CHAPTER IV

DISCUSSION AND CONCLUSION

Though blue green algae do not dominate in saline especially marine habitats, Dosikachary (1959) has recorded as many as fifty species of this group from saline environment. More over as already said in the first chapter of this investigation, the present agricultural practice of using large quantity of inorganic fertilizer subject the soil algae to unnatural stress of salts. Now this exigency of sudden flux in salt concentration in the environs of blue greens is met inter alia of physiological adoption is the subject of this preliminary investigation.

Talpasyal (1979) has worked out the physiology of blue-green algae in saline condition. He, however, has chosen filamentous and heterocystous forms such as *anabaena* for his investigation. Ability of this organism to grow in high salt concentrations is of great interest to the workers studying physiology of stress. By studying the ecophysiological aspects of the dominant blue-green forms, some methods can be divided to control the growth of this alga so that the salt may be extracted in a more economical way. This alga can also be grown in mass scale perhaps in a sea water with a view to utilizing the algae mass as biofertilizers.
Results discussed here are on investigation with unicellular form such as Chroococcus. Growth is the reflection of the physiological status of the cells and hence to get an over all picture of the growth pattern of Chroococcus under varying conditions, growth studies have been carried out. Results given in tables 1 and 2, and plate I-A and I-B clearly indicates that the alga not only with standing the varying external concentrations of salts but also enhanced the growth when the algal environ has 0.5 % sodium chloride and potassium chloride. 0.5 % NaCl and KCl are optimum concentration for the best growth in Chroococcus. Nutritional requirement in nitrogen metabolism has biochemical basis of inorganic substances. In Anabena cylindrica, Williams (1960) showed that Na⁺ inhibit the N₂ fixation. Brownell and Croxalland (1972) used NaCl and compared it to KCl at 1 mM concentration and have shown to stimulate markedly the uptake of PO₄ in the unicellular green algae. Anabaena cylindrica. Sodium ion also prevent chlorosis. Raven (1971) showed the active K⁺ influx in Hydrodictyon africanum, is supported by cyclic phosphorylation.

Our experiment showed that high concentrations of sodium or potassium hamper the growth of the algae and these may be due to competitive effect of the elements on each other. Barret and Jeffrey (1964) concluded that potassium added was not the cause of the increase in algal population.
If K, P and Na supplied together, the colonies of *Microcystis* *sphaeroides* disintegrates, but altering the mono and bivalent salts of Na and K give the better results. Williams (1960), Brownell and Crossland (1972) have made the survey for Na⁺ requirement in the plant metabolism.

Williams (1960) showed that the nitrate reductase activity was greatly increased in cells grown without sodium, whereas Brownell and Crossland (1972) found out that the sodium is micronutrient for species having C₄ di-carboxylic photosynthetic pathway. They studied six species having C₄ type respond to Na⁺ ions. Na⁺ deficient plant have chlorosis and necrosis effect. *Peyularia* failed to get flowers. In contrast *Poa annua* L. (Kentucky blue grass) having characteristic C₃ photosynthetic pathway, which made normal growth and did not respond to the additions of sodium. It is concluded by them that sodium is essential for species having C₄ pathway but not for species with the C₃ path way. The correlation between potassium of the C₄ pathway and essentiality of Na⁺ content contribute to the understanding of the role of sodium in metabolism of plants, for which it is essential. It is interesting to note that sulphate of these two element via. sodium and potassium however, do not encourage the growth even at low concentration, tables 3 and 4, plate 1-C and 1-D. Thus whatever promotory effect on growth observed is not due to the sodium and
The results also corroborate the fact that entire metabolic status of the cells of *Chroococcus* is boosted up by 0.5% sodium chloride and potassium chloride, as can be seen from the following account.

Ascorbic acid is universal in occurrence in plant tissue and it plays a very important role in electron transfer system in plants. There is a good amount of free ascorbic acid and its turnover is also remarkable. Chinoy (1967, 1969) has postulated that enhancement in the energy transfer mechanism is mediated by free radicals of ascorbic acid. Macromolecules like nucleic acid are thereby activated and various metabolic processes are hastened, ultimately resulting in tissue differentiation in plants.

Ascorbic acid turnover is least studied in algae as can be seen from the review. Velidya et al. (1971) demonstrated that some blue-green algae give out considerable quantity of ascorbate in environment. This is only possible if the algae have capacity to produce large quantity of ascorbic acid. Talapaseyi (1967) also detected ascorbic acid in the heterocyst. Patel (1974) showed that ascorbic acid content of *Nostoc* is higher than that of * Oscillatoria* and *Anabaena*. The present study clearly indicate that ascorbic acid content of *Chroococcus* (Tables 9 and 10, Plate III-A, III-B) is
quite high and is comparable with the results obtained by Patel (1974). The most remarkable fact that it remains in tune with the growth of *Chroococcus* when the alga is subjected to salinity. The ascorbic acid content increased in presence of 0.5% NaCl but when the salinity rises to 2.0% the ascorbic acid content of the cells drops down.

In the algal cells the chain reaction of synthesis (metabolic process) continuously proceeds as a result of water absorption. Due to the catabolic process the food materials are degraded and the energy is released. This energy is utilized to carry out the process of metabolism. The bound form of AA i.e. ASC is also high in the alga grown in 0.5% NaCl and KCl (Tables 9 and 10, Plates III-A, III-B). AAU increased in alga grown in low concentration of NaCl and KCl. It indicates that mobilization of food materials can be governed by energy provided by utilization of ascorbic acid during growth.

As ascorbic acid (AA) and ascorbigen (ASC) content are high and ascorbic acid utilization (AAU) being high, the redox balance is maintained by the AA turnover and do not shift toward the oxidative side since the free as well as the bound form of the AA increases during growth under saline condition. This further strengthens the belief that low salinity level cause the decrease in oxidative process, as the peroxidase is also found at low level (Tables 11 and 12, Plates III-C, III-D).
Since ascorbic acid is utilized as a protecting agent there is not enough of it for the formation of charge transfer complex (CTC) with macromolecules and hence all the biosynthetic process as well as enzymatic processes are at a higher level under inadequate water, (Salt stress).

Ascorbic acid is a powerful electron donor and activate anabolic process by increasing redox potential. Thus higher content of ascorbic acid in Chroococcus at 0.5% salinity stress further support the view that this much salinity stress the growth and metabolism of the alga are enhanced but this organism cannot tolerate further stress of salinity as indicated by drop in growth and also in ascorbic acid content at 2.0% salinity. Results of bound ascorbic acid and ascorbic acid utilization (Table 9 and 10 Plate III-A, III-B) further support the view that entire ascorbic acid turnover is enhanced at low salinity stress while it is depressed at high salinity stress at least in case of Chroococcus. The picture of redox potential of the cells of Chroococcus in saline condition could be visualized by the study of ascorbic acid, but to understand the status of oxidising metabolic mechanism of the cells in saline condition the peroxidase enzyme has been studied. Such a study would give us a picture as to whether the reserved material is degraded faster in saline condition.
Result shown in the table 11 and 12, Plate III-C, III-D indicates that peroxidase activity is not enhanced at 0.5 % salinity. This may be due to the utilization of ascorbic acid at faster rate against the peroxidase damage. Thereby indicating that disintegration process is not set in at this stage. This may also be due to the high level of ascorbic acid content of the cell at 0.5 % salinity. Ascorbic acid would slow down the activity of oxidising enzymes and then by protect the cell against the demand for the use of reserve material.

In our results we also found reduced peroxidase activity in low salinity stress. Chinoy et al. (1970-a) have also reported a decrease in peroxidase activity under water stress condition. Siegel and Galston (1966) ascribed the significant role of peroxidase in the regulation of cell growth and differentiation. The picture of physiological status of the cell, so far as peroxidase is concerned, changed adversely when the cells of the Chroococcus were subjected to high salinity (2.0 % NaCl and KCl), the peroxidase activity was increased and thus indicates that the catabolic activities have set in the cells. This is also reflected on the growth of the cells at 2.0 % salinity level. At this juncture we are tempted to heralded a hypothesis that high salinity enhance the
catabolic activities of the cells by increasing the activity of oxidising enzymes such as peroxidase. However, if redox potential of the cells is maintained by more synthesis of ascorbic acid or even by exogenous supply of ascorbic acid, the catabolic process set in the cells by high salinity can be stopped and there by the cells can be made to withstand the high saline conditions. This hypothesis is yet to be checked by experiments with algae.

Protein content depends upon the mode of nutrition. Sancjia and Myers, (1953) showed that *Chlorella pyrococca* when grown on glucose, it had 49% of proteins, 36% of carbohydrates and 15% of lipids. Bergmann (1955) also found the variation in protein content in different species of *Chlorella*. It can be said that respiratory and photosynthetic mechanism in the algae are responsible for the protein status of species. In the algal metabolism protein level in a cell is the main deciding factor. Calvin and his coworkers established that proteins of *Chlorella* and *Scenedesmus* are in a state of rapid dynamic equilibrium with simple intermediary metabolites, since carbon atom from $^{13}C\text{CO}_2$ carbon dioxide enters in protein molecules in very short period of time. Bidwell (1957) considered that protein synthesis may be utilized and not drawn from the amino acid pool within the cell.
In present study the protein content is reduced under saline condition as compared to that in control. Quantitative reduction in protein content was also observed in the water stressed corn seedlings (West, 1962). Positive correlation between water content of the tissue and protein level and inverse correlation between protein and rate of water loss were also found by Chen et al. (1964). Genkel et al. (1967) reported that protein synthesis was disrupted in wilted plants. Number of workers have reported that protein is hydrolyzed in stress condition (Petrie and Wood, 1938; Savitskaya, 1968). The proteins are hydrolyzed by a number of proteolytic enzymes. A marked increased in proline has been reported by Chen et al. (1964), Barnett and Naylor (1966), Falfi and Juhasz (1968). They suggested that proline and asparagine favour the increase in relative hydration of protoplasm and in this way play a protective role during unfavourable conditions like stress.

Protein content of *Chromococcus* under saline conditions remained at low level (Table 13 and 14, Plate IV-B). This is perhaps, because in order to meet exigent situation created by salinity, the cell would like to keep more and more amino acids in free pools. This would not mean that free pool of amino acids will increase considerably in saline condition, because the amino acids of free pool are
soon utilized to fight the saline environment, and hence neither free pool of amino acid is increased nor protein content of the cell go up.

There is reason to believe that protein synthesis is not very much enhanced by saline condition in this alga because even the RNA content of the cells which is responsible for protein synthesis, goes down in saline environment (Table 17 and 18, Plate V-B). Talapsayi (1979) has also observed that free pool of sugar increases and hydrolysable sugar content decreases in Anabaena when kept in saline condition. This observation may serve an evidence for the disturbances in the carbohydrate metabolism under stress conditions. Our results are also in accordance with above observations, as shown in Table 15, Plate IV-D, free sugar increases in Chroococcus with increasing intensity of salinity, while total sugar which largely represent hydrolysable sugar falls down. The decrease in the hydrolysable sugar content may be due to their conversion into amino acids (Stewart, et al., 1966). According to these authors the oxidation of sugar furnishes -ketoglutarate and NADP (H) which are responsible for proline synthesis under water stress which favour the hydration of protoplasm. Our results in Chroococcus lead us to believe that on setting of saline conditions perhaps the reserved carbohydrates
might have been converted into free sugars to fight the adverse conditions created by saline environment or possibly converted into AA, since AA is the conversion product of sugar, Loewus (1959).

Although, contrary to general observation, that the hydrolysing enzymes are stimulated under water stress, hence depression of both amylase and invertase activity may be seen, further, despite these lowered enzymatic activities, sugars are increased. This lead to possibility that some other enzyme such as phosphorylase might have been activated so that sugars can be formed to increase stress resistance. This may be consider as biomechanism for alga growing continuously under salt stress.

The results on amino acid contents of the cells in saline conditions are very much chakered (Table 16). Even at 0.5% salinity one important amino acid viz. glutamic acid begins to drop down in their content in free pool. Aspartic acid content in protein also drops down, cysteine, tyrosine, phenylalanine and iso-leucine disappear on onset of salinity. Proline is the only amino acid which increases in its content at both the level of salinity studied here. Stewart (1977) has shown that in barley, when cells become less and less turgid because of water stress, the proline oxidation slows down, that is to say that the conversion of
proline into glutamate is reduced. The high content of proline enables the cells to fight against water stress. Thus playing role in osmoregulation. Juhász (1970) has shown that when crop plant are subjected to cold, water stress or salinity, the proline content in the leaves increases very much. Thus there is an evidence to show that high content of proline is consequence of stress to which the cell is subjected. In our investigation also, it has been found that on increasing the sodium chloride and potassium chloride in the medium the cells of Chlorella begins to show high content of proline. Talpasayi (1979) observed in Anabaena that the total amount of amino acid increased with the increase in salinization of the medium while the number of amino acids decreased. It is presumed that the accumulation of amino acids is due to increased hydrolysis of storage proteins as well as a decreased synthesis of new proteins under salt stress. The decrease in number of amino acids is perhaps due to the fact that some free amino acids that accumulated during stress conditions are metabolized and consequently disappear while others accumulate in excessive amounts.

Schematic behaviour of amino acids on subjecting cells to saline conditions i.e. appearance and disappearance of certain amino acids on changing conditions of salinity in
the cell protein and free pool is really interesting. Preliminary experiments such as ours cannot fully explain the cause and effect of this phenomenon.

Considering the nucleic acid metabolism in Chlorella, the content of RNA decreased under the saline environment (Table 17 and 18, Plate V-B). This may be the reason for reduced charge transfer complex in the algal cells with inadequate water. At the same time decrease in the RNA content may be due to decrease RNA synthesis and increased RNA break down. Quite a few workers have tried to study nucleic acid metabolism in plants under water stress. Absence of normal synthesis was due more to the failure of formation of ATP under stress rather than the deficiency of inorganic phosphorous. Gates and Bonner (1959) also were of the view that loss in turgidity of plant cells brought about a disruption of ATP synthesis. Retardation of ATP formation adversely affected photosynthesis and uptake of nutrients including phosphorus. Barskaya and Oknina (1959) have ascribed the important role of nucleic acid metabolism in the adoption of plants to adverse environmental conditions. All these observations are of interest in that they lend support to the idea that environment may influence plant growth and metabolism by interacting with genetic control at the level of RNA. Our results (Table 17 and 18, Plate V-B) also confirm the low level of RNA under
stress and yet we have no sufficient experimental data to give reasons for the low content of DNA in saline condition. Thus due to chemical and ecological environment many of the biological and physiological processes are affected. In order to investigate effect of salinity on photosynthesis an experiment was designed in the laboratory conditions.

Sodium $^{14}$C bicarbonate was assimilated by blue green alga *Chlorella* in the presence or absence of sodium chloride. However, the rate of uptake was greater in untreated cells. This perhaps may be due to the limitation of energy in form of ATP, in 0.5 % NaCl treated cells, where it is bifurcated for two simultaneous processes; one, it is utilized to drive the sodium chloride ions into the cells from surroundings; and two, it is also required for the transport of sodium $^{14}$C bicarbonate into the cells. But this is not true in case of 2.0 % NaCl treated cells where sodium chloride itself seems to have harmful effect on alga which is also revealed by the growth data presented in previous chapter.

The ratio of alcohol soluble to alcohol insoluble fractions remained higher than unity indicates the uncoupled nature of rate of incorporation of radioactivity into alcohol insoluble fraction to that of assimilation. The fluctuation in aforesaid ratio is probably the reflection of dynamic turnover of compound into insoluble products.
The ratio of distribution of radioactivity from sodium $^{14}$C bicarbonate into TCA soluble to TCA insoluble fractions remained less than unity in presence of 0.5 % NaCl and even less than that of control through out the experimental period of 60 min. This probably reflects onto the higher rate of synthesis of macromolecules especially some type of protein under saline condition. Kozlowski (1964) observed that the synthesis of macromolecules reduced and hydrolysis was accelerated under low water potential. In Anabaena talpasyi (1979) also presumed that it may be due to increased hydrolysis of storage proteins as well as decreased synthesis of new proteins under salt stress. In fact most of the evidences for this view is derived from vascular plants after lengthy exposure to low water potentials and little is known about blue-green algae. In present study also the total protein content was less in 0.5 % NaCl treated cells. However, the higher rate of incorporation in treated cells probably suggests the core synthesis of some type of protein.

In presence of 0.5 % NaCl, alanine was more heavily labelled than that of control. This can be the result of greater flow of radioactivity into pyruvate which subsequently gives rise to alanine by transamination of pyruvate. Similar observations have been made by Kennedy and Laetsch (1974) in *portulaca oleracea*, a C₄ plant, where pyruvate is
one of the earliest labelled photosynthetic products. Besides this comparatively heavy labelling was found in sugar phosphate and less in certain organic acids and other amino acids of TCA cycle. This perhaps may be due to the reduced rate of glycolysis as suggested by Greenway and Setter (1977). They have observed the increased concentration of fructose-1-6, diphosphate and triose phosphate in the extract of peas in the presence of KCl or NaCl. Moreover in peas, grown in high NaCl concentrations the Cl\textsuperscript{-} inhibition of aldolase and/or GAPDH leading to the shift from tricarboxylic acid cycle to the pentose phosphate pathway (cf. Porath, and Poljakoff-Mayber, A. 1964).

Though we have insufficient data at present to derive any final conclusion, however it does give some indications of shift of metabolism under saline conditions. Thus the metabolism of cell might respond to high salinity but rather widespread increase in level of intermediates, yet the metabolic control of the cell would remain reasonably well balanced (Greenway and Sims).
CONCLUSION

This investigation leads us to conclude that:

1. Wide uses of inorganic fertilizers and irrigation water subject the soil flora and plants to unnatural salt stress. Both blue-green and green algae could tolerate increased saline conditions which suggest the halotolerant nature of both the algae.

2. More synthesis of ascorbic acid and its utilization under stress probably protects algae against stress. High level of ascorbic acid reduce the peroxidase activity and protect algae against peroxidase damage. Thus ascorbic acid acts as a powerful electron donor playing important role in electron transfer system in algae.

3. Reduced protein content under stress probably suggests the inhibition of protein synthesis or increase rate of protein hydrolysis.

4. Since glucose and galactose are precursors of ascorbic acid, increased quantity of ascorbic acid, free sugar and simultaneous decrease in total reserve carbohydrates suggests the probable conversion of free sugar...
into ascorbic acid and thus it helps in protecting alga against physiological stress.

5. Comparatively higher content of proline in free pool of amino acids under stress suggests its probable role in osmoregulation. It also functions as a storage compound which helps the alga to withstand the adverse conditions.

6. The reduced charge transfer complex and the ATP synthesis under stress caused retardation in RNA synthesis, as low level of ATP adversely affect the uptake of nutrients including phosphorous.

7. Heavy labelling in alanine and reduced ratio of TCA sol./TCA insoluble suggest the increased synthesis and incorporation of alanine into insoluble product. It also suggests the greater flow of radioactivity into pyruvate. This shows that alanine has a key role to play as an initial product of photosynthesis.