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Microbiological Study and Contaminant Analysis of Sambhar Lake, Jaipur (Rajasthan), for Evaluating Toxicity Risk to Birds, Aquatic Life, and Humans

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Abstract
Sambhar Lake, located near Jaipur in Rajasthan, is India's largest inland saline wetland and supports significant biodiversity as well as salt production activities. However, escalating anthropogenic pressures have resulted in extensive ecological degradation. This study presents an integrated assessment of microbiological and chemical contamination in Sambhar Lake to evaluate toxicity risks to birds, aquatic life, and humans. Seasonal water samples were collected from four representative sites and analyzed for physico-chemical parameters, heavy metals, and microbial indicators following standard protocols. Elevated concentrations of Pb, Cd, and Cu were detected at several locations, particularly in zones affected by domestic and industrial activities. High total and fecal coliform counts indicated substantial organic and sewage contamination. The concomitant presence of heavy metals and pathogenic microorganisms suggests multiple stressors acting on the lake ecosystem. Reported avian mortality events in the region are consistent with deteriorating water quality and potential toxin exposure. Human health risks are associated with contaminated groundwater and salt derived from polluted brine. The findings underscore the urgent need for continuous monitoring, effective pollution control, and integrated lake management. Conservation of Sambhar Lake is essential to protect its biodiversity and to safeguard the health and livelihoods of local communities.
Keywords*—Sambhar Lake, microbiological contamination, heavy metals, aquatic toxicity, avian risk, saline wetland.*

I. Introduction

Sambhar Lake is situated approximately 80 km southwest of Jaipur, Rajasthan, India, and is recognized as the country's largest inland saline wetland. The lake has been designated as a Ramsar site due to its ecological significance, particularly for migratory avifauna and halophilic microbial communities. Sambhar Lake hosts large populations of migratory birds, including flamingos, and contributes notably to India's salt production.

In recent decades, rapid and largely unregulated anthropogenic activities have resulted in severe degradation of the lake ecosystem. Major stressors include the discharge of untreated domestic effluents, industrial runoff, and unplanned or illegal salt extraction operations. These pressures have altered the physico-chemical properties of the lake water and increased contaminant loads, raising concerns about ecological and human health impacts.

A catastrophic mass mortality event in 2019, in which more than 18,000 migratory birds were reported dead, highlighted the critical condition of Sambhar Lake. Investigations linked the event to avian botulism, associated with eutrophic, low-oxygen environments that favor the growth of toxin-producing Clostridium species. Such conditions are typically promoted by high organic loads and deteriorating water quality.

Several studies have been conducted on Sambhar Lake. Bhat and Sharma [1] examined the physico-chemical characteristics of groundwater in areas adjoining the lake, reporting contamination of selected metals above World Health Organization (WHO) limits. Chakravati et al. [2] explored the halophilic bacterial diversity of the lake, while Pradhan et al. [3] utilized remote sensing to analyze algal bloom dynamics and environmental change. Despite these contributions, there remains a limited number of integrated studies that simultaneously address microbiological contamination, heavy metal pollution, and associated toxicity risks to birds, aquatic life, and humans.

This work seeks to fill this gap by combining microbiological, physico-chemical, and toxicological assessments to evaluate the current contamination status of Sambhar Lake and to infer potential ecological and public health risks.

II. Objectives

The primary objectives of the present study are:
1. To evaluate microbial contamination in Sambhar Lake water, with particular focus on total and fecal coliforms and halophilic bacterial populations.
2. To analyze heavy metal concentrations and key physico-chemical parameters at different sites within the lake.
3. To assess potential toxicity risks to birds, aquatic life, and humans by comparing the observed contaminant levels with relevant environmental quality standards and by applying standard risk assessment models.

III. Materials and Methods

A. Study Area

Sambhar Lake (26°52′N, 75°07′E) lies between Jaipur and Nagaur districts in Rajasthan. It is a shallow, elliptical depression encompassing an area of approximately 230 km^2. The lake exhibits high salinity, with values typically ranging from 20 to 40 $g \cdot L^{-1}$, depending on seasonal and spatial variations.
The region is characterized by a semi-arid climate with an annual rainfall of about 400–500 mm. Summer temperatures may exceed 45 °C. The lake receives inflows from several seasonal streams and surrounding catchments, which also serve as conduits for domestic and industrial discharges.
Four sampling stations were established based on dominant land-use patterns and anticipated pollution sources:
- **S1**: Northern inlet region influenced by anthropogenic runoff and inflow of domestic effluents.
- **S2**: Central saline zone containing active salt pans and brine concentration areas.
- **S3**: Western outlet zone located near human settlements and associated wastewater discharge.
- **S4**: Relatively undisturbed site considered as a local control region with minimal direct anthropogenic inputs.

B. Sampling Strategy

Water samples were collected seasonally during pre-monsoon, monsoon, and post-monsoon periods to capture temporal variability. Standard sterilized polyethylene and glass bottles were used for sample collection. For microbiological analyses, bottles were pre-sterilized and handled aseptically. Samples were transported to the laboratory under cooled conditions and processed within the recommended holding time.

C. Physico-Chemical Analysis

Physico-chemical parameters were determined following the standard methods prescribed by the American Public Health Association (APHA, 2017) [4]. The parameters analyzed included:
- pH
- Electrical conductivity (EC)
- Total dissolved solids (TDS)
- Biological oxygen demand (BOD)
- Chemical oxygen demand (COD)
- Major ions, including chloride (Cl^-), sulfate (SO_4^{2-}), and nitrate (NO_3^-)

pH, EC, and TDS were measured using calibrated portable meters. BOD and COD were determined using standard incubation and titrimetric methods, respectively, while major ions were assessed through titrimetric and spectrophotometric techniques as applicable.

D. Heavy Metal Analysis

For heavy metal determination, water samples were first filtered to remove suspended matter and then digested with concentrated nitric acid. The digested samples were analyzed using Atomic Absorption Spectrophotometry (AAS). The metals quantified included lead (Pb), cadmium (Cd), copper (Cu), chromium (Cr), zinc (Zn), nickel (Ni), and iron (Fe). Quality control was maintained through calibration with certified standards, reagent blanks, and replicate analyses.

E. Microbiological Examination

Microbiological assessment comprised:
1. **Total heterotrophic bacterial count:** Determined by the nutrient agar pour plate method, with incubation at appropriate temperature for colony development and enumeration.
2. **Total and fecal coliforms:** Estimated using the Most Probable Number (MPN) technique, employing selective media for presumptive and confirmatory tests to differentiate total coliforms from fecal coliform (thermotolerant) populations.
3. **Halophilic bacteria:** Isolated and enumerated using halophilic nutrient agar supplemented with NaCl concentrations ranging from 5% to 15% (w/v) to target moderate to extreme halophiles.

Representative isolates were characterized based on colony morphology, Gram staining, and a series of biochemical tests. Identification was guided by Bergey's Manual of Systematic Bacteriology.

F. Risk Assessment

To interpret contamination levels in terms of ecological and human health risks, the following indices and models were applied:

1. **Heavy Metal Pollution Index (HPI):** HPI was calculated as a composite indicator of heavy metal contamination in water, incorporating measured concentrations, standard permissible values, and unit weights for each metal.
2. **Ecological Risk Index (ERI):** ERI, as proposed by Hakanson, was used to estimate the potential ecological risk posed by individual metals and their combined effect. The index incorporates contamination factors and toxic response factors for each metal.
3. **Human Health Risk Assessment (HHRA):** Human exposure to metals through ingestion and dermal contact was evaluated using the United States Environmental Protection Agency (USEPA) (2011) models [5]. Hazard Quotients (HQ) were computed for key metals, particularly Pb and Cd, for relevant exposure groups such as local residents and salt workers. An HQ value greater than 1.0 was interpreted as indicative of potential non-carcinogenic risk.

IV. Results

A. Physico-Chemical Characteristics

The physico-chemical data revealed pronounced spatial and seasonal variability across the four sampling stations. Elevated EC and TDS values were observed throughout the lake, reflecting high salinity and substantial dissolved ionic loads, especially in the central saline zone (S2).

Stations S1 and S3, which are under the influence of domestic effluents and runoff from human settlements, showed notably higher BOD and COD values compared to S2 and S4. These elevated organic load indicators suggest significant inputs of biodegradable organic matter and sewage-related contaminants.

Major ion concentrations (chloride, sulfate, nitrate) were consistent with the saline nature of the lake but were further modulated by local inputs and evaporation effects. Nitrate levels at S1 and S3 indicated possible contributions from domestic wastewater and agricultural runoff.

B. Heavy Metal Concentrations

Heavy metal analysis demonstrated that concentrations of Pb and Cd at several sites exceeded commonly accepted permissible limits for surface water and, in some instances, for irrigation and drinking water guidelines. Elevated Pb and Cd levels were most pronounced at S3 (near residential areas and salt-processing activities) and at specific points within S2 where industrial and salt extraction operations are concentrated.

Cu levels were also appreciable and, in combination with high nutrient loads, may favor algal bloom formation, in agreement with previous remote-sensing observations reported by Pradhan et al. [3]. Other metals such as Cr, Zn, Ni, and Fe were present at varying concentrations but generally exerted lower contributions to the overall risk indices compared to Pb and Cd.

C. Microbiological Profile

Microbiological analysis indicated substantial microbial loads, particularly at S1 and S3. Total heterotrophic counts were markedly higher in these zones, implicating continuous inflow of untreated or partially treated domestic waste.

Total and fecal coliform counts significantly exceeded standard thresholds for recreational and potable waters, confirming severe fecal contamination. This suggests the direct discharge of sewage or inadequately treated wastewater into the lake.

The halophilic bacterial community included genera such as *Halomonas*, *Bacillus*, *Pseudomonas*, and *Vibrio*. These organisms are adapted to high-salinity conditions and may participate in both beneficial biogeochemical processes and pathogenic interactions. The presence of *Vibrio* species is particularly noteworthy from a public health perspective due to their potential pathogenicity.

D. Risk Evaluation

The Heavy Metal Pollution Index (HPI) values ranged from approximately 86.4 at S2 to 121.5 at S3. The HPI value at S3 exceeded the widely accepted safe limit of 100, indicating significant heavy metal pollution in that area.

The Ecological Risk Index (ERI) analysis identified Cd and Pb as the primary contributors to potential ecological toxicity among the assessed metals. Their relatively high contamination factors and toxic response values resulted in elevated ERI scores at sampling locations receiving substantial anthropogenic inputs.

Human Health Risk Assessment (HHRA) revealed that the Hazard Quotient (HQ) for Pb exceeded 1.0 in salt workers exposed through dermal contact and incidental ingestion of contaminated brine and salt. This finding suggests a potential non-carcinogenic risk associated with long-term occupational exposure. Other metals exhibited HQ values below unity for most exposure scenarios but still warrant attention due to possible additive or synergistic effects.

V. Discussion

The results confirm that Sambhar Lake is subject to multiple, interacting pollution pressures, combining natural salinity with anthropogenic inputs of organic matter and toxic metals. Elevated EC and TDS are characteristic of hypersaline environments, yet the superimposed influence of domestic and industrial effluents has altered the ecological balance.

The coexistence of high salinity, elevated BOD and COD, and heavy metal contamination creates a complex stress environment for aquatic biota. Metals such as Pb and Cd are known to accumulate in sediments and biota, causing oxidative stress, reproductive impairment, and growth inhibition in aquatic organisms. Long-term exposure can also lead to bioaccumulation and biomagnification through food webs, ultimately affecting higher trophic levels, including fish-eating and filter-feeding birds.

The substantial organic and fecal contamination recorded, especially at S1 and S3, is consistent with the inflow of untreated domestic sewage from nearby settlements. High coliform counts and the presence of potentially pathogenic bacterial genera represent a direct threat to aquatic life and indirect risks to birds that feed on invertebrates, plankton, and other organisms inhabiting contaminated waters.

The 2019 mass bird mortality event at Sambhar Lake, attributed to avian botulism [6], is compatible with the degraded water quality observed in this study. Eutrophic and anoxic conditions favor the proliferation of toxin-producing *Clostridium* species, which can cause botulism in birds consuming contaminated invertebrates or carcasses. Elevated BOD values and high organic loads at impacted sites provide suitable conditions for such anaerobic processes, reinforcing the link between pollution and wildlife disease outbreaks.

From a human health perspective, the contamination of groundwater in areas surrounding Sambhar Lake and the accumulation of metals in locally produced salt are of particular concern. Bhat and Sharma [1] reported Pb and Cr levels above WHO guidelines in adjacent groundwater, which, coupled with the present findings, suggest ongoing migration of contaminants from the lake system to connected aquifers. Long-term ingestion of metal-contaminated water and salt may increase the risk of chronic health problems in local communities.

The findings are in broad agreement with previous studies addressing specific components of the Sambhar Lake ecosystem [1]–[3], [7]–[11], but provide an integrated perspective that simultaneously considers microbial contamination, physico-chemical stressors, and toxicological indices. This holistic view is important for designing effective management and conservation strategies.

VI. Conclusion

The present study demonstrates that Sambhar Lake is experiencing severe and multifaceted contamination involving both microbiological and chemical components. Elevated concentrations of Pb, Cd, and Cu, together with high total and fecal coliform counts, indicate that the lake is under substantial ecological stress.

These contaminants threaten aquatic organisms, contribute to conditions favorable for disease outbreaks such as avian botulism, and pose potential health risks to nearby human populations reliant on the lake for livelihoods, salt production, and, indirectly, for water resources. The calculated HPI and ERI values highlight the significance of heavy metal pollution, while HHRA results indicate potential non-carcinogenic risks for occupationally exposed groups.

Given the ecological importance of Sambhar Lake as a Ramsar site and its socio-economic value, urgent interventions are required. These should include the implementation of robust pollution control measures, comprehensive monitoring programs, and integrated management frameworks involving all relevant stakeholders.

VII. Recommendations

Based on the findings of this study, the following measures are recommended:

1. **Continuous Monitoring:** Establish a long-term monitoring program for microbiological indicators and heavy metals in lake water, sediments, and biota to detect trends and evaluate the effectiveness of interventions.
2. **Wastewater Treatment Infrastructure:** Construct and operate sewage treatment plants for nearby settlements to prevent the discharge of untreated domestic effluents into the lake and its tributaries.
3. **Quality Control of Salt and Groundwater:** Implement regular testing of locally produced salt and groundwater for toxic elements, particularly Pb and Cd, and enforce regulatory limits to protect consumer health.
4. **Integrated Management Authority:** Form a dedicated Sambhar Lake Conservation and Management Authority with clear jurisdiction and authority to coordinate conservation, pollution control, and sustainable livelihood activities.
5. **Eco-Friendly Salt Production and Bioremediation:** Promote environmentally sound salt production practices and explore bioremediation approaches using native halophilic bacteria to reduce pollutant loads in hypersaline niches.

6. **Community Awareness and Participation:** Conduct awareness programs for local communities, salt workers, and stakeholders to encourage responsible waste disposal, support conservation efforts, and improve compliance with environmental regulations.

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