CHAPTER-2

2.0. ISOLATION, ENUMERATION AND IDENTIFICATION OF PLANKTONS FROM THE SELECTED SALTPAN WATER

2.1. INTRODUCTION

Saltpan ecosystem is highly dynamic where the organisms are subjected to vulnerable physico chemical disturbances. Saltpans are unique enclosed ecosystems that are characteristically exposed to a wide range of environmental stress and perturbations manifest mainly through salinity changes. In the extreme a static physico – chemical conditions of these hypersaline habitats only a few plant and animal species can live. Saltpan ecosystem offers a number of unique ecological niches having a strange combination of environmental factors. Biodiversity is a measure of the health of ecosystems. In terrestrial habitats, tropical regions are typically rich whereas polar region support fewer species (Litchfield et al. 2009).

A microbe is any living thing that spends its life at a size visible sometimes only with a microscope. It is too tiny to be seen with the naked eye. Microbes are the oldest form of life on earth. Some types have existed for billions of years. They may live as individuals or cluster together in communities. Microbes include bacteria, viruses, fungi, algae, and protozoa. The microbial world is the largest unexplored reservoir of biodiversity on the Earth. Interest in the exploration of microbial diversity has been spurred by the fact that microbes are essential for life since they perform numerous functions essential for the
biosphere that include nutrient cycling and environmental detoxification. The vast array of microbial activities and their importance to the biosphere and to human economics provide strong rationale for understanding their diversity, conservation and exploitation for society (Hughes et al., 2001; Curtis et al., 2002; Curtis and Sloan, 2005). Though the oceans are invariably considered as largest saline body, environments are particularly those containing salt concentrations in excess of sea water (3.5% total dissolved salt). Many hypersaline bodies derive from the evaporation of sea water and are called thalassic (Das Sharma and Arora, 2001).

The solar pans are man-made seasonal ponds constructed mainly for the production of raw salt along sides of creeks and other low lying coastal areas. These ponds offer an experimental system with an extreme environmental conditions and strong gradient in biodiversity of primary and secondary producers. It is among the most simplified ecosystem for the simple reason that the number of species at any trophic level is low (Williams, 1991). While some biological studies of salt pan is carried out elsewhere (Rahman, 2006), there is very little information available on the plankton community of the west coast of India (Ramamoorthy and Thangaraj, 1980; Mustafa et al., 1999) Marine salterns are habitats for a large variety of halophilic or halotolerant bacteria that develop throughout the entire gradient of salt concentration (Ollivier et al., 1994). While some biological studies of salt pan is carried out elsewhere (Rahman, 2006), there is very little information available on the plankton community of the west coast of India (Ramamoorthy and Thangaraj, 1980; Mustafa et al., 1999).
Halophiles include fungi, diatoms, bacteria and cyanobacteria, which occur as free forms or in associations called “mats”. From the biological point of view, the ponds in a salt pan can be divided into three main categories namely primary condensers, secondary condensers and crystallizers. Here the brine is evaporated to about three times its original salinity. These ponds are characterized by micro flora similar to that of seawater, with a great species diversity and low number of individuals of each species. In the first ponds most bacteria are slightly halophilic, whereas in the intermediary ponds, where the seawater is concentrated to a salinity of about 10 to 20% NaCl, most of the bacteria are moderately halophilic. This intermediate environment contains the greatest numbers of organisms. The last ponds are inhabited by extremely halophilic organisms including aerobic members of the archaea (Zeikus, 1983).

It is well established by the observations of numerous investigators that the size and shape of halophilic bacteria may be influenced by the concentration of the salt present. As the concentration of the salt is varied, rod-like forms become elongated or bulbous, or even appear as cocci (Flannery, 1956).

Spruit and Pijper (1952) found even flat, curved, ribbon forms. When other salts are substituted for sodium chloride, extreme pleomorphism may result (Flannery, 1953). At higher salinities (3 to 7 times of seawater) the brine becomes dark cloured and supports dense algal populations (mainly Dunaliella), on which the brine shrimp *Artemia* sp. Bacteria in this brine are mainly moderate halophiles and present as a very heterogeneous community with many species (Rodriguez *et al.*, 1981). *Aphanothece halophytica* are blue-green algae (cyanobacteria), photosynthetic, and exist as single cells or colonies of cells,
whose colors range from green to blue-green to yellow. *Aphanothece halophytica*, harmless in desired biological systems, is common in both communities of the concentrating ponds, but grows and reproduces best in intermediate salinities and do not survive in crystallizers. Disturbances often cause *Aphanothece halophytica* to reproduce at high rates, exclude competing species, and release massive quantities of mucilage highly damaging in the ponds of intermediate and high salinity, and crystallizers (Davis, 2009).

At moderately high salinities dense populations of green algae are supported. These are aerobic, photosynthetic, unicellular eukaryotic microorganisms, some species of which produce large quantities of orange-coloured b-carotene at high salinities. Most species of green algae are moderate halophiles, with only a few extremely halophilic species, e.g. *Dunaliella salina* and *Asteromonas gracilis*, which can grow even in saturated NaCl. Diatoms are algae surrounded by silica cell walls and are commonly found but rarely abundant in hypersaline environments (Das Sarma and Priya Arora, 1997).

Zooplankton are often an important link in the transformation of energy from producers to consumers due to their large density, drifting nature, high group or species diversity and different tolerance to the stress (Tatrai *et al.*, 1997).

The objective of this part of the work is to survey the important biological organisms namely halophilic bacteria, phytoplankton and zooplankton in the selected saltworks of Kanyakumari, Thoothukudi and Ramnad saltpan.
2.2. MATERIAL AND METHODS

2.2.1. Collection of sample

The water samples were collected from the selected salt pans of Kovalam, Puthalam, Swamythoppu, Thoothukudi and Ramnad. The samples were collected by aseptically in sterile bottles and transported to the lab for further study.

2.2.2. Isolation of halophilic bacteria

The collected samples were diluted by serial dilution technique. From the serially diluted samples \(10^{-4}\) to \(10^{-6}\), 0.1 ml was aseptically transferred and spread plated on halophilic agar medium. The plates were incubated at 37°C for 10 - 15 days. After incubation period, the colonies were picked up and transferred to halophilic agar plate for the purpose of pure culture and then the isolates were streaked in slants and were incubated at 39°C until growth was observed and then the slants were stored at 4°C for further studies.

2.2.3. Identification of the isolate

The isolated bacterial strains were identified by the detailed procedure described by Holt et al., (1996) and are as follows:

2.2.3.1. Biochemical test

i. Gram’s staining

The different bacterial cultures (16 to 18 hrs) were smeared on a clean glass slide and heat fixed. The smears were flooded with crystal violet for a minute and the stain was washed off using distilled water. The smears were flooded with Gram’s stain iodine solution (fixative) for a minute and rinsed with
distilled water, decolourized with acetone alcohol and rinsed out and the smears were counter stained with saffranin, air dried and examined under the oil immersion objective. Gram positive bacteria were purple or violet and Gram negative bacteria were red when observed.

ii. Motility test

A depression slide and the cover glass were located well. A small drop of bacterial culture was spreaded around the edges of the cover glass and inverted carefully by lowering it towards the depression slide until it made contact with vaseline, which was spread around the concavity slide. The slide was turned upright and the drop of culture remained suspended from the cover glass, which was then observed under oil immersion microscope.

iii. Indole production test

Tryptone broth tubes were prepared and sterilized at 121°C for 15 minutes. The culture was then inoculated and incubated at 37°C for 24 – 48 hours. After the incubation period, a few drops of (0.2 ml) Kovac’s reagent was added into the tubes and the result was observed. The development of bright red colour at the interface of reagent and medium indicates the presence of indole and constitutes positive test. The absence of colour change at this interface indicates the negative test.

iv. Methyl red test

The MR-VP broth tubes were prepared and sterilized at 121°C for 15 minutes. The culture was then inoculated into the tubes containing sterilized MR-VP broth medium and incubated at 37°C for 24 – 48 hrs. After the incubation
period, about 5 – 6 drops of methyl red indicator solution was added. The
development of stable bright red colour of the indicator indicates sufficient acid
production and constitutes a positive reaction. A weekly positive test will be red
orange. Yellow orange colour indicates a negative reaction.

v. Citrate utilization test

Simmons citrate agar was prepared and dispensed on test tubes and
sterilized at 121°C for 15 minutes and allowed to set as slope. The culture was
then inoculated into the tubes containing Simmons citrate agar slants (Stabbed
into the bud and streaked on the surface of slants) and incubated at 37°C for 24 –
48 hours. After the incubation period, the development of intense blue colour
from the original green colour of the medium indicates the ability of the organism
to utilize citrate as carbon source and constituted the positive reaction. The
absence of blue colour indicates negative reaction.

vi. Urea hydrolysis

The isolates were inoculated in to urea agar slant and incubated at 37°C
for 24 to 48 hours. After incubation the results were observed.

vii. Nitrate reduction test

Nitrate broth was prepared and 5ml was dispensed in test tubes. The
isolates were inoculated into nitrate broth. The tubes were then incubated at 37°C
for 96 h. After incubation, 0.1 ml of sulphonilic acid and alpha naphthalamine
were added and mixed well. Finally, the results were observed.
viii. Starch hydrolysis test

Starch agar plates containing loopful of culture was incubated for 24 hours at 37°C. After incubation, the plates were flooded with iodine solution clear zone around the growth indicated positive hydrolysis and no change of starch resulted in blue colour was negative.

ix. Casein hydrolysis test

Skim – milk agar plates were prepared and streaked with the bacterial isolates. The plates were then incubated at 37°C for 24 – 48 hours and the zone formation was observed.

x. Catalase test

A nutrient agar slant was inoculated with the test culture and was incubated at 37°C for 24 hours. Then, 1 ml of 3% hydrogen peroxide was trickled down the slant and was examined immediately and after 5 minutes observation for the evolution of bubbles, which indicates a positive test.

xi. Carbohydrate fermentation test

The test organism was inoculated in a basal medium with glucose and incubated at 37°C for 48 hours. Acid production indicates colour change from green to yellow and gas production was detected by Durham’s tube.

2.2.4. Fixation of sample

The samples were fixed at the estuarine field itself using 4% formalin for further analysis.
2.2.5. Concentration of sample

The samples were concentrated by centrifugation method. Here 50 ml of sample from each bottle were transferred into a clean centrifuge tube. The tubes were centrifuged at 4000 rpm for 10 minutes using REMI-R 8C Laboratory Centrifuge. The supernatant was discarded and 1 ml of pellet was transferred into a fresh eppendorff’s tubes and the tubes were marked.

2.2.6. Observation of phytoplankton

The observation of phytoplankton was done by exposing the concentrated sample to the Light microscope (COSLAB). Here one drop of concentrated and thoroughly mixed sample was transferred to a clean glass slide. The sample was gently covered by applying a clean cover slip over it. Then the slide was placed on the microscopic stage and the phytoplankton present was observed under 40X magnification. The Phytoplankton observed under microscope were captured with the help of a digital camera (MDCE-5C) and the pictures were saved to the attached computer. These captured pictures were further analyzed for identification of Phytoplankton.

2.2.7. Observation of zooplankton

The observation of Zooplankton was done by exposing the concentrated sample to the Light microscope (COSLAB). Here one drop of concentrated and thoroughly mixed sample was transferred to a clean glass slide. The sample was gently covered by applying a clean cover slip over it. Then the slide was placed on the microscopic stage and the zooplanktons were observed under 10X magnification. The Zooplankton observed under microscope was captured with
the help of a digital camera (MDCE – 5C) and the pictures were saved to the attached computer. These captured pictures were further analyzed for identification of Zooplankton.

2.2.8. Identification of phytoplankton and zooplankton

The identification of Phytoplankton and Zooplankton were carried out with the help of standard books and identification manuals. The standard books followed for identification are:


- Zooplankton of the Atlantic and Gulf coasts –Johnson and Allen and the website, WORMS (World Register of Marine Species).

2.2.9. Counting of bacteria

Calculate the number of bacteria (CFU) per millilitre of sample by dividing the number of colonies by the dilution factor multiplied by the amount of specimen added to liquefied agar.

\[
\text{Number of colony} = \frac{\text{Number of cells counted}}{\text{Volume of sample taken}} \times \text{dilution factor}
\]
2.2.10. Counting of phytoplankton cells

The concentrated samples were mixed well before being exposed to counting. The counting of cells was performed by Haemocytometer method (H-Slide method) and the values were calculated using the following formula;

\[
\text{Total no. of cells/ml} = \frac{\text{No. of cells counted}}{\text{No. of chambers counted}} \times \text{dilution factor} \times 10000
\]

2.2.11. Counting of zooplankton

The quantitative enumeration of the zooplankton was carried out with the help of a Sedgwick-Rafter (S-R) counting cell which is 50mm long, 20mm wide and 1mm deep. Before filling the S-R cell with sample, the cover glasses were diagonally placed across the cell and then samples were transferred with a large bore pipette so that no air bubbles in the cell covers were formed. The S-R cell was stunned for at least 15 minutes to settle zooplankton. The plankton on the bottom of the S-R cell was enumerated by compound microscope. By moving the mechanical stage, the entire bottom of the slide area was examined carefully. To achieve a random sampling, each time 3 fields were examined for each sample and an average of the counts had been recorded. The organisms thus counted, were expressed as cells per litre of the sample. From each sample 20 cells counts in 3 slides have been made to achieve random counts and an average of the counts has been recorded. Number of plankton (zooplankton) in the S-R cell was derived from the following formula

\[
\text{No./ml} = \frac{C \times 1000 \text{mm}^3}{L \times D \times W \times S}
\]
Where, \( C \) = Number of Organisms Counted; \( L \) = length of each strip (S-R cell length) in mm; \( D \) = depth of a strip (Whipple grid image width) in mm; \( W \) = Width of each strip (mm); \( S \) = Number of strip counted. The number of cells per mm was multiplied by a correction factor to adjust the number of organisms per litre (APHA, 1976).

### 2.2.12 Gene sequencing of halophilic bacteria

The bacterial strain *Paracoccus haloplilus* and *Paracoccus saliphilus* were finally identified by 16S rRNA sequence analysis. All the PCR reaction was carried out by Genei Biotech Ltd (Bangalore). The obtained nucleotide 16S rRNA sequence of the bacterial isolate was analyzed using Basic Local Alignment Search Tool (BLAST) and the phylogenetic tree was constructed using 16S rRNA sequence of others obtained from the NCBI data base.
2.3. RESULTS

The biological organisms in the selected saltpans of Kovalam, Puthalam, Swamythoppu, Thoothukudi and Ramnad were observed and recorded for a study period from May 2013 to April 2015. The results are given below.

2.3.1. Identified halobacterial species in the reservoir ponds of the selected saltpans

Survey of halobacteria was undertaken in all the 5 sampling stations during the study period. Twenty two species of halobacteria namely Serratia sp., *Shewanellamarisflavi* sp., *Halobacillus* sp.(1), *Halobacillus* sp.(2), *Halobacterium* sp.,(1), *Halobacterium* sp.(2), *Halococcus* sp.(1), *Staphylococcus* sp.(1), *Staphylococcus* sp.(2), *Paracoccus saliphilus*, *Staphylococcus* sp.(3), *Vibrio* sp., *Halobacillus* sp.(3), *Aeromonas* sp., *Bacillus* sp., *Pseudomonas* sp., *Salinococcus* sp., *Comamonas* sp., *Staphylococcus* sp.(4), *Paracoccus halophilus* and *Natranobacterium* sp. were identified in most of the saltpans (Table 2.1).

2.3.1.1. Total halobacterial density in the reservoir pond of kovalam saltpan (Station-1)

The total halobacterial numbers observed were ranged between 5.0 and 26.5 x $10^3$ CFU/ml during the study period in the Kovalam Saltpan. No halobacteria were observed during the months of November, December, January and February in the year 2013 and 2014. In 2014 and 2015 also during the months of November, December, January and February, there were no halobacteria were observed. A low number of 5.0 x $10^3$ CFU/ml was observed in
the year 2014 during the month of August. A high number (26.5 x 10^3 CFU/ml) was observed during May 2014 (Table 2.2).

2.3.1.2. Station-II (Puthalam)

A total halobacterial count of 11.3 – 26.2 x 10^3 CFU/ml was observed in Puthalam Saltworks. A low count of 11.3 x 10^3 CFU/ml was observed during the month of March 2014. A maximum number of 26.2 x 10^3 CFU/ml was observed during September 2013. No halobacteria were observed during the months of October, November, December, January and February 2013 and 2014. In 2014-2015, no halobacteria were observed during the months of October, November, December, January and February (Table 2.2).

2.3.1.3. Station-III (Swamythoppu)

In Swamythoppu Saltpan, a total halobacterial count of 6.6 – 27.2 x 10^3 CFU/ml was observed. A low number of 6.6 x 10^3 CFU/ml was observed during the month of August 2014. A maximum number of 27.2 x 10^3 CFU/ml was observed during May 2013. No halobacteria were observed during the months of July, August, September, October, November, December, January and February 2013-2014. In 2014 also there were no halobacteria observed during the months of October, November and December. In 2015 also there were no halobacteria during the month of January and February (Table 2.2).

2.3.1.4. Station-IV (Thoothukudi)

In Thoothukudi saltpan a total halobacterial count of 7.6 to 20.2 x 10^3 CFU/ml was observed. In the year 2013 during the month of August, October, November and December no halobacteria were observed. In the year 2013 a
maximum number of $19.8 \times 10^3$ CFU/ml was observed during the month of July. In the year 2014 the occurrence of the halobacteria was different. No bacteria were observed during the months of January, February, November and December. In the year 2014, a maximum number of $20.2 \times 10^3$ CFU/ml was observed in June. In 2015, there were no halobacteria were recorded during the months of January and February (Table 2.2).

2.3.1.5. Station-V (Ramnad)

The total halobacterial count observed was ranged between $3.4$ and $19.1 \times 10^3$ CFU/ml during the study period in the Ramnad Saltpan. No halobacteria were observed during the months of September, October, November, December, January, February and March in the year 2013-2014. In 2014-2015 also there were no halobacteria recorded during the months of October, November, December, January and February. A low number of $3.4 \times 10^3$ CFU/ml was observed in the year 2014 during the month of July. A high number of $19.1 \times 10^3$ CFU/ml was observed in June 2013 (Table 2.2).

2.3.2. Total halobacterial density in the condenser pond of the saltpans

Survey of halobacteria was undertaken in all the 5 sampling stations during the study period. Seven species of halobacteria namely *Halobacillus* sp.(1), *Halobacterium* sp.(1), *Paracoccus halophiles*, *Halobacterium* sp.(2), *Paracoccus saliphilus*, *Halobacillus* sp.(2) and *Halococcus* sp. were identified in most of the saltpans (Table 2.3).
2.3.2.1. Station-I (Kovalam)

Kovalam Saltpan had the highest halobacterial count (20.1x10^4 CFU/ml) during the month of August 2013. The lowest halobacterial count (9.8 x 10^4 CFU/ml) was observed during the month of March 2015. No halobacteria were observed during the months of October, November, December, January and February in the year 2013 and 2014.

2.3.2.2. Station-11(Puthalam)

In Puthalam Saltpan, a total halobacterial count observed was 12.5-20.7 x 10^4 CFU/ml. There were no halobacteria during the month of October, November and December 2013, January, February, October, November and December 2014 and January and February 2015. The lowest halobacterial count (12.5 x 10^4 CFU/ml) was observed during the month of March 2015. The highest count (20.7 x 10^4 CFU/ml) was observed during the month of June 2014.

2.3.2.3. Station-111(Swamythoppu)

A total halobacterial count observed in Swamythoppu Saltpan was 8.3-29.3 x 10^4 CFU/ml. Halobacteria were not observed during the month of October, November and December 2013, January, February, October, November and December 2014 and January and February 2015. The lowest halobacterial number (8.3 x 10^4 CFU/ml) was observed during the month of March 2015. The highest number of 29.3 x 10^4 CFU/ml was observed during the month of July 2013.
2.3.2.4. Station-IV (Thoothukudi)

In Thoothukudi Saltpan, a total halobacterial count observed was 9.7-24.1 x 10^4 CFU/ml. The lowest number (9.7 x 10^4 CFU/ml) was observed during the month of March 2014. The highest number (24.1 x 10^4 CFU/ml) was observed during the month of May 2013. No halobacteria were observed during the rainy season i.e., October, November and December 2013 and 2014. During January and February 2014 and 2015 also no halobacteria were observed.

2.3.2.5. Station-V (Ramnad)

Ramnad Saltpan had the highest count (20.4 x 10^4 CFU/ml) during the month of September 2013. The lowest halobacterial count (9.3 x 10^4 CFU/ml) was observed during the month of April 2014. No halobacteria were observed during the months of October, November and December 2013 and 2014. During January and February 2014 and 2015 also no halobacteria were observed.

2.3.3. Total halobacterial density in the crystallizer pond of the saltpans

The halobacterial density was measured in the crystallizer ponds of the five sampling stations during the study period. There was no halobacteria observed during the months of October, November, December, January and February. But the halobacteria were observed in other months (Table 2.4).

2.3.3.1. Station-1(Kovalam)

In the crystallizer pond of Kovalam saltpan a total halobacterial count of 11.1 - 31 x 10^5 CFU/ml was observed. A high number of 31 x 10^5 CFU/ml was observed during the month of March 2015 and a low number of 11.1 x 10^5
CFU/ml was observed during the month of March 2014. There were no halobacteria observed during the rainy season (October, November, December, January and February).

2.3.3.2. Station-II (Puthalam)

A total halobacterial count observed in the crystallizer of Kovalam Saltpan was $8.6 - 34.4 \times 10^5$ CFU/ml. A high number of $34.4 \times 10^5$ and a low number of $8.6 \times 10^5$ CFU/ml were observed during May 2013 and March 2015 respectively. No halobacteria were observed in the months of October, November, December, January and February.

2.3.3.3. Station-III (Swamythoppu)

The number of total halobacterial found in the crystallizer pond of Swamythoppu Saltpan was 4.5 and $32.9 \times 10^5$ CFU/ml. A high number ($32.9 \times 10^5$ CFU/ml) was observed during September 2013 and a low number of $4.5 \times 10^5$ CFU/ml was observed during the month of March 2014. No halobacteria were observed during October, November and December 2013-2014 and January and February 2014-2015.

2.3.3.4. Station-IV (Thoothukudi)

The total halobacterial count in the crystallizer pond of Thoothukudi fluctuated between 6.3 and $34.1 \times 10^4$ CFU/ml and no halobacteria were observed during October, November and December 2013-2014 and January and February 2014-2015. The low number of $6.3 \times 10^4$ CFU/ml was observed during the month of March 2015. A high number of $34.1 \times 10^4$ CFU/ml was observed during the month of June 2013.
2.3.3.5. Station-V (Ramnad)

A total halobacterial count observed in the crystallizer of Ramnad saltpan varied between $5.8 - 38.5 \times 10^5$ CFU/ml. A high density of $38.5 \times 10^5$ CFU/ml was observed during March 2014 and a low number of $5.8 \times 10^5$ CFU/ml was observed during March 2015. Halobacteria were not observed during the months of October, November and December 2013-2014 and January and February 2014-2015.

2.3.4. Survey of microalgae in the reservoir of kovalam saltpans

Survey of Phytoplankton was undertaken in all the 5 sampling stations (Reservoir) during the study period. The results are given below.

2.3.4.1. Kovalam Saltpan

Survey of microalgae in the reservoir of Kovalam saltpan during 2013-2014 is given in table-5. A total of 24 genera were identified which come under 4 divisions such as Bacillariophyta, Chlorophyta, Dinophyta and Cyanophyta.

During this study period, which extended from May 2013 to April 2014, *Pleurosigma* sp. was present in maximum numbers with an average cell count of $1.837 \times 10^5$ cells ml$^{-1}$ and *Bidulphia* sp. was present in the minimum numbers with an average cell count of $0.118 \times 10^5$ cells ml$^{-1}$. The other species present were *Asterionella* sp. (0.722), *Coscinodiscus* sp. (1.115), *Chaetoceros* sp. (0.674), *Thalassionema* sp. (0.333), *Surirella* sp. (1.284), *Nitzschia* sp. (1.376), *Fragilaria* sp. (0.377), *Navicula* sp. (0.414), *Pinnularia* sp. (0.801), *Rhizosolenia* sp. (1.037), *Spirulina* sp. (0.568), *Ceratium* sp. (1.409), *Ceratium fusus* (0.463), *Pronoctiluca acuta* (0.29), *Prorocentrum* sp. (0.291), *Diploneis* sp. (0.434),
Chlamydomonas sp. (0.767), Prorocentrum micans (0.767), Dunaliella sp. (0.195), Chlorella sp. (0.892), Pediastrum sp. (0.402), Chroococcus sp. (0.323), sp. (0.661), Cocchochloris sp. (0.195), Oscillatoria sp. (0.455), Anabaena sp. (0.221) and Microcystis (0.693×10^5 cells ml^-1) (Table 2.5).

During the study period of May 2014 to April 2015, a total number of 24 genera were observed which comes under 4 divisions such as Chlorophyta, Cyanophyta, Bacillariophyta and Dinophyta.

During this period, Nitzschia sp. was present in maximum number which was 1.613×10^5 cells ml^-1 and Cylindrotheca sp. was present in minimum number (0.112no/ml). The species present were Asterionella sp. (0.609), Coscinodiscus sp. (0.641), Chaetoceros sp. (0.617), Thalassionema sp. (0.409) Surirella sp. (1.581), Fragilaria sp. (0.222), Navicula sp. (0.827), Pinnularia sp. (0.596), Rhizosolenia sp. (0.893), Spirulina sp. (0.596), Ceratium sp. (0.291), Pronoctiluca acuta (0.453), Diploneis sp. (0.540), Prorocentrum sp. (0.344), Prorocentrum micans (0.307), Dunaliella sp. (0.782), Chlorella sp. (0.811), Pediastrum sp. (0.424), Cocchochloris sp. (0.112), Oscillatoria sp. (0.528) and Microcystis sp. (0.707×10^5 cells ml^-1) (Table 2.6).

2.3.4.2. Puthalam Saltpan

The total genera identified were 25 which come under 4 divisions such as Bacillariophyta, Chlorophyta, Dinophyta and Cyanophyta (Table 2.7 and 2.8).

During the study period of May 2013 to April 2015, Nitzschia sp. was present in maximum numbers with an average cell count of 1.505×10^5 cells ml^-1.
and *Dunaliella* sp. was present in minimum number with an average cell count of $0.110 \times 10^5$ cells ml$^{-1}$.

### 2.3.4.3. Swamythoppu Saltpan

A total of 28 genera were identified during the study period of May 2013 to April 2015, which comes under 4 divisions such as Bacillariophyta, Chlorophyta, Dinophyta and Cyanophyta.

During the study period May 2013 to April 2014, *Navicula* sp. was present in maximum numbers with an average cell count of $1.584 \times 10^5$ cells ml$^{-1}$ and *Bidulphia* sp. was present in minimum numbers with an average cell count of $0.333 \times 10^5$ cells ml$^{-1}$ (Table 2.9).

During the study period May 2014 to April 2015, *Pleurosigma* sp. was present in maximum numbers with an average cell count of $1.654 \times 10^5$ cells ml$^{-1}$ and *Coccochloris* sp. was present in minimum numbers with an average cell count of $0.110 \times 10^5$ cells ml$^{-1}$ (Table 2.10).

### 2.3.4.4. Thoothukudi Saltpan

The result of the survey of phytoplankton in the reservoir of Thoothukudi saltpan, during the study period of May 2013- April 2015 is given in table 2.11 and 2.12. In total, 28 genera were identified, which comes under 4 divisions such as Bacillariophyta, Chlorophyta, Dinophyta and Cyanophyta.

During the study period of May 2013 to April 2015, *Surirella* sp. was present in maximum numbers with an average cell count of $1.733 \times 10^5$ cells ml$^{-1}$
and *Nitzschia* sp. was present in minimum numbers with an average cell count of $0.108 \times 10^5$ cells ml$^{-1}$ (Table 2.11 and 2.12).

### 2.3.4.5. Ramnad Saltpan

A total of 28 genera were identified during the study period of May 2013 to April 2014 and 26 genera were identified during the study period of May 2014 to April 2015 which comes under 4 divisions such as Bacillariophyta, Chlorophyta, Dinophyta and Cyanophyta.

During the study period of May 2013 to April 2014, *Navicula* sp. was present in maximum numbers with an average cell count of $2.728 \times 10^5$ cells ml$^{-1}$ and *Coccochloris* sp. was present in minimum numbers with an average cell count of $0.158 \times 10^5$ cells ml$^{-1}$ (Table 2.13).

During the study period of May 2014 to April 2015, *Pleurosigma* sp. was present in maximum numbers with an average cell count of $1.686 \times 10^5$ cells ml$^{-1}$ and *Prorocentrum* sp. was in minimum numbers with an average cell count of $0.153 \times 10^5$ cells ml$^{-1}$ (Table 2.14).

### 2.3.5. Survey of microalgae in the condenser of selected saltpans

Survey of Phytoplankton was undertaken in all the 5 sampling stations (condenser) during the study period. The results are given below.

#### 2.3.5.1. Kovalam saltpan

In Kovalam saltpan, a total of 11 and 8 genera of phytoplankton were identified during 2013-2014 and 2014-2015 respectively. They include 4 divisions such as Bacillariophyta, Chlorophyta, Dinophyta and Cyanophyta.
During the study period of May 2013 to April 2014, *Dunaliella* sp. was maximum numbers with an average cell count of $1.996 \times 10^5$ cells ml$^{-1}$ and *Chlorococcum* sp. was present in minimum numbers with an average cell count of $0.237 \times 10^5$ cells ml$^{-1}$. The other species present were *Pleurosigma* sp. (0.964), *Chaetoceros* sp. (0.978), *Nitzschia* sp. (1.093), *Pediastrum* sp. (0.873), *Navicula* sp. (0.499), *Chlorococcum* sp. (0.237), *Coccochloris* sp. (0.810), *Prorocentrum* sp. (1.090), *Oscillatoria* sp. (0.620) and *Microcystis* sp. (0.329 $\times 10^5$ cells ml$^{-1}$) (Table 2.15).

During the study period of May 2013 to April 2014, *Dunaliella* sp. was present in maximum number ($2.229 \times 10^5$ cells ml$^{-1}$) and *Chaetoceros* sp. was present in minimum numbers ($0.666 \times 10^5$ cells ml$^{-1}$). The other species present were *Pleurosigma* sp. (0.719), *Nitzschia* sp. (0.668), *Pediastrum* sp. (0.990), *Prorocentrum* sp. (1.595), *Oscillatoria* sp. (1.072) and *Microcystis* sp. (0.754 $\times 10^5$ cells ml$^{-1}$) (Table 2.16).

### 2.3.5.2. Puthalam Saltpan

During the study period 2013-14 and 2014-15, a total of 8 and 9 genera respectively were identified which comes under 4 divisions such as Bacillariophyta, Chlorophyta, Dinophyta and Cyanophyta.

During the study period of 2013 – 2014, *Dunaliella* sp. was maximum with an average cell count of $2.251 \times 10^5$ cells ml$^{-1}$ and followed by *Nitzschia* sp. (1.388), *Coccochloris* sp. (1.111), *Chaetoceros* sp. (1.041), *Pleurosigma* sp.(0.925), *Prorocentrum* sp. (0.849), *Pediastrum* sp. (0.408) and *Oscillatoria* sp.( 0.327 $\times 10^5$ cells ml$^{-1}$) (Table 2.17).
During the study period of 2014 – 2015, *Dunaliella* sp. was found maximum with an average cell count of $2.014 \times 10^5$ cells ml$^{-1}$ which was followed by *Pleurosigma* sp. (0.952), *Coccochloris* sp. (0.772), *Nitzchia* sp.(0.756), *Anabaena* sp.(0.740), *Prorocentrum* sp. (0.554), *Oscillatoria* sp. (0.221), *Chaetoceros* sp.(0.212) and *Pediastrum* sp. ($0.152 \times 10^5$ cells ml$^{-1}$) (Table 2.18).

### 2.3.5.3. Swamythoppu Saltpan

In the condenser of Swamythoppu saltpan, the survey of phytoplankton during the study period of May 2013- April 2015 is given in table 19 and 20. A total of 7 genera were identified which comes under 3 divisions such as Bacillariophyta, Chlorophyta and Cyanophyta (Table 2.19 and 2.20).

During the study period of May 2013 - April 2014, *Dunaliella* sp. was found in maximum numbers with an average cell count of $1.757 \times 10^5$ cells ml$^{-1}$ and the other species present were *Chaetoceros* sp. (0.342), *Nitzschia* sp. (0.575), *Pleurosigma* sp. (0.744), *Chroococcus* sp.(0.761), *Anabanaena* sp.(1.298) and *Oscillatoria* sp.( $0.332 \times 10^5$ cells ml$^{-1}$).

During the study period May of 2014 - April 2015, *Dunaliella* sp. was found in maximum numbers with an average cell count of $1.163 \times 10^5$ cells ml$^{-1}$ and the other species present were *Chaetoceros* sp. (0.768), *Nitzschia* sp. (1.336), *Pleurosigma* sp.( 0.460), *Chroococcus* sp.(0.494), *Coccochloris* sp. (1.959 ) and *Oscillatoria* sp. (0.504 $\times 10^5$ cells ml$^{-1}$).
2.3.5.4. Thoothukudi saltpan

A total of 9 genera were identified during the study period of May 2013 to April 2015 which comes under 4 divisions such as Bacillariophyta, Chlorophyta, Dinophyta and Cyanophyta.

During the study period of May 2013 to April 2014, Dunaliella sp. was present in maximum numbers with an average cell count of $1.709 \times 10^5$ cells ml$^{-1}$. Coccolithus sp. was present in minimum numbers with an average cell count of 0.292 no/ml. The other species were Pleurosigma sp. (0.333), Chaetoceros sp. (1.420), Nitzschia sp. (0.447), Pediastrum sp. (0.299), Prorocentrum sp. (0.638), Oscillatoria sp. (0.938) and Microcystis sp. ($0.541 \times 10^5$ cells ml$^{-1}$) (Table 2. 21).

During the study period of May 2014 to April 2015, Dunaliella sp. was present in maximum numbers with an average cell count of $1.789 \times 10^5$ cells ml$^{-1}$ and Nitzschia sp. was in minimum numbers with an average cell count of $0.355 \times 10^5$ cells ml$^{-1}$. The other species were Pleurosigma sp. (1.091), Chaetoceros sp. (0.515), Pediastrum sp. (0.511), Chroococcus sp. (0.648), Chlorococcum sp. (1.314), Coccolithus sp. (0.419), Prorocentrum sp. (0.493) Oscillatoria sp. (0.788) and Microcystis sp. ($0.571 \times 10^5$ cells ml$^{-1}$) (Table 2.22).

2.3.5.5. Ramnad Saltpan

A total of 9 genera were identified during the study period of May 2013 to April 2014 and 11 genera were identified during the study period of May 2014 to April 2015 which comes under 4 divisions such as Bacillariophyta, Chlorophyta, Dinophyta and Cyanophyta.
During the study period of May 2013 to April 2014, *Dunaliella* sp. was present in maximum numbers with an average cell count of $1.343 \times 10^5$ cells ml$^{-1}$. *Pleurosigma* sp. was present in the minimum numbers with an average cell count of $0.192 \times 10^5$ cells ml$^{-1}$ (Table 2.23).

During the study period of May 2014 to April 2015, *Dunaliella* sp. was present in maximum numbers with an average cell count of $1.759 \times 10^5$ cells ml$^{-1}$ and *Pleurosigma* sp. was present in the minimum numbers with an average cell count of $0.333 \times 10^5$ cells ml$^{-1}$ (Table 2.24).

### 2.3.6. Survey of microalgae in the crystallizer of selected saltpans

Survey of Phytoplankton was undertaken in all the 5 sampling stations (crystallizer) during the study period 2013-2015. The results are given below.

#### 2.3.6.1. Kovalam saltpan

The study of phytoplankton in the crystallizer of Kovalam saltpan during 2013-2015 is given in table 2.25. A total number of 6 genera were identified which comes under 3 divisions such as Bacillariophyta, Chlorophyta and Cyanophyta.

During the study period of May 2013 to April 2014, *Dunaliella* sp. was present in maximum numbers with an average cell count of $4.400 \times 10^5$ cells ml$^{-1}$ (42.960%) and *Coccochloris* sp. was present in minimum numbers with an average cell count of $0.560 \times 10^5$ cells ml$^{-1}$ (5.462%). The other species were *Chaetoceros* sp. (0.893, 8.719%), *Pediastrum* sp. (2.99, 29.262%), *Chroococcus* sp. (0.779, 7.606%) and *Oscillatoria* sp. (0.613 $\times 10^5$ cells ml$^{-1}$, 5.985%).
During the study period of May 2014 to April 2015, *Dunaliella* sp. was present in maximum numbers with an average cell count of $5.209 \times 10^5$ cells ml$^{-1}$ (49.941%) and *Chaetoceros* sp. was present in minimum numbers with an average cell count of $0.63 \times 10^5$ cells ml$^{-1}$ (5.695%). The other species were *Pediastrum* sp. (3.023, 27.242%) *Chroococcus* sp. (0.641, 5.776%), *Coccochloris* sp. (0.945, 8.516%) and *Oscillatoria* sp. (0.646 $\times 10^5$ cells ml$^{-1}$, 5.821%).

### 2.3.6.2. Puthalam saltpan

A total of 4 genera were identified which comes under 3 divisions such as Bacillariophyta, Chlorophyta, and Cyanophyta (Table 2.26).

During the study period of May 2013 to April 2014 and May 2014 to April 2015, *Dunaliella* sp. was present in maximum numbers with an average cell count of 6.770 and $5.699 \times 10^5$ cells ml$^{-1}$. The other species were *Chaetoceros* sp. (0.493, 5.384%), *Coccochloris* sp. (0.984, 10.747%) and *Oscillatoria* sp. (0.909 noml, 9.928%) during 2013-2014 and *Chaetoceros* sp. (0.345, 4.526%), *Coccochloris* sp. (0.921, 12.082%) and *Oscillatoria* sp. (0.658 $\times 10^5$ cells ml$^{-1}$ (8.632%) during 2014-2015.

### 2.3.6.3. Swamythoppu saltpan

During the study period, A total of 4 genera were identified which comes under 3 divisions such as Bacillariophyta, Chlorophyta and Cyanophyta.

During the study period of May 2013 to April 2014 and May 2014 to April 2015, *Dunaliella* sp. was present in maximum numbers with an average cell count of 4.147 (62.118%) and $5.113 \times 10^5$ cells ml$^{-1}$ (76.530%) and *Coccochloris* sp.
sp. was present in minimum numbers with an average cell count of 0.549 (8.223%) and $0.313 \times 10^5$ cells ml$^{-1}$ (4.685%) during 2013-2014 and 2014-2015 respectively (Table 2.27).

2.3.6.4. Thoothukudi saltpan

The study of phytoplankton in the crystallizer of Thoothukudi saltpan during 2013-2015 is given in table 2.28. A total of 6 genera were identified which comes under 3 divisions such as Bacillariophyta, Chlorophyta and Cyanophyta.

During the study period of May 2013 to April 2014 and May 2014 to April 2015, *Dunaliella* sp. was present in maximum numbers with an average cell count of 5.100 (52.110%) and $5.578 \times 10^5$ cells ml$^{-1}$ (55.652%). *Chroococcus* sp. and *Coccochloris* sp. were present in minimum numbers with an average cell count of 0.421 (4.302%) and $0.608 \times 10^5$ cells ml$^{-1}$ (6.066%) respectively.

2.3.6.5. Ramnad saltpan

In Ramnad saltpan, a total of 5 and 4 genera were identified during 2013-2014 and 2014-2015 respectively which come under 3 divisions such as Bacillariophyta, Chlorophyta and Cyanophyta (Table 2.29).

During the study period of May 2013 to April 2014, *Dunaliella* sp. was in maximum numbers with an average cell count of $2.486 \times 10^5$ cells ml$^{-1}$ (51.630%) and *Coccochloris* sp. was present in minimum numbers with an average cell count of 0.221 no/ml (64.496%). The other species present were *Chaetoceros* sp. (0.595, 12.357%), *Chroococcus* sp. (0.644, 13.375%) and *Oscillatoria* sp. ($0.869 \times 10^5$ cells ml$^{-1}$ (18.048%).
During the study period of May 2014 to April 2015, *Dunaliella* sp. was in maximum numbers with an average cell count of $4.160 \times 10^5$ cells ml$^{-1}$ ($64.496\% \times 10^5$ cells ml$^{-1}$) and *Chroococcus* sp. was present in minimum numbers with an average cell count of $0.457 \times 10^5$ cells ml$^{-1}$ ($7.081\%$). The other species present were *Chaetoceros* sp. (1.088, 16.858%) and *Oscillatoria* sp. (0.749 $\times 10^5$ cells ml$^{-1}$, 11.605%).

### 2.3.7. Zooplankton density in the reservoir ponds of the selected saltpans

A study of zooplankton was undertaken in all the 5 sampling stations of Kovalam, Puthalam, Swamythoppu, Thoothukudi and Ramnad saltpans during the study period of 2013-2014 respectively are given in table 2.30-2.34.

During the study period 2013-2014, the maximum mean zooplankton number of 1913 no/lit and the minimum mean zooplankton numbers of 230 no/lit were recorded in Kovalam saltpan during the month of October and August respectively. In Puthalam saltpan, the maximum mean zooplankton number of 1277 no/lit and the minimum mean zooplankton number of 123 no/lit were observed during the month of December and August respectively. The maximum mean zooplankton density of Swamythoppu saltpan was 997 no/lit and the minimum mean zooplankton density was 113 no/lit, which were observed during the month of November and July respectively. The maximum mean zooplankton density of Thoothukudi saltpan was recorded as 1692 no/lit and the minimum mean zooplankton density was 683 no/lit, which were observed during the month of October and June respectively and the maximum mean zooplankton density of Ramnad saltpan was recorded as 1023 no/lit and the minimum mean zooplankton
density was 204 no/lit, which were observed during the month of December and July respectively.

During the study period 2014-2015, the maximum mean zooplankton density was 1577 no/lit and the minimum mean zooplankton density was 89 no/lit, which were observed during the month of December and August respectively. In the Puthalam saltpan, the maximum mean zooplankton density of 1437 no/lit and the minimum mean zooplankton density of 216 no/lit were recorded in the month of October and July respectively. The Swamythoppu saltpan recorded a maximum of 1314 no/lit and the minimum mean density of 143 no/lit was recorded during the month of October and July respectively. In Thoothukudi saltpan, a maximum of 1870 no/lit and the minimum mean density of 486 no/lit were recorded in the month of December and July respectively. The maximum mean density recorded in Ramnad saltpan was 916 no/lit and the minimum mean density was 163 no/lit, which were noted in the month of November and July respectively.

2.3.8. Zooplankton density in the condenser ponds of the selected saltpans

During the study period 2013-2014, the maximum mean zooplankton density of 54444 no/lit and the minimum mean zooplankton density of 21328 no/lit were recorded in Kovalam saltpan during the month of April and July respectively. In the Puthalam saltpan, the maximum mean zooplankton density of 53703 no/lit and the minimum mean zooplankton density of 24343 no/lit which were observed during the month of March and July respectively. The maximum mean zooplankton density of Swamythoppu saltpan was 54492 no/lit and the
minimum mean zooplankton density was 21336 no/lit which were observed during the month of April and July respectively. The maximum mean zooplankton density of Thoothukudi saltpan was 53719 no/lit and the minimum mean zooplankton density was 24383 no/lit, which were observed during the month of March and July respectively and the maximum mean zooplankton density of Ramnad saltpan was recorded as 50519 no/lit and the minimum mean zooplankton density was 23399 no/lit, which were observed during the month of April and August respectively.

During the study period of 2014-2015, the maximum mean zooplankton density of 57238 no/lit and the minimum mean zooplankton density of 28689 no/lit were recorded in Kovalam saltpan during the month of March and September respectively. In Puthalam saltpan, the maximum mean zooplankton density of 53617 no/lit, and the minimum mean zooplankton density of 18503 no/lit were observed during the month of April and July respectively. The maximum mean zooplankton density of Swamythoppu saltpan was 57289 no/lit and the minimum mean zooplankton density was 19411 no/lit, which were observed during the month of March and July respectively. The maximum mean zooplankton density of Thoothukudi saltpan was 53617 no/lit and the minimum mean zooplankton density was 18470 no/lit, which were observed during the month of April and July respectively and the maximum mean zooplankton density of Ramnad saltpan was recorded as 57772 no/lit and the minimum mean zooplankton density was 20823 no/lit, which were observed during the month of March and August respectively (Table 2.35-2.39).
2.3.9. Zooplankton density in the crystallizer ponds of the selected saltpans

During the study period 2013-2014, the maximum mean zooplankton density of Kovalam saltpan was 58199 no/lit and the minimum mean zooplankton density was 39388 no/lit, which were recorded during the month of April and August respectively. In Puthalam saltpan, the maximum mean zooplankton density was 57346 no/lit and the minimum mean zooplankton density was 37413 no/lit were observed during the month of March and August respectively. The maximum mean zooplankton density of 67421 no/lit and the minimum mean zooplankton density of 40045 no/lit were observed in Swamythoppu saltpan during the month of March and August respectively. The maximum mean zooplankton density of 57402 no/lit and the minimum mean zooplankton density of 37369 no/lit were observed in Thoothukudi saltpan during the month of March and August respectively and the maximum mean zooplankton density of Ramnad saltpan was 57646 no/lit and the minimum mean zooplankton density was 35471 no/lit, which were observed during the month of March and August respectively.

During the study period 2014-2015, the maximum mean zooplankton density of 59907 no/lit and the minimum mean zooplankton density of 37193 no/lit were observed during the month of April and August respectively. In Puthalam saltpan, the maximum mean zooplankton density recorded was 62609 no/lit and the minimum mean zooplankton density recorded was 35691 no/lit, which were observed in the month of April and August respectively. In Swamythoppu saltpan, a maximum of 62921 no/lit and the minimum mean density of 40298 no/lit were noted in the month of March and December respectively. In Thoothukudi saltpan, a maximum zooplankton density of 68872
no/lit and the minimum mean density of 39080 no/lit were recorded in the month of March and August respectively. The maximum mean density of zooplankton in Ramnad saltpan was recorded as 66572 no/lit and the minimum mean density was 38585 no/lit, which were observed in the month of March and August respectively (Table 2.40-2.44).

**Gene sequencing of halophilic bacteria**

The 16s ribosomal RNA gene was sequenced for selected strains about 1370bp and submitted in the National Center for Biotechnology Information (NCBI) in the following Accession Numbers KT962230 and KT962231.

GCTACGGCAGGCTTAACACATGCAAAGTGACGGAAGACTTCCGCTTTAGCGG
CGGACGGGTAAGTAGAACGCTGAGAAACGCCCTCTACATCACGAAATGCCAGGG
ACTTTGATTATACGGTATACGCCCCCTCCGGGAAAAAGAACTTTCGTTGAAGAGCTGGCC
CGGCTGGATTATGGAGTTGGGTATATGGCTACTCAAGGCCAGATTTCACTGGTAA
GGTATTGAGGATGCTACGCCAAGCTGGAAGTGAACAGCTGCACTTACGGG
AGGACGAAGTTGATAGAAATGGGACACCCGTATATCGGTGCACAGCCGGCAGTAAAG
GCTAGGCTTTTGTCGGAATACTTGGCGTAAGGCGCACTGAGGCGCGACGGAAGATTG
GGGGTTAAATCCCCGGAAGCTCGTCAAAACTACGTCTGGAGTT
TCGAGAGAGTGGATGATGGAATTCCAGATGTGAAATTCGTTAGATATTCGGAG
AACACAGTGCGAGAAGCGGCCTACCGCTGATACGACGCTGAGTTCCAAA
CTACTCGTGAGGGTACAGAGGTGATGGAGAGTGAATTCCGAGTGAAGTTAAAAT
GTAGATTTGGAGAACAAGCCAGAAAGCGGCTACCTGGGCTAGTACACTGGCAGC
TGAGGTTGGCAGAAAGTGGGGAAGGAAACAGGATTAAGATACCCCTGGTAGTTCAAGCCG
TAAACAGTGATGTCGATGCGGTAGCATTGCTATTCGTTAGCAGACACTTAGGGAAT
TAAAGCTACCCCGCTGGAGGTACGTTGCAAGAATTTACCTAAGAGATTTGGG
GGGCGACCAACAGGGTGAGCTGTTTTAATTGAGACCAAGGGCGGAGAACTTAC
CAAACCTGGTCACTCGGACAGCCGCTGTTTTTCTGGTAAGAGCCAG
AGCAAGGTGTGCAATGGTGCTCCCTAGTGACGAATCCGAAGAAAGTGTGAAATG
GCAAGCGGAAACCAGTCCTCAGTGGCCAGATCAAGTTGCCCAGTCTCTAGTGA
ACTCGGGAAGTAGATAGGGAGACTGGGATAGCTAAGCTTACCCGCTTTA
CCGTTGGGCTACACGCTACAAAGTGTGTTCAAGATGGGTATTATCCAAAAC
CATCTCGTCTGGATTGTCCCTGCAACTGACGGGAGAATTGGGAAATCGCTAGTA
ATCCGCGAAGACAGATGGCGCTGTTAATACCTTCCCGGCTTTTGCACACCCGGT
CAACACATGGGAGTTGTTTACCCGACGGGCCGTCGCTAACCTTTGGAAGGACACG
GACCACGGTACGATCCGTCGAAGCT
Figure: Dendogram of the sequenced data and retrieved sequence data
Paracoccus halophiles

GAA CGTTT GAT ACC TTG CT CA G A A C G A C G T C G C C G A G C C T A A C
A C A C G T G G G A A T A T G C C T C T C A G G G A A T G T C T G G G A A T C T G G
C G T G C G C C A A C C T T T T G G A G
Figure: Dendogram of the sequenced data and retrieved sequence data
2.4. DISCUSSION

The remarkable power of adaptive evolution of nature has permitted living organisms to extract nutrients and energy from a wide range of environments. In a moderate environment competition from other species is the most serious restriction to exploitation whereas in extreme environments physical conditions detrimental to essential biological process are the serious limitations. Hypersaline environment represents one such unique and interesting challenge to biological survival. A group of halophilic archaea is capable of outliving others in hypersaline environment; remarkably, these extremely halophilic organisms have abandoned the cell envelope (Lanyi, 1974).

The survival and the growth of halobacteria depend on the physico-chemical structure of a habitat. In the present study the total halobacterial density in reservoir ponds range from $3.4 \times 10^3$ CFU/ml to $26.6 \times 10^3$ CFU/ml in the saltworks chosen for investigations. The reservoir ponds of Kovalam saltwork had the highest halobacterial density ($26.5 \times 10^3$ CFU/ml) during the month of May in 2013. The lowest halobacterial density ($11.3 \times 10^3$ CFU/ml) was recorded in the reservoir ponds of Puthalam saltworks during the month of March in 2014. In Swamythoppu saltwork, the lowest halobacterial density ($6.6 \times 10^3$ CFU/ml) was recorded during the month of August in 2014. In reservoir ponds of Thoothukudi saltworks also the lowest halobacterial density of $7.6 \times 10^3$ CFU/ml was recorded during the month of April in 2015. In the reservoir ponds of Ramnad saltwork, the lowest halobacterial density ($3.4 \times 10^3$ CFU/ml) was observed during the month of July in 2014. In the same station the highest
halobacterial density \((19.1 \times 10^3 \text{ CFU/ml})\) was recorded during the month of July in 2014. These results clearly prove the ability of halobacteria to tolerate different salinities. The data also demonstrate the adaptability and ability of extreme halobacteria to withstand changes in residual salinities. Probably this is the reason for the persistence of halobacteria even in reservoir ponds where the salt concentration is rather low \((40 – 60.3 \text{ ppt})\) compared to that of crystallizer ponds.

Nissenbaum (1975), Aoust and Gerber (1974) and Rodriguez-Valera et al. (1981) have reported that extreme and moderate halophiles are found in hypersaline environments such as hypersaline lakes, solar salterns and seawater. Hypersaline lakes are geographically discontinuous and generally result from the concentration of seawater by solar evaporation. It seems possible that seawater could contain halobacteria of different types and thereby serve as the medium of dispersal between hypersaline sites.

During the present study period, the highest halobacterial density was observed at higher salinity in reservoir, condenser and crystallizer ponds and the reservoir ponds had the highest halobacterial density \((26.6 \times 10^3 \text{ CFU/ml})\). In the condenser ponds, the highest halobacterial density observed was \(29.3 \times 10^4 \text{ CFU/ml}\). But in the crystallizer ponds a high halobacterial density \((34.4 \times 10^5 \text{ CFU/ml})\) was recorded. This observation indicates that salinity is an important factor that determines the halobacterial survival and propagation in saltworks.

Forsyth et al. (1971) have reported the survival of a halotolerant bacterium in seawater at 35 ppt salinity level. Del moral et al. (1987) has isolated extremely halobacteria from seawater. Such bacteria were isolated from
freshwater rivers at least in relatively high salt concentrations (Rodriguez-Valera et al., 1979).

In saltworks pond systems may differ gradually in their biological properties. The solar systems worldwide are generally assumed to support microbial communities. A comparison of total biomass showed great differences in different ponds of saltworks. Javor (1989) has made a comparison of the nutrient rich Western Salt Co. (California, USA) and nutrient poor Exportadera de sal (California, Mexico) which showed great differences in the total biomass.

In the present study the halobacterial density ranged from $3.4 \times 10^4$ CFU/ml to $8.3 \times 10^4$ CFU/ml in condenser ponds. A highest distribution of halobacteria was observed in the month of May in 2013 ($29.3 \times 10^4$ CFU/ml) in the condenser ponds of Swamythoppu Saltworks. The low percentage of halobacteria was observed in the condenser ponds of all the five saltworks studied the total halobacterial density fluctuated according to salinity level and temperature. These results might explain the role of physic-chemical parameters in hypersaline environments such as solar saltworks.

In some cases the range for microbial growth might change at different temperatures. For instance, this happens in extreme halophiles, but in the case of *Halococcus morrhuae* and *Halobacteria* species, usually there are fewer growth differences (Prado et al., 1993). Delmoral (1987) has reported that temperature seems to be the decisive factor within a range of 20 to 30% salt concentrations in the population densities of halobacteria in an ecosystem.
The result reported here support the development of dense communities of halobacteria in a solar saltern when the water column is stratified. In the reservoir, condenser and crystallizer ponds the difference in temperature causes the stratification. In the Dead Sea prolonged stratification of water column results in the persistence of dense communities of halobacteria. An overturn of the water column would greatly reduce the biomass until conditions become suitable for the development of algae and bacterial bloom (Oren, 1985).

Oren (1983) has concluded that temperature and salinity are the important determining factors for halobacterial growth. The bacterial bloom is more likely to develop in the summer season. The salinity in water layer increase due to increase in temperature and evaporation of brines. In the present study, the peak in halobacterial density in condenser ponds was observed in the summer season, May 2013 and 2014. Oren (1983) has reported similar results for the Dead Sea where the salinity of surface water plays a key role in the development of halobacteria. The density of halobacteria generally shows higher values in surface or near surface water in saltworks. Maximum values were found to be reached in or above the thermocline (Ferguson and Palumbo, 1979).

Heterotrophic bacteria mostly utilize organic matter for their growth and their distribution to some extent depends on pH and salinity (Fukami et al., 1983). The density and activity of halobacteria showed correlation to seasonal variations and relationship to the concentration of suspended solids (Goulder, 1977; Wilson and Stevenson, 1980; Bent and Goulder, 1981).
Saltwater is concentrated to obtain sodium chloride through solar evaporation. The evaporation rate is normally controlled by temperature, salinity, vapour pressure, dissolved solids, biological assemblage etc. Solar salterns consist of a series of shallow ponds connected in a sequence of increasingly saline brines. Crystallizer ponds are the last ones and have salinity above 30% (Benlloch et al., 1995). Quantitative studies on the microbial ecology of hypersaline ecosystems such as salterns are not many and most of them deal with plate counts followed by isolation of halobacteria present (Rodriguez-Valera, 1981). Systematic quantitative studies undertaken in the present investigations show that suitable conditions for the development of archaeal blooms existed in all the five saltworks during the experimental period of two years i.e., May 2013 to April 2015.

The overall comparison of data obtained in all the five crystallizer ponds revealed that the total halobacterial density ranged from $4.5 \times 10^5$ CFU/ml to $34.4 \times 10^5$ CFU/ml. Goulder (1977) has reported that density and activity of halobacteria show good correlation to seasonal fluctuation.

Norton et al. (1993) has found that spreads of brine in media produced diverse populations after incubation at $37^\circ$C for 2 to 3 weeks. Viable counts of Winsford brine (British) ranged from $5.0 \times 10^5$ CFU/ml to $5.0 \times 10^6$ CFU/ml. The high densities of halobacterial community would make the saltern crystallizer an ideal site for research (Tamar kis-papo and Aharon, 2000). Oren (1985) has reported similar changes in bacterial densities in response to variation in environmental.
Phytoplankton

During studies carried out by various authors have proved beyond doubt that microalgae are the vital component of the biological system in a saltpan. The diversity of the various biological organisms present in the five salt pans of Kovalam, Puthalam, Swamythoppu, Thoothukudi and Ramnad were carried out for a period two years (May 2013 to April 2014 and May 2014 to April 2015). The diversity studied in a monthly basis for both the years. The microalgae diversity was studied in different water bodies such as reservoir, condenser and the crystallizer. In this present investigation, which was carried out from May 2013 to April 2015, a maximum of 29 genera belonging to 4 divisions such as Bacillariophyta, Chloropyhta, Dinophyta and Cyanophyta were observed in the saltpans of Kovalam, Puthalam, Swamythoppu, Thoothukudi and Ramnad. The 29 genera observed during the above study period included Chlorella sp., Dunaliella salina., Pleurosigma sp., Coscinodiscus sp., Spirulina sp., Thalassiothrix sp., Chaetoceros sp., Surirella sp., Fragillaria sp., Pediastrum sp., Nitzchia sp., Coccochloris sp., Ceratium sp., Ceratium fusus., Biddulphia sp., Prorocentrum sp., Oscillatoria sp., Microcystis., Navicula sp., Nitzschia sp., Chroococcus sp. and Anabaena sp. During 2013-2014 the reservoir of Kovalam, Puthalam, Swamythoppu, Thoothukudi and Ramnad saltpan had 24, 25, 28, 28 and 28 genera of microalgae where as in the condensers there were 11, 8, 7, 9 and 9 genera and in the crystallize ponds the number of genera was 6,4,5,6 and 5 respectively. During 2014-2015, the reservoir of Kovalam, Puthalam, Swamythoppu, Thoothukudi and Ramnad saltpan had 24, 25, 28, 28 and 26 genera of microalgae where as in the condensers there were 8, 9, 7, 9 and 11
genera each and the crystallizer ponds the number of genera were 6, 4, 5, 6 and 4 respectively. The distribution of microalgae in the reservoir, condenser and crystallizer ponds showed a definite pattern at the five selected saltpan. It was observed that as the salinity of the water increased, the genera diversity decreased. This decrease in the diversity at higher salinities may be attributed to the environmental stress due to increased salinity (Ortega and Martinez, 1987; Rahaman et al., 1990). It was also observed that *Dunaliella salina* and *Coccochloris* were able to survive in all the systems namely the reservoir, condenser, and crystallizers, which indicates that they can tolerate wide fluctuations of salinity. The present study also indicates the fact that *Dunaliella salina* was the dominant in the condenser ponds as well as the crystallizers. This definitely will attribute positively towards the efficiency of salt production in the salt pans. *Dunaliella salina*, whose cells accumulate carotenoids, when grown in high salt concentrations and therefore appear red. The red colour of the concentrated saltern pond is often regarded as a significant contribution to the solar salt production process (Reginald et al., 2004; Reginald et al., 2009). The coloured microorganism present increase the absorption of solar energy which raises brine temperature and these enhance evaporation. *Dunaliella salina* releases organic carbon under different conditions also improves the salt quality (Giordano et al., 1994).

On the other hand, it was also observed that along with *Dunaliella salina* and *Coccochloris* sp. was also present in the condensers and crystallizer ponds of the selected saltpan. According to Persoone et al. (1979), *Coccochloris* sp. present in the saltworks coloured the water into deep green. The cells of the
Coccochloris secreted sufficient mucilage to make the brine quite viscous. Abnormally high concentrations of organic matter, particularly excessive Coccochloris mucilage, decrease sodium chloride crystal size and increase coloured inclusions in the crystals. Mark Coleman and Bindy Datson (2005). However, the abundance of *Dunaliella salina* was always higher than *Coccochloris sp.* in all the ponds at the selected saltpan. Moreover, it has also been reported by Reginald *et al.* (2009) that *Dunaliella salina* and *Coccochloris* sp. present in the crystallizer pond played an important role in quality salt production. Study is needed to know the significance microbial biodiversity in the solar salterns. Similar results have been observed for other salterns (Rodriguez *et al.*, 1999; Litchfield, 2005). This solar salterns, worldwide, are an excellent reservoir of untapped microbial diversity an must be preserved.

**Zooplankton**

The coastal ecosystems are better understood now but little attention has been paid to the salterns. The hydrology play determinant role in the development of biological community of salterns (Davis, 2000). During 2013-2014 the reservoir of Kovalam, Puthalam, Swamythoppu, Thoothukudi and Ramnad saltpan had the maximum zooplankton population (1913, 1277, 997, 1692 and 1023 no/l), where as in the condensers had the maximum of 54444, 53703, 54492, 53719 and 50519 no/l and in the crystallize ponds had the maximum number of 58199, 55150, 67421, 57402 and 57646 no/l respectively. During 2014-2015 the reservoir of Kovalam, Puthalam, Swamythoppu, Thoothukudi and Ramnad saltpan had the maximum number of 1577, 1437, 1314, 1870 and 916 no/l respectively during the month of October, November and December.
as in the condensers that had the maximum number of 57238, 53617, 57289, 53617 and 57772 no/lit respectively and in the crystallize ponds, the maximum number of 59907, 62609, 62921, 68872 and 66572 no/lit respectively during March and April (Premonsoon period). Zooplankton are susceptible to variation in a wide number of environmental factors including water temperature, light, pH, Oxygen, salinity, toxic contaminants, food availability and predation by fish and invertebrates. Though this is true zooplankton, no drastic changes or shifts to extreme levels were observed in the different physico-chemical parameters that were measured leading to little or no impact on zooplankton population. Valuable information on nutrient availability, algal biomass, macrophyte density and zooplankton predation may give a more comprehensive account on its effect on the zooplankton population of the selected saltpan. The maximum mean population was found in all the reservoirs during the month of March and April. The highest reading was recorded during post monsoon season (March and April) this might be due to the dilution effect of rains and settlement of sediment after monsoon season (Tlusty, 2002).

Seasonal variation indicated peak for biomass and population during post monsoon period (av 0.7g/m$^3$:2512 no/m$^3$). In the reservoirs (sts B2 and B3) the maximum biomass (av 5.8 g/m$^3$) and population (av 180011 no/m$^3$) was observed during the post monsoon period (Mustafa et al., 1999). In all the selected salt pans, the protozoan ciliate Fabrea salina was maximum. The micro zooplankton community was mainly comprised of protozoans, rotifers and tintinids. Protozoan dominated the total population as normally observed in hypersaline salt lake (Rattan and Ansari, 1982).
The distribution of microzooplankton suggested poor community assemblage which may be attributed to the extreme high and low environmental conditions. Frontier (1985) has suggested that extreme environmental conditions in the salt pans could be expected to be less diverse. Similarly Mustafa et al. (1999) reported that the salt pans ecosystem permits the growth and survival only selectively adapted organisms which can withstand the extreme variations in environmental parameters. The salterns give impression of high biomass system due to the high biomass of *Dunaliella salina*. It has been reported that *Dunaliella salina* is an important factor controlling the abundance of *Fabrea salina* in the ponds (Rattan and Ansari, 1982). Hypersaline ecosystems are generally inhabited by a limited variety of life forms. The upper limit of salt concentration for vertebrates is about 10%, above which, only invertebrates such as brine shrimp (*Artemia salina*), algae (*Dunaliella salina*), bacteria (members of the families Halobacteriaceae and Haloanaerobiaceae, methanogens, etc.), and Cyanobacteria (*Oscillatoria sp.*) have been reported (Lavens and Sorgeloos, 1996). These solar salterns, worldwide, are an excellent reservoir of untapped microbial diversity and must be preserved.
2.5. SUMMARY

Saltpans are unique enclosed ecosystems that are characteristically exposed to a wide range of environmental stress and perturbations manifest mainly through salinity changes. In the extreme physico-chemical conditions of these hypersaline habitats, only a few plant and animal species can live. Saltpan ecosystem offers a number of unique ecological niches having a strange combination of environmental factors. The nutrient rich seawater in saltworks favours algal blooms in reservoirs and evaporators. The biological process that develops along with the increasing salinity in the evaporating ponds of saltern forms a unique saline ecosystem which is yet to be better understood. Organisms developing in saltern that operate efficiently constitute a biological system or ecosystem, which interacts with the physicochemical process and is vital to the production of salt.

The biological system is in admirable harmony with the production process of the saltern. The biological organisms produces the appropriate quantity of organic matter, which is a source of energy for the various organisms and reduces the permeability of the bottom of the ponds, thus minimizing brine losses, particularly at low concentrations. In this chapter is given an overview of microorganisms in the saltpan. In the present study the distribution of halophilic bacteria, phytoplankton and zooplankton were studied at different water bodies such as reservoir, condenser and crystallizer at the saltpans of Kovalam, Puthalam, Swamythoppu, Thoothukudi and Ramnad. The major findings of this study are highlighted under this section.
In the reservoir pond of the selected saltpan, the maximum number of halobacteria was recorded during May 2013 at Swamythoppu saltpan (27.2×10^3 CFU/ml), whereas the minimum number (3.4×10^3 CFU/ml) of halobacteria was recorded during July at Ramnad saltpan.

In the condenser pond of the selected saltpan, the maximum number (29.3×10^4 CFU/ml) of halobacteria was recorded at Swamythoppu saltpan during July 2013, whereas the minimum number of halobacteria (8.3×10^3 CFU/ml) was recorded at Swamythoppu saltpan during March 2015.

In the crystallizer pond of the selected saltpan, the maximum number (38.5×10^5 CFU/ml,) of halobacteria was recorded at Ramnad saltpan during March 2014, whereas the minimum number (4.5×10^5 CFU/ml) of halobacteria was recorded at Swamythoppu saltpan during March 2014.

Coscinidiscus sp. was abundantly (1.837×10^5/ml) seen in the reservoir pond of Kovalam saltpan during 2013-2014 and Nitzschia sp. was maximum (1.613×10^5 CFU/ml) during 2014-2015.

Nitzschia sp. was abundantly seen during the study period 2013-2014 but in the next study period (2014-2015) Nitzschia sp. was maximum in the reservoir pond of Puthalam saltpan.

Navicula sp. was abundantly (1.584×10^5/ml) seen during the study period 2013-2014 but in the next study period between 2014-2015, the Pleurosigma sp. was maximum(1.654×10^5 CFU/ml) in the reservoir pond of Swamythoppu saltpan.
- *Surirella* sp. was abundantly (1.622 and $1.733 \times 10^5$/ml) seen in the reservoir pond of Thoothukudi saltpan during the study period 2013-2015.
- *Navicula* sp. was abundantly ($2.728 \times 10^5$/ml) seen during the study period 2013-2014 but in the next study period of 2014-2015, *Pleurosigma* sp. was maximum ($1.686 \times 10^5$/ml) in the reservoir of Ramnad saltpan.
- *Dunaliella* sp. was abundantly seen in the condenser pond of Kovalam, Puthalam, Thoothukudi and Ramnad during the study period of 2013-2015.
- *Dunaliella* sp. was abundantly seen in the condenser pond of Swamythoppu during the study period of 2013-2014 but the next study period (2014-2015), *Coccochloris* sp. was recorded maximum.
- *Dunaliella* sp. was abundantly seen in all the crystallizers of the selected saltpans during the study period 2013-2015.
- The zooplankton *Fabrea salina* was abundantly seen in all the reservoirs, condensers and crystallizers of the selected saltpans during the study period 2013-2015.

During the total study period 2013-2015, the halophilic bacterial population varied based on the salinity and area location. The species abundance in the halobacterial population did not show direct relationship with salinity and more over a particular halophilic population present in a particular saltpan during a particular time period also varied, when the study was carried out for two subsequent years. The species abundance also varied between one saltpan and others. A particular algal species found in maximum in the reservoir of one saltpan was not seen as abundant in the reservoir of the other saltpan.