# BIOLOGICAL MANAGEMENT OF SAMBHAR LAKE SALTWORKS (RAJASTHAN, INDIA)

**S. SUNDARESAN[1], K. PONNUCHAMY [2] and ABDUL A. RAHAMAN[3]**

[1]Salt Commissioner, Government of India, Ministry of Industry,
Lavan Bhawan, Jhalana, Dungari, Jaipur,302 004, India.
[2] Chairman & Managing Director,
Hindustan Salts Limited & Sambhar Salts Limited
( A Government of India Enterprise), Jaipur , 302017 India.
[3]21, Vidhya Nagar, Erode , 638009, India.
E-mail:rahaman@ath.net

## EXTENDED ABSTRACT

The Sambhar salt lake is the largest single salt source situated in Rajasthan state which experiences desert conditions. It is situated in latitude $26^0$58'N and longitude $75^0$5' E on the east of the Aravalli hills. The lake bed varies from 1181 feet to 1196.76 feet above the sea level. The initial source of brine in the lake is catchment water about 12-13 inches of rainfall annually mostly during April to September which results in the accumulation of about 35 inches depth of water in the lake in normal year. The density water is less than1°Be which rises to about 3°Be during September. This water is pumped into the main reservoir and the salinity increases 6-8°Be and later this is pumped into condenses and further density increases to 22-24°Be. During the process micro algae appear and impart different colors. At higher concentrations micro algae are destroyed. The temperature varied between 7.12 (December) to 44.5(June) 2005-2006. The humidity was highest in July '05, 81.49% when the rainfall was also maximum 204.5mm. Low percentage of humidity of 37.34% was recorded in May '05 when there is no rainfall which corresponds with high temperature in May 43.28°c. The total rainfall for the year 2005-2006 was 391.0 mm out of which the maximum rain of 204.5mm was recorded in July 05. The sodium chloride content available in the subsoil brine at 16°Be,17°Be and 21°Be are 82%,81.74% and 78.82% respectively. However in the lake brine at 3°Be NaCl was reported as 88.74%. The bacterial load in the different brine samples were analysed by dilution plating technique which shows higher concentration of 1000 cfu/ml in 100 ppm salts. The study indicated the isolates belong to *Bacillus* and *Halobacterium.* The samples indicated the percentage of occurrence and the presence of several species of saltern bacteria such as B*acillus subtilis, B.amyloliquefaciens, B.sphaericus, B.licheniformis, Halobacterium sp;Micrococcus sp and Staphylococcus capitis.* It was observed that brine algae *Dunaliella salina* blooms making the brine red in the crystallisers. It is assumed that *Dunaliella* population in the hypersaline crystallizers of 24-28°Be. supplies the organic compounds required for the growth of halophilic archaea and probably the glycerol is the most important nutrient available to the halobacterial community. Taking advantage of the role of *Artemia*, there is ample scope for mass management of extensive area of salt work for biological management in a balanced way. This will result in quality and quantity of production of salt and its by products.

**Keywords** : Saltern bacteria, Bacillus sp., Halobacterium sp., Dunaliella salina, Artemia.

## INTRODUCTION

The solar evaporation of sea water to produce brine is not only a physical process but there is also an organic contribution from the biological communities with in the pond ecosystem. Hypersaline water, previously thought to be lifeless, contains primary producers, consumers and decomposers like any other ecosystem (Borowitzka, 1981).This organic contribution to the evaporation process influences production of salt (Rahaman *et al* 1993). Thus the quantity and quality of salt production are strongly influenced by the hydrobiological activity in solar salt operations (Davis, 1980). It was reported that in Sambhar lake during salt manufacturing seasons 1984-85 and 1985-86, the precipitation of very fine salt particles had taken place in the crystallisers. The report further states that normal crystal growth had been adversely affected, besides, the production of salt had also declined which appeared to be largely due to algal contamination of ponds(Lall,1987).

The biological communities in the salt works are beneficial as well as harmful to salt production. It has been reported that the organic impurities will contaminate salt due to the formation of small crystals (=poor quality) where as introduction of brine shrimp in the brine stream controls the algal bloom(= improved quality (Sharma,1992).The organic impurities as algal agglomerations, may contaminate salt as black due to oxidation and reduce the size of the crystals and reduces salt quality. It is further stated that higher viscosities may inhibit salt crystal formation and precipitation whereas lower viscosity levels promote the formation of larger crystals and thereby improve the salt quality(Sorgeloos,1983;Haxby and Tackaert,1987).

High salinity, physico-chemical instability and low productivity have been the most characteristic features of the solar salt works. The variation in ionic proportion with increasing salinity was also studied(Williams1964). Extreme saline waters were reported to contain high concentrations of $Mg^{2+}$ $So^{2}_{4}$ and $HCO^{-}_{3}$ (Volcani,1944;Bayly and Williams,1966;Oren and Shilo,1982). A basic relationship between salinity and faunal occurrence (Bauld,1981)and in extreme saline waters only two or three algal species and bacterial species to grow and multiply were recorded(Davis,1978) .The mucilaginous secretions of algae found to increase brine viscosity , hamper salt crystallization process and contaminate the salt with organic impurities(Sorgeloos,1983).

Further,high salt concentration, high light intensity and high temperature parameters were stated to be responsible to stimulate *D.salina* to accumulate carotenoids(Lerche,1937). Similar observation was made on *Dunaliella salina* blooms that colored the water in Vedaranyam salt works at salinities above 200ppt(Rahaman *et al*.,1993a).

It is known that with 15% salinity moderate halophiles and salinity 25% extreme halophiles or halotolerant microorganisms change the brine into brown color. These salinities include phototrophs (cyanobacteria), chemotrophs (sulfur oxidizers) and heterotrophs (aerobes, anaerobic fermenters and sulfur reducers) and methanogens and halobacteria. It is evident that number of bacteria increases with an increasing the amount of impurities in the solar salt (Nakashizuka and Arita 1993).

It is well known that the biological system in salt ponds is essential for salt production, an unbalanced system creates problems for salt precipitation. In the Indian salt works photosynthetic algae harm salt production. These organisms utilize nutrients and produce metabolites which adversely influence salt production .

Due to the inflow of rain water into the basin of Sambhar Lake, the nutrients in the soil contributes to the algal development at lower salinity. It was pointed out that the sub-terranean brine is free from algae and consequently the salt production was not suffered. On the other hand substantial deterioration in the quality and quantity had taken place in the crystallizer using lake brine and main reservoir brine in which algae were present. For salt production 66% from the lake brine and 34% subterranean brine are used. The precipitation of very fine salt particles in the crystallizers and there by normal crystal growth had been adversely affected besides the production of salt has also

declined(Lall,1987). The present study was undertaken as the water gets evaporated, the water in lake basin turns into brine resulting due to percolation, evaporation and crystallization processes(Shukal and Rahaman,2006) resulting algal contamination of ponds needs the biological management of ponds for quality salt production

## MATERIAL AND METHODS

It is the largest single salt source in Rajasthan State, India. It is situated in latitude $26^0$58'N and longitude $75^0$5' E on the east of the Aravalli hills. The lake bed varies from 1181 feet to 1196.76 feet above the sea level(Fig. 1). There are two brine samples viz.,lake brine and subterranean brine.

The temperature, (maximum and minimum) humidity, and rainfall were recorded during 2005-2006. The sodium chloride content in the sub soil and lake brine were determined by conventional methods(Fig. 2) shows the presence avian fauna in the Sambhar lake.

Brine samples were collected from various locations of Sambhar lake and analysed for chemical(Strickland and Parsons,1972) and for bacteriological studies.The observations were made with reference to the visual coloration of pond(Fig. 3) and the micro algae being the source in the brine which are removed by adding alum and the treated brine is used for salt production in the experimental model farm at Nawa-Rajasthan, near Sambhar lake(Fig. 4).

Growth of saltern bacteria in different halophillic media:

All the isolates of bacteria from salt ponds after identification were inoculated into different halophillic media to analyse the effect of media components on the growth of the Marine bacteria. This was done to standardize and detect the suitable media for the isolation and cultivation of bacteria from salterns. The media used were Alkaline peptone water, Halobacteria medium, Synthetic sea water medium, Alakaline Bacillus medium, Marine broth and Halophilic halobacterium medium. The synthetic sea water (SSW) medium was found to be the suitable media and hence used.

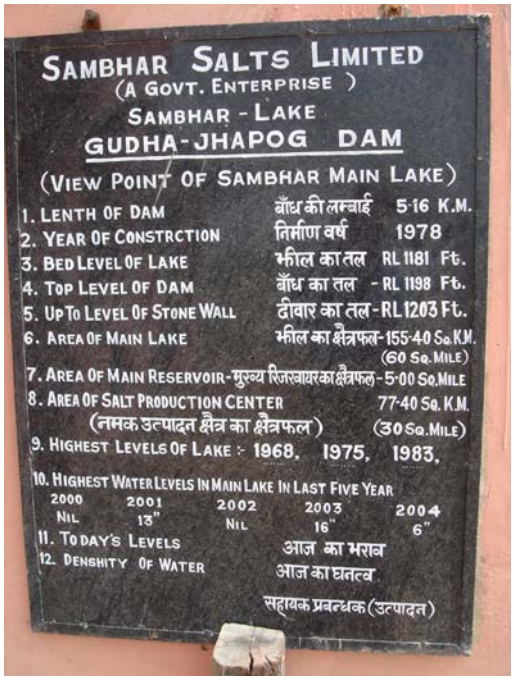


**Figure 1:** Showing the view point of Sambhar lake.


**Figure 2:** Showing the check list of migratory birds visiting Sambhar lake.

**Figure 3:** Showing the varying shades of pigments of saltern bacteria.


**Figure 4:** Model salt farm Nawa-Rajasthan.Pigment production by saltern bacteria:

All the isolates of saltern bacteria showed varying pigmentation in the primary isolation plates and hence a study was done to visually observe the pigments produced by them in broth cultures. The isolates were inoculated into synthetic sea water medium taken in tubes and incubated at $37^0$c for 24 hours. The pigments produced were visually observed and noted. The results are presented.

**RESULT**

The temperature varied between 7.12 (December) to 44.5(June)2005-2006.The humidity was highest in July '05,81.49% when the rainfall was also maximum 204.5mm. Low percentage of humidity of 37.34% was recorded in May '05 when there is no rainfall which corresponds with high temperature in May 43.28°c.

**Table 1:** Showing monthly variation in temperature,humidity, rainfall and sodium chloride content in the Sambhar lake salt works during 2005-2006

| Month and year | Temperature | | | Total rain fall | Nacl.Contents. available in brine | |
|---|---|---|---|---|---|---|
| | Max. | Min. | Humidity | | | |
| Apr.,05 | 40°c | 21.69°c | 45% | 21 MM. | Subsoil Brine 17°Be | 81.74% |
| May.,05 | 43.28°c | 29.41°c | 37.34% | Nil | - | - |
| Jun.,05 | 44.5°c | 32.11°c | 47.32% | 36 MM. | - | - |
| Jul.,05 | 37.55°c | 29.69°c | 81.49% | 204.5 MM. | - | - |
| Aug.,05 | 36.85°c | 28.65°c | 74.77% | 36.5 MM. | Subsoil Brine (Kyar 7-8) 21°Be | 78.82% |
| Sep.,05 | 37.29°c | 27.75°c | 77.93% | 93 MM. | Lake Brine (New Kyar) 3°Be | 88.74% |
| Oct.,05 | 40.07°c | 22.35°c | 61.70% | Nil | - | - |
| Nov.,05 | 35.94°c | 13.69°c | 65.81% | Nil | - | - |
| Dec.,05 | 29.4°c | 7.12°c | 69.48% | Nil | Perculation canal 13°Be | 78.85% |
| Jan.,06 | 31.57°c | 7.77°c | 73.25% | Nil | - | - |
| Feb.,06 | 37°c | 15.61°c | 66.96% | Nil | - | - |
| Mar.,06 | 36.27°c | 16.57°c | 66.44% | Nil | Subsoil Brine 16°Be | 82.00% |

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The total rainfall for the year 2005-2006 was 391.0mm out of which the maximum rain of 204.5mm was recorded in July 05. Rainfall was recorded during summer month from April to June extending upto September 2005,however, there was no rainfall during October to March 2006. However during this period migratory birds are reported to have visited Sambhar lake being their feeding ground.

The Sodium Chloride available in the subsoil brine at 17°Be it was 81.74% during April, where as at 21°Be during August 2005 it was 78.82%. However during March,2006 the sub soil brine at 16°Be with 2% NaCl whereas during December 2005 in the perculation

canal at 13°Be the NaCl content was 78.85%. During September 2005 using lake brine at 3°Be the NaCl content was recorded as 88.74%.

The total bacterial load in each of the sample was analysed by dilution plating technique, which shows higher concentration of 1000 cfu/ml in 100ppm salt. These isolates belong to Bacillus and Halobacterium. The samples indicated the percentage of occurrence and the presence of several species of saltern bacteria such as B*acillus subtilis, B.amyloliquefaciens, B.sphaericus, B.licheniformis, Halobacterium sp;Micrococcus sp and Staphylococcus capitis*(Table-2). Most of isolates produced varying shades of pigments which can be visually observed(Fig-3.). The growth of Halobacterium in 200ppt of salt concentration with a production of orange pigment a bacterial rhodopsin, makes the bacteria consider for commercial exploitation of halorhodopsin.

**Table 2:** % of saltern bacteria and pigment colouration produced by the isolates

| S.No. | Bacteria Identified | Percentage of Occurrence | Pigment produced |
|---|---|---|---|
| 1. | *Bacillus substilis* | 69 | Cream |
| 2. | *Bacillus licheniformis* | 78 | No pigment |
| 3. | *Bacillus sphaericus* | 80 | Light brown |
| 4. | *Bacillus amyloliquefaciens* | 60 | Dull cream |
| 5. | *Staphylococcus capitis* | 18 | Pale yellow |
| 6. | *Halobacterium species* | 40 | Pink or orange |
| 7. | *Micrococcus species* | 10 | Red |

**DISCUSSION**:

As has been stated that the sub-soil brine is free from algae, and consequently the quality of salt production has not suffered .On the other hand substantial deterioration in the quality and quantity of salt has taken place in the crystallisers using lake brine and main reservoir brine in which algae are present. The density of this brine does not go up beyond 28°Be(Lall, 1987). The present study indicated that the NaCl content in lake brine at 3°Be was 88.74% when the rainfall was 93 mm during September 2005 whereas in the sub soil brine at 21°Be it was 78.82% when the rainfall was 36.5 mm during August 2005. The appearance of salt produced at Sambhar lake varied as inherently pink colour(Kyar salt) pink colour(Reshta salt) whitish colour( Summer and Winter crops of Pan salt) and off white with greenish tinch(Summer and Winter crops of Pan salt Bundewals).(Jonwal, personal communication, 2006). The test report provided by the Indian Institute of Technology Kanpur on the total organic content of the Reshta salt and Kyar(Sambhar)salt were 32.59 ppm and 42.70 ppm respectively suggest the algal contamination in the salt.

It has also been observed that the concentrated brine whose density does not go beyond 28°Be in the lake brine which is oily and viscous in nature(Lall, 1987).Such condition was also reported in *Artemia* experimental farm at Adirampattinam Tamil nadu Salt work wherein *Tetraselmis* sp. produced intense blooms coloring the water green and making it oily and viscous. When *Artemia* introduced into this system the water became clear (M. Ambika devi, Personal communication,1991).

The micro algae which are of greeny grey colour when alive, die at varying densities and turn to a deep pink, and their presence tends to impart a pink colour to the salt which crystallizes out. The present observation in Sambhar salt work led to the experimental model salt form near Sambhar lake salt work in which micro algae are removed by using 30 mg of alum per litre of brine and the clear brine is used for quality salt production(Fig 4).

The result of the study will be made available to the salt works. It is however, the sludge produced from such operation may need further research. Such experimental study will

have an impact on the quality of salt produced. However, in the Sambhar lake it is observed that since *Dunaliella salina* blooms making the brine red in the crystallizes at 24□Be. Hence the introduction of right kind of *Artemia* strain will feed on the micro algae and thus quality in the salt could be achieved.

The finding of the discolourisation of salt owing to the presence of green algae *Dunaliella salina* resulted to manage the Sambhar Salt lake biologically although the quality of salt from the point of chemical purity is good.

There is also some discoloration in the bed which is soft and silty. The brine is drawn into the salt farm from lake and when the salt concentration exceeds 25$^{o}$Be., the salt works is found inhabit by dense communities of the autotrophic red halophillic archaea (genera Halobacterium, Halococcus).These archae impart red colour to the crystallizers. It is assumed that *Dunaliella* population in the hypersaline crystallizers of 24-28$^{o}$Be. supplies the organic compounds required for the growth of halophilic archaea and probably the glycerol is the most important nutrient available to the halobacterial community.

Biological management is aided by the presence of *Artemia* which acts as vacuum cleaners and feed on *Dunaliella salina.* It is reported that *Artemia* do not interfere with the salt production. However, *Artemia* ingest suspended particles less than 50□ in size and the excreta produced by the animals settles at the bottom and provides a suitable substratum for the development of Halobacterium which imparts red coloration to the brine, and enhances evaporation of brine and reduce concentrations of organics.Lower viscosity levels promote the formation of larger salt crystals and thereby improve the salt quality(Sorgeloos,1983;Haxby and Tackaert,1987). Further, the study has suggested the presence of Halobacterium in the salt works improves in the quality of salt production.

It is observed that in the crystallizer the density of halobacteria was found to be low when compared to the brine algae *Dunaliella* was high. In the absent of *Artemia* the natural resource could not utilized either biologically or techno-commercially in the form of β-carotene.However, the organic load in the sediment has increased and hampers in the quality of salt production. It is reported that the bacteria acted not only as food for the *Artemia* ,but also assisted in the digestion of the algae(Intriago and Jones,1993). Under Indian field conditions Vietnam *Artemia* strain seems to be suitable in view of its adaptability to high temperature besides its biomass and cysts productions. Since native strain of *Artemia* is absent introducing a foreign strain could be attempted. Each situation needs to be analysed for specific requirements with regard to selection of *Artemia* strain. It is to be decided in consideration of the water retention times, rate of evaporation food concentrations and water temperature(Sorgeloos, *et al*,1986). Taking advantage of the role of *Artemia*, there is ample scope for mass management of extensive area of salt work for biological management in a balanced way. This will result in quality and quantity of production of salt and its byproducts.

**CONCLUSIONS:**

Sambhar Lake is only one such biotope in Rajasthan desert region, India ,which is done at present only for salt production, but there is unlimited scope for commercial exploitation of quality salts. The role of *Artemia* in management of salt work in controlling the production of microalgae and thereby the quality of salt production has been brought out. The Sambhar Lake has unlimited resource for techno-commercial production such as halobacterium, β- Carotene extraction, *Artemia* biomass and cysts production. The commercial exploitation of orange pigment, a bacterial rhodopsin could lead to value added by product thereby making the salt as nil value.

## ACKNOWLEDGEMENT

The facilities provided by the Chairman and Managing Director of Hindustan salt Limited Jaipur to Abdul A.Rahman is gratefully acknowledged

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