

Assessment of Physicochemical Properties of Samples Collected from District Jaipur

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The microbial diversity and ecological dynamics of extreme environments such as Sambhar Lake, Rajasthan, are of significant interest due to their unique characteristics and potential biotechnological applications. In this study, Physicochemical analysis is done from various sites in Jaipur district. Different parameters include colour, temperature, pH, Total solid suspension (TSS), total dissolved solids (TDS), Turbidity, Total alkalinity, Total hardness, calcium, and presence of chloride by prescribed methods. The results highlight the presence of the extreme conditions of Sambhar Lake and marble slurry site, contributing to the overall microbial diversity of the ecosystem. The insights gained from this assessment contribute to our knowledge of physical-chemical differences in extreme environments and hold potential implications for biotechnological applications, environmental monitoring, and conservation efforts in saline ecosystems.

Keywords: Physicochemical properties, Sambhar Lake, extreme environment, Jaipur.

1. Introduction

Saline habitats have hypersaline conditions and are harsh settings. Both thalassohaline and athalassohaline environments may be found there(1). Earth's environments that fit this description are unique and have qualities such a neutral pH, moderate temperature, plenty of oxygen, salinity in both freshwater and saltwater, and pressure in a few atmospheres(2). Extremophiles in the type of halophiles are those that are always present at extreme amounts of salt. It may be found in saline environments all around the globe. To regulate the osmotic pressure of the surrounding medium, they stop salts from denaturing and gather inorganic ions like K⁺, Na⁺, and Cl⁻ in the cytoplasm. They fall into two primary categories: eukaryotic microorganisms and prokaryotic microorganisms (bacteria and archaea). The hypersaline

habitats of bacteria and archaea are harsh circumstances with high salinity, fluctuating temperatures, little oxygen, and a wide pH range. The bacteria are categorized according to the amount of salt they contain: mild halophiles grow best at 0.2-0.85M (2–5% NaCl), moderate halophiles grow best at 0.85-3.4 M (5–20%) NaCl, and severe halophiles grow best at 3.4-5.1 M (20–30%) NaCl(3). Certain halophiles thrive in moderate environmental circumstances, which include 20–40°C temperatures, neutral pH values, water availability, salts, and certain macro- and micronutrients that are necessary for mesophile development. Osmotic pressure is controlled by utilizing two different strategies: the first balances osmotic pressure in the cell by accumulating K^+ and Cl^- ions, and the second strategy involves accumulating organic solutes like ectoine, hydroxyectoine, Nε-acetyldiaminobutyrate β-glutamine, betaine, trehalose, and proline to maintain osmotic balance in the cell so that they can survive in saline environments. The isolation of somewhat halophilic bacteria has been reported by researchers. 2,4,6-trinitrotoluene (TNT), which this bacteria may collect, contributes to its detoxification⁸. Growing in saltwater environments, halophilic bacteria astonish us with their physiological characteristics, growth parameters, and ability to produce bioactive compounds. Halophiles are used in the synthesis of biomolecules for medical applications. Saline and alkaline conditions may be found in Rajasthan at the Sambhar Lake. Sambhar Lake has a wide variety of halophilic microorganisms. Because of the hypersaline environment, this lake has a high concentration of Na^+ and Cl^- ions. Polyhydroxyalkanoate (PHA) is produced by the *Vibrio proteolyticus* strain that was isolated from the maritime environment in Korea⁽⁴⁾.

Sambhar Lake

In Rajasthan's metamorphic rock, there is a naturally formed lake with an alkaline and hypersaline environment (Latitude 26°58'N, Longitude 75°05'E). Since it is alkaline, it is also known as Sambhar Soda Lake. Its height is 360 meters above mean sea level. The Aravalli hills encircle the Sambhar Lake basin from the north and west, dividing it into an eastern subhumid region and a semi-arid region⁽⁵⁾. The Thar Desert is another name for the semi-arid region. These lakes have maximum and lowest depths of 3 and 1 meters, respectively. A horizontally developed bed split illustrates the sedimentary layers in Sambhar Salt Lake. Tropical weather is typical to Sambhar Lake⁽⁶⁾. Seasons like cold, rain, and summer determine its climate. Because of the desiccation of Sambhar Lake's surface, which leads to the production of an efflorescent crust but does not display total desiccation, the temperature drops below 4 °C in the winter and rises to 45 °C in the summer (May to June). The two primary constituents of the crust are calcite and halite. The solute (Ca^+ , Mg^+ , and Na^+) content of the lake is increased by these concentrations⁽⁷⁾. During the monsoon season, the Sambhar Salt Lake was supplied with water by rivers including the Roopangarh and Mendha rivers. Sambhar Lake saw an annual rainfall range of around 50 cm in 1983–1985, and 39 cm in 2005–2006. The average annual rainfall in Lake was 30 to 40 cm. This lake's chemical and physical properties make it very significant geologically⁽⁸⁾. Geochemical features include monsoon, climatic fluctuation, the hydrological system in Sambhar Salt Lake water, oxygen isotope-assisted water evaporation from brine, and mineralogical research. The lake spans around 225 square kilometers and has a shallow form, measuring 22.5 km in length and 11.2 km in breadth⁽⁹⁾. Water is dependent upon Mendha and Rupangarh, the two main streams of water that conjugate. The Mendha River flows southwestward after entering from the northeast. The

Rupangarh River emerges from the city of Ajmer and goes into the southern hilly region's lake(10).

Features of Sambhar Lake

Sambhar Lake, characterized by its shallow depth averaging about 3 meters, is a saline waterbody renowned for its brackish waters, with salt content ranging from 8 to 10 grams per litre. This lake serves as a favored breeding ground for flamingos and various other migratory bird species. Dotted across its expanse are numerous small islands, some reachable by boat, adding to the charm of the landscape. The surrounding geography of Sambhar Lake is adorned with picturesque beach formations, attracting visitors keen on sightseeing and exploration(11).

Ecological and Economic Significance of Sambhar Lake

Sambhar Salt Lake, Rajasthan's largest saline lake, stands as a vital hub for salt production in India. Annually, it yields a staggering 196,000 tonnes of high-quality salt, contributing about 9% to the nation's total output. Governed by the government-owned entity Sambhar Salts Ltd. (SSL), formed as a partnership between Hindustan Salts Ltd. and the state government, salt production primarily relies on brine evaporation methods. SSL manages 3% of the eastern lake area.

Designated as a Ramsar site, Sambhar Lake holds immense ecological significance. During winters, it transforms into a sanctuary for tens of thousands of pink flamingos and migratory birds from northern Asia and Siberia. The lake's unique algae and bacteria species infuse vibrant hues into its waters, fostering a delicate ecosystem that sustains these migrating waterfowl.

Surrounding the lake, diverse forests provide habitat to a rich array of wildlife, including freely roaming Nilgai, deer, and foxes. This harmonious blend of salt lake and adjacent forests creates a haven for both avian and terrestrial species, enriching the region's biodiversity and contributing to its ecological balance(12).

2. MATERIALS AND METHODS

Soil & Marble slurry sample collection

After analysis and a continuous literature survey, Shambhar Lake has been selected for the collection of samples. The Sambhar Lake is considered as one of the largest saline depression land in western desert areas of Rajasthan, India. It is the largest salt source lake available in Rajasthan. The geographical location of it is (26°52'–27°2'N, 74°53'–75°13'E). Soil samples were collected from four points of Sambhar Lake and two marble slurry areas from Jaipur, India (Table 1). These samples were collected in sterilized screw cap plastic bottles and sterile polyethylene bags. Samples were kept in 4°C and future work was done in aseptic conditions. Further, these samples were used for analyzing various physicochemical parameters such as pH, Temperature, TDS, TSS, alkalinity, and various ion concentrations).

Table 1: Sample collection sites with their locations

Sl. No.	Sites	Location	Geographical coordinates
1.	Site1	Sambhar Lake (Main area)	26.946778456985722, 75.03496112141154
2.	Site2	Sambhar Lake, Salt production (side ridge area)	26.91502156109291, 75.12087764039624
3.	Site3	Outer salt production area	26.910429463030972, 75.14027537458605
4.	Site4	Outridge (salt production area)	26.909051797183224, 75.11126460398357
5.	Site5	Marble slurry sample 1	27.05495859290586, 75.91558926898483
6.	Site6	Marble slurry sample 2	27.05495859290586, 75.91558926898483

Sample pre-treatment

These samples were collected in sterilized screw cap plastic battles and sterile polyethylene bags. Samples were kept in 4°C and future work was done in aseptic conditions.

Physicochemical analysis parameters

All collected soil samples and marble slurry samples from Sambhar Lake were estimated for validation of several physicochemical attributes viz. colour, Total Solids (TS), pH, Total Dissolved Solids (TDS), temperature, Total hardness, Turbidity (NTU), total chloride, Total Suspended Solids (TSS) and Alkalinity. The protocols used for the estimation of the above attributes were per the guidelines of APHA 2005 and considered to be within the WHO permissible limit of potable water (APHA, 2005).

Statistical validation

All the physicochemical test results were provided in the form of mean $\pm$ SD taking a minimum of three replicates. The data collected from soil and marble slurry samples were validated statistically by using the GraphPad Prism 6.05 version taking the least significant difference for mean comparison at 5 % level. A statistical study was used to validate and describe the significant differences at P value level that has to be lower than 0.05 ($P < 0.05$).

3. RESULTS AND DISCUSSION

Sambhar Lake is also known as Salt Lake. The concentration of salts and minerals was very high. Water from this area does not fall in the permissible range of the World Health Organization (WHO) and APHA. Various quantitative biochemical and physicochemical parameters of soil and slurry samples were processed within 48 hours to avoid errors (Nivedha et al., 2023). Though several conventional techniques are available for remediating hazardous chemicals and toxicants from different sources like industrial and/or domestic sectors (Saksena

et al., 2008; Mandal et al., 2010; Misra, 2010).

Physicochemical analysis of soil samples and marble slurry samples

To understand the biochemical potency of the collected soil samples and marble slurry samples, collected from Sambhar Lake, Jaipur, Rajasthan, certain physicochemical parameters like colour, pH, Temperature, Total hardness, Total Solids (TS), Total Dissolved Solids (TDS) (2540 C), Turbidity (NTU) (2130), total chloride (4500-CL), Total Suspended Solids (TSS) and Alkalinity (Table 2, Figure 1-9). The results depicted significant P value ($P < 0.0001$ and $P < 0.0021$) for all the six selected sites soil and slurry samples at 95% confidence interval. Similar types of studies have been validated by many other researchers (Jeevanantham et al., 2019; Aghalari et al., 2020; Nivedha et al., 2023).

Analysis of Colour

All the samples, collected from various sites, were analyzed to have different colors. Colour was analyzed based on physical appearance at the time of collection. Sample collected from site 1 were showed whitish brown in appearance while site 2 samples was whitish green, site 3 is brownish-black, site 4 was dark brown, site 6 was slurry sample and appeared creamish white and site 6 samples colour was greyish white yellowish.

Analysis of Temperature

In case of temperature access of all the collected samples, the range lay between 17.16-21.33°C. The lowest temperature of $17.16 \pm 0.2^\circ\text{C}$ was seen in case of soil samples from site 1; while the highest temperature of $21.33 \pm 0.8^\circ\text{C}$ was seen in case of slurry samples from site 6 (Figure 1).

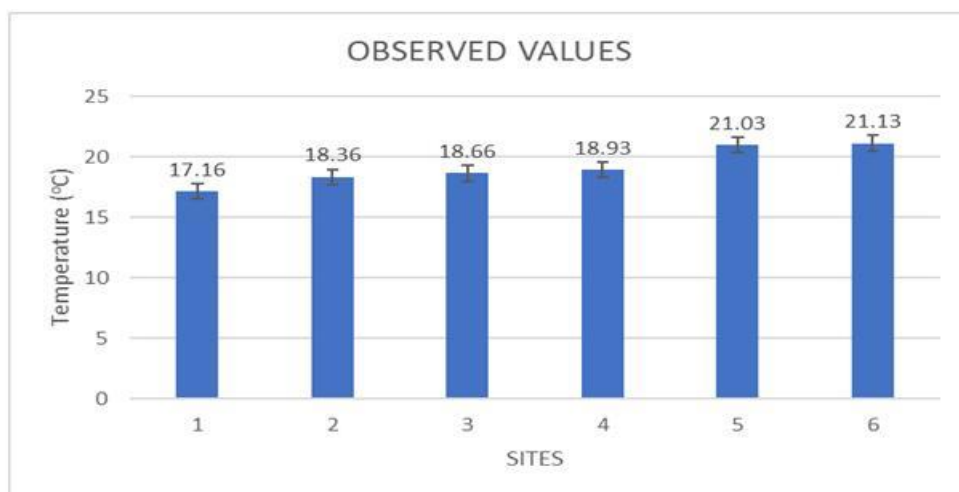


Figure 1: Temperature analysis of soil and slurry samples

Analysis of pH

The pH of the soil samples collected from Sambhar lake was found in the range 9-9.9 where as marble slurry sample temperature ranges from 8-9. Minimum pH was observed from site 5 (8.93) and maximum pH was recorded from site 2 i.e 9.64 (Table 2; Figure 2).

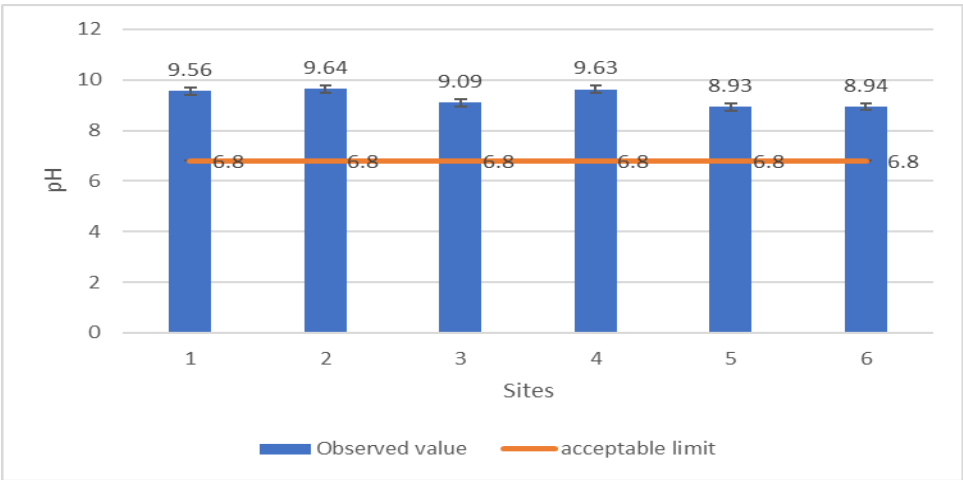


Figure 2: pH analysis of different sites samples

Table 2: Various physicochemical parameters of different sites

Site/ Parameters	limit	Site 1 Mean ± SE	Site 2 Mean ± SE	Site 3 Mean ± SE	Site 4 Mean ± SE	Site 5 Mean ± SE	Site 6 Mean ± SE	Mean (n = 3)	SD
Colour	colourless	Whitish brown	Whitish green	Brown black	Dark brown	Cream- white slurry	Grey-white slurry	-	-
Temperature (°C)	-	17.16± 0.2 ^{b*}	18.36±0.14	18.66±0.14	18.93±0.14	21.03±0.17 ^a	21.33± 0.8 [#]	19.21	1.56
pH	6.5-8.5	9.54±0.27	9.64± 0.01 ^{a#}	9.13±0.05	9.63±0.17	8.93± 0.03 [*]	8.94±0.037 ^b	9.29	0.347
TSS (mg/l)	5-10	9268.87±2.6 ^{0^a #}	8259.67±1.4 ⁵	6510± 3.78 [*]	6016±2.64 ^b	8625.67±1. 2	8322.33±2.0 ²	7833.718	1277.71
TDS (mg/l)	50-500	2562.33±1.2	3390.67±1.2 ^{a #}	2793.33±1. 76	2247±0.57	2109.67±2. 9	1853.67±2.0 ^{2^{b*}}	2492.77	550.82
Turbidity (NTU)	1	359± 2.08 [#]	212± 2.18	175±2.08	149± 1.52 ^{b*}	219±2.08	206±1.52	377.72	197.34
Total Alkalinity (mg/l)	200	6793± 2.08 ^{a#}	4049± 2.5	3567±2.64	3182±2.57	1993±2.51	1988±2.64 ^{b*}	3728.66	1767.79
Total hardness (CaCO ₃) (mg/l)	200	410.83±1.01	327.33±0.81	304.16±0.7 ^{6^{b*}}	425±0.79	475.16±0.8 ^{4^a #}	359.33±0.63	383.63	64.66
Calcium (CaCO ₃ mg/l)	4.0	409± 0.95 [*]	501±1.80	470±0.91	463±1.60 ^b	656± 1.41 ^{a#}	511±1.55	501.5	83.87
Chloride (mg/l)	75	663± 0.97 ^{a#}	548±0.96	497±0.91	598±0.98	413± 1.17 ^{b*}	439±1.06	526.33	95.514

*Minimum value, # Maximum value, SE = Standard error, SD = Standard deviation; a depicts P < 0.0001; b depicts P < 0.002

Analysis of Total Suspended Solids

Total suspended solids are responsible for turbidity of water. TSS values were recorded in the range of 6016-8625 mg/ L for all samples. The highest values were observed in samples collected from site 6 whereas the lowest value was noted for site 4. APHA prescribed 5-10 mg/L permissible limit of TSS for drinking water. All the samples of the seven sites were much higher compared to the permissible limit level. Mean of TSS was 7833.71 mg L-1 (Figure 3).

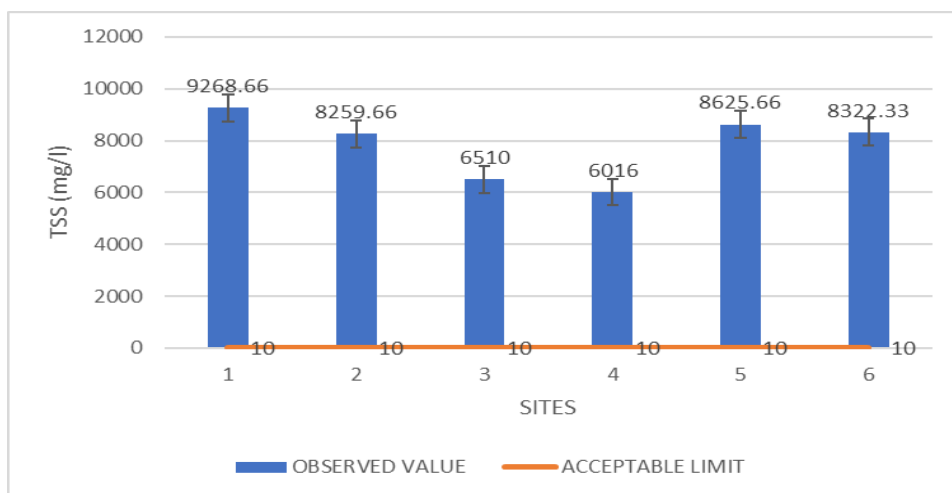


Figure 3: TSS of sample collected from different sites

Analysis of Total Dissolved Solid

Total dissolved solid that was recorded more than 2000 mg L⁻¹ for all the samples collected from all 6 sites. Samples collected from site 2 showed the maximum TDS while site 6 samples showed the minimum concentration. TDS values were observed in the range of 1850-3400 mg L⁻¹ (Table 2). 500 mg L⁻¹ is the maximum permissible limit for TDS as per APHA. All the samples were not permissible range (Figure 4).

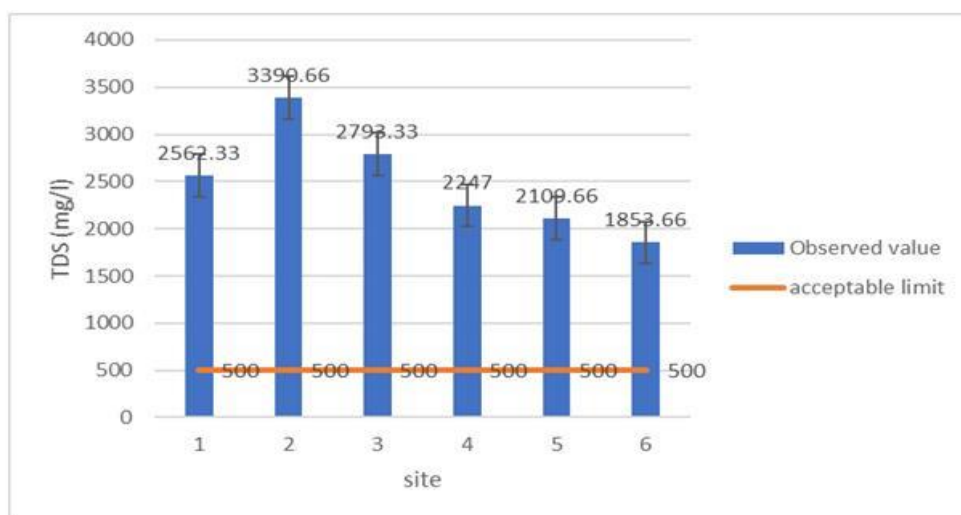


Figure 4: Variation in TDS values of different Site samples

Analysis of Turbidity

Samples collected are from the lake area and the presence of a high number of solid particles shows high turbidity compared to the limit of APHA. Maximum turbidity was observed from site 6 whereas minimum turbidity was recorded from site 3 (Figure 5).



Figure 5: Variation in turbidity from 6 sites

Analysis of Total Alkalinity

The sample collected from Sambhar Lake shows a higher range in alkalinity. Samples collected from Site 1 (main area, Sambhar Lake) showed maximum alkalinity as compared to other sites. The minimum value was recorded from site 5 as shown in Figure 6.

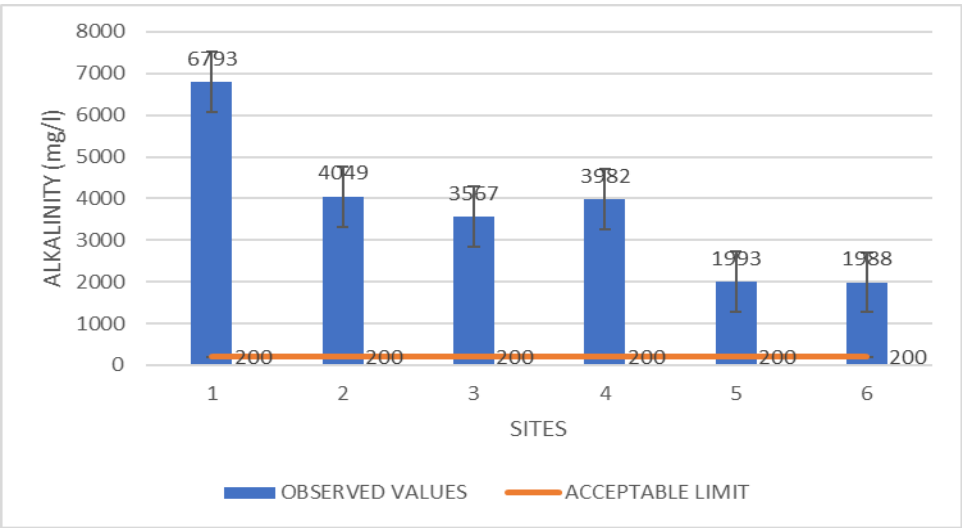


Figure 6: Various sites with their alkalinity (mg/L)

Analysis of Total hardness

Samples were collected from 6 different sites. Maximum value observed from site 5 whereas site no 3 showed lower value. Values observed were very high as compared to the limit for water given by APHA i.e. 200 (Figure 7).

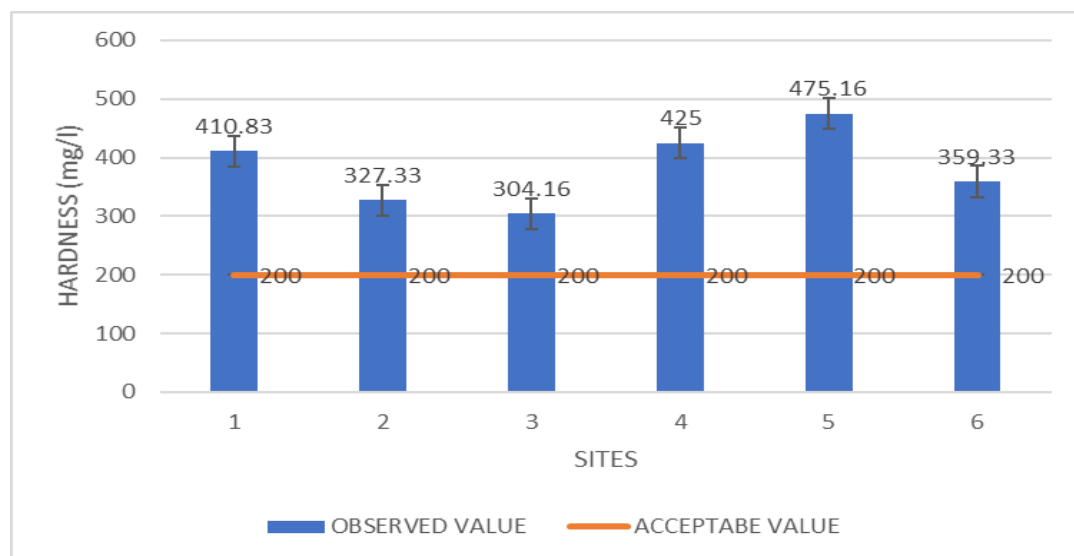


Figure 7: Sample sites with their hardness compared with permissible limits

The concentration of calcium in the form of carbonates was recorded and the maximum value was observed from site 5 i.e. 656 mg/L (marble slurry sample) and the lowest value was from site 1 i.e. 409 mg/L. The permissible limit of APHA was much lower as compared to the observed value (Figure 8).

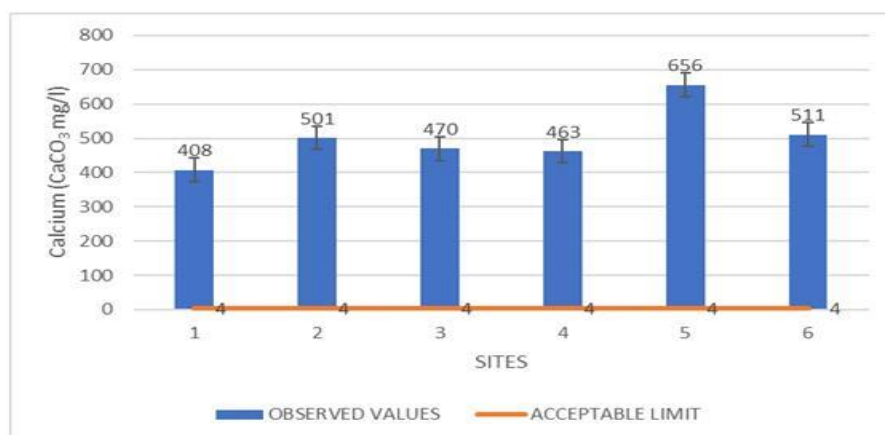


Figure 8: Concentration of CaCO₃ (mg/l) in soil and slurry samples collected from different sites

Analysis of Chloride

Site no. 1 samples reflect the highest value i.e. 663 mg/L while the lowest chloride value was recorded 413 mg/L collected from site 5. The permissible limit of chloride in water prescribed by WHO was 75mg/L. The mean value of collected samples was observed as 526.33 mg/L (Figure 9).

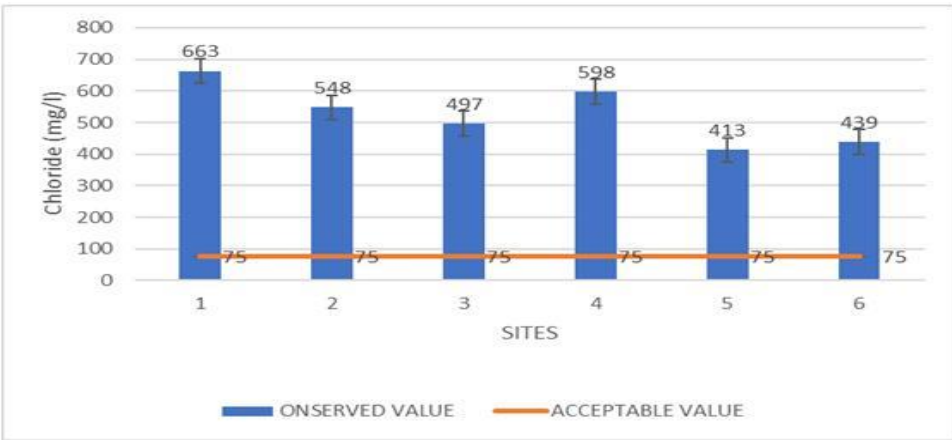


Figure 9: Sample sites with concentration of chloride ions

Statistical validation:

The statistical validation was done by taking mean (triplicate readings per sample) and calculating the standard deviation and standard error of various parameters which was illustrated in Table 2.

4. Conclusion

Measuring various physicochemical parameters of selected soil and marble slurry samples from different sites in Jaipur compared with prescribed limits shows diversity in environmental conditions. Samples taken from high-alkaline sites show a higher level of alkalinity as well as higher pH which results in the presence of diverse microflora where whereas samples which were collected from marble sites showed lower pH which propagates the growth of different varieties of microorganisms compared to other parts of Jaipur wastewater (Arslan et al., 2005; Bakshi et al., 2020; Chidichimo et al., 2024). In the future, this type of analysis will help to identify a variety of microflora, their ability to thrive in such harsh conditions helps to utilize their potency in bioremediation and other sustainable solutions.

References

1. Cui HL, Dyall-Smith ML. Cultivation of halophilic archaea (class Halobacteria) from thalassohaline and athalassohaline environments. *Marine Life Science and Technology*. 2021.
2. Rodriguez-Valera F. Introduction to Saline Environments. In: *The Biology of HALOPHILIC BACTERIA*. 2020.
3. DasSarma P, Coker JA, Huse V, DasSarma S. Halophiles, Industrial Applications. In: *Encyclopedia of Industrial Biotechnology*. 2010.
4. Mitra R, Xu T, Xiang H, Han J. Current developments on polyhydroxyalkanoates synthesis by using halophiles as a promising cell factory. *Microbial Cell Factories*. 2020.
5. Upasani VN. Microbiological Studies on Sambhar Lake (Salt of Earth) Rajasthan , India Microbiological Studies on Sambhar Lake (Salt of Earth) Rajasthan , India . *Res Gate*. 2015;
6. Yadav DN, Sarin MM, Krishnaswami S. Hydrogeochemistry of Sambhar Salt Lake, Rajasthan:

- Implication to recycling of salt and annual salt budget. J Geol Soc India. 2007;
7. Sinha R, Raymahashay BC. Evaporite mineralogy and geochemical evolution of the Sambhar Salt Lake, Rajasthan, India. Sediment Geol. 2004;
8. Kumar O, Devrani R, Ramanathan A. Deciphering the Past Climate and Monsoon Variability from Lake Sediment Archives of India: A Review. J Clim Chang. 2017;
9. Pathak AP, Cherekar MN. Hydrobiology of hypersaline Sambhar salt Lake a Ramsar site, Rajasthan, India. Indian J Geo-Marine Sci. 2015;
10. Yadav DN, Sarin MM. Ra-Po-Pb isotope systematics in waters of Sambhar Salt Lake, Rajasthan (India): geochemical characterization and particulate reactivity. J Environ Radioact. 2009;
11. Singh P, Khadim R, Singh AK, Singh U, Maurya P, Tiwari A, et al. Biochemical and physiological characterization of a halotolerant *Dunaliella salina* isolated from hypersaline Sambhar Lake, India. J Phycol. 2019;
12. Preparation I. Sambhar Lake - Learn about its Features, Physiography, Topography, Significance & Types Of Soil Found. testbook [Internet]. 2023; Available from: <https://testbook.com/ias-preparation/sambhar-lake#:~:text=Sambhar Lake is located about,cranes%2C and numerous other waterfowl.>
13. Binayke A, Ghorbel S, Hmidet N, Raut A, Gunjal A, Uzgaré A, et al. Analysis of diversity of actinomycetes from arid and saline soils at Rajasthan, India. Environ Sustain. 2018;
14. Singh RP, Jha PN. A halotolerant bacterium *Bacillus licheniformis* HSW-16 augments induced systemic tolerance to salt stress in wheat plant (*Triticum aestivum*). Front Plant Sci. 2016;
15. Gaur A, Sharma G, Mohan D. Isolation and characterization of moderate halophilic *Salinicoccus* sp. producing extracellular hydrolytic enzymes. J Glob Biosci. 2015;
16. Gupta M, Aggarwal S, Navani NK, Choudhury B. Isolation and characterization of a protease-producing novel haloalkaliphilic bacterium *Halobiforma* sp. strain BNMIITR from Sambhar lake in Rajasthan, India. Ann Microbiol. 2015;
17. Sundaresan S, Ponnuchamy K, Abdul R. Biological management of sambhar lake saltworks (rajasthan, india). Managing. 2006;
18. Upasani V, Desai S. Sambhar Salt Lake - Chemical composition of the brines and studies on haloalkaliphilic archaeobacteria. Arch Microbiol. 1990;
19. DUSSAULT HP. An improved technique for staining red halophilic bacteria. J Bacteriol. 1955;
20. Margesin R, Schinner F. Potential of halotolerant and halophilic microorganisms for biotechnology. Extremophiles. 2001.
21. Cuadros-Orellana S, Pohlschröder M, Durrant LR. Isolation and characterization of halophilic archaea able to grow in aromatic compounds. Int Biodeterior Biodegrad. 2006;
22. Sabet S, Diallo L, Hays L, Jung W, Dillon JG. Characterization of halophiles isolated from solar salters in Baja California, Mexico. Extremophiles. 2009;
23. Hezayen FF, Tindall BJ, Steinbüchel A, Rehm BHA. Characterization of a novel halophilic archaeon, *Halobiforma haloterrestris* gen. nov., sp. nov., and transfer of *Natronobacterium nitratireducens* to *Halobiforma nitratireducens* comb. nov. International Journal of Systematic and Evolutionary Microbiology. 2002.
24. Xin H, Itoh T, Zhou P, Suzuki KI, Nakase T. *Natronobacterium nitratireducens* sp. nov., a haloalkaliphilic archaeon isolated from a soda lake in China. Int J Syst Evol Microbiol. 2001;
25. Sharma AK, Walsh DA, Bapteste E, Rodriguez-Valera F, Ford Doolittle W, Papke RT. Evolution of rhodopsin ion pumps in haloarchaea. BMC Evol Biol. 2007;
26. Karan R, Khare SK. Purification and characterization of a solvent-stable protease from *Geomicrobium* sp. EMB2. Environ Technol. 2010;
27. Karbalaee-Heidari HR, Amoozegar MA, Hajighasemi M, Ziaee AA, Ventosa A. Production, optimization and purification of a novel extracellular protease from the moderately halophilic bacterium *Halobacillus karajensis*. J Ind Microbiol Biotechnol. 2009;
28. Aghalari Z., Dahms H.U., Sillanpää M., Sosa-Hernandez J.E., Parra-Saldivar R., (2020), Effectiveness of wastewater treatment systems in removing microbial agents: a systematic review, Global Health, 16(1), 1-11. <https://doi.org/10.1186/s12992-020-0546-y>
29. Ankita Saxena, Varsha Gupta, 1 Sonika Saxena, Identification and characterization of Microbial Consortia *Nanotechnology Perceptions* Vol. 20 No.5 (2024)

- present in sewage samples collected from Sewage Treatment Plant, Jaipur, (Raj.), *J. Integr. Sci. Technol.* 2023, 11(3), 516
30. APHA. "Standard Methods for Examination of Water and Wastewater", American Public Health Association WWA, Washington, D.C. (2005)
 31. Arslan, E. I., Aslan, S., Ipek, U., Altun, S., & Yazicioğlu, S. (2005). Physico-chemical treatment of marble processing wastewater and the recycling of its sludge. *Waste management & research : the journal of the International Solid Wastes and Public Cleansing Association, ISWA*, 23(6), 550–559. <https://doi.org/10.1177/0734242X05059668>
 32. Bakshi, P.; Pappu, A.; Patidar, R.; Gupta, M.K.; Thakur, V.K. Transforming Marble Waste into High-Performance, Water-Resistant, and Thermally Insulative Hybrid Polymer Composites for Environmental Sustainability. *Polymers* 2020, 12, 1781. <https://doi.org/10.3390/polym12081781>
 33. Chauhan, A. and Jindal T. (2020) 'Biochemical and Molecular Methods for Bacterial Identification. In: Microbiological Methods for Environment, Food and Pharmaceutical Analysis', Springer
 34. Chidichimo, F.; Basile, M.R.; Conidi, C.; De Filipo, G.; Morelli, R.; Cassano, A. A New Approach for Bioremediation of Olive Mill Wastewaters: Combination of Straw Filtration and Nanofiltration. *Membranes* 2024, 14, 38. <https://doi.org/10.3390/membranes14020038>
 35. Dragone, N. B., Henley, J. B., Holland-Moritz, H., Diaz, M., Hogg, I. D., Lyons, W. B., Wall, D. H., Adams, B. J., & Fierer, N. (2022). Elevational Constraints on the Composition and Genomic Attributes of Microbial Communities in Antarctic Soils. *mSystems*, 7(1), e0133021. <https://doi.org/10.1128/msystems.01330-21>
 36. Enebe, M.C.; Babalola, O. The Impact of Microbes in the Orchestration of Plants' Resistance to Biotic Stress: A Disease Management Approach. *Appl. Microbiol. Biotechnol.* 2019, 103, 9–25
 37. Jeevanantham S., Saravanan A., Hemavathy R.V., Kumar P.S., Yaashikaa P.R., Yuvaraj D., (2019), Removal of toxic pollutants from water environment by phytoremediation: A survey on application and future prospects, *Environ. Technol. Innov.* 13, 264–276
 38. Malla M. A., Dubey A., Yadav S., Kumar A., Hashem A., Abd Allah E. F., (2018), Understanding and Designing the Strategies for the Microbe-Mediated Remediation of Environmental Contaminants Using Omics Approaches, *Frontiers in Microbiology*, 9, 1-18. doi:10.3389/fmicb.2018.01132
 39. Nisha Chakravati, Varsha Gupta¹, Devki¹, Ravi Kant Rahi¹ and Deepesh Kumar Neelam, *Halophilic Bacterial Diversity of Sambhar Salt Lake, Rajasthan, India, SVOA microbiology*, 2022, 3:4, 59-69.
 40. Rafeef A. Yass , Sherko A. Mohammed , Trifa K. Jalal , and Haider M. Hamzah, Assessment of Chemical and Bacteriological Parameters of Leek in Sulaymaniyah City, Iraq, *Journal of Advanced Zoology*, Volume 44 Special Issue -02 Year 2023 Page 725:739
 41. Ravi Kant Rahi, R. N. Prasad and Varsha Gupta, Analysis of physico-chemical properties of textile effluents collected from Sanganer, Jaipur, *International Journal of Advanced Scientific Research and Management*, Volume 3 Issue 7, July 2018
 42. Tabacchioni, S.; Passato, S.; Ambrosino, P.; Huang, L.; Caldara, M.; Cantale, C.; Hett, J.; Del Fiore, A.; Fiore, A.; Schlüter, A.; et al. Identification of Beneficial Microbial Consortia and Bioactive Compounds with Potential as Plant Biostimulants for a Sustainable Agriculture. *Microorganisms* 2021, 9, 426. <https://doi.org/10.3390/microorganisms9020426>
 43. Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S., (2013) 'MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0', *Molecular Biology and Evolution*, 30(12), pp.2725-2729.
 44. Zhou, J., Huang, Y., & Mo, M. (2009). Phylogenetic analysis on the soil bacteria distributed in karst forest. *Brazilian journal of microbiology : [publication of the Brazilian Society for Microbiology]*, 40(4), 827–837. <https://doi.org/10.1590/S1517-838220090004000013>
 45. Elhenawy S., Khraisheh M., AlMomani F., Al-Ghouti M., Hassan M.K., (2022), FromWaste toWatts: Updates on Key Applications of Microbial Fuel Cells in Wastewater Treatment and Energy Production. *Sustainability*, 14, 955. <https://doi.org/10.3390/su14020955>
 46. Nie A., Kung S.-S., Li H., Zhang L., He X., Kung C.-C., (2021), An environmental and economic assessment from bioenergy production and biochar application, *J. Saudi Chem. Soc.*, 25, 10117