Chapter-6

Conclusions
Highly alkaline pH of Lonar lake non-significantly varied throughout the period of investigation. pH of Lonar soda lake water ranged between 9.0 to 10.5.

Moderate temperature of lake remained stable in the range of 30°C – 35°C during daytime for recorded days.

Considerably more concentration of total solids was recorded. Total dissolved solids (TDS) recorded in present investigation was higher as compared to the very well studied African soda lake and Kenyan soda lake.

Alkalinity of lake water was not significantly variable throughout all seasons. The cause of alkalinity is the minerals which dissolve in water and geological reactions carried out in lake water.

Remarkable variation in dissolved oxygen was recorded in all seasons of lake water samples when compared with previous reports of Lonar lake and other soda lakes.

Biological oxygen demand (BOD) levels were significantly different. The high BOD value was found at season where decomposition occurs which is indicative of assimilation of organic load and occurrence of more microorganisms.

Green cyanobacterial mass was observed indicating eutrophication. High phosphate content could be another reason for eutrophication.

Total viable count (TVC) was recorded as $2.2 \times 10^3$ cfu/ml for surface samples and $2.3 \times 10^4$ cfu/ml for sediment samples at optimum pH 10.5. Highest bacterial count with enriched composite samples ($2.5 \times 10^3$ cfu/ml) was recorded at pH 10.5 and temperature 35°C.
Out of six media used, modified Horikoshi medium (mHk) (F) supported highest diversity hence it was used to isolate various types of organisms. However modified Horikoshi and Tindal’s medium were also implicated for isolation of various organisms.

Estimation of cultivable bacterial diversity of Lonar soda lake yielded 107 types of morphotypes.

Microbial diversity of soil samples collected from Lonar shore was also studied. But only isolates belonging to the Firmicutes were identified.

Selected isolates were capable of producing more than four different alkaline enzymes, like amylase, protease, caseinase and lipase etc and they are resistant to many antibiotics like rifampicin, nitrofurantoin, erythromycin etc.

Effect of pH on growth of the isolates showed that maximum isolates had an optimum pH of 10.0-11.0 and temperature at 30⁰C-40⁰C.

Out of 34, 14 isolates showed luxuriant growth in pH 8.0-11.0 range and 8 isolates showed in pH 8.0-10.0. While six isolates showed growth in 9.0-11.0 pH ranges. Only 3 isolates were able to show luxuriant growth at 7.0-12.0 and 7.0-11.0 pH ranges. pH tolerance capacity of isolates clearly indicates that these microbial isolates belong to native microflora of soda lake.

Out of 107, 34 were thoroughly characterized, identified and classified. Representative of different morphotype, tropic level, physiological and biochemical groups were isolated and cultivated.
Isolates were identified as *Planococcus maritimus*, *Bacillus cohnii*, *Bacillus subtilis*, *Bacillus licheniformis*, *Alcanivorax* sp., *Oceanobacillus iheyensis*, *Bacillus cohnii*, *Alcanivorax* sp., *Alcanivorax* sp., *Bacillus subtilis*, *Haloalkaliphilic bacterium*, *Xanthomonas* sp., *Devosia yakushimensis*, *Alcaligenes* sp., *Paracoccus* sp., *Halomonas* sp., *Halomonas venusta*, *Paracoccus* sp., *Pseudomonas aeruginosa*, *Stenotrophomonas* sp., *Bacillus alkologaya*, *Pseudomonas aeruginosa*, *Planococcus maitriensis*, *Halomonas* sp., *Bordetella* sp., *Alkalimonas delamerensis*, *Halomonas* sp., *Achromobacter* sp., *Halomonas* sp., *Halomonas* sp., *Bacillus alkologaya*, *Bordetella petrii*, *Halomonas venusta* and *Halomonas hydrothermalis*.

All these 34 sequence clones were classified into 4 group's viz. Firmicutes, α-proteobacteria, β-proteobacteria, γ-proteobacteria.

In present study Phylogenetic analysis of isolates indicated that most of the isolates were related to phylum γ-proteobacteria followed by Firmicutes.

Out of the 34 isolates, eleven belonged to phylum Firmicutes, three to α-Proteobacteria, four to β-Proteobacteria and sixteen to γ-Proteobacteria. In the present study, Gram negative Proteobacteria group were more diverse and abundant.

Presence of *Planococcus maritimus* (KBDL1), *Oceanobacillus iheyensis* (KBDL6), *Alcanivorax* sp. (KBDL8), *Xanthomonas* sp. (KBDL12), *Devosia yakushimensis* (KBDL13), *Alkalimonas delamerensis* (KBDL26) from Lonar lake is a new finding, which extend our knowledge of diversity of the soda lake.
Halophilic bacteria belonging to genera *Halomonas*, *Alkalimonas*, *Stenotrophomonas* were obtained from Lonar lake water.

The DNA sequences of isolates KBDL14 (showed 79% similarity with *Alcaligenes* sp.), KBDL28 (showed 92% similarity with *Achromobacter* sp.) and KBDL32 (showed 82% similarity with *Bordetella petrii*) showed very less similarity with previously known sequences in GenBank database. Thus these could be novel organisms which need to be further confirmed by fatty acid analysis, DNA-DNA hybridization etc.

Chemotaxonomic characterization of a haloalkaliphilic isolate of *Halomonas* KBDL16 and KBDL23 was studied.

*Oceanobacillus iheyensis* has at least 29 'proteolytic' enzymes, which could be important in future industrial applications.

Alkaliphilic bacteria isolated from the Lonar soda lake exhibited protease activity. *B. licheniformis* KBDL4 and *Pseudomonas aeruginosa* (KBDL19) were could be considered as promising strains for biotechnological applications.

The strains were found to produce proteases; which was highly stable and active at high pH and showed optimum activity at pH 8 – 12 and at 60°C.

The *B. licheniformis* KBDL4 and *Pseudomonas aeruginosa* KBDL19 crude protease showed excellent stability and compatibility with various commercial detergents.

Considering the high activity and stability in high alkaline pH and temperature, stability in the presence of surfactants and stability in the presence of various commercial detergents, the KBDL4 and KBDL19 protease may find potential application in laundry detergents.
> The alkaline protease could decolorize a blood stain on cotton fabrics indicating its potential use in detergent formulation.

> KBDL4 and KBDL19 protease has the commercial application in decomposing of gelatinous coating on the X-ray films which is useful in recovery of silver.

> The ability of alkalophilic *Bacillus subtilis* (KBDL3), *Xanthomonas* sp. (KBDL12) and *Bacillus licheniformis* (KBDL4) amylase enzyme to withstand a pH up to 11.0 for 30 min, and its high pH and temperature optimum for activity and stability, and resistance to SDS could suggest that the enzyme has a potential in starch liquefaction and detergent industry.

> *Halomonas* sp. KBDL16 and *Halomonas* sp. KBDL24 were Gram-negative, non-spore-forming, motile singly curved rods, 1.2-1.4 X 0.7-0.8 μm.

> *Halomonas* sp. showed growth at 30–50°C (optimally at 40°C), at pH values of 8.0–11.0 (optimally at pH 10.0) and in 5.0–20.0% (w/v) NaCl (optimally in 15.0% NaCl).

> The fatty acid profiles of the strain KBDL16 and KBDL24 were similar to those of related taxa; the fatty acids C\textsubscript{18:1ω7c}, C\textsubscript{16:0} and C\textsubscript{16:1ω7c} were predominant. Q-9 is the predominant respiratory lipoquinone.

> Hydrocarbon degradation activity was also performed using novel isolates as *Alcanivorax* sp. (KBDL8) and *Pseudomonas* sp. (KBDL19) of our collection. Napthelene degradation rate of *Alcanivorax* sp. and methanol degradation rate of *Pseudomonas* sp. was high.
➢ *Alcanivorax* sp. (KBDL8) and *Pseudomonas* sp. (KBDL19) showed highest degradation of napthalene and methanol at 144 hrs respectively. Benzene and toluene degradation rate of *Alcanivorax* sp. (KBDL8) and *Pseudomonas* sp. (KBDL19) were very slow.

➢ To understand role and structure of microbial communities, only sequence data is not enough but cultivability of microorganisms is also very important. Lonar lake harbors a wealth of diverse microorganisms with useful commercial properties.