# **BACTERIAL AND TOXIC POLLUTANTS IN LAKES OF RIVER INDUS**

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#### Abstract

Indus river water gets polluted through three sources *viz.*, municipal wastewater, industrial wastewater and agricultural runoff through drainage structure. The lakes in Sindh (fed by the river Indus), constitute the important source of drinking water, recreation and fish, etc. and offer employment for many. A large number of chemicals that either exist naturally in the land dissolve in the water, or human excreta added due to human activity thereby, contaminating and leading to various diseases. In order to assess the microbial contamination, detection of pollutant indicator organisms (coliform group), using Coliform test was performed by Most Probable Number technique and total bacterial count by Pour Plate method. The level of various heavy metals (arsenic, calcium, cadmium, chromium, copper, iron, lead, mercury, potassium, magnesium, manganese, sodium, selenium & zinc) and electrolytes (Cl<sup>-1</sup>, HCO<sub>3</sub><sup>-1</sup>) was monitored in water and fish meat samples collected from Haleji and Keenjhar lakes to assess the impact of toxic pollutants. Metal concentrations in water and fish samples were estimated by atomic absorption spectrophotometry. Total coliform organisms were found in both the lake water samples, exceeded in 38% samples than the acceptable limits, while total average aerobic bacterial count analyzed in both the lakes was  $10^2 \text{ CFU/ml} - 10^{10} \text{ CFU/ml}$ . Toxic chemical contaminants were estimated below the detection limit, while other several (essential) metal ions were found within the range set by WHO, except arsenic, cadmium and iron that exceeded slightly in 12.5% water samples. This study was designed to ensure the access of safe and potable water to urban & rural areas of Sindh. Further, the findings will help public/private enterprises and public health institutions to work for the people health friendly policies.

#### Introduction

The Indus is the longest and the most important river of Pakistan which ultimately falls into Arabian Sea after distributing its water to some lakes adjacent to its bank, like Haleji and Keenjhar lakes (Mehta, 1988). Lakes are large bodies (i.e., greater than 20 acres) of inland water. Most of our drinking water, as well as water used for irrigation, industry, and hydropower, comes from freshwater lakes and reservoirs. In addition, freshwater lakes also offer as resources for recreation activities and habitats for wildlife (Anon., 2006).

Keenjhar lake is the biggest man-made freshwater lake in Asia. Its source of water KB feeder starts its journey from Kotri barrage, constructed on Indus. Keenjhar lake is situated in Thatta, adjacent to the national highway on the right bank of river Indus. It is about 122 km from Karachi with a length of 32 km and width of 11 km. Whereas, Haleji lake lies in Sindh province some 75 km east of Karachi and 16 km from the town of Thatta. Keenjhar lake is one of the major sources of water supply to the increasing population of Karachi (Nasir *et al.*, 2002).

Toxic substances when enter lakes, streams, rivers, oceans and other water bodies, get dissolved or remain suspended in water or get deposited on the bed. This results in the pollution of water whereby the quality of the water deteriorates, affecting aquatic ecosystems. Raffaelli *et al.*, (1989) observed that the elevated level of nutrients had increased the density of weeds. The enrichment of nutrients also occur due to disposal of domestic and industrial effluents from surrounding areas, which support the growth of a variety of macrophytes and microbes in aquatic system.

Microbial contamination of drinking water poses a potential public health risk in terms of acute outbreak of diseases. The illness associated with contaminated drinking water is mainly gastro-intestinal in nature, although some pathogens eg., *Salmonella* spp., *Shigella* spp., etc., are capable of causing severe and life-threatening illness (Bianchi & Giulaino, 1996).

The chemical pollutants of concern in the lakes include metals like arsenic, lead chromium etc., that are known to cause adverse health effects in animals and humans. These chemicals do not break down easily, persist in the environment and bioaccumulate in aquatic biota, animal and human tissues; thus they are called Persistent Bioaccumulative Toxic chemicals (PBTs), tend to accumulate in organs, muscle and flesh. Food is the primary route of human exposure to these PBT chemicals, and consumption of fishes is the most important source of exposure originating directly from the lakes (Anon, 2006). In order to make improvements in water quality of the lakes, a better understanding was needed to obtain the fundamental information related to drinking water and sources of contamination and to check the suitability of water quality for its intended use. This study was specifically designed to assess chemical and microbial water quality of the two lakes.

## **Materials and Methods**

A two year (June 2006-May 2008) field study was undertaken at a regular interval of three months. In order to analyze the bacterial and toxic pollutants in the lake waters, certain sites (i.e., 1, 2, 3 & 4) were selected for the collection of water samples from both Haleji and Keenjhar lakes, viz, shore water, surface water, 75-80 m away from the shore and 3-4 ft. (including the bed of Keenjhar lake) deep water respectively. The water samples for heavy metal ions estimation, from all the locations were collected in acid rinsed polyethylene bottles and preserved by decreasing the pH up to 2. Live fish samples taken from the two lakes were immediately preserved in ice after sampling along with water samples that were collected in sterile tubes and bottles, transported to the laboratory and processed within 24 hours after sampling. All treatments after sampling were carried out under aseptic conditions (Hahn, 2003).

For the analysis of water samples, 10-fold serial dilutions were used to inoculate the lactose broth and nutrient agar (NA). All the tubes and plates were incubated at 37°C for 24 h. Coliform test was performed by the most probable number (MPN) technique (Benson, 1998) and total bacterial count (aerobic) by Pour Plate method (Sugita *et al.*, 1993). After incubation, the lowest-dilution plates containing countable bacterial colonies were scored and reported as colony forming units (CFU/ml).

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For the estimation of heavy metals *viz.*, arsenic, calcium, cadmium, chromium, copper, iron, lead, mercury, potassium, magnesium, manganese, sodium, selenium and zinc, the water samples were digested by using HNO<sub>3</sub> - HCl on hot plate. The finally obtained residue was then dissolved and filtered (Whatman no. 1). The soft tissues of fish samples from both the locations were carefully removed. All samples were digested in 5 ml HNO<sub>3</sub> (conc.) in Griffin beaker. Following acid digestion, all samples (water and fish) were analyzed for heavy metal content by atomic absorption spectrophotometer (Anon., 1976). Cadmium was analyzed by NOVA 6 method, whereas, mercury determination was performed by using dithiozone method as reported by Anon., (1998). These methods quantitatively determine the soluble concentration of the elements in the water samples.

## Results

The observations of the two year research work are based on monitoring the water quality of the selected lakes of river Indus (w. p. r. to Haleji and Keenjhar lakes). The results encompass certain heavy metal ions and total bacterial load including total coliforms and thermotolerant coliforms in the two lake water samples.

The results pertaining to coliforms (total and the thermotolerant) are depicted in Fig. 1. The highest load refers to total coliforms, determined in Haleji lake, while the lowest count has been found with reference to the fecal coliforms from Keenjhar lake water samples.

Total average bacterial (aerobic) count in both the lake samples reflects seasonal variation in bacterial count (Fig. 2).

> 1<sup>st</sup> Quarter (June – August): Haleji and Keenjhar Total average aerobic bacterial count  $10^8 - 10^{10}$  CFU/ml

2<sup>nd</sup> Quarter (September – November): Haleji Number increased to infinity Keenjhar Bacterial number decrease 10<sup>3</sup> CFU/ml

3<sup>rd</sup> Quarter (December – February): Haleji and Keenjhar Same as 2<sup>nd</sup> Quarter

> 4<sup>th</sup> Quarter (March – May): Haleji and Keenjhar Count increase to 10<sup>4</sup> CFU/ml

> 5<sup>th</sup> Quarter (June – August): Haleji Increased to 10<sup>5</sup> CFU/ml Keenjhar Increased to 10<sup>6</sup> CFU/ml

6<sup>th</sup> Quarter (September – November): Haleji and Keenjhar Decreased to 10<sup>2</sup> CFU/ml

7<sup>th</sup> Quarter (December – February): Haleji and Keenjhar Decreased to 10<sup>3</sup> CFU/ml

> 8<sup>th</sup> Quarter (March – May): Haleji 10<sup>4</sup> CFU/ml Keenjhar 10<sup>3</sup> CFU/ml

The results of the concentration of all the metal ions and electrolytes are depicted in Figs. 3a-i and 4a-f respectively. Accordingly, the values were found within the WHO maximum acceptable limits with the exception of arsenic, cadmium and iron, which were recorded in slightly elevated concentrations in a few water samples, collected during the pre monsoon seasons. Further, mercury was found below the detectable limit. However, copper and manganese exceeded the WHO limits in a few fish tissue samples from the two lakes (Fig. 5b&e).

# Discussion

The freshwater bodies like rivers, lakes etc. are the major sources of drinking water along with other purposes. However, over exploitation of the lakes such as fishing, swimming, boating, picnicking, littering, churning up sediments by motorboats in shallow areas and spilling the fuel and its exhaust into lakes (all these activities results in deterioration of water quality).

**Fecal coliform testing:** Total coliforms include a general group of bacteria, encompassing *E. coli*, fecal coliforms, as well as common soil microorganisms and contrary to the name, fecal coliform bacteria are not limited to fecal sources, but also are commonly found in pulp and paper-mill effluents, textile processing-plant effluents and cotton mill and sugar beet processing wastewaters (Dufour, 1976).

The relative abundance of the indicator organisms in a sample can serve as an indicator of the presence of pathogens in the water (*Salmonella, Shigella*). The indicator organisms are reflection of fecal coliforms or enterococci. The presence of indicator organisms from the two lakes revealed striking results, (Fig. 1). The seasonal variation in the coliform count was observed, as higher values were estimated during summer season before the onset of monsoon, which may also be due to enhanced human activities (during these days lake waters are also used for recreational purposes). The coliform load was decreased in the samples collected after the rainy season as the water gets diluted due to heavy rainfall.

The elevated number of fecal coliforms was observed from deep water samples of Haleji lake. It may probably be due to vertical mixing of the water body. Generally, in many basins of the lakes, a number of notable patterns of circulation are encountered. This circulation is mostly a vertical mixing of the water. It has been called an "over-turn" or "turn-over" of the lake. The circulation or mixing is usually wind driven and is facilitated when the lake has a uniform (or near uniform) temperature from top to bottom. This upper layer (epilimnion) is eventually warmed and mixed to a depth of about 5-6 meters (Kevern *et al.*, 2004).

The South Carolina freshwater standard states for fecal coliforms: "If only one sample is collected in a 30-day period, then that single sample should not exceed 4 CFU/ml" (South Carolina Department of Health and Environmental Control, 1992). While, North Carolina also uses a freshwater fecal coliform standard i.e., "fecal coliforms shall not exceed 4/ml in more than 20 percent of the samples examined during such period." (North Carolina Department of Environment and Natural Resources, 1997). From both the lakes, higher values for fecal coliforms were determined from shore samples, possibly due to human activities.





Fig. 1. Cumulative load of total and fecal coliforms in Haleji and Keenjhar lakes during the two years collection periods (June 2006 - May 2008).







Fig. 3c. Copper in water samples of Haleji and Keenjhar lakes.

Fig. 2. Total Average (aerobic) bacterial load in the two sampling sites during the two years collection period.



■M.A.L. □1st Qtr. ■2nd Qtr. ■3rd Qtr. ■4th Qtr. □5th Qtr. ■6th Qtr. ■7th Qtr. ■8th Qtr.

Fig. 3b. Chromium in water samples of Haleji and Keenjhar lakes.





Fig. 3d. Iron in water samples of Haleji and Keenjhar lakes.









■M.A.L. □1st Qtr. ■2nd Qtr. ■3rd Qtr. ■4th Qtr. □5th Qtr. ■6th Qtr. ■7th Qtr. ■8th Qtr.







■M.A.L. □1st Qtr. ■2nd Qtr. ■3rd Qtr. ■4th Qtr. □5th Qtr. □6th Qtr. ■7th Qtr. ■8th Qtr.









Fig. 3i. Cadmium & Mercury in water samples of Haleji & Keenjhar lake.

Fig. 4a. Bicarbonate in water samples of Haleji and Keenjhar lakes.







500

60

50

40

30

■M.A.L. □1st Qtr. ■2nd Qtr. ■3rd Qtr. ■4th Qtr. □5th Qtr. ■6th Qtr. ■7th Qtr. ■8th Qtr.

Fig. 4b. Calcium in water samples of Haleji and Keenjhar lakes.





■M.A.L. □1st Qtr. ■2nd Qtr. ■3rd Qtr. ■4th Qtr. □5th Qtr. ■6th Qtr. ■7th Qtr. ■8th Qtr.

Fig. 4d. Magnesium in water samples of Haleji and Keenjhar lakes.







20 10 WHO Max. Acceptible Limit Haleji Lake Keenjhar Lake

Sampling sites M.A.L. □1st Qtr. =2nd Qtr. =3rd Qtr. =4th Qtr. =5th Qtr. =6th Qtr. =7th Qtr. =8th Qtr.





■T.C.L. □1st Qtr. ■2nd Qtr. ■3rd Qtr. ■4th Qtr. □5th Qtr. ■6th Qtr. ■7th Qtr. ■8th Qtr.

Fig. 5a. Calcium in fish samples of the two lakes.







Fig. 5d. Magnesium in fish samples of the two lakes.





Fig. 5c. Iron in fish samples of the two lakes.





Fig. 5e. Manganese in fish samples of the two lakes.



■T.C.L. □1st Qtr. ■2nd Qtr. ■3rd Qtr. ■4th Qtr. □5th Qtr. ■6th Qtr. ■7th Qtr. ■8th Qtr.

Fig. 5g. Zinc in fish samples of the two lakes.

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Total aerobic bacterial count: Rapid detection and enumeration of highly diluted indicator cells are challenging, particularly in the drinking water field. For the water samples investigated, total cell counts ranged from approximately 10<sup>6</sup> cells per ml in raw water to 10<sup>5</sup> cells per ml in sand-filtered water and chlorinated water were estimated by Baudart et al., (2002). While in our study, the possibility of the fluctuation in Total average aerobic bacterial count might be due to increased eutrophication (as observed in Haleji lake) and dilution of water body (as samples were collected after monsoon season) respectively. The total aerobic bacterial count (8.9 x 10<sup>10</sup> cfu/ml & 2.5 x 10<sup>8</sup> cfu/ml) observed, in Haleji and Keenjhar lakes respectively reveals the human activity as bacterial population was estimated in higher concentration from water samples collected from the bank of the lakes. There is a wide variety of disease-causing bacteria, parasites and other microorganisms that can enter the water and be transmitted to humans. Some microorganisms are indigenous to natural waters. Others are carried from wastewater sources including runoff from animal and wildfowl areas. Infected swimmers may also by a source of pathogens (Dufour, 1984).

Toxic pollutants: Metals occur naturally in the environment, but industrial processes have also been responsible for the distribution of metals in the environment. In most reported cases of metal contamination, high concentrations of metals appear in fish tissues rather than the water column because the metals accumulate in greater concentrations in predators near the top of the food chain (Anon., 2006). During the current work, toxic chemical contaminants like mercury, selenium etc., were estimated to be in negligible amount i.e., below the detection limit, while other several essential metal ions were found within the range recommended by WHO guideline of drinking water (Anon, 2004). This is an indication of a favorable (potability of the water) reflecting the drinking water safety profile. This is a welcome relief as the certain estimated metal ions are nonessential and toxic to aquatic life as well as for human beings even at low concentration. The present results are in agreement with other workers, who reported lower concentration of metal ions in water samples from freshwater bodies (Frits, 1990; Lu, 1995; Cheung et al., 2003).

Arsenic, however, was detected in elevated concentration compared to the maximum acceptable limits (Fig. 3a) in 5<sup>th</sup> quarter (summer season) of both the lake water samples which may be due to discharge from different industries like pharmaceuticals, pesticide, mining and metallurgy industrial effluents etc., as samples were collected during July i.e., before the rainy season. However, according to Ipinmoroti (1993), Tariq et al., (1996) and Ponta et al., (2002), elevated concentrations of arsenic and certain other metal ions in some freshwater sample sources could be attributed to industrial (including agricultural) activities. According to National Standard for Drinking Water Quality, the proposed Standard Values for Pakistan for arsenic, cadmium and iron are  $\leq 0.05$ , 0.01 & 2.0 ppm respectively. In the current investigation only arsenic was found in exceeded concentration during 5<sup>th</sup> quarter (before the rainy season), that may be due to industrial effluents. As far as the presence of heavy metal ions in fish samples is concerned, the captured fishes were relying on herbivorous and omnivorous mode of feeding; therefore, increased level of these (Cu & Mn) metal ions may have reached the fish tissues via aquatic weeds and insects.

## Conclusion

It is concluded that Haleji lake was found more polluted, compared to the Keenjhar lake. This is possibly due to excessive growth of aquatic weeds in this lake, visitors (picnickers) misuse of water by washing of cloths, utensils, vehicles etc., and disposal of the garbage along with other recreational activities. All these activities contribute to increase the microbial load. Slightly elevated concentrations of arsenic, cadmium and iron in a few water samples indicate that untreated discharge of industrial effluents and agricultural run off, either directly or indirectly flow into the lake waters. Several other metal ions *viz.*, chromium, copper, lead, manganese, selenium and zinc were found within the WHO maximum acceptable limits. The water bodies no doubt are polluted (viz a viz to total bacterial count/load) and hence not safe for human consumption. Such polluted water requires effective and proper treatment before its supply to urban and rural areas.

# Acknowledgment

The authors acknowledge with thanks the WWF PAK for funding this research project.

#### References

- Anonymous. 1976. Environmental Protection Agency (EPA). Methods for chemical analysis of water and wastes. EPA 625/6-74-003A.
- Anonymous. 1992. South Carolina Department of Health and Environmental Control. Water classifications and standards (Regulation 61-68): p 35.
- Anonymous. 1997. North Carolina Department of Environment and Natural Resources. Classifications and water quality standards applicable to surface water and wetlands of North Carolina: Division of Water Quality, p 41.
- Anonymous. 1998. Standard method for the examination of water and wastewater. Washington DC, USA.
- Anonymous. 2004. World Health Organization. Guidelines for drinking water quality. 3rd edition, Vol. 1: Recommendations (W.H.O., Geneva).
- Anonymous. 2006. EPA (US Environmental Protection Agency). Morbidity and Mortality: How Do We Value the Risk of Death and Illness? National Center for Environmental Economics, US Environmental Protection Agency [online]. Available: <u>http://yosemite.epa.gov/ee/epa/eerm.nsf/</u>vwRepNumLookup/ EE-0497? Open Document.
- Baudart, J., J. Coallier, P. Laurent and M. Prévost. 2002. Rapid and sensitive enumeration of viable diluted cells of members of the family *Enterobacteriacae* in freshwater and drinking water. *Appl. Environ. Microbiol.*, 68(10): 5057-5063.
- Benson, H.J. 1998. Microbiological applications: *Laboratory manual in general Microbiology*, seventh edition, pp. 208-211.
- Bianchi, A. and L. Giulaino. 1996. Enumeration of viable bacteria in the marine pelagic environment. *Appl. Environ. Microbiol.*, 62(1): 174-177.
- Cheung, K.C., B.H.T. Poon, C.Y. Lan and M.H. Wong. 2003. Assessment of metal and nutrient concentrations in river water and sediment collected from the cities in the Pearl River Delta, South China. *Chemosphere*, 52(9): 1431-1440.
- Dufour, A.P. 1976. *Escherichia coli*: the fecal coliform, in bacterial indicators/health hazards associated with water, In: (Eds.): A.A. Hoadley and B.J. Dutka. ASTM STP 635: American Society for Testing and Materials, pp 48-58.
- Dufour, A.P. 1984. Health effects Criteria for Fresh Recreational Waters. Cincinnati, Ohio, U.S. Environmental Protection Agency.
- Frits, V. L. 1990. The Water Encyclopedia., Lewis Publishers, UK.
- Hahn, M.W. 2003. Isolation of strains belonging to the Cosmopolitan *Polynucleobacter necessarius* Cluster from Freshwater Habitats Located in Three Climatic Zones. *Applied and Environmental Microbiology*, 69(9): 5248-5254.
- Ipinmoroti, K.O. 1993. Water quality of shallow wells located close to dump sites in Akure, Nigeria. *Pak. J. Sci. Ind. Res.*, 36(4): 137-141.

- Kevern, N., D. King and R. King. 2004. Lake classification systems (part 2), <u>http://www.mlswa.org/lkclassf.htm</u>
- Lu, R. 1995. *Handbook of regulations on environmental protection in China*. Resources for the Future, Washington, DC.
- Mehta, Jagat S. 1988. The Indus water treaty: a case study in the resolution of an international river basin conflict: opinion/Jagat S. Mehta. Druk van: *Natural Resources Forum*; 12: (1): 69-77.
- Nasir, A., A. Ali and N. Jamat. 2002. Management of wetlands in Pakistan using remote sensing GIS techniques a case study of Kalri and Haliji lakes (Presented at the International Seminar on Natural Hazards Monitoring, Karachi, Pakistan): 29.
- Ponta, M., T. Frentiu, A. Sarkany-kiss and E.A. Cordosa. 2002. Traces of Cu, Mn and Zn in aquatic animals, water and sediments from the Cris river basin-West Romania. Part II:

Distribution study. CROATICA CHEMICA ACTA CCACAA 75(1): 307-317.

- Raffaelli, D., S. Hull and H. Milne. 1989. Long term changes in nutrients, weed mats and shorebirds in estuarine system. *Can. Biol. Mar.*, 30: 259-270.
- Sugita, H., N. Okamoto and T. Nakamura. 1993. Characterization of microaerophilic bacteria isolated from the coasted waters of of Tokyo Bay, Japan. *FEMS Microbiology Ecology*, 13: 37-46.
- Tariq, J., M. Ashraf, M. Jaffar and M. Afzal. 1996. Pollution status of the Indus River, Pakistan, through heavy metal and macronutrient contents of fish, sediment and water. *Water Research*, 30(6): 1337-1344.

(Received for publication 30 August 2010)