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Impact of pH on Cell Morphometry of *Scenedesmus abundans*

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Global climate change is expected to dramatically impact the structure and function of freshwater aquatic ecosystems. Regional climate change in the upper and lower lake of Bhopal, India is expected to increase air and stream temperatures, modify hydrologic regimes, and increase the amount and frequency of disturbance events (debris flows, landfill, and human encroachment to the catchments area). These climatic changes combined with species-specific tolerances to regime extreme (e.g., pH variability) will likely result in significant changes in the distribution, abundance, and diversity of many aquatic species, particularly *Scenedesmus* species, a phenotypic plastic microalga. In the present study, the effect of pH on cell morphometry *Scendesmus abundans var. brevicauda* was investgated. In the low pH (below 5.0) range, no growth of *Scenedesmus abundans var. brevicauda* was detected and the measured cell dimensions were 6.0×2.7µm.The coenobium became almost colourless and lost its obvious green colour. The cells were shrunken and perforated. The cells became quadratic in their outline. At the alkaline pH, the cell dimensions increased to 7.5×3.3µm with an increase in chlorophyll content.

Key words: *Scenedesmus*, pH, phenotypic plastic

Introduction

The continuing rapid growth in the requirement for potable water has increased the importance for studying and understanding the interactions between chemical substances used in the treatment of drinking water. One of the major environmental factors of freshwater ecosystem is pH. It is impacted by biological processes, such as photosynthesis or respiration[26] as well as influencing the morphological characteristics of freshwater algae both in experimental and natural conditions [3, 5, 14, 23]. Major eco physiological traits and sinking parameters of phytoplankton can be related to the cell size and cell morphology[20]. Shift in size structure has been observed in planktonic organisms over centennial time scales [12, 22], and these shifts have been linked to the changing climate. This suggests that shift in the cell size is a function of changing physical conditions and supports other observations that abiotic fluctuation plays an important role in maintaining algal structure in natural communities [10].

The environmental pH, along with other factors, such as high acidity and high ion concentrations, strongly affects the biota, due to which algal species diversity becomes quite low and decrease as the pH level decreases[7, 25]. However, certain species of algae flourish[15, 25]. Lund *et al.*[21] demonstrated that the member of chlorophyceae seems to prefer neutral pH range (i.e. 5-7).

Brock[8] observed a wide variety of acidic environments, both natural and artificial, and revealed that blue-green algae (*Cyanophyta*) is completely absent from habitats in which the pH is less than 4 or 5, whereas eukaryotic algae flourishes. With increasing acidity, there was decline in the number of plankton species, and pronounced change in the taxonomic composition [16, 17].

The pH level was related to the nutrient dissolution, which caused a change in species composition and biomass of the phytoplankton[9]. Amount of nutrient determines the phytoplankton growth rate,

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and phytoplankton growth is defined by its cell size. Cell size is a key morphometric parameter, which determines the species composition[2].

Material and method

Lake environment is characterized by different trophic states, morphology, and presumably by different phytoplankton assemblages. Bhopal, the capital city of Madhya Pradesh, is known as the city of lakes as it has two beautiful artificial lakes, the Upper Lake and the Lower Lake. The Upper Lake and the Lower are divided from each other by an over bridge. They are spread over an area of 6km^2 of land. The Lower lake of Bhopal is enclosed by human settlements from all sides. The salient features of upper and lower lake of Bhopal are:

Salient features	Upper lake	Lower lake
Longitude	77°18'-77°24' E	77°24'-77°26' E
Latitude	23°13'-23°16' N	23°14'30''-23°15'30'' N
Catchment area	361 km^2	9.6 km^2
Submergence area	30.72 km^2	1.287 km^2
Maximum depth	11.7 m	9.4m
Storage capacity	101.5 M.cum	3.5 M.cum

The upper lake and lower lake of Bhopal were identified as the sampling locations from where surface samples were collected including the "Boat Club" and "Hamidia College, Bhopal". Bi-weekly to monthly sampling is necessary to capture the seasonal dynamics of phytoplankton. Lake should be sampled during mid-day to optimize the light transparency. The investigated algal strain *Scenedesmus* was isolated from the sampled plankton. The isolation was based on bacteriological technique[4]. Small quantities of water samples were kept in sterilized petridishes enriched with a pinch of a KNO_3. The samples were exposed to fluorescent light for incubation without any disturbance.

The pure cultures of test organism were isolated from collected water sample. In present studies ward and perish media[2,4] were found to be most suitable for algal growth. Hence, the mother cultures and experimental cultures were raised in this medium. The pure culture of algae obtained was used to establish stock culture. All the cultures were illuminated with daylight fluorescent tube with an intensity of 2000-lux. The ambient temperature ranged from 20-30°C. The pH of the culture medium should be around 7.5. The duration of light and dark periods was 12:12 hrs. The cultures were shaken manually 3-5 times a day to provide uniform suspension. The photo periods of 12 hrs of light and 12 hrs of dark were considered the optimum light regime to sustain algal growth. Clean sterilized 200 mL borosil conical flasks were filled with 100 mL culture medium. Required concentration of NaCl was added to the medium with the help of micro pippetes.

A known number of microalgae cell was inoculated from a stock culture kept in exponential growth phase. The pH of the medium was adjusted, ranging from 5 to 10. The flasks were shaken thoroughly and incubated. All the cultures were hand shaken to keep the cell in suspension. Duration of experiment was 24 hours to measure the cell dimensions.

Results

The presence of NaCl and changes in pH cause different qualitative changes according to their concentrations, such as the chlorophyll content. The observations indicate that the cells plasticity of *Scenedesmus* is slightly affected with the basicity of the pH, but with high level of salt (acidity) it causes a far-reaching and astonishing effect on the morphology and growth of the coenobia.

Prior to the placement of sample with various salt concentrations, the samples were observed under light microscope to evaluate the actual cell shape and size of *Scenedesmus abundans* var. *brevicauda* through micrometry. Colonies of 4 celled were attached side-by-side and arranged linearly. Cells are oval in shape, 7µm in length and 3µm in width (Fig 2). The cell wall was smooth or occasionally very finely sculptured with irregular net like structure.

At pH 6-8, there was no significant difference in comparison to controlled sample but variations in the cell density of the microalga *Scenedesmus* were observed with the pH 5 of sodium chloride concentration. The coenobia was perforated between the cells. The cells were quadratic in their outline.

The variations in shape were typical of *Scenedesmus* and related to the size and numbers of cells within the individual coenobia. The disparity was observed in the colony of *Scenedesmus* from pH8 - 10 with an increase in chlorophyll content in microalga. The graph represents (Fig 1) the cell dimensions variation of *Scenedesmus abundans* with respect to change in pH.

At pH 8-10, the alkalinity caused growth in colony resulting high chlorophyll and high photosynthetic rate. The cell size appeared to be significantly related to both width and the length of the cells. The size of the cells from particular pH level is illustrated in Fig 2. The cells were smaller at acidic pH level.

The morphological variation in relation with pH cannot be described only to the change in cell size as it also affects the shape characteristics. Therefore, it is likely that *Scenedesmus abundans* probably presents the ecomorph expression that occurs most frequently in low pH environment. At pH 5, the cell sizes were gradually reduced due to the acidity of the medium. At pH 6 and pH7 *Scenedesmus* coenobia slightly regained their original continuity (Table 1). At pH 10, the size increased up to 7.5μm in length and 3.3μm in width.

Table 1: Variation in cell dimensions of *Scenedesmus abundans* at varied pH ranges

pH	Cell Length (μm)	Cell Width (μm)
Control	7.0	3.0
5	6.0	2.7
6	6.5	2.9
7	6.7	2.9
8	6.7	3.0
9	7.1	3.1
10	7.5	3.3
Mean	6.7	2.9

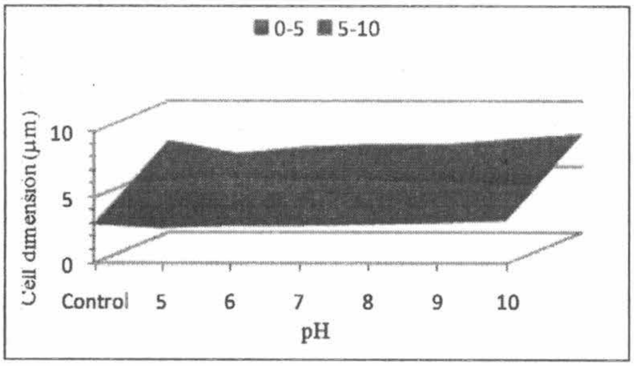

Fig 1: The cell dimension variations of *S. abundans* with respect to pH. (0-5= Cell width (μm); 5-10 = Cell length (μm)).

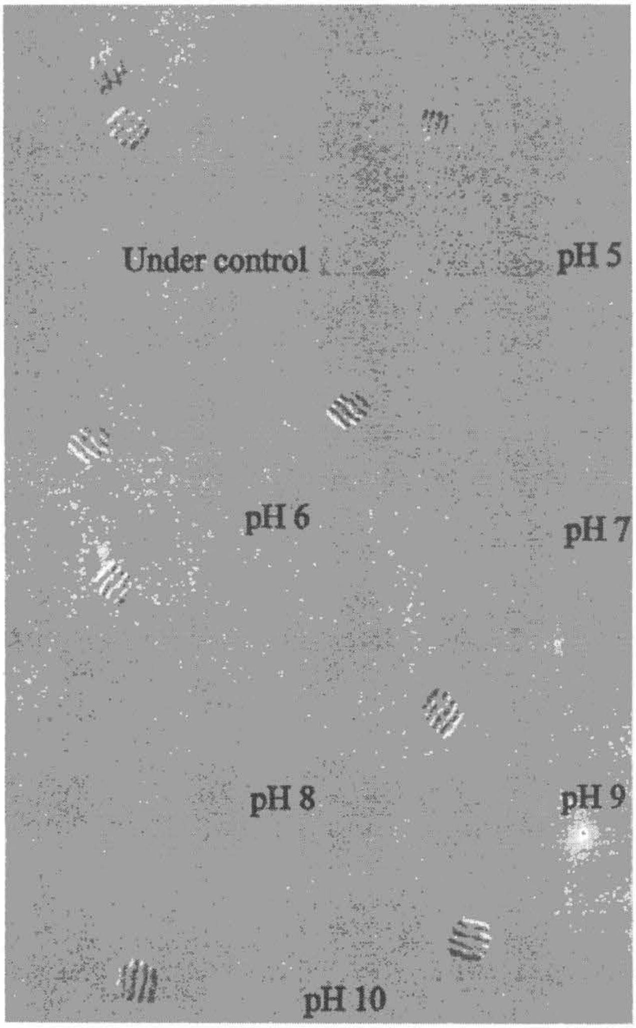

Fig 2: The phenotypic variation in *Scenedesmus abundans* cells dimension under various pH ranges

Discussion

A change in pH plays an important role in the metal toxicity, and pH seems to be a major cause of inhibition of growth [13]. El-Dib *et al.*[11] reported that increase in the pH value of the algal culture was always associated with the growth of *Scenedesmus* cells and cell morphometry is also subject to change due to change in pH of water. The results point out that in *Scenedesmus abundans* var. brevicauda, the cell length at pH 5 was measured 6μm and cell width was 2.7μm which was reduced from its actual cell dimension i.e.7×3 μm, but at pH 9 and 10, the cell significantly increased dimension which was measured 7.1×3.1μm and 7.5×3.3μm. The present study along with the findings to detect the cell morphology on

Mougeotia sp. in response to pH, found that growth form, cell dimension, chloroplast morphology and cell wall ultrastructure were significantly different in culture grown at pH 5 and pH 8. Low to moderate salinities (50 and 100 mM NaCl) stimulated the growth of both the species while higher levels (150-250 mM NaCl) reduced the growth of *C. humicola* only[1].

The results of present studies are in accordance with the outcomes that different salinities considerably affect the morphology of green algae. An increase in cell volume with increasing pH was observed[19], which indicates that salinity fluctuations are responsible for 64% of the cell volume changes in *Scenedesmus armatus,* but marked difference was found to exist in the cell size before and after cell division. pH value of the water correlated to the biomass of *Scenedesmus quadricauda*, *Microcystis aeruginosa*, *Lyngbya limnetica* and *Chlorella vulgaris* significantly[27]. Cell morphometry is also altered in response to changing pH [6].

The present study spells out that the disparity was observed in the colony of *Scenedesmus* at pH 8-10, i.e. the cell dimensions increase with an increase in chlorophyll content of *Scenedesmus*, relevant with the result of [18] which indicates that the content of chlorophyll a and chlorophyll 'c' in *P.pavonica* was significantly related to pH. Both the chlorophyll 'a' and 'c' contents increased with declining pH.

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