4. ALGAL DISTRIBUTION, ABUNDANCE AND PHYTOPLANKTON COMPOSITION IN PUTHALAM SALTWORKS

4.1. INTRODUCTION

Saltpans are one of the hypersaline extreme environments exhibit wide range of environmental stress through salinity changes (Sugumar et al., 2011). Halophiles can survive that limit the growth of most organisms. Among halophilic microorganisms, bacteria, cyanobacteria, green algae, fungi and diatoms are abundant in saltpans (DasSarma and Arora, 2002) and form a biological pad (Zhiling and Guangyu, 2009). The highly adverse biological system of solar salterns, with evaporation ponds and crystallizer ponds of different salinities, with often high densities of phototrophic microorganisms, planktonic as well as benthic, makes the salterns excellent model systems for the study of primary production and other microbial processes (Oren et al., 2009). Many halophiles and holotolerant microorganisms can grow over a wide range of salt concentrations with requirement or tolerance for salts sometimes depending on nutritional and environmental factors (Meltem and Numan, 2008). These environments are generally highly productive, but most of the oxygen produced during day time by the photoautotrophs appears to be recycled within the mats rather than exchanged with the overlaying water and the atmosphere (Oren, 2009). The halophilic communities are denser in high salt concentration zones. They withstand extreme saline conditions and regulate the osmotic pressure, thereby resisting the denaturing effects of salt in their environment (Kerkar, 2004).
Hypersaline environments are ubiquitous and are vital to beginning to estimate the compartment of biodiversity (Litchfield et al., 2009). Biodiversity is high along the specific gravity 1.120 to 1.140, but the variety of species begins to decrease at higher salinities where gypsum precipitates thickly on pond floors. At saturation with sodium chloride, a few species that exist are often present in high concentrations (Oren and Dubinsky, 1994). Gypsum deposition occurs not only as firm to soft sheets on pond floors, but also as powdery layers of individual microscopic crystals which are carried downstream (Burnard and Tyler, 1993; Magana et al., 2005). Upon reaching crystallizers, nutrient-rich viscous brine is detrimental to salt quantity and quality, and promotes development of large populations of *Dunaliella salina*.

The diversity of microorganisms is very interesting at the beginning of the system where the brine concentration initiates the differentiation and selection of resistant organisms to particular condition. In the system two great groups of microorganisms are developed, one of them in the column of brine (plankton) and another one in the floors or bottoms of pools (benthos). Both groups interrelate giving as a result the different conditions of health of the salt ponds (Ortiz-Milan and Davis, 2009). The physico-chemical parameters of the brines and salts contain sufficient ions and hardness to support the growth of halophilic bacteria such as extreme environments (Birbir and Sesal, 2005).

**4.1.1. Phytoplankton from a healthy solar saltwork ecosystem**

Microbial cells attach to the surfaces and develop a biofilm. Biofilm is an assemblage of the microbial cells that is irreversibly associated with a surface and
usually enclosed in a matrix of polysaccharide material (Kokare et al., 2009). Biofilm formation occurs step by step such as formation of conditioning layer, bacterial adhesion, bacterial growth and biofilm expansion (Kumar, 1998). Biofilm associated cell is differentiated from suspended counterparts by reduced growth rate, up and down regulation of gene and generation of extracellular polymeric matrix (Donlan, 2002). Light can have very significant effects on the growth and internal composition of marine algae. The effects of varying light intensity range from the seasonal slowing/acceleration of growth rates in marine ecosystems, or marine microalgae sinking through the water column and out of the photic zone due to light attenuation (Barnes and Mann, 1999).

Characteristics of benthic communities favourable to salt production include development and maintenance of mats firmly attached to pond floors (Oren, 2009) that sustain desired thickness, preserve biodiversity, remove and permanently sequester nutrients from the overlying water and control leakage (Reginald and Banu, 2009), seal seepages (Jhala, 2009) and infiltration (Moosvi, 2006) through pond floors to a great extent and maintains desired thickness. The benthic mats in the solar salt ponds are important because as a beneficial effect, the mat reduces loss of brine from the field, but it unfortunately also supports species which can have a serious detrimental effect on the halite crystallization process. Planktonic communities develop red colour and it helps in better solar energy absorption and increases the rate of evaporation and hence causes faster precipitation of the halite crystals (Litchfield et al., 2009; Rahaman and Jeyalakshmi, 2009a).
Apart from the physico-chemical process in the saltworks, biological process is also of great importance for the process of salt production. If the care is taken at the design stage itself, encouraging results in this regard can be achieved as biological process is in admirable harmony with the production process of saltworks comprises of a large variety of organisms, and produce the most biomass (Moosvi, 2006).

Phytoplankton diversity was lower in the low salinity ponds in comparison to the adjacent marine area of Kalloni Gulf, whereas abundance and biomass were higher in the initial ponds in comparison with the marine area and declined downstream the pond sequence (Evagelopoulos et al., 2006).

Diatoms commonly constitute the dominant group of algae in saltpan biofilm of Thamaraikulam saltworks, Kanyakumari District. Bacillariophyceae are represented by a large number of species and play a fundamental role in salt production (Wilsy et al., 2008). Their ubiquitous distribution, well known taxonomy and high representativeness in benthic consortia have made them a valuable and widely used tool to assess the variable environmental conditions and characteristic of aquatic systems (Stoermer and Smol, 1999). According to Caric et al. (2011), the diatom was most abundant in November to January when temperature and salinity were low.

4.1.2. Biochemical composition of microalgae

Microalgae have the ability to create the biomass by use solar energy in combining water with carbondioxide. Halophiles produce variety of stable and unique biomolecules which are useful for practical applications. The current
commercial uses of the halophiles are quite significant and many novel and unique properties of these organisms suggest that they have been greater potential for biotechnology (Rodriguez-Valera, 1992). Solar saltern contains rich and varied communities of phototrophic microorganisms along the saltern gradient, and the photosynthetic primary production by these communities largely determines the properties of the saltern system (Oren, 2009). Organic osmotic solutes that have to be produced in large amounts and accumulated in the cells which serve as osmolytes (Kraegeloh et al., 2005) to provide osmotic balance according to the salinity of the brines (Oren, 2000; 2006). Several studies have given information on the composition and ecology of phytoplankton in solar saltworks located in various countries (Ayadi et al., 2004; Segal et al., 2006).

Algal protein either as a supplement or as an alternative source has received worldwide attention. Microalgae produce vast array of natural products including proteins, enzymes, bioactive compounds and carotenoids (Ausich, 1997). A large number of marine nitrogen-fixing cyanobacteria to serve as a complete aquaculture feed source (Thajuddin and Subramanian, 2005). Proteins of halobacteria are either resistant to salt concentrations or require salts for activity. Extracellular proteins, such as those secreted into the medium and probably those in the periplasmic space and exposed to external saline environments and must adapt to variable salinities (Meltem and Numan, 2008). Chlorella was investigated for the wide-scale production and used for nutritional purposes, such as a source of protein, lipids, carbohydrates, vitamins and minerals to help fill the “protein gap” and feed an ever expanding world population (Becker, 2007).
Microalgae are very diverse (Harwood and Guschina, 2009). They can create a range of useful products and there is a variety of ways in which they can be cultivated, manipulated, harvested and utilized (Harun and Singh, 2010). They have the ability to produce large amounts of lipids, including triacylglycerides (TAGs), a high energy density storage molecule (Courchesne and Parisien et al., 2009). According to Ak et al. (2008), cell densities and pigment yields of Dunaliella strains strongly depend on salinity, temperature and light intensity. The dense microbial communities and in the microbial mats of hypersaline lakes often exhibit high activities of photosynthesis, dissimilatory sulphate reduction and other microbial processes, thereby exerting a profound influence on the biogeochemical cycles of carbon, nitrogen, sulfur and other elements (Javor, 1989; Oren, 1988).

Chlorophyll ‘a’ is a biomass indicator of aquatic microalgae which support food webs in the sea; it is probably the most frequent measured biochemical parameter in oceanography. The chlorophyll content is an index of photosynthetic potential, its decrease or increase represents the multiplication or decline of the microalgae in a culture system. In the green algae, chlorophyll ‘a’ makes up about two third of the total pigment in the chloroplast. The chlorophyll pigments are formed from a substance called protochlorophyll, which is synthesized in the dark. It is changed to chlorophyll only in the light. Chlorophyll ‘a’ being exclusively a plant constituent, has naturally been used extensively to express phytoplankton biomass, which has been proved by HPLC analysis (Brown et al., 1981).
Puthalam saltworks is the largest saltworks in Kanyakumari District. This saltworks is a major source of solar salt for food, hide and other industries locally. Due to the economic importance of salt obtained from the saltworks, a microbial survey has been conducted. For the estimation of biomolecues the standard procedures were followed. The objective of this chapter was aimed at identifying the different species of microalgae along its abundance and phytoplankton composition found in different ponds of Puthalam saltworks in different seasons during the study period (from March 2009 to February 2011).
4.2. MATERIALS AND METHODS

4.2.1. Algal sampling and analysis

For the present study, the microalgae and its abundance (%) were studied throughout the study period. The investigation period was divided into four seasons (Season I, II, III and IV). The collection was made in early morning. In each season water samples were collected four times per season from the surface waters of different ponds of Puthalam saltworks with clear polythene cans. A circular hand net of ½ m length with 15 cm mouth diameter of a mesh size of 2 µ was used for the collection of phytoplankton samples by filtering 100 litres of water. Then the filtrate was put into clean labeled plastic container and also fixed in Lugol’s iodine soon after the collection for further analysis. The fixed samples were transferred to the laboratory and kept undisturbed until analysis. Later the biomass were concentrated to 10 ml or 50 ml (depending on the abundance of plankton) by siphoning out the supernatant solution with a plastic tubing, one end of which was closed with a blotting silk (30 µm) to prevent the loss of buoyant phytoplankton. From the collected and concentrated filtrate 1 ml of the sample was taken and the concentrate was shaken in order to get an even distribution of phytoplankton for identification. The analysis was repeated for ten times. Drop method was applied for counting and identification of phytoplankton species from different samples (APHA, 1992). The cell number was determined by direct counting under a compound microscope (10 x objective) and the cell number was counted with haemocytometer using light microscope. Photographs were taken with a digital camera (Pentax - 6.0 mega pixels). The collected microalgae were
identified as per the observations made up by Venkataraman (1939), Prescott (1962) and Desikachary (1986). Phytoplankton abundance in different ponds was found out (Plate 4) and the results were declared as % abundance/unit area. Benthic semi-dried algal mat of the littoral marshes were estimated with the help of microscope (Plate 4a). Portions of benthic mats and submerged growth were removed from the substrate with a scapel. For the estimation of biomolecules the microalgae from various ponds of Puthalam saltworks were collected and stored in airtight pearlpet container labeled with details. Then they were used for the estimation of biomolecules such as protein and chlorophyll – a.

4.2.2. Estimation of total protein (Lowry et al., 1951)

The Lowry method (Lowry et al., 1951) was used to estimate the total protein content. The carbamyl group of protein reacts with the copper iron of the alkali and when this complex reacts with phosphomolybdic acid of folin phenol reagent gets reduced with tyrosine and tryptophan.

a. Reagents

i. 10% TCA:

10 g of Tri Chloroacetic Acid (TCA) was dissolved in 100 ml distilled water.

ii. Sodium hydroxide (1N)

4 g of NaOH was dissolved in 100 ml distilled water.

iii. Sodium hydroxide (0.1 N)

400 mg of NaOH was dissolved in 100 ml distilled water.
iv. Solution A

1 g of sodium carbonate was dissolved in 50 ml of 0.1 N NaOH solution.

v. Solution B

5 mg of copper sulphate was dissolved in 1 ml distilled water and to this 10 mg sodium potassium tartarate was added.

vi. Solution C

Mixed 50 ml of solution A with 1 ml of Solution B.

vii. Folin ciocalteu phenol

Mixed 1 ml of folin ciocalteu phenol with 2 ml of distilled water.

e. Preparation of samples for estimation

Algal samples of different ponds at different seasons were collected. 20 mg of each algal sample were weighed separately and were homogenized in 2 ml of 10% TCA and centrifuged at 5000 rpm for 10 – 15 minutes. After centrifugation, precipitate were dissolved in 1 to 2 ml of 1 N sodium hydroxide solution and used as sample for total protein estimation.

c. Procedure

To 250 ml of sample, standard (Bovine Serum Albumin) and blank (1N NaOH) added 2.5 ml of solution C. After 10 min, added 2.5 ml of folin ciocalteu
phenol reagent and kept undisturbed for 20 minutes. The absorbency of blue
colour developed was measured at 640 nm in a UV Spectrophotometer.

d. Calculation

The amount of protein was calculated as

\[
\frac{\text{Optical density of sample}}{\text{Optical density of standard}} \times \text{concentration of standard} = \mu g \text{ mg/gram sample}
\]

4.2.3. Estimation of chlorophyll (Jorgensen, 1969)

The algal sample was filtered through whatman filter paper No. 42 and the
chlorophyll pigments were extracted from the algae by using 90% acetone. The
resulting coloured acetone extract was measured in the Spectronic20.

Reagent - 90% acetone

Mixed 90 ml of acetone with 10 ml distilled water to get 90% acetone.

Procedure

The algal samples were filtered through the filter paper individually. For the
extraction of chlorophyll – a, a known quantity of the algal sample was taken in a
test tube and 10 ml of 90% ice cold acetone was added to this. The tube was kept
in a refrigerator for 20 – 24 hours for complete extraction. At the time of
extraction, blank was prepared by acetone. After extraction period, the sample was
taken out from the refrigerator and was allowed to warm to room temperature.
Then, the sample and blank were centrifuged for 10 minutes at 5000 rpm into a
screw cap tube and the colourless biomass was discarded. Pigment was analyzed with comparing sample of unknown transmission against a blank of 100% transmission. The concentration of chlorophyll – a is in the supernatant was spectrometrically at 630, 645 and 655 nm wavelength. Then the chlorophyll – a content was calculated using the formula

\[
\text{Chl a} = 11.6 \times \text{O.D.}_{(665)} - 1.31 \times \text{O.D.}_{(645)} - 0.14 \times \text{O.D.}_{(630)}
\]

Where \( \text{O.D.}_{(655)} \), \( \text{O.D.}_{(645)} \) and \( \text{O.D.}_{(630)} \) are absorbency at 630, 645 and 655 respectively.
4.3. RESULTS

4.3.1. Microalgae and its abundance in the reservoir pond during first year

The microalgae and its abundance (%) recorded in the reservoir pond of Puthalam saltworks during the first year study (from March 2009 to February 2010) is shown in the Table 4.1 and Fig. 4.1. The samples were collected in different salinities of the saltpan showed variations in their numbers. The decline in the number of algal species was noticed in the higher salinities.

 Totally 18 different genera of phytoplankton were identified in four divisions such as Bacillariophyta, Chlorophyta, Cyanophyta and Dinophyta. Among these Bacillariophyta contributed more number of genera (7 numbers) followed by Cyanophyta (6 numbers), Chlorophyta (3 numbers) and Dinophyta (2 numbers). During season I, the *Pleurosigma* species was found in maximum number (45.62%), next to *Dunaliella* (41.86%) followed by *Oscillatoria* (7.35%) and *Chlorella* (3.07%). The species of *Navicula* (0.55%) and *Cyclotella* (0.52%) were almost same in their contribution. *Pinnularia* and *Lyngbya* registered the equal number of cells (0.34%). *Closterium* and *Amphora* expressed their representation 0.14 and 0.09% respectively. Species such as *Spirulina* and *Chroococcus* represent the equal number of cells 0.06%. The species which showed no representation were *Nitzschia*, *Coscinodiscus*, *Anabaena*, *Gloeocapsa*, *Exuviella* and *Peridinium*. 

97
In season II, among the phytoplankton generic composition, *Oscillatoria* species showed maximum number of 26.40%. It was followed by the microalgal species such as *Lyngbya* (22.71%), *Dunaliella* (20.52%) and *Navicula* (14.50%). The other species representations were *Pleurosigma* (7.11%), *Cyclotella* (2.87%) and *Nitzschia* (2.19%). Here *Amphora* and *Chlorella* showed the equal number of 0.96% cells. Species of *Pinnularia*, *Anabaena*, *Spirulina* and *Closterium* showed 0.95, 0.54, 0.42 and 0.41% respectively. *Coscinodiscus*, *Gloeocapsa*, *Chroococcus*, *Exuviella* and *Peridinium* had no representation.

In season III also *Oscillatoria* represented maximum number with 26.53%. Next to this *Dunaliella* 21.0, *Pleurosigma* 17.72 and *Navicula* 15.82%. Species such as *Amphora* (4.93%), *Spirulina* (3.80%), *Cyclotella* (2.94%), *Pinnularia* (2.77%), *Chlorella* (1.64%), *Anabaena* (1.38%), *Coscinodiscus* (1.31%), *Gloeocapsa* (0.09%) and *Exuviella* (0.08%) showed maximum representation. No representation of microalgae such as *Nitzschia*, *Closterium*, *Lyngbya*, *Chroococcus* and *Peridinium* were noted during this season.

In season IV, dominance of *Pleurosigma* with the contribution of 56.73%. Next to *Pleurosigma*, the percentage abundance of *Oscillatoria* (10.72%), *Navicula* (7.24%), *Chlorella* (6.86%) and *Dunaliella* (5.64%) were observed. The other algae presented were *Cyclotella* (4.51%), *Lyngbya* (4.33%) and *Gloeocapsa* (1.03%). But the algal species *Amphora* and *Spirulina* contributed the equal number of 0.85% cells. Other species were *Closterium* 0.75%, *Peridinium* 0.65% and *Exuviella* 0.47%. Species like *Anabaena* showed 0.09% only in this season.
The algal species which showed no representation were *Pinnularia*, *Nitzschia*, *Coscinodiscus* and *Chroococcus*.

It is clearly evident and notable that after the study on the first year, microalgal species like *Nitzschia* was recorded only in season II, *Cosinodiscus* in season III, *Chroococcus* in season I and *Peridinium* in season IV. But in this year, *Gleocapsa* and *Exuviella* were recorded in season III and IV. And also it is a point of consideration.

4.3.2. **Microalgae and its abundance in the reservoir pond during second year**

In this study period, only 17 genera were identified in four different divisions such as Bacillariophyta (7 genera), Chlorophyta (3 genera), Cyanophyta (6 genera) and Dinophyta (1 genera). Table 4.2 and Fig. 4.2 showed the abundance of phytoplankton in all four seasons of the second year study period (from March 2010 to February 2011).

During the I season, *Navicula* species was important contributing highest level (59.36%) of cells. The contribution of other species and their percentage were *Pleurosiga* 9.93, *Cyclotella* 9.36, *Dunaliella* 6.55 and *Oscillatoria* 4.49%. Followed by *Amphora* (3.75%), *Pinnularia* (3.00%) and *Chlorella* (2.25%). Species like *Spirulina* (0.75%) and *Lyngbya* (0.56%) showed minimum representation. The microalgal species such as *Nitzschia*, *Coscinodiscus*, *Closterium*, *Anabaena*, *Gloeocapsa*, *Chroococcus* and *Peridinium* showed no representation in this season.
In season II, the leading microalgae *Oscillatoria* had the percentage of 19.76. Next to this, the percentage existence of *Cyclotella, Navicula, Nitzschia, Lyngbya, Gloeocapsa, Coscinodiscus* with 18.00, 12.68, 10.98, 9.29, 8.29 and 5.12% respectively. The percentage abundance of species such as *Dunaliella, Peridinium, Closterium, Spirulina* and *Pleurosigma* were 4.39, 4.37, 2.44, 1.95 and 1.71% respectively. The species like *Amphora, Chlorella, Anabaena* and *Chroococcus* had no representation.

In season III, *Spirulina* contributed more number of 33.12% followed by *Oscillatoria* (12.62%), *Pinnularia* (12.30%), *Cyclotella* (11.36%), *Dunaliella* (9.78%) and *Navicula* (5.99%). The percentage of *Amphora, Pleurosigma, Lyngbya, Gloeocapsa, Coscinodiscus, Chroococcus* and *Nitzschia* were 3.47, 3.18, 2.40, 2.02, 1.58, 1.26 and 0.95% respectively. Species such as *Chlorella, Closterium, Anabaena* and *Peridinium* showed no representation.

Among the species representation in season IV, *Dunaliella* species showed the maximum number of 32.89% cells. Next to *Dunaliella*, the percentage abundance of *Cyclotella* 19.73, *Pleurosigma* 8.55 and *Lyngbya* 7.89%. In this season *Navicula, Chlorella* and *Oscillatoria* had the equal number of 6.57% cells. Species like *Nitzschia, Spirulina, Amphora, Chroococcus, Coscinodiscus, Anabaena* and *Pinnularia* had minimum contribution were 3.28, 2.63, 1.97, 1.40, 1.22, 0.72 and 0.58% respectively. The species which showed no representation were *Closterium, Gloeocapsa* and *Peridinium*.

*Closterium* and *Peridinium* were recorded only in season II and also in the study of second year.
4.3.3. Microalgae in the condenser pond during the first year of study

Phytoplankton abundance (%) in the condenser pond of Puthalam saltworks during March 2009 to February 2010 is presented in Table 4.3 and Fig. 4.3. During the study period, only seven genera from three divisions were identified such as Bacillariophyta (3 genera), Cyanophyta (2 genera), Chlorophyta and Dinophyta with one genera each.

During season I, the percentage of *Dunaliella* species reached to 79.10% of the planktonic microalgae population. Next to this, *Synechococcus* showed 18.30%. The other species representations were *Pleurosigma* 2.23%, *Chroococcus* 0.32% and *Pinnularia* 0.04%. Species such as *Navicula* and *Peridinium* had no representation. But in season II, *Synechococcus* species were found to be dominant maximum number (78.16%), next to *Dunaliella* (14.94%) were sub-dominant. The contribution of other identified species was *Pleurosigma* 4.98% and *Chroococcus* 2.04%. Species of *Navicula*, *Pinnularia* and *Peridinium* showed no representation.

In season III, *Dunaliella* showed maximum of 98.05%. The other species such as *Pleurosigma*, *Navicula*, *Chroococcus*, *Pinnularia* and *Synechococcus* reached minimum density of 1.20, 0.31, 0.28, 0.12 and 0.03% respectively. The *Peridinium* species showed no representation. In season IV, again *Dunaliella* contributed the maximum percentage of 90.14%. Next to this, the number of other species were *Pleurosigma* 4.93, *Peridinium* 2.46, *Synechococcus* 1.76 and *Navicula* 0.70%. But the species of *Pinnularia* and *Chroococcus* had no representation.

In the condenser pond, *Dunaliella* was dominant in all the seasons except season II. At the same time, *Synechococcus* had dominated only in season II.
Peridinium, one of the members of Dinophyta was recorded only in season IV which is a notable fact.

4.3.4. Microalgae in the condenser pond during the second year study

Microalgae and its abundance (%) in the condenser pond of Puthalam saltworks during March 2010 to February 2011 is presented in the Table 4.4 and Fig. 4.4. Here also seven different genera distributed in four divisions such as Bacillariophyta, Cyanophyta, Chlorophyta and Dinophyta with 3, 2, 1 and 1 generic forms respectively.

In season I, the Synechococcus species showed the maximum percentage of 79.49% and followed by Dunaliella which contributed 16.93%. The other species were Peridinium 1.90, Pleurosigma 1.64 and Navicula 0.03% respectively. But Pinnularia and Chroococcus had no representation. In season II, the contribution of Dunaliella was the highest (73.47%) followed by Synechococcus 25.45%. But the Peridinium (0.59%) and Pleurosigma (0.48%) species had the minimum contribution to this season. Species of Navicula, Pinnularia and Chroococcus showed no representation.

In season III, again Dunaliella registered its maximum abundance of 97.95% and the other species Pleurosigma showed 2.05% only. When compared to other seasons, only two microalgal species were found in this season, at the same time the other species had no contribution. In season IV also Dunaliella has highest percentage of occurrence of 89.04%. Next to Dunaliella, the number of other species were Pleurosigma 3.65, Chroococcus 3.32, Navicula 2.66, Synechococcus 1.00 and Pinnularia 0.33%. The Peridinium species had no
representation. Also only in this season except *Peridinium*, all the microalgal species were present.

### 4.3.5. Phytoplankton in the crystallizer pond during first year study period

Phytoplankton abundance in the crystallizer pond of Puthalam saltworks during the first year study period (from March 2009 to February 2010) is tabulated in Table 4.5. Here only two genera were found in one in each of the divisions Chlorophyta and Cyanophyta.

*Dunaliella* was the dominant one in all the seasons in the first year study and registered the abundance of 96.21 (season I), 98.73 (season II), 98.96 (season III) and 99.37 % (season IV) respectively. The another species *Chroococcus* showed the lowest abundance of 3.79, 1.27, 1.04 and 0.63% in the seasons (Fig. 4.5).

### 4.3.6. Phytoplankton in the crystallizer pond during second year study period

The abundance of phytoplankton in the crystallizer pond during second year study period (from March 2010 to February 2011) is given in the Table 4.6 and Fig. 4.6. Just like last year, *Dunaliella* was the dominant one than *Chroococcus* and the percentage occurrence was 97.79, 94.34, 94.15 and 78.92% in the seasons (I to IV) and the abundance of *Chroococcus* was 2.21, 5.66, 5.85 and 21.08% respectively.
With regard to crystallizer pond, *Dunaliella* dominates both the years of study. Since *Dunaliella* grows in high salinity, it was found abundant.

**4.3.7. Benthic semi-dried algal mats in the reservoir pond during the first year (March 2009 to February 2010)**

Algal abundance per unit area during various season of the first year study is presented in Table 4.7 and Fig. 4.7. In the benthic semi-dried algal mats the microalgae such as *Pleurosigma* (23.79%), *Navicula* (9.65%), *Cyclotella* (9.42%), *Nitzschia* (15.56%), *Pinnularia* (8.91%), *Dunaliella* (19.68%) and *Peridinium* (5.00%) showed maximum abundance in season I and registered minimum abundance in season IV with 15.40, 5.59, 5.03, 10.29, 5.36, 10.25 and 2.80% respectively. At the same time, the microalgae *Amphora* recorded highest abundance (11.85%) in season II and lowest (7.14%) in season IV. The other microalgae *Coscinodiscus* registered maximum abundance (6.59%) in season III and minimum (4.08%) in season IV. *Chlorella* had highest abundance 12.48% in season III and lowest 6.57% in season I. During season I, *Oscillatoria* showed the maximum abundance of 26.10 ± 3.36% and the minimum abundance was during the III season (21.14 ± 2.78%).

The statistical analysis by two way ANOVA for the data on benthic semi-dried algal mats in the reservoir pond as a function of sampling microalgal species and seasons showed that the variation between the microalgal species and seasons were statistically significant (F = 37.53256; P < 0.05 and F = 5.496337; P < 0.05) during the first year.
4.3.8. Benthic semi-dried algal mats in the reservoir pond during the second year (March 2010 to February 2011)

Microalgal species abundance during the second year study in various seasons was studied and presented in Table 4.8 and Fig. 4.7. During second year study, Pleurosigma and Dunaliella recorded maximum abundance (25.11 and 9.12%) in season I and the minimum (19.02 and 5.12%) in season II. Navicula showed maximum abundance in III season (12.52 ± 1.21%) and minimum abundance in II season (8.30 ± 1.58%). During season IV, Nitzschia showed the maximum abundance (13.61 ± 2.38%) but minimum abundance was expressed in season I (10.04 ± 2.36%). The maximum abundance of 5.55% in season II and the minimum of 3.85% in season IV was observed in Pinnularia. In season I Cyclotella (8.42%), Amphora (9.27%), Coscinodiscus (6.19%), Chlorella (15.56%) and Oscillatoria (8.05%) reached maximum abundance and attained minimum (5.09, 6.12, 3.07, 10.43 and 5.49%) in season IV. Finally the species of Peridinium showed the maximum abundance in season II (3.91 ± 0.09%) and minimum (2.26 ± 0.05%) in season I.

Two way ANOVA for the data on benthic semi-dried algal mats in the reservoir pond as a function of sampling microalgal species and seasons showed that the variation between species were statistically significant (F = 52.82918; P < 0.05). But variation between seasons were statistically not significant (F = 2.421552; P < 0.05) during the second year study.
4.3.9. Protein content in the algal samples during the first year study 
(March 2009 to February 2010)

From the recorded data on the results of protein content present in the algal samples in all the ponds of Puthalam saltworks expressed the maximum protein content in season II and the minimum protein content in season IV throughout the first year study presented in Table 4.9. and Fig. 4.8.

The highest protein content 1.79 ± 0.003 mg/g and the lowest 1.08 ± 0.004 mg/g was calculated in the algal samples of the reservoir pond. Protein content ranged from a minimum of 2.16 ± 0.009 mg/g to a maximum of 2.84 ± 0.008 mg/g in condenser pond. In crystallizer pond, it was ranging from 0.575 ± 0.006 mg/g to 0.917 ± 0.001 mg/g.

Statistical analysis (Two-way ANOVA) conducted for the data on protein content in the algal samples as a function of sampling ponds and seasons revealed that the variation between ponds and the variation between seasons were statistically significant (F = 169.2465; P < 0.05 and F = 8.96726; P < 0.05).

4.3.10. Protein content in the algal samples during the second year study (March 2010 to February 2011)

The results on the protein content in the algal samples from various ponds of Puthalam saltworks during different seasons of the second year study is given in Table 4.10 and Fig. 4.9.

Also in the second year study, the protein content present in the algal samples expressed the maximum value in season II and minimum in season IV.
The highest protein content 4.63 ± 0.018 mg/g and the lowest 3.75 ± 0.073 mg/g was calculated in the reservoir pond. Protein content ranged from a minimum of 8.63 ± 0.116 mg/g to a maximum of 5.34 ± 0.159 mg/g in condenser pond. In crystallizer pond, it was ranging from 1.42 ± 0.251 mg/g to 1.25 ± 0.001 mg/g.

Two-way ANOVA was used for the data on protein content as a function of sampling ponds and seasons showed that the variation between ponds were statistically significant (F = 55.33076; P < 0.05) and the variation between seasons were statistically non-significant (F = 2.287504; P < 0.05).

4.3.11. Chlorophyll - a content in the algal samples during the first year study

Chlorophyll - a content (mg/g) in the algal samples collected from the different ponds of Puthalam saltworks during the first year study is given in Table 4.11 and Fig. 4.10.

The reservoir pond showed the higher chlorophyll – a content of 0.49 ± 0.007 mg/g in season II and lower of 0.11 ± 0.002 mg/g in season IV. Likewise, in the condenser pond there was maximum chlorophyll - a content of 0.93 ± 0.006 mg/g observed in the algal samples during season II and minimum of 0.60 ± 0.004 mg/g observed in season IV. Similarly the crystallizer pond showed the maximum chlorophyll - a content (0.50 ± 0.009 mg/g) in the season II and showed minimum chlorophyll - a content (0.10 ± 0.00 mg/g) in the season IV.

From the result of the statistical analysis (Two-way ANOVA) it is inferred that the variation in chlorophyll – a content of algal samples between ponds and
between seasons were statistically significant ($F = 411.53001; P < 0.05$ and $F = 103.7845; P < 0.05$) during the first year.

4.3.12. Chlorophyll - a content in algal samples during the second year study

Chlorophyll – a content in the algal samples during the second year study is presented in Table 4.12 and Fig. 4.11. The algal samples in the reservoir pond showed the highest chlorophyll - a content of $0.24 \pm 0.001$ mg/g in season II. But the lowest chlorophyll - a content $0.17 \pm 0.00$ mg/g were noticed in the algal samples during season IV. Likewise, in the condenser pond, the algal samples showed their maximum chlorophyll - a content of $1.69 \pm 0.074$ mg/g and the minimum of $1.00 \pm 0.023$ mg/g in seasons II and IV respectively. The algal samples of crystallizer pond expressed the higher chlorophyll - a content of $0.78 \pm 0.015$ mg/g in season II but it was lower in season IV ($0.40 \pm 0.008$ mg/g).

The two way ANOVA expressed for the data on chlorophyll - a content in the algal samples as a function of sampling ponds and seasons showed that the variation between ponds were statistically significant ($F = 78.74305; P < 0.05$) and the variation between seasons were statistically not significant ($F = 4.251961; P < 0.05$). Throughout the study period the chlorophyll – a content of the algal samples from the various ponds of Puthalam saltworks was present as follows season II > season I > season III > season IV.

With regard to biomolecules (protein and chlorophyll – a) the maximum value was recorded in season II and the minimum was recorded in season IV throughout the study period.
4.4. DISCUSSION

4.4.1. Saltern ecosystem and their biota related to ecological factors

Phytoplankton in the algal samples collected from the study area Puthalam saltworks during both years were identified into a total number of genera consisted of Bacillariophyta, Chlorophyta, Cyanophyta and Dinophyta. Hypersaline environments pose a number of ecological and metabolic challenges to the organisms that live in them.

The abundance of phytoplankton showed fluctuation during different seasons of the study period. The population density estimated in reservoir pond of Puthalam saltworks showed the major variation among microalgal species. Although Bacillariophyta were dominant in respect to species numbers and it contributed 54.06% of the total phytoplankton community. The other divisions such as Cyanophyta and Chlorophyta constitute 22.79 and 22.42% respectively. The Dinophyta comprised of 0.74% in the total population.

The richness of phytoplankton in species were related to the season. There were no equal representation and population density during different seasons of the study period in the condenser pond of Puthalam saltworks. The seasonal variation in phytoplankton density was in relation to physico-chemical properties. Examination of phytoplankton population revealed that the density of phytoplankton varied in relation to salinity. Some of the saltern phytoplankton are gradually adapts to the high salinity as the concentration of the brine increasing. The lower pH during rainy season may be due to dilution of alkaline substances.
and resulting increase in turbidity of water which in turn reduced photosynthetic activity of algae (Pawar, 2010). The nutrient rich water source in saltpan favours algal blooms in reservoirs as well as evaporators. The physico-chemical characters such as pH, light, temperature, salinity and nutrients play an important role of phytoplankton density during the study periods.

The population size of different groups and species of phytoplankton at various ponds during different seasons were highly variable. Primary producers of the studied saltworks ecosystem consist of phytoplankton community such as Bacillariophyceae, Chlorophyceae, Cyanophyceae and Dinophyceae. Bacillariophyceae and Cyanophyceae have their maximum growth at pH 7 to 8.0. (Touliabh et al., 2010). Marine microbial mats may grow to a few centimeters in thickness of which only the top few millimeters are oxygenated (Che et al., 2001). The upper most layers are generally dominated by aerobic photosynthesizing cyanobacteria while the lowest layers are generally dominated by anaerobic sulphate-reducing bacteria (Risatti et al., 1994). The mat also helps in reducing the seepage and preventing infiltration through pond floors to a great extent and maintains desired thickness. The organic matter accumulation is due to the laterally and vertically extensive growth of microbial mats (Gerdes et al., 1991; Cornee et al., 1992). Cyanobacteria members are dominant in planktonic biomass and form microbial mats in many of the saltpan ecosystem as stated by DasSarma and Arora (2002). The top most layer of microalgal mat contains the cyanobacteria Aphanothece which grow well over a wide range of salt concentrations. Britten and Johnson (1987) stated that diatoms constituted the bulk of benthic algal
biomass in low salinity which was in agreement with the results of the present study but did not occur more than 150 ppt salinity.

Higher abundance of phytoplankton in all ponds were recorded during season IV. This may be due to induced by changes in salinity, temperature, pH and light during the season. Salinity showed a significant relation with Bacillariophyceae and Cyanophyceae which was abundant in low salinity ponds followed by Chlorophyta and Dinophyta. These results are agreement with the results of Reginald (2003). Khomayis (2002) reported that high nutrients content was accompanied by low phytoplankton biomass during summer. In addition, inorganic micronutrients considered as limiting factors affecting the growth of phytoplankton. This same trend was observed in the present study. Species of Exuviella was found during the first year study in the reservoir pond but which was not recorded in the survey of the second year study. Dinophyceae represented rare group in the investigated ponds with abundance of 1.27 and 0.20% respectively during the first and second year of investigation.

The genera Pleurosigma, Nitzschia and Navicula were the most dominant genera of the diatom assemblage. Oscillatoria and Pleurosigma were the dominant benthic algal groups in the present study. Solar light penetration in the bottom of the brines activating the metabolic process of the benthic microorganism increases the competition by the capture of nutrients. Seasonal changes of benthic semi-dried algal mats of the littoral marshes in the reservoir pond of Puthalam saltworks during the study period showed that, some species were abundant in some seasons
and were not present as same as in all seasons. A study conducted by Reginald (2003) also confirmed the same findings.

Diatoms were abundant and most frequently encountered in the microscopic survey of present study. It was in accordance with the findings of Oren (2009) and Khan et al. (2009). During spring where diatoms showed a good flourishing with lowest silicate level also confirmed with the findings of Touliabah et al. (2010). Diatoms are normally not characteristic members of phytoplankton associations in late summers (Padisak et al., 2003).

In the reservoir pond, among the most abundant microalgae identified were species of the genera *Pleurosigma, Navicula, Cyclotella, Amphora, Dunaliella, Chlorella, Oscillatoria* and *Spirulina*. Although Bacillariophyta were dominant in respect to species numbers, Cyanophyta and Chlorophyta type phytoplankton were registered in terms of population density (Elif and Arif, 2010). Blooming of phytoplankton occur in season II induced by changes in temperature and nutrients supply during summer seasons. Planktonic diatoms as *Pleurosigma, Navicula, Cyclotella, Pinnularia* and *Amphora* were observed permanently throughout the study period while *Amphora* sp. was not detected during Autumn of second year study.

The cyanobacterial species occupied the second dominant position in the present study was *Oscillatoria, Spirulina, Lyngbya, Anabaena, Gloeocapsa* and *Chroococcus*. Thajuddin and Subramanian (1992) reported to species of 19 genera in saltpans with salinity of over 50 ppt. Cyanobacteria are grown to tolerate and acclimate to high salt concentrations. Desikachary (1959) suggested that probably
20% of all known Cyanobacteria occur in saline conditions and a majority of them are truly marine. Bass and Becking (1931) found that cyanobacteria were sensitive to increased Ca and Mg concentrations at higher salinities. The presence of *Oscillatoria, Spirulina* and *Lyngbya* were countered throughout the larger part of the study period. However *Nitzschia, Coscinodiscus, Chlorella, Closterium, Anabaena, Gloeocapsa, Chroococcus, Exuviella* and *Peridinium* were observed only during low saline periods. In general, the number of organisms found to decrease with an increase in salinity. The two algal species of Dinophyceae were rarely recorded during the study period indicate high nutrient concentrations in that season. In general, the reservoir pond showed the higher average population more than the other studied (condenser and crystallizer) ponds.

The population density and species abundance were very low in the condenser pond of Puthalam saltworks. Here only three species of Bacillariophyta were identified followed by two species of Cyanophyta. The others were one species each from Chlorophyta and Dinophyta. The salinity was higher in the condenser pond than the reservoir and also condenser pond receives brine from the reservoir. The presence of *Pleurosigma, Navicula, Dunaliella* and *Chroococcus* throughout the study period indicates that they can tolerate wide fluctuations in salinity. As the salinity increases the species density decreases mostly due to environmental stress, while the various population densities increase. The increase in phytoplankton population during higher salinities was mainly due to the abundance of hypersaline algal species like *Cocochloris* and *Dunaliella* reported by Sundararaj *et al.* (2006). The same trend of population density was noticed in
the above said microalgae in as well as other halophilic species showed lower density in the present study even in the subsequent study period.

One organism that is the cyanobacterium *Aphanothece* (also related to as *Euhalothece*, *Halothece*, *Cyanothece*, *Aphanocapsa*, *Coccochloris* or *Synechococcus* in the literature - Margheri et al., 2008) was present in the condenser pond which was not observed in reservoir pond and crystallizer pond throughout the study period. *Aphanothece* is found in worldwide salterns in the upper layers of the benthic microbial mats. It is a slime producing algae grow and reproduce best in intermediate salinities (Oren, 2000) but they do not survive in crystallizers. Disturbances often cause to reproduce at high rates, exclude competing species and release massive quantities of mucilage highly damaging in the ponds of intermediate, high salinity and crystallizers. In that time more attention must be paid on the saltern ecosystem. *Aphanothece* and *Dunaliella* species are the key organisms in condenser pond and common in most solar saltworks. The unicellular green algae of the genus *Dunaliella* are among the most widespread eukaryotic organisms in hypersaline environments. It is an obligatory phototrophic, organic, aerobic unicellular organism. *Dunaliella* species lack a rigid cell wall, ovoid in shape and contain large cup-shaped chloroplast with two equal flagella. Significant size differences with smaller cells at higher salinities as well as changes in shape were reported for *Aphanothece* strain from Eliat, Israel (Yopp et al., 1978). Their presence or absence, colours and concentrations, performance under favourable and adverse conditions are highly important to salt production also require special attention and management efforts. *Aphanothece* sp. are
photosynthetic and exist as single cells or colonies of cells. It is harmless in desired biological systems or common in both communities of the concentrating ponds (Davis, 2009). It is an indicating organism of the quantity of the brines and that in favourable conditions of growth form mucilage that aggravates the optimal condition for the solar salt production. However, the halophilic microalgae _Dunaliella_ could tolerate extreme high salinity and they become the dominant species in the high salinity area of the saltworks. The other species were contributed very low percentage abundance in the condenser pond during both the study periods.

Increased salinity in the crystallizer ponds has eliminated some non-tolerant species. There are only two micro algal species were noticed even in the crystallizer pond. They were _Dunaliella_ and _Chroococcus_. One ml water sample of crystallizer pond has 70% of such algae present were dead and remaining 30% alive. The population density of these algae in the crystallizer pond varies due to geographic location, nutrient status and management of the salterns. At the higher salinity (more than 140 ppt) slightly higher values of total algal density were observed due to the abundance of halobiont species like _Dunaliella salina_ (Borowitzka, 1981). The most important phytoplankton species in the study area at the time of sampling was the chlorophycean member _Dunaliella_ species. This species was present in all ponds and constituted the bulk of phytoplankton in the crystallizer ponds. Higher abundance of _Dunaliella_ sp. in the crystallizer ponds in Thamaraikulam saltworks of Kanyakumari District, South Tamil Nadu was observed in the past by Reginald (2003). According to Davis (2009) the algal
species of *Dunaliella* remain alive in high salinity and gradually change colour to bright orange-red, enlarge, become spherical and accumulate glycerol and beta carotene. The pigment responsible for the red colouration displayed by *Dunaliella* often designated in the older literature as “hematochrome” was recognized already very clearly as a carotenoid (Teodoresco, 1906). The red pigment was distributed all over the cells ‘cytoplasm’ (Labbe, 1921). The colour of the brine usually indicates the predominant phytoplankton species. Chlorophyta algae of the genus *Dunaliella* are found in nature in salt fields. Even though the algal blooms are beneficial to the salt former, their decomposed products act as chemical traps and finally prevent early precipitation of gypsum. *Dunaliella* species release organic molecules such as enzymes, nitrogen compounds and proteinaceous substances into the water which help the growth of halophilic bacteria that in turn helps quick evaporation (Giordano, 1997). During the present study a gradual reduction in number of microalgal species were observed with an increase in salinity.

4.4.2. Changes in biomolecules of microalgal samples in response to saltern environment

Microalgae are a large and diverse group of photosynthetic eukaryotes with a simple cellular structure ranging from unicellular to multicellular forms; they can be found anywhere water and sunlight co-occur, including soils, ice, lakes, rivers, hot springs and ocean (Parker *et al*., 2008), and they have the ability to capture solar energy in combing water with CO$_2$ to create biomass. They are among the fastest growing autotrophs on the earth, which assimilate CO$_2$ as the carbon source for growth (Pokoo-Aikins *et al*., 2009). Algae are at the bottom of the aquatic food
chain. The primary determinant in establishing the food quality transferred through successive levels of the food web appears to be the biochemical composition of the algae (amino acids, fatty acids, pharmaceutical products and protein) (Morimoto et al., 1995; Cardozo et al., 2007). Microalgae are rich in pigments like chlorophyll, carotenoids and phycobiliproteins.

In the present study, the algal samples present in the condenser pond contain large amount of protein when compared with other ponds. Light, temperature, salinity and dissolved nutrient concentrations are the important factors which are influencing the chemical composition of microalgae (Scott, 1980; Mortensen et al., 1988). For example, pigment content (e.g. chlorophyll a), polysaturated fatty acid content and profile, carbohydrates and protein content can all change in response to increased or decreased light intensity (Thompson et al., 1990). Phytoplankton population is mainly dependent on temperature, solar illumination and the availability of certain essential nutrients which are important for their growth and reproduction. In solar saltworks, the nutrient rich seawater favours algal blooms. Saltern crystallizer ponds at different geographical locations, although superficially similar in biological properties may show significant differences in the structure of the microbial communities inhabiting them (Oren and Rodriguez-Valera, 2001).

The dense communities of halophilic microorganisms in the condenser ponds represent large amount of protein. Proteins of halobacteria are either resistant to salt concentrations or require salts for activity. As a group, they contain an excess ratio of acidic to basic amino acids, a feature likely to be required for
activity at high salinity (Kerkar, 2004). Extracellular proteins, such as those secreted into the medium and probably those in the periplasmic space are exposed to external saline environments and must adapt to variable salinities (Meltem and Numan, 2008). Microorganisms gradually increase from initial ponds (reservoir) to condenser then they disappear as they move from the condenser ponds to crystallizer. In hypersaline environments however, *Dunaliella* species often predominate. Thus *Dunaliella* species are often the major primary producers in hypersaline salt lakes and in the evaporation ponds of saltworks throughout the world. Proteins make up a large fraction of the biomass of actively growing microalgae and cyanobacteria, although they are generally undervalued compared to minor products such as omega fatty acids. Protein content of microalgal biomass is determined either by calorimetric methods (Lowry *et al*., 1951). Algal pigments such as chlorophyll also contain a significant amount of nitrogen and the biomass often contains inorganic nitrogen (Fujihara *et al*., 2001). For microbial protein to be measured accurately, the cells must be pretreated to fully release the intracellular proteins. Pre-treatments typically involve disrupting the cells by physical or chemical means (Barbarino and Lourenco, 2005). Now-a-days the Lowry method is one of the most accurate methods for quantifying proteins (Peterson, 1979). Bovine serum albumin (BSA) is the most commonly used standard in this method. Total protein content in the biomass depends strongly on the microbial species. Present investigation showed that the protein content of microalgae had maximum in the condenser pond and it falls gradually towards the crystallizer pond, probably due to salinity stress.
Solar saltern contains rich and varied communities of phototrophic microorganisms along the saltern gradient, and the photosynthetic primary production by these communities largely determines the properties of the saltern system. *Dunaliella salina* contains a mixture of natural carotenoids including extremely high quantities of beta and alpha-carotene, a deep orange-red pigment. A rich combination of carotenoids and green chlorophyll give *Dunaliella salina* its orange-red colour. Large amounts of β-carotene are accumulated as globules between the thylakoids of the single chloroplast and the pigment protects the cells from damage by high light intensities. Pond depth is an one-factor for carotene production which has relationship with light quality and intensity absorbed by the algae (Bhumibhamon *et al*., 2003). *Dunaliella* is one of the causes of the pink-red colouration of saltern crystallizer brines; however although β-carotene may be present in the biomass in amounts exceeding those of the bacterioruberin pigment of the halophilic Archaea, the algal pigment contributes only a minor part to the overall colour of the brines because of its dense packaging (Oren and Dubinsky, 1994). From the data occurred on the present study, season II had the highest protein in the algal samples throughout the study period. This may be due to blooming of phytoplankton occurred by changes in temperature, nutrients and other physico-chemical parameters. Highest nutrient contents was accompanied by the reduction of phytoplankton biomass during season I and III is the major reason for the reduction of protein in these seasons. Lowest protein content was observed during season IV is followed by the heavy rainfall, lowest temperature, variations in pH and salinity along with addition of inorganic micronutrients considered as
limiting factors affecting the phytoplankton to reduce their protein content. These results agree with the result of Reginald (2003) for Thamaraikulam saltpan, Kanyakumari District, India and Touliabah et al. (2010) for Jeddah Coast – Red Sea, Saudi Arabia.

Pigment composition is an important factor in the physiological behavior and can be used to measure growth as well as productivity of algae. Pigments are one of the cellular compounds specially chlorophyll, that is used for estimating biomass of microalgae in culture (Gireesh, 2009). Light intensity is a key induction parameter for chlorophyll production in algae. The chlorophyll - a content in an algal cell is not constant and varies with the nutritional state of cell (Creswell, 2010). Chlorophyll - a is the principal pigment in plants. As a biomass indicator of aquatic microalgae which support food webs in the sea. It is probably the most frequently measured biochemical parameter in Oceanography.

Chlorophyll is heterogenically bound to other compounds in the chloroplast and at least two or even three times fractions of chlorophyll exist in the chloroplast. Therefore different solvents having different polarities can extract different types of chlorophyll (Deroche and Briantais, 1974). Chlorophyll content were extracted from algal pellets with ice cold acetone and assayed (Arnon, 1949). The cell membrane gets ruptured because of organic solvent (acetone), thus extracting β-carotene along with chlorophyll. Chlorophyll molecule contains four nitrogen atoms in its structure and therefore it becomes very difficult for the cell organelles to synthesize chlorophyll into the absence of nitrogen. When a cell is depleted of water due to high salinity, photosynthesis also gets adversely affected. Hence the
chlorophyll content was decreased with the increase in salinity. A gradual reduction and density of plankton were observed with an increase in salinity and temperature which coincided with decrease in dissolved oxygen concentration (Rahaman, 2006). Chlorophyll catalyzed singlet oxygen production under high salinity. The decrease in chlorophyll content was prominent which might be due to sudden exposure of cells to high irradiation were reported by Pisal and Lele (2005). The most common function among all carotenoids is that of an accessory pigment, serving in the process of light absorption by the highly organized chlorophyll – carotenoid antenna protein. Accumulation of carotenoids in intracellular of Dunanliella salina to the photoprotective function of these compounds which that chlorophyll - a can be protected from bleaching in high light by β-carotene, which quenches the singlet oxygen generated during photo-oxidation (Borowitzka and Borowitzka, 1986; Foote et al., 1970; Bar et al., 1995). According to Singh and Chandra (1988) the green alga Chlorella is one of the richest chlorophyll sources which contain 50 – 60% protein. Turpin (1991) reported that the increase in chlorophyll – a content under nitrogen deficiency shows the relationship between the chlorophyll and nitrogen. Based on the results obtained in the present study, the maximum amount of chlorophyll – a was estimated in the condenser ponds than the other ponds throughout the study period. Chlorophyll - a revealed their maxima during season II which is considered the most productive season. Next to that season I showed the highest chlorophyll - a content almost same as season II. This may be due to the algal blooming occurring during summer by the high temperature, pH, salinity and other parameters. There was a good positive
correlation between the temperature and salinity which lead the photosynthetic activity of the microalgae for the production of chlorophyll. Lowest chlorophyll - a content was expressed in season IV attributed to the rainy season. Likewise the season III values also almost same as season IV. The lowest chlorophyll - a content was influenced by the heavy rainfall. The rain water mix with the brine influence the variations in pH, salinity, temperature and BOD which create unfavourable condition for the growth and multiplication of microalgae and leads the disappearance of microalgae. It was the major cause of the lowest chlorophyll observation during the study period. The present study was confirmed with the reports of Reginald (2003),Aktan et al. (2005) and Touliabah (2010).

From these observations, the present study of surveying in Puthalam saltworks gives an idea about microalgal population, provide first hand knowledge about the co-existence of various phytoplankton to the peculiar environment offered by the solar saltpan, evaluate their interaction among themselves and provide useful information regarding their nature in various salinities of the ponds in order to survive for quality salt production. Another important fact learned through this study was, most of the marine algae prefer shelf-shade for its survival in salinity. More economically, ecologically and evolutionarily important microalgal species exist in the saltworks ecosystem, if the solar salterns are destroyed for other purpose, we will lose as significant source of microbial diversity. From this study, it is understood that, it is our prime duty to protect the wetlands and the microalgal species which are useful in saltworks.