



Sambhar lake: Some physical - chemical features and composition of biological communities

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Abstract

The present paper deals with biological studies of natural salt lakes located in the Rajasthan. There were investigated for first time the halophilic microorganisms (bacteria and archaea) and also phyto- and zooplankton species in relation with physical-chemical parameters as control factors. The chemical composition of all tested stations is marked by the abundant presence of chlorides (sodium, magnesium, potassium) and also some relatively important concentrations of aluminum. The biological diversity was represented by several types of archaea and halophilic bacterial species and some species of cyanobacteria and diatoms. There were also identified ciliates, rotifers and copepods mainly in the samples taken from Sambhar Lake. The results revealed the presence of magnesium and calcium ions and a chloride content that influences the phytoplankton and zooplankton diversity in these saline environments.

Keywords: salt lakes, halophilic archaea, plankton communities, saline ecosystems

1. Introduction

Hypersaline ecosystems are widely distributed habitats including a variety of terrestrial lakes and deep-sea basins with salt concentrations exceeding three times seawater up to saturation [15]. In addition to being hypersaline, these ecosystems are often characterized by other extremes in environmental conditions such as high alkalinity, low oxygen concentration and high UV irradiation [12, 6, 7, 25].

Salt lakes attracted researchers in the last period as a spring of novel microorganisms capable to grow and develop in extreme conditions, namely high ionic strength due to high salt content of water body of such environments. Following this approach, more than 150 species of halophilic archaea distributed in 40 valid published genera were found to populate saline and hypersaline environments [16, 19]. Regarded initially as low diversity areas, saline lakes appear to harbour a rich endemic biological diversity represented by brine shrimp *Artemia salina*, brine fly *Ephydra*, photosynthetic flagellates belonging to the genera *Dunaliella*, *Asteromonas*, *Synechococcus* and a lot of prokaryotes either Bacteria or Archaea, which represent the predominant organisms [28]. The biodiversity of saline habitats, typical examples of extreme environments, is determined by several physical-chemical parameters like the chloride content, salinity and iron content, temperature and oxygen solubility, pH value [10, 13, 29]. These parameters act as factors that can control the diversity towards physiological mechanisms [27, 29, 18].

It is difficult to attribute a spectral characteristic of salinity for a salt lake in the absence of generally agreed classification. On the base of their taste, the lakes are divided in fresh water lakes and salt lakes. The salinity of salt lakes may be different. The species richness in salt lakes decreases with the increasing of salinity [29]. Thus, in the range of salinity below 50 g L⁻¹ there could be found vertebrates, invertebrates, angiosperms,

macrophytes, phytoplankton and prokaryotes. Over the salinity of 50 g/l, the vertebrates and angiosperms are not generally able to survive. On the other hand, at a salt content over 120 g L⁻¹, saline waters harbour only some invertebrates like *Dunaliella* sp., sulphate reducing bacteria, cyanobacteria and archaea. At a salinity level between 170-220 g L⁻¹, sulphate reducing bacteria and cyanobacteria cannot be found, and over 220 g L⁻¹ the biodiversity is limited only to *Dunaliella* sp. and archaea [3]. In order to cope with high ionic strength from hypersaline and saline environments, prokaryotes developed two strategies, namely "salt-in" and "compatible solutes" [8, 19, 21].

In the first strategy the salts are accumulated in high concentrations inside cells to equalate the osmolarity with external environment. The enzymes and proteins present special adaptations i.e. the increasing of acidic amino-acid residues to their surface [14, 9, 10, 11]. The second strategy is based on the synthesis or accumulation in the cell of some organic molecules named compatible solutes in order to face external osmolarity [19].

The present paper presents the biological studies of lakes with natural genesis located in the sambhar, being investigated for the first time the presence of halophilic microorganisms both bacteria and archaea, phytoplankton and zooplankton species in relation with physical-chemical parameters as control factors.

2. Materials and Methods

A. Study Area

Sambhar Lake is situated at an altitude of 360 m AMSL in a relatively obscure habitat some 60 Km west of Jaipur at 26° 52' N – 27° 02' N and 74° 54' E – 75° 14' E just outside prosaically named Salt Lake City – Sambhar. This vast body of glacial saline is somewhat elliptical in shape having its long axis towards east-northeast to west-southwest. The lake basin

is spread at the confluence of three districts of Rajasthan namely Jaipur, Nagaur and Ajmer close to the desert fringe line. To the northwest and west of the basin, the Aravalli Ranges rise abruptly to a height exceeding 700 m in the form of hillocks, scattered along northern and southern periphery of the lake.

B. General Morphometric Features of Sambhar Lake

Parameters	Values
Surface Area (Sq. Kms.)	190
Max. Length (Kms.)	22.5
Max. Width (Range Kms.)	11.2 – 3.2
Max. Depth (Mts.)	3 (at its full)
Average Depth (Cms.)	> 0.61

The Sambhar Lake has a vast catchment area encompassing over 7560 sq. kms most of which lies to the north and north-east of the wetland extending up to Sikar District of Rajasthan. Four ephemeral streams Mendha, Rupangarh, Kharian and Khandel, numerous rivulets and surrounding surface run-off drains into the lake basin. Many faunistic surveys were undertaken during 1994-1997 to collect and identify the faunal diversity of the Sambhar Lake. Data on various other parameters of importance to the wetland were also categorized so as to evolve strategies for the conservation of various valuable taxa and the wetland ecosystem. The avian diversity recorded during 1994-1997 is based on seven survey conducted by the author in this region during different seasons twice every year.

C. Physiography of the lake

Sambhar Lake is divided by a five kms dam into two unequal parts. The eastern divide of the lake is a brine reservoir covering an area of 76.8 sq. km. This area comprises number of salt pans (kyars) for the manufacture of salt through natural evaporation method, by Sambhar Salt Limited. Though the process of concentrating salt from the lake brine involves several steps, the lake water is pumped into these reservoirs through sluice gates western part of the lake is open water undisturbed natural lake ecosystem. It is also sometimes altered by dredging channels to obtain water for salt manufacturing. Along the southern length of open waters of the lake are situated small clusters of "Dhanis" which are distantly placed in the region. About 20 km from the dam towards south-west direction of the lake, there is a hilly outcrop on which a temple of Mata Shakambari was constructed long back. It is a regular pilgrimage site for the people of Rajasthan. The northwestern length of the Sambhar Lake is fragmented into three major zones. On the northern side of the dam there is a small settlement called Gudha and a railway station adjoining the lake margin. Sambhar Salt Limited has their salt yard in that area. There is a continuous railway track along the northeastern side of the lake, which is distantly placed from the margins of the lake. The land in between is raised to create a sort of bund formation (dam) to restrict water to spread beyond certain land area. From Gudha settlement onwards up-till Nawa, a zone of about 10-15 km just adjacent and along the length of the lake is occupied by small units of private salt industries. At Nawa also Sambhar Salt Limited has brine reservoirs for salt extraction. The lake

still extends further with scanty shrub vegetation zone and touches the interrupted Aravalli hill-outcrops on the western end of the lake. The south-eastern length of the lake area is little elevated and extensively used for crop cultivation by the local farmers. Sambhar Lake is scattered surrounded by sparse and very scanty scrub vegetation. The elevated wasteland around the Sambhar Lake harbors grassy vegetation, which is extensively grazed by high-density livestock population. Since the actual inundation of the lakebed varies from year to year, the marginal demarcation of the lakebed remains variable. This is also accentuated due to high evaporation rate and water recedes faster shrinking the lake area leaving behind actual active zone with very shallow water.

D. Field Stations

Filed Stations Since the morphometry of sambhar Lake is complex with a lot of dendritic structures along its whole length, and the reservoir in general has an undulating topography, accounting of spatial variation was not easy in this system. However, five permanent sampling stations were fixed in the lake for convenient monitoring and systematic field study and regular sampling of waters. These stations were fixed after preliminary survey in the lake and according to differences in degrees of human interactions. The study stations were designated as Sambhar Lake Stations (SLS) - SLS-1, SLS-2, SLS-3, SLS-4 and SLS-5 respectively. The samples have been taken in summer and autumn period as it follows: the end of June 2013 and the end of August 2013; beginning of August 2013; beginning of September 2013. The physical aspect of the lakes was recorded using a digital camera Canon Power Shot model Pro 1.

Physical-chemical analysis of the water samples. The estimation of pH values, density and chloride content has been performed following previously described protocols^[3]. The content in mono and divalent cations or anions was determined using a Supermini X-Ray Fluorescence Spectrometer, following the semi quantitative method for light elements analysis in helium atmosphere. 10 ml of the surface water sample were weighted and placed in the spectrometer. The percent of elements was transformed into mg/ml dates, considering the weight of the sample^[2, 17, 24]. The UV-VIS investigations were performed using a Nano drop spectrophotometer conducted to record the absorbance and protein content as mg/ml at 280 nm.

E. Isolation of halobacterial strains.

The halobacterial strains were isolated from samples in a MH medium^[2, 17, 24]. 1 ml of the sample was placed in a Petri dish and mixed with 30 ml of the autoclaved molten agar culture medium (cooled to 55-60°C). After solidification, the plates were incubated at 37°C and 28°C for 7-10 days and after this period the bacterial colonies were counted.

F. Phyto and zooplankton samples.

The phytoplankton and zooplankton samples were taken on water column with a Patalas Schindler plankton trap, filtered through plankton net mesh with 65µm Ø and preserved in 4% formaldehyde. Species identification was made using a Zeiss inverted microscope according to the method described by^[21].

22, 23, 27] and specific taxonomic keys.

3. Result and Discussion

The recorded data revealed the salinity in investigated lakes is due to the presence of chloride and sulphur salts with ions from alkaline and alkaline-earth groups. Table 1 show that potassium quantities varying from 0.5 mg mL⁻¹ in station - 5, to 12 mg mL⁻¹ in (station-1), similarly, silica was present in a different content in all stations. In station - 1 and station - 3 the sodium was very less identified. In opposition, concentrations of 25 mg mL⁻¹ in station - 5 and 18 mg mL⁻¹ in (station-4) were recorded. Various chloride concentrations were determined as showed in table 1 arguing to consider the investigated stations as hyposaline or hypersaline. The water in station - 1 contained 21 mg mL⁻¹ sulphur, station - 3, 7 mg mL⁻¹ and the remaining lakes (station - 4, 5) contained only traces. The samples taken from station - 1 and station - 5 presented some magnesium content. In other stations, the concentration of these ions was recorded as 2 mg mL⁻¹ in station - 1, 0.1 mg mL⁻¹ in (station - 4) and 4 mg mL⁻¹ in station - 5. Aluminium was detected as trace element in all tested samples. (Station - 4 sample harboured phosphorus and bromide content.

Table 1: The ionic composition and physical-chemical characteristics of the investigated surface water samples from hyposaline and hypersaline stations. The presence of fungi is marked by “+”.

	SLS- 1	SLS- 2	SLS-3	SLS-4	SLS-5
K (mg mL ⁻¹)	12	13	11	2	0.5
Si (mg mL ⁻¹)	0.04	0.3	0.4	0.3	0.6
S (mg mL ⁻¹)	21	21	7	7	0.4
Absorbance at 280 nm	0.18	0.18	0.17	2.6	0.05
Protein content (mg mL ⁻¹)	0.2	0.2	0.16	3	0.05
pH	8.6	8.7	9.2	8.9	6.7
Chloride content (g L)	17	15	13	80	252
Density (g mL ⁻¹)	1.01	1.01	1.02	1.1	1.2
Presence of fungi	+	+	+	+	+

Based on these results presented in table 1, stations of sambhar lake may be classified in hyposaline and hypersaline. The results recorded in table 1 showed a protein content of 0.05 mg mL⁻¹ in station - 5, 0.16 mg mL⁻¹ in station - 3, 0.2 mg mL⁻¹ in station - 1, and 3 mg mL⁻¹ in the hypersaline lake station - 4. The values observed for protein content are related with the absence of sodium ions (station - 1 and station - 3—hyposaline stations) and a low level of chloride content around 15 g L⁻¹. In the case of station - 4 and station - 5 (hypersaline lakes) the protein content is influenced by the presence of sodium ions and high values of chloride content - 80 and 252 g L⁻¹, respectively. Station - 4 is characterized by a green (yellow- green) colour influenced by the intensity of sun light. The presence of algae from Bacillariophyceae and Chlorophyceae contributed to this colour. The water colour of the other stations showed no particular aspect being transparent (station - 3 and station - 5) or turbid, in case of station-1, due to human impact by sapropelic mud exploitation and extensive touristic use. The registered pH values varied from 6.7 in station - 5 to 9.2 in station - 3. The physical-chemical data presented in table 1 showed that station - 3 salt

lake harbours high salinity and alkalinity, which argue for considering this lake to have polyextremophilic conditions. The values recorded for density are correlated with the chloride content (Table 1). There were not observed any significant differences between hyposaline and hypersaline lakes. The microbiological research revealed that all investigated stations were inhabited by populations of halophilic microorganisms, both bacteria and archaea. The sodium ions content in the culture medium influences the number of total colony forming units (c. f. u.). By increasing sodium content, the number of c. f. u. decreased (Table 2). Station - 1 hyposaline lake was characterized by a high number of c. f. u. and the absence of magnesium ions. The hypersaline lakes station - 4 and station - 5 are inhabited by a low number of microbial strains and characterized by the presence of magnesium ions. A similar behaviour is observed in case of station - 3 hyposaline lake in which magnesium ions were not present. In spite of having different physical-chemical conditions, the obtained c. f. u. number is close to previously reported data for several man-made salt lakes [4]. Fungal species belonging to *Basipetospora* or *Walemia* genera were observed and isolated from all stations. Several numbers of microbial strains were selected for further investigations, as showed in table 2. Data from table 2 revealed that halophilic bacteria are abundant in the investigated lakes dominating the archaeal strains, both in hyposaline or hypersaline lakes. Four strains isolated from station - 1 showed the capacity to grow both in the presence of sodium deoxycholate and chloramphenicol. The same behaviour was noted for one strain from station - 3 and one from station - 5. The number of strains capable to produce hydrogen sulphide was significant in station-4. There is noted that such kind of microbial strains is closely related to the mechanism of sapropelic mud formation [26]. The use of mud for treatment of various diseases constitutes the main economic value of salt lakes [1, 4]. The investigation of phytoplankton taxonomical structure revealed a wide spectrum of cyanobacterial species in station - 1, characterized by the presence of magnesium and calcium ions, in station - 3 being detected only *Merismopedia tenuissima* (Table 3a). The high salinity of Station - 4 is reflected in the absence of cyanobacterial species. Diatoms were present in all stations with the higher richness in Station - 3 hyposaline lake where aluminium was detected in higher concentrations than in the other stations. A similar result was recorded in the case of green algal species (Table 3a). *Phacus* sp. Was observed only in Station - 1. Due to the high chloride content in Station - 5, no phytoplanktonic and zooplanktonic species were recorded. Among the investigated stations, Station - 1 presented the higher zooplanktonic richness (Table 3b), being detected protist, rotifer and crustacean species. The distribution of zooplankton species was related to the chemical composition of stations, at high chloride content (Station-4) being detected predominantly crustaceans and cladocerans. The Station - 4 is characterized by the presence of sodium, bromide and phosphorous. On the other hand, these species were observed in Station-3 hyposaline stations characterized by the absence of sodium, magnesium and calcium ions, the salinity of this lake being a consequence of the presence of sulphur salts.

These data are supported also by the relatively high numbers of halophilic microorganisms from this lake capable to produce hydrogen sulphide (Table 2). In the case of phytoplankton, the species richness appears to be double than

in the case of zooplankton. The salted environments investigated in this study are represented by salt lakes of natural origin located in Rajasthan.

Table 2: Distribution of colony forming units number (c. f. u.) in investigated salt lakes.

Stations	Total cfu. number	Selected strains number	Strains growing in the presence of bile salt	Strains growing in the presence of chloramphenicol	Strains producing H ₂ S
Station - 2	33x10 ²	18	10	4	1
Station - 3	43x10 ²	7	4	1	3
Station - 4	73x10 ²	10	8	0	7
Station - 5	69	7	5	1	2

Table 3: Phytoplankton diversity in investigated hyposaline and hypersaline environments

	Station-1,2	Station-3	Station-4
Cyanobacteria			
<i>Merismopedia tenuissima</i>	-	+	-
<i>Merismopedia</i> sp.	+	-	-
<i>Oscillatoria chalybea</i>	+	-	-
<i>Gloeocapsa</i> sp.	+	-	-
<i>Chroococcus</i> sp.	+	-	-
<i>Crucigenia</i> sp.	+	-	-
<i>Microcystis</i> sp.			
Bacillariophyceae			
<i>Asterionella formosa</i>	-	+	+
<i>Melosira granulata</i> var.	+	+	+
<i>Navicula</i> sp.	+	+	-
<i>Cymbella</i> sp.	-	+	+
<i>Fragillaria</i> sp.	-	-	+
<i>Cyclotella</i> sp.	+	-	-
Chlorophyceae			
<i>Pediastrum</i> sp.	+	-	-
<i>Scenedesmus</i> sp.	+	+	+
Euglenophyceae			
<i>Phacus</i> sp.	+	-	-

Table 3b: Zooplankton diversity in investigated saline and hypersaline environments.

	Station - 1,2	Station - 3	Station - 4
Protozoa			
<i>Protozoa</i> g. sp.	+	+	-
<i>Flagelata</i>	+	-	-
<i>Testacea</i>	+	-	-
<i>Diffugia</i> sp.	+	-	-
Ciliata			
<i>Vorticella</i> sp.	+	-	-
Rotifera			
<i>Keratella</i> sp.	-	-	+
<i>Cephalodella</i> sp.	+	-	-
<i>Brachionus</i> sp.	+	-	-
<i>Rotifera</i> g. sp.	+	-	-
Crustacea			
Copepoda			
Diaptomida			
Harpacticoida			
<i>Harpacticoida</i> g. sp.	-	+	-
Cladocera			
<i>Moina salina</i>	-	+	+
<i>Bosmina</i> sp.	+	-	-

4. Conclusions

Based on the investigation from this work, the chemical composition of all tested stations resulted to be marked by abundant presence of chlorides (sodium, magnesium, potassium) and some relatively important concentrations of aluminium and strontium in the particular case of Station - 5. Biological diversity was represented mostly by several types of archaeal and halophilic bacterial species. Several species of Cyanobacteria, Bacillariophyta, Ciliata, Rotifera and Copepoda were detected mainly in the samples taken from Station - 1 and Station - 3 hyposaline lakes, where the total chloride concentrations did not exceed 20 g L⁻¹ and sodium appeared to be absent. The data from this study revealed that the presence of magnesium and calcium ions and the chloride content have the role to control the phytoplankton and zooplankton populations in these saline environments.

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