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Evaluation of the Physico Chemical Parameters and the Halobacterial Density in Brine Samples of Different Saltworks

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Abstract: The physico chemical parameters are an important factor that determines halobacterial survival and propagation in brine samples. Isolation of halophilic bacteria from the brine samples were collected at different sampling stations (reservoir, condenser and crystallizer and wet salt) from the Kovalam, Swamithoppu and Thamaraiikulam saltworks of Kanniyakumari district, were drawn from December to March. The temperature, salinity and pH of crystallizer ponds and wet salt greater than reservoir and condenser ponds. The total halobacterial counts of brine samples showed bacterial density sizes much greater than those in sea water and thus indicating an increase of halobacterial number with increase in salinity. The halobacterial densities of crystallizer are much greater than reservoir pond and also the highest bacterial biomass was observed at higher salinity brines during summer month March.

Keywords: Brine samples, physico chemical parameters, halobacterial density, Kovalam saltwork, Swamithoppu saltworks and Thamaraiikulam saltwork.

I. INTRODUCTION

To understand water ecosystems, study of the physico chemical parameters and biological relations are very essential. Physico chemical characteristics may play an important role in the rate of microbial attachment to the surfaces. The bacterial attachment are different aqueous systems affected by season. This may be due to the temperature or water or other seasonally affected parameters (Kokare *et al.*, 2009). Physico chemical disturbances can affect the quality and quantity of salt (Coleman and White, 1992). Climate and parameters need to be taken into consideration, when determining the technique parameters. It needs to stress that this physical process is balanced with biological community existed in brine water ecosystem (Zhiling and Guangyu, 2009).

The organisms living in salt solutions more concentrated than seawater are termed as halophiles. In salterns, the halobacteria in the brine samples reduces the formation of surface salt crusts, hence for salt productivity, quality improvement and other environmental needs many biological process are important.

The research was aimed for the quantitative analysis of physico chemical parameters and the population dynamics of halobacteria in different ponds of Kovalam, Swamithoppu and Thamaraiikulam saltworks due to montly variations.

A. Description of the study area

- 1) *Kovalam saltworks:* The Kovalam saltwork is situated 5Km away from Kanniyakumari towards northwest direction. The total area of the saltwork is 27.38 ha. Kovalam saltworks receive water from the Arabian sea.
- 2) *Swamithoppu saltworks:* The Swamithoppu saltwork is situated about 10Km from Kanniyakumari. The total area of the saltwork is 21.0 ha. Swamithoppu saltwork receive water from the underground water.
- 3) *Thamaraiikulam saltworks:* Thamaraiikulam saltwork is situated near Keelamanakudy sea coast in the eastern direction. The total area of the saltwork is 70.14 ha. The sub soil water is used for the manufacture of salt water from the ground.

B. Materials and Methods

- 1) *Collection of samples:* The brine samples and 100 gram of wet salts were collected at different sampling stations (reservoir, condenser and crystallizer pond) from the saltworks Kovalam, Swamithoppu and Thamaraiikulam of Kanniyakumari District. The samples were drawn from December to March, at monthly intervals.

C. Evaluation of the physico chemical parameters and the density of the halophilic bacteria of brine samples

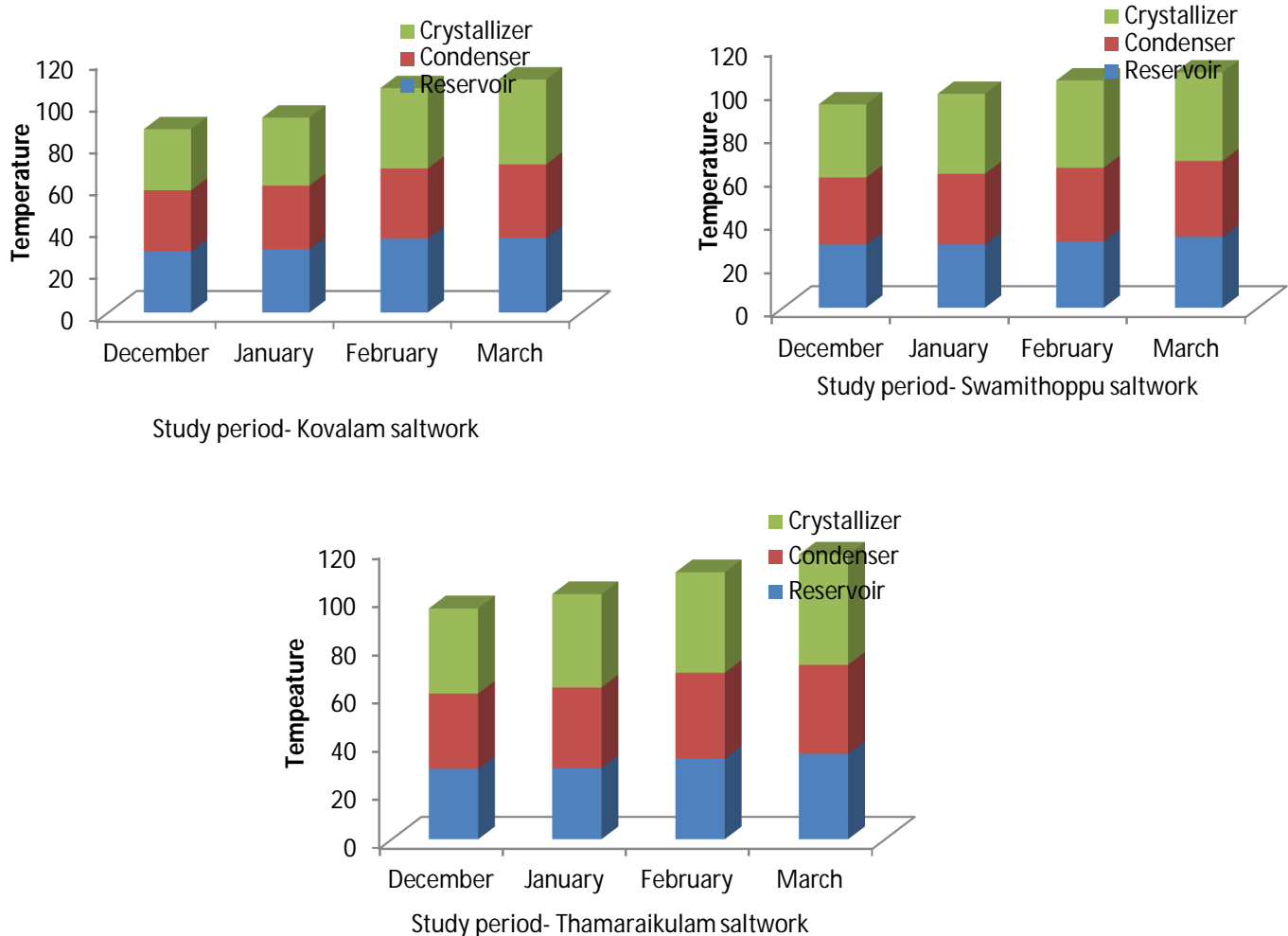
- 1) *Temperature*: The surface temperatures of the brine water in all the three sampling sites were recorded using a field thermometer.
- 2) *Salinity*: The salinity of brine water was measured with the help of salinity refractometer.
- 3) *Ph*: The pH of brine water samples was measured using a calibrated pH meter.
- 4) *Isolation of halophilic bacteria in brine samples*: The sterilized halophilic agar medium was poured in petriplates. The petriplates were incubated in an incubator in inverted position at 37°C for 15-20 days till they attained sufficient growth. Finally a single colony was separated out and streaked on the already prepared agar slants.

D. Result

1) *Physico chemical parameters of brine in saltworks*

- 2) *Temperature*: The surface brine water temperature ranged between 29°C-35.5°C, 29°C-36.8°C and 29°C-45.5°C in reservoir pond, condenser pond and crystallizer pond respectively, among the three saltworks investigated.

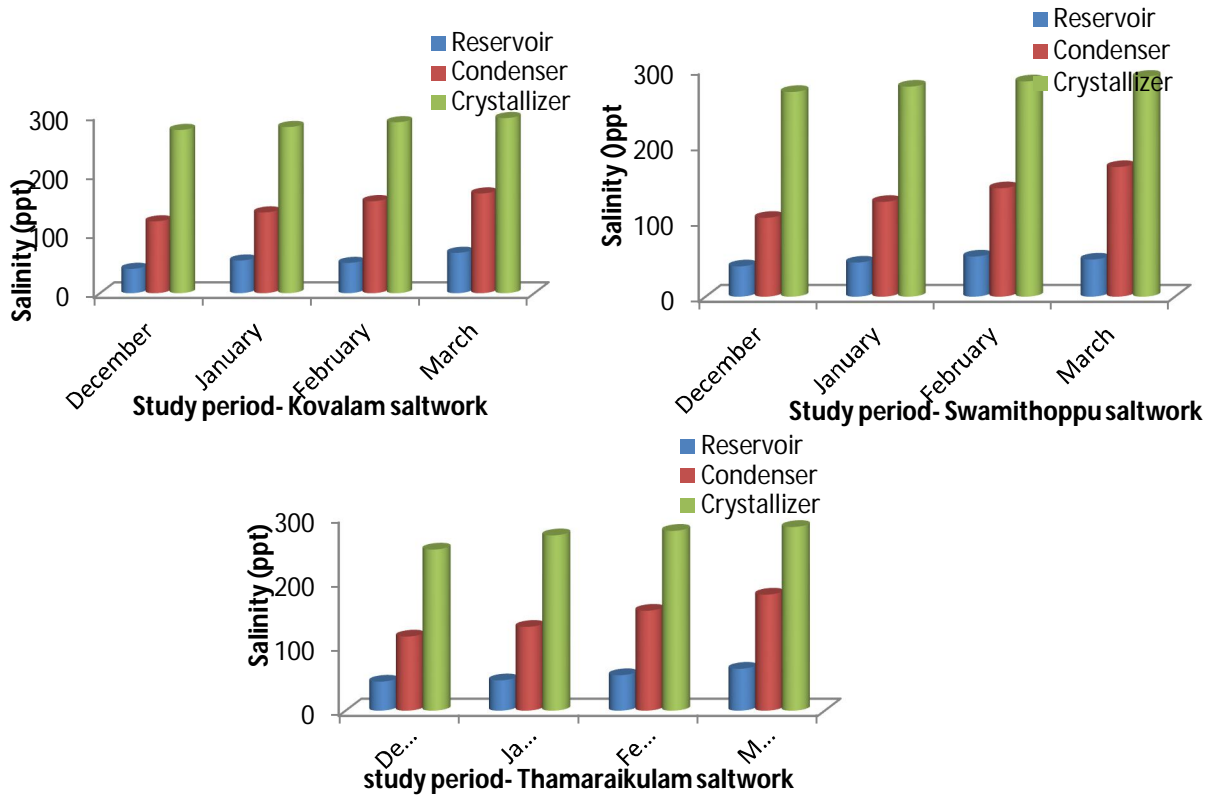
Fig 1. Fluctuation of temperature (°C) in brine samples of Kovalam, Swamithoppu and Thamaraiikulam saltwork during the experimental period



E. Salinity

The salinity ranged from 40ppt to 68ppt, 104ppt to 180ppt and 250ppt to 295ppt in reservoir, condenser and crystallizer ponds respectively, among the three salt works investigated.

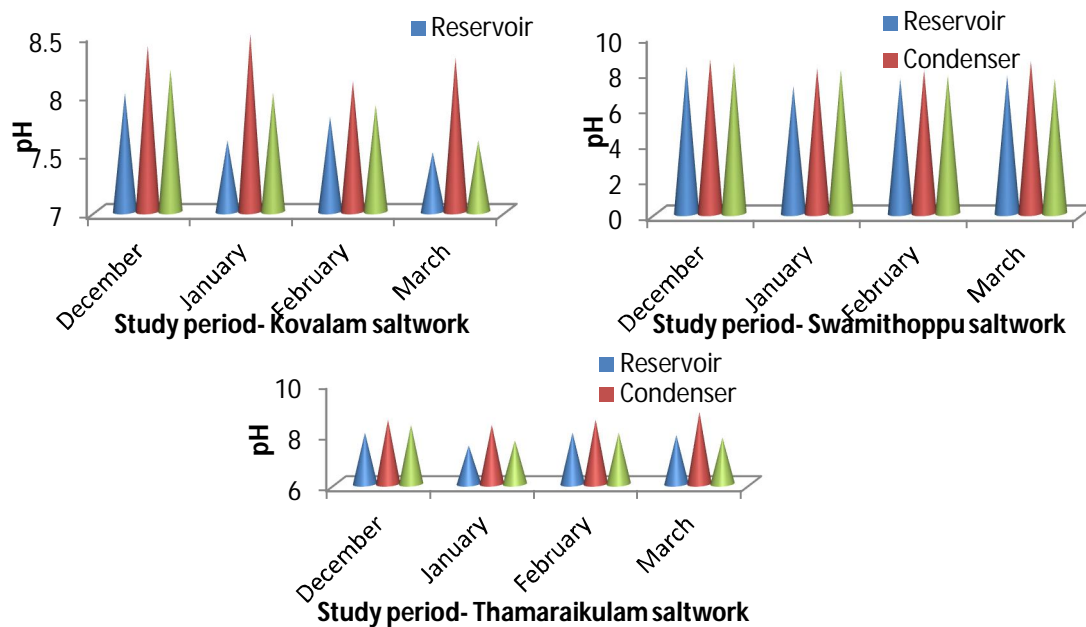
Fig 2. Fluctuation of salinity (ppt) in brine samples of Kovalam, Swamithoppu and Thamaraiikulam saltwork during the experimental period



F. Ph

The pH ranged from 7.1 to 8.2, 8.0 to 8.8 and 7.5 to 8.4 in reservoir pond, condenser pond and crystallizer pond respectively of Kovalam, Swamithoppu and Thamaraiikulam saltwork during the experimental period from December to March.

Fig 3. Fluctuation of salinity (ppt) in brine samples of Kovalam, Swamithoppu and Thamaraiikulam saltwork during the experimental period

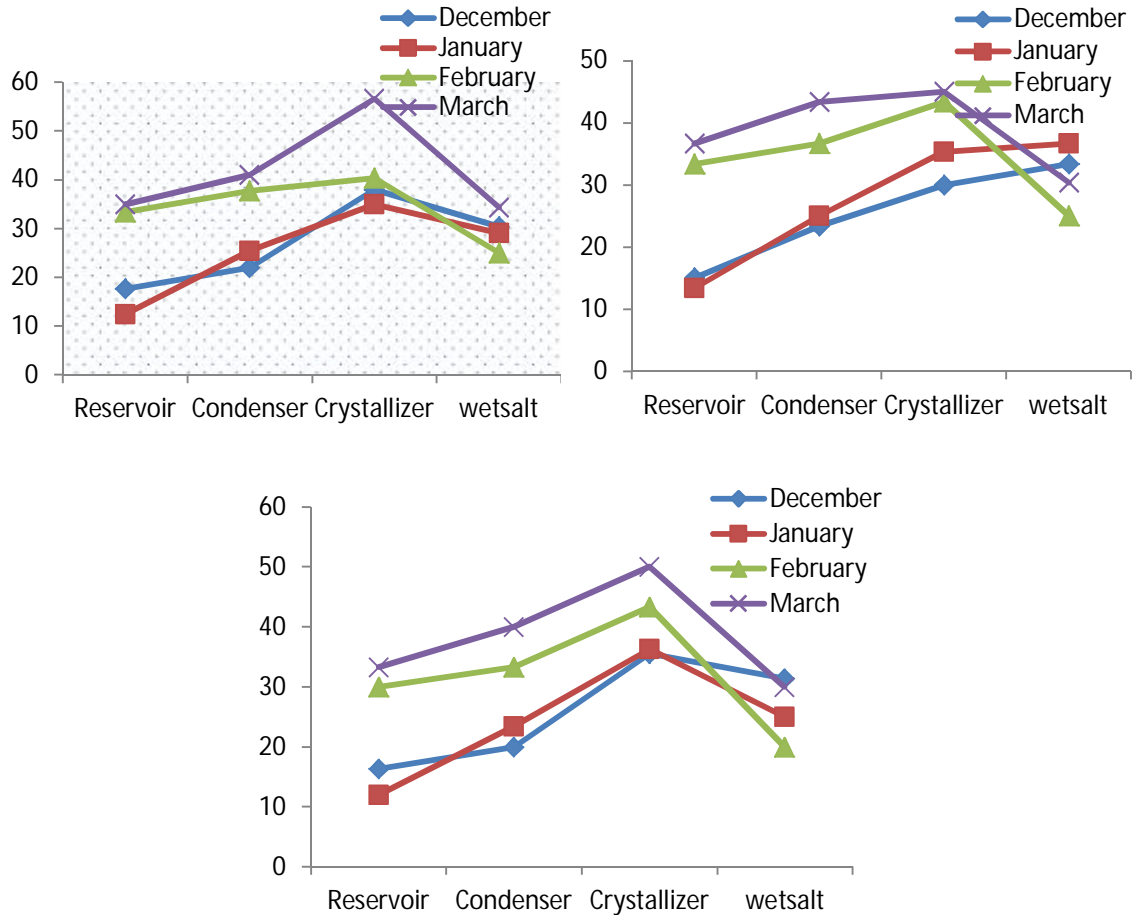


G. Total halobacterial density in the brine samples and wet salts of saltwork

The total halobacterial counts of brine samples taken from various ponds showed bacterial density sizes much greater than those in sea water and thus indicating an increase of halobacterial number with increase in salinity. The halobacterial density ranged from 12.00×10^2 CFU/ml to 36.66×10^2 CFU/ml, 20.00×10^2 CFU/ml to 43.33×10^2 CFU/ml and 30.00×10^2 CFU/ml to 56.66×10^2 CFU/ml in reservoir pond, condenser pond and crystallizer pond respectively, among the three (kovalam, swamithoppu and thamaraiikulam) saltworks investigated.

In wet salt, the halobacterial density ranged between 20.00×10^2 CFU/ml to 36.66×10^2 CFU/ml in kovalam, swamithoppu and thamaraiikulam saltwork respectively.

Fig 4. Total halobacterial density (10^2 CFU/ml) in the brine samples of reservoir, condenser and crystallizer pond and wet salt of kovalam, swamithoppu and thamaraiikulam saltwork during the experimental period



II. DISCUSSION

Hyper saline environments represent one such unique and interesting challenge to biological survival. The growth and survival of living organisms is controlled by variety of physical and chemical factors viz., temperature, pH, salinity etc.

The halobacterial density of reservoir pond ranged between 12.00×10^2 CFU/ml and 36.66×10^2 CFU/ml. The highest bacterial biomass was observed at higher salinity brines during the summer month (March) and the lowest bacterial density was observed at lower salinity brine during winter months (December and January). The salinity of reservoir ponds varied from 40ppt to 68ppt. This clearly reinforces that solar saltworks, the salinity is an important factor that determines halobacterial survival and propagation in brine sample. Many researches viz., Forsynth *et al.* (1971); Nissenbaum (1975); Rodriguez- Valera *et al.* (1979) and Delmoral *et al.* (1986) have reported such relationship between halophilic bacteria and salinity (35 ppt) in sea water. In case of pH, halobacteria density was high at 7.0-7.1 pH during the experimental period. Similar trend was observed by Norton and Grant 1988, they suggested that the halobacteria are neutrophilic organisms, which grow best in 7.0 ± 0.2 pH.

In the present work, the halobacterial density of condenser pond ranged between 20.00×10^2 CFU/ml to 43.33×10^2 CFU/ml. the maximum occurrence of halobacterial communities was observed at salinities 140.00ppt and 180.00ppt and the minimum number of halobacteria was noted at 100.00ppt and 130.00ppt in condenser ponds. Similarly, the surface brine water temperature ranged from 29°C to 36.8°C . All the three study areas, the total halobacterial numbers encountered were fluctuated according to their salinity ranges. In some cases, the optimal salinity for microbial growth changes with temperature (Prado, 1987; Delmoral, 1986) inferred that the temperature is the decisive factor within the range of 20% to 30% salt concentration on the population densities of halophilic bacteria in an saltwork ecosystem. Oren (1983) had also reported the temperature and salinity as important determining factors for halobacterial growth. During the study period, the salinity of water layer increased due to the increase in temperature and evaporation rate of brines with the halobacterial bloom was high in the summer seasons. From these data, it could be concluded that density and activity of halophilic bacteria in the saltworks are influenced by short term and long term salinity changes and physicochemical parameters.

Crystallizers are last stage ponds and have the salinity above 30%. An overall comparison of data obtained in all the three study area pertaining to crystallizer pond reveal the total halobacterial density that ranged from 30.00×10^2 CFU/ml to 56.66×10^2 CFU/ml. The optimum brine water temperature for halophilic bacterial development in crystallizer pond ranged from 29°C to 45.5°C . The bacterial density in the wet salt ranged from 20.00×10^2 CFU/g to 36.66×10^2 CFU/g. In the case of pH the halobacteria attained the maximum growth at pH 7.5-8.4. The halobacteria can thrive in concentrated brine, nine times higher than the salinity of sea water and even remain alive in dry salt crystal for years (Norton and Grant, 1988). Among the selected saltwork, the crystallizer ponds had higher halobacterial biomass when compared to reservoir and condenser ponds. The observations pertaining to the incidence of high halobacterial densities in crystallizer pond in the present study correlated with earlier investigations, which include Oren (1990), Litchfield *et al.*, 2000 and Tamerkisspapo (2000). Litchfield *et al.*, 2000 had remarked that the biodiversity of saltern is due to the seasonal differences in an ecosystem.

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