (0.3 and 0.4M). [S. Hiremath and P. Mathad., 2010].



Material & Method

For this experiment, *Chara globularis* Algae selected, which was collected from Damanganga river, Silvassa, dadra and nagar haveli and grown in beakers in laboratory condition.

Table.1 Solubility parameters of *Chara globularis*

Sr. No.	Solvents	<i>Chara globularis</i>
1	Distilled Water	Soluble
2	Dilute Hydrochloric acid	Soluble
3	Ethanol	Soluble
4	Pyridine	Insoluble
5	Benzene	Insoluble
6	Chloroform	Insoluble

[M. Karpakavalli et al., (2018)]

Table.2 Phyto-chemical analysis of *Chara globularis*

Sr.No.	Phytoconstituents	<i>Chara globularis</i>	
		Acid extraction	Alkali extraction
1	Carbohydrates	+	+
2	Glycosides	-	-
3	Proteins	+	+
4	Fixed oil and Fats	-	-
5	Tannins and Phenolic compounds	+	+

[M. Karpakavalli et al., (2018)]

Basically this experiment was divided into two parts:

1. Effect of different NaCl(salt) concentration on *Chara globularis* fresh water green algae growth and pH of water.
2. Estimation of Chlorophyll content.

To study the effect of different concentration of NaCl on *Chara globularis*. Prepared of 0.2% , 0.4% , 0.6%, 0.8% and 1.0% NaCl solution , and each solution have five set. One set was kept as control. 2gm of chara put in each set. Algae was treated with NaCl (salt) for 15 days of duration. Equal amount of NaCl solution poured in all sets. All experiment done in under laboratory condition, and all set covered with aluminium foil.



Estimation of Chlorophyll

It include the estimation of Total Chlorophyll content in Algae.

Total chlorophyll: (Arnon , 1949)

Principle

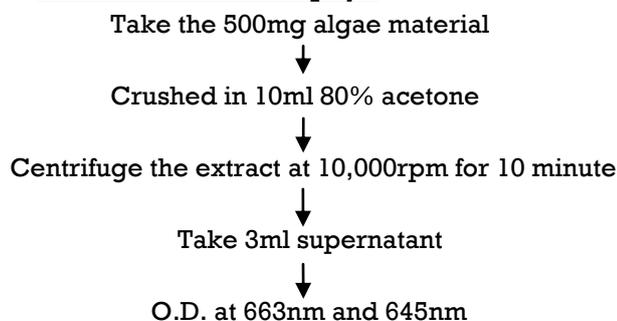
In algae Chlorophyll is present as a green pigment. Chlorophyll-a and Chlorophyll-b occur in higher plant and chlorophyll-b is bound loosely to protein and extracted in organic solvents such as acetone and ether.

Chemically each chlorophyll molecule contains a porphyrin nucleus with a chelated Mg⁺ ion at the centre and long hydrocarbon side chain attached through a carboxylic acid group.

Procedure

Grind the algae material (500mg) in 10ml 80% acetone in pestle and mortar. Centrifuge the extract at 10,000 rpm for 10 minutes. Use the supernatant for estimation of chlorophyll content. Read the absorbance of the solution at 663nm and 645nm.

Flow chart for chlorophyll



The formula of chlorophyll is determined by following formula:

$$\text{Chlorophyll-a mg/g} = \frac{12.7 \times A_{663} - 2.69 \times A_{663} \times V}{a \times 1000 \times w}$$

$$\text{Chlorophyll-b mg/g} = \frac{22.9 \times A_{645} - 4.68 \times A_{663} \times V}{a \times 1000 \times w}$$

$$\text{Total chlorophyll mg/g} = \frac{20.2 \times A_{645} + 8.02 \times A_{663} \times V}{a \times 1000 \times w}$$

Result & Discussion:

Salt stress is correlated to growth of algae. When algae are given treatment, they showed the changes and respond to the effect. This affects algae morphologically and changes are seen in the content of metabolites.

The experiment actually showed the effect of different concentration of NaCl on the algae.

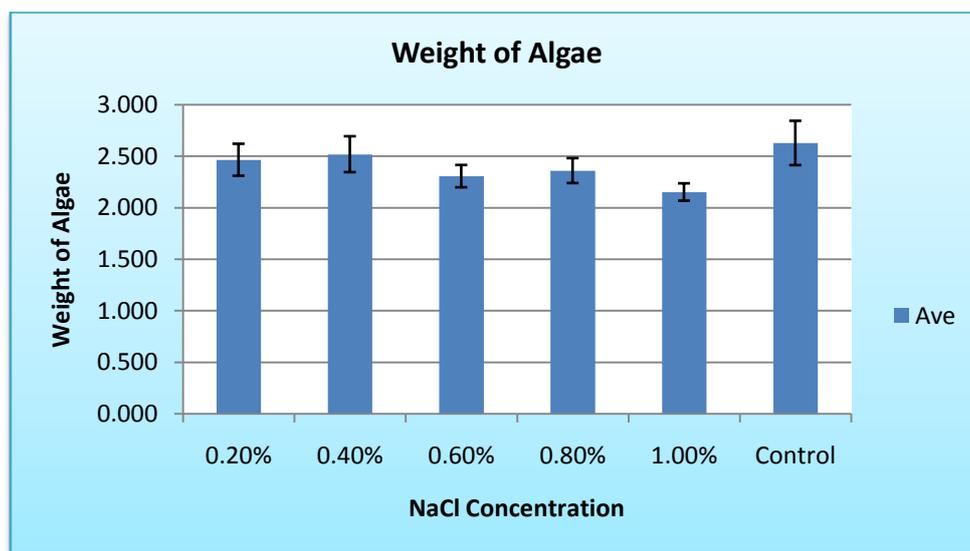
Different parameters like weight of algae, pH of water and Chlorophyll content were studied for the experiment.



Weight of Algae

Table.3 Weight of Algae

Sr. No.	Concentration	Average Weight (gm)
1	Control	2.464±0.190
2	0.2%	2.464±0.155
3	0.4%	2.450±0.157
4	0.6%	2.305±0.108
5	0.8%	2.359±0.121
6	1.0%	2.151±0.084



Graph.1 Weight of Algae in different concentration of NaCl

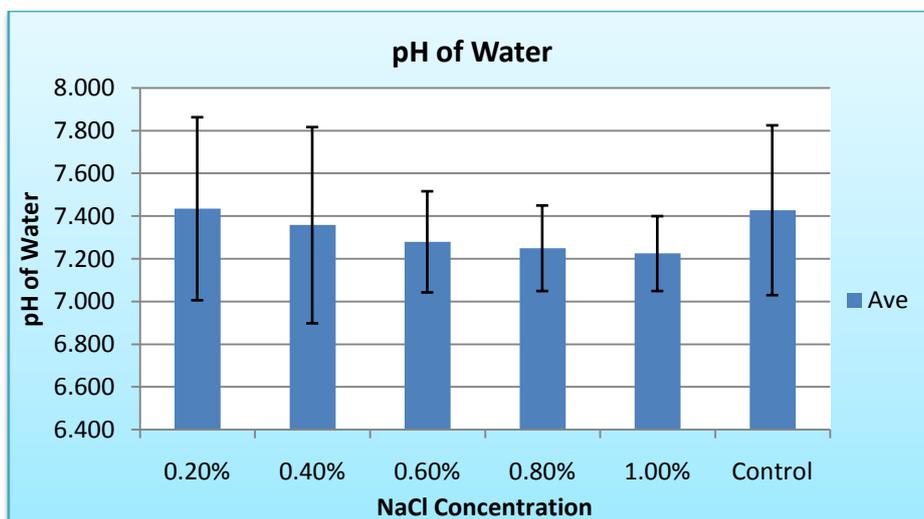
This shows that, at high concentration of NaCl, weight of Algae Was decrease because of toxic effect of NaCl.



ph of Water

Table.4 ph of Water

Sr.No.	Concentration	Average pH
1	Control	7.428±0.397
2	0.2%	7.435±0.428
3	0.4%	7.358±0.334
4	0.6%	7.280±0.237
5	0.8%	7.250±0.200
6	1.0%	7.225±0.175



Graph.2 pH of Water in Different concentration of NaCl

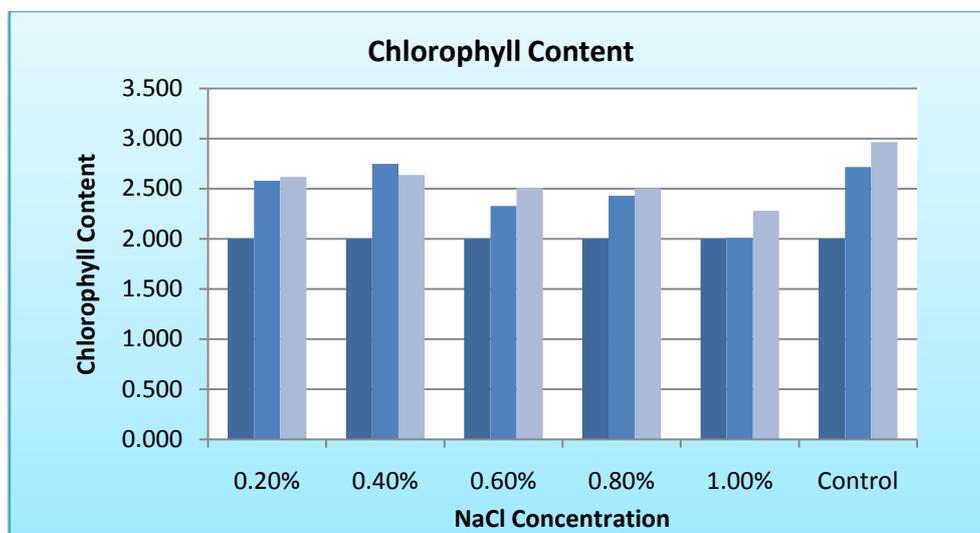
This shows that, at high concentration of NaCl, pH of Water is decrease. NaCl affect the pH of water and Chara also reduce the pH of Water.

Chlorophyll Content

Green pigment Chlorophyll found in cyanobacteria and the chloroplasts of algae and plants. It's a extremely important biomolecule, critical in photosynthesis, Which allows plants to absorb energy from light.



Sr.No.	Concentration	Chlorophyll a	Chlorophyll b	Total Chlorophyll
1	Control	0.116	0.211	0.327
2	0.2%	0.131	0.238	0.366
3	0.4%	0.117	0.213	0.329
4	0.6%	0.054	0.099	0.152
5	0.8%	0.104	0.189	0.293
6	1.0%	0.108	0.197	0.303



Graph.3 Chlorophyll Content in Different concentration of NaCl

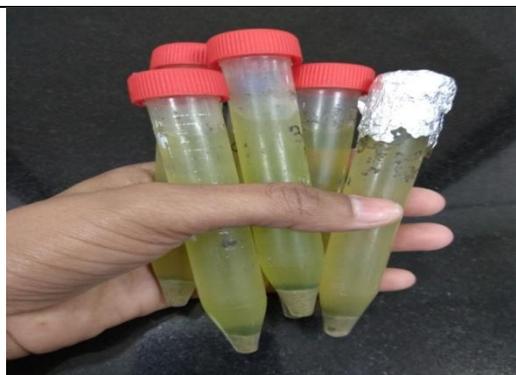
This graph Shows that at high concentration of NaCl Chlorophyll content of algae is decrease. Algae suffer from high NaCl concentration. At high concentration give toxic effect to algae.





Set of Different concentration

Filtration of extract



Chlorophyll Test

Discussion

From soil or through Water Sodium Chloride (NaCl) micronutrient which available to Algae. Na and Cl inhibit Algal growth and development. Salinity affect to the algal growth because of interference of high concentration of salt. Salinity and temperature are major factor controlling growth rate of algae. (N.S.Adenan *et al.*, 2013). Optimum growth of algae at lower salinity and temperature reported by N.S.Adenan *et al.*, (2013).

Conclusion

NaCl or Salt has sodium and Chloride which are indirectly absorbed by Algae and change metabolites content and effect on algae growth. NaCl salt decrease the weight of algae at high concentration and at 0.10% NaCl concentration *Chara* was almost death. And at low concentration *Chara* growth was slow. At high concentration pH was increase and high pH indirectly affect to *Chara* growth. High concentration also affect chlorophyll content decrease compare to control. It concluded that high level of salinity stress gives toxic effect to *Chara globularis* and decrease the growth and chlorophyll.



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