Coliforms and *Halophiles* pollution in surface and sub-surface water of Salt Range Wetlands, Punjab, Pakistan

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K E Y W O R D S

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A B S T R A C T

Faecal Coliforms (FC) have traditionally been regarded as indicators of microbial contamination of water resource. Pathogens still pose a threat to human health, despite the fact of development in the field of microbiology and bioengineering. Although attention and management efforts have focused on treating drinking water by the Pakistan Wetlands Programme to prevent microbial contamination in Salt Range Wetlands Complex (SRWC), human activities and technological development continue to contribute directly and indirectly to contamination of surface and ground water with microbial pathogens. For example, Uchali, Namal and Jahlar Lakes in the Salt Range area of Pakistan are surrounded by agricultural lands and hence agricultural run-off from these lands end up eventually in the lakes. Agricultural practices are often contaminated with nutrients and pathogens. The major source of pathogens in agricultural run-off is farm and grazing livestock which either end up in lakes or leach down in soil profile to ground water resources. These lakes are very critical and important in terms of its consumptive and non-consumptive uses by humans and ecology in a region which already had limited freshwater resources. In order to investigate the impacts of agricultural activities on the microbial water quality of the Salt Range water resources (lakes and ground water), water samples were collected from 56 different locations each for surface and sub-surface bacteriological testing of water quality including open dug wells, hand pumps and lakes of Salt Range Wetlands Complex. Membrane filtration technique was used for the assessment of the microbiological quality of different water sources. The results were compared with WHO standards for water quality. All 56 sampling sites were found contaminated with *Coliforms*/*Halophiles* and were found unsafe for drinking and not suitable for human consumption. Processing and compilation of data reveals that 100 % samples were found contaminated with *Coliforms* and *Halophiles* even exceeding WHO Guidelines for developing countries (0-10 E-coli / 100 ml). In order to manage microbial contamination, continuous microbiological monitoring of water is essential to ensure compliance with quality standards and to protect public health. Buffer strips (grasses and wetlands) between agricultural lands and the lakes are also recommended to hold on to the run-off during rainfall and irrigation events to reduce and/or completely immobilise the FC before it reaches to the lakes. Moreover, proper livestock manure management is required to minimise the contact of rain and/or irrigation water with the manure to avoid loss of the manure slurry into surface and ground water resources. This could significantly reduce *faecal* contamination of water resources in the Salt Range, Pakistan.

Introduction

The importance of potable (drinking) surface and sub-surface water supplies cannot be overemphasised (Boyer, 2008). With increasing industrialisation, water resources available for consumptive and non-consumptive uses such as bathing, cleaning and recreation have been adulterated with industrial, agricultural, animal and human wastes as well (Miernik, 2004). Sewerage effluents contain a wide variety of pathogenic micro-organisms that may pose a possible health hazard to human population (Lewis *et al.,* 2007). The effluents which are discharged directly into recreational water without proper treatment (Hill *et al.,* 2006), it may contaminate the water by providing a growing media for microbes. The density and variety of these pathogens are related to the size of the human population, the seasonal incidence of the illness, and dissemination of pathogens within the community (Pipes, 1982). Some types of *Coliforms* bacteria also naturally live in soils and surface waters, lakes, rivers and ponds (Giannoulis *et al*., 2005). The presence of *Coliforms* bacteria in open well water indicates that urban sewerage or some other type of surface contaminated water is entering and contaminating the water supply (Van *et al*., 2007). An open well contaminated with *Coliforms* bacteria requires immediate attention. According to WHO (2003); 80 % of sickness and disease in the world are caused by inadequate sanitation and polluted water. Children below 5 years of age are more affected due to diarrhoeal diseases in Pakistan where mortality rate is 109 per 1000 live births. Many waterborne pathogens are difficult to detect and/or quantify and the specific methods to detect them in environmental water samples has still to be developed (Borrego, 1994). While *Faecal streptococci* are suggested as the recommended indicator for salt water, either *Faecal streptococci* or *Escherichia coli* can be used for monitoring freshwater (Hardina and Fujioka, 1991). Additional variables can be investigated if, they are considered relevant, such as the spores of *Clostridium perfringens* in tropical water where the traditional indicators may increase in number in water (Anon, 1996).

In order to test *coliforms* and *faecal coliforms* and more effectively recover stressed *coliforms*, a variety of simpler and more specific test have been developed among which membrane filtration method has become a common and preferred method of evaluating the microbiological characteristics of water (WHO, 2003). According to WHO for drinking water to be safe a 100 ml sample of water should not contain any *coliforms* bacteria.

Halophiles are aerobic bacteria that grow in high saline/salty environments and form a diverse group of micro-organisms (Ramos, 1993). Bacterial *Halophiles* can be found in a range of environments i.e. saline water lakes and sediments (Rodriguez, 1986). These bacteria maintain their intra-cellular ionic

concentration at low-levels while producing organic solutes to provide osmotic equilibrium of the cytoplasm with the surrounding medium (Rodriguez, 1988). The aerobic *halophilic* bacteria have not been studied extensively while in comparison to other bacteria i.e. *archaea* (Kushner, 1989).

Majority of freshwater lakes and their surrounding wetlands in Pakistan are mainly surrounded by agricultural, industrial and residential areas. Wastewater treatment is not widely practiced in rural and suburban areas of Pakistan, which lead to the discharge of wastewater into water bodies. The practices of discharging untreated wastewater into water bodies and livestock grazing around water resources may pose a serious threat to the water quality. Water in lakes is used for human and livestock drinking, bathing, cleaning and irrigation purposes. These uses with no regulatory or water treatment facilities may have deteriorated water quality and contaminated with pathogens. Keeping in view the urgency of safe drinking water, the presence of *faecal coliforms/halophiles* in surface and sub-surface water of different lakes in Punjab province, Pakistan was investigated with the following specific objectives:

1. Investigated the presence of *faecal coliforms* and *Halophiles* in water resources of the lakes and ground water arising from agricultural run-off and to educate the stakeholders involved in waterways and wetlands management for consumptive and non-consumptive uses of water resources.
2. Recommend remedial actions, if any, to address potential health risk due to *faecal coliforms* presence in the water.

Material and Methods

Site selection for sampling

Key wetlands and wells located in Punjab Province of Pakistan have significant amount of salt. Saline nature of water is suitable for growth of *Halophiles* bacteria; in addition to the unhygienic practices involved in the wetlands keep stimulating the *faecal* contamination. The vulnerable status of the region's water resources and high rate of recorded water-borne diseases in the locality has helped investigate the pertaining issue. A total 56 sites were selected for water sampling in SRWC, including five lakes (Fig. 1), four open dug wells and four hand pumps. All these water resources (surface and ground water are currently in use by the custodian community for drinking purposes and other domestic uses).

Collection of samples

Water sampling was carried out according to the recommended procedures and outlines as published in the literature. The samples for the microbiological analysis were collected in small sterilised sample bottles sized 100 ml and transported in ice bags within 6 hours of time interval from collection up to refrigerator in the laboratory (Lewis *et al*., 2007). All the samples were analysed immediately after their collection for microbiological variables. The samples were kept cold under 4°C in a refrigerator till the time of analysis.

Bacteriological analysis

Membrane Filter (MF) technique was used for bacteriological analysis. The MF technique offers advantage of isolating discrete

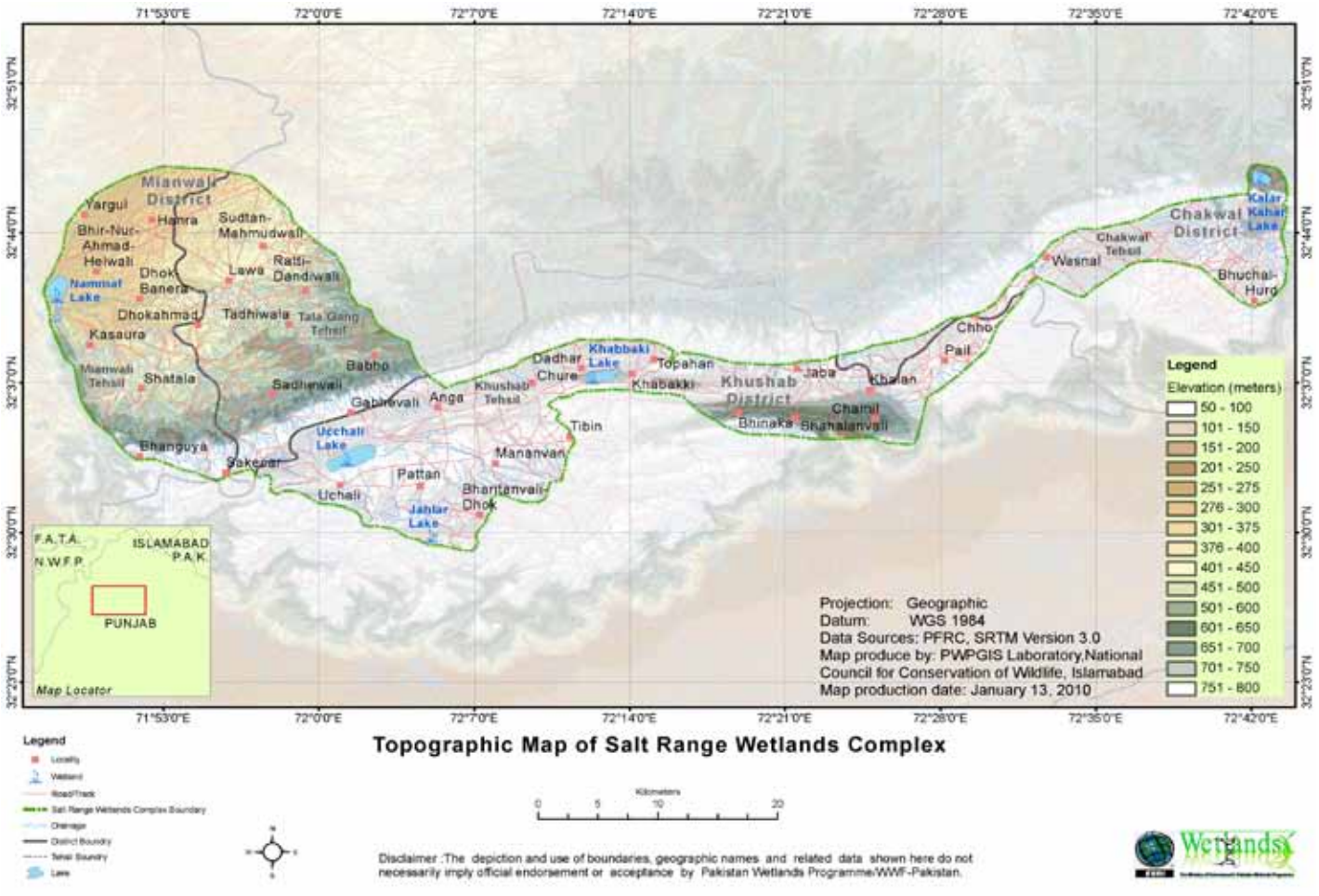

Figure 1: Five lakes of Salt Range Wetlands Complex, Pakistan

colonies of bacteria. US EPA declared that MF technique is an easy and standard technique and this was adopted for the bacteriological analysis of the water samples collected during the study.

Identification of *E-coli*

For identification of *E-coli,* brilliant green agar was used on which numbers of yellow colonies were counted using standard colony counter. The green agar is a culture medium, which contains lactose. Thus by degradation, acid raise the pH of the media which changes the colour of colonies to yellow.

Confirmation of *E-coli*

The presence of *E-coli* was confirmed on EMB (Eosin Methylene Blue) and MacConkey agar. *E-coli* exhibit green metallic sheen on EMB and pinkish white colonies on MacConkey agar (Table 1).

Identification of *Halophiles*

Halophilic bacteria were identified by providing MSA (Mannitol Salt Agar) as a growing medium. The colonies of the *Halophiles* on culture media were counted through colony counter.

Results

Analysing water samples and after deeply envisaging the drastic number of *Faecal*/*Halophiles* counts conclude a high *faecal* contamination. The results suggest a higher *Faecal*/*Halophiles* counts than WHO and US EPA standards for developing countries.

Status of *faecal coliforms*/*Halophiles* in selected water bodies

Kallar Kahar Lake

All of the samples were detected positive and show high concentration of *faecal coliforms* 324CFU/10ml in the sample labelled (KKWS2). On the other hand, the concentration of 213CFU/10ml *Halophiles* are high in number in the sample labelled (KKWS3). The lowest concentration of the *faecal coliforms* and *Halophiles* are 209CFU/10ml and 111CFU/10ml respectively (Table 2 & 2.1).

Khabeki Lake

The analysis show positive results for concentration of *faecal coliforms*, 2012CFU/10ml of sample KBWS4 to 320CFU/10ml for sample KBWS3 while concentration of *Halophiles* in a range between 156CFU/10ml in KBWS4 t 301CFU/10ml in KBWS1. The result explains that Khabeki Lake exceeds the WHO guidelines of the bacteriological standards of water quality and therefore the water is not suitable for human consumption (Table 3).

Khabeki Wells

Water extracted from wells are considered safe to drink but the detection level of aforementioned bacteria are positive and beyond WHO permissible level. High concentrations of *faecal coliforms* have been detected in the sample KBWWS3 viz 283CFU/10ml and concentration of *Halophiles* in sample KBWWS2 with 212CFU/10ml. While the lowest concentration of the *faecal coliforms* and *Halophiles* are 198CFU/10ml and 103CFU/10ml respectively (Table 4).

Uchali Lake

The analysis of samples from Uchali Lake demonstrate a range of 212CFU/10ml in sample ULWS2 to 269CFU/10ml in ULWS4 of *faecal coliforms* and the range of 101CFU/10ml in ULWS4 sample to 312CFU/10ml in ULWS2 sample of *halophiles.* The results are well above the permissible water standards of WHO (Table 5).

Uchali Well Water

High Concentration of *faecal coliforms* in the sample ULWWS4 is 267CFU/10ml of water, while concentration of *Halophiles* in sample ULWWS2 is 188/10ml, while the lowest concentration of the *faecal coliforms* and *Halophiles* are 197/10ml and 99/10ml respectively (Table 6).

Jahlar Lake

The situation of *faecal coliforms* and *Halophiles* at Jahlar Lake is not different than the other lakes of the Salt Range. The analysis of samples show the *faecal coliforms* are in the range of 233CFU/10ml in sample JLWS1 to 333CFU/10ml in sample JLWS3. The *Halophiles* concentration range determined is 142CFU/10ml in sample JLWS1 to 366CFU/10ml in sample JLWS2. This is higher according to WHO standards. Comparatively, Jahlar Lake reveals higher bacterial contamination, which could be caused due to its high salinity ratio and direct discharge of human and animal waste and defecations from the surrounding settlements (Table 7).

Table 1: showing *E-Coli* reaction with various growth media

Media	Organism identified	Selective/deferential media
Brilliant-green agar	*E-coli spp*	Brilliant green dye inhibit the growth of gram positive bacteria, *E-coli* with yellow colonies
EMB	Gram negative enteric pathogen	*E-coli* colonies purple and have metallic sheen
MacConkey	Gram negative enteric pathogen	Pinkish white colonies of *E-coli*

Table 2: *Faecal coliforms* and *Halophiles* count at Kallar Kahar Lake

Sample #	**FC**			***Halophiles***		
	EMB Media	**Qty**	**Usability**	**MSA Media**	**Qty**	**Usability**
Kkws1	Positive	314CFU	No	Positive	111 CFU	No
Kkws2	Positive	324CFU	No	Positive	203 CFU	No
Kkws3	Positive	303CFU	No	Positive	213 CFU	No
Kkws4	Positive	209CFU	No	Positive	144CFU	No

Table 3: *Faecal coliforms* and *Halophiles* count at Khabeki lake site

Sample #	**FC**			***Halophiles***		
	EMB Media	**Qty**	**Usability**	**MSA Media**	**Qty**	**Usability**
KBWS1	Positive	265CFU	No	Positive	301CFU	No
KBWS2	Positive	318CFU	No	Positive	219CFU	No
KBWS3	Positive	320CFU	No	Positive	197CFU	No
KBWS4	Positive	212CFU	No	Positive	156CFU	No

Table 4: Showing *faecal coliforms and Halophiles* count at Khabeki Wells

Sample #	FC			*Halophiles*		
	EMB Media	**Qty**	**Usability**	**MSA Media**	**Qty**	**Usability**
KBWWS1	Positive	198CFU	No	Positive	103CFU	No
KBWWS2	Positive	230CFU	No	Positive	212CFU	No
KBWWS3	Positive	283CFU	No	Positive	123 CFU	No
KBWWS4	Positive	247CFU	No	Positive	189CFU	No

Table 5: Showing *faecal coliforms and Halophiles* count at Uchali Lake

Sample #	FC			*Halophiles*		
	EMB Media	**Qty**	**Usability**	**MSA Media**	**Qty**	**Usability**
ULWS1	Positive	265CFU	No	Positive	133CFU	No
ULWS2	Positive	212OCFU	No	Positive	312CFU	No
ULWS3	Positive	219OCFU	No	Positive	299CFU	No
ULWS4	Positive	269OCFU	No	Positive	101CFU	No

Table 6: Showing *faecal coliforms and Halophiles* count at Uchali wells

Sample #	FC			*Halophiles*		
	EMB Media	**Qty**	**Usability**	**MSA Media**	**Qty**	**Usability**
ULWWS1	Positive	197CFU	No	Positive	99CFU	No
ULWWS2	Positive	201CFU	No	Positive	188CFU	No
ULWWS3	Positive	236CFU	No	Positive	171CFU	No
ULWWS4	Positive	267CFU	No	Positive	111CFU	No

Table 7: Showing *faecal coliforms* and *Halophiles* count at Jahlar Lake

Sample #	FC			*Halophiles*		
	EMB Media	**Qty**	**Usability**	**MSA Media**	**Qty**	**Usability**
JLWS1	Positive	233CFU	No	Positive	142CCFU	No
JLWS2	Positive	269CFU	No	Positive	366CFU	No
JLWS3	Positive	333CFU	No	Positive	199CFU	No
JLWS4	Positive	301CFU	No	Positive	213CFU	No

Table 8: Showing *faecal coliforms and Halophiles* count at Namal Lake

Sample #	FC			*Halophiles*		
	EMB Media	**Qty**	**Usability**	**MSA Media**	**Qty**	**Usability**
NLWS1	Positive	233CFU	No	Positive	323CFU	No
NLWS2	Positive	349CFU	No	Positive	99CFU	No
NLWS3	Positive	278CFU	No	Positive	297CFU	No
NLWS4	Positive	199CFU	No	Positive	256CFU	No

*CFU- Colony Forming Unit, *EMB-Eosin Methylene Blue, *MSA-Mannitol Salt Agar

Namal lake

One of the beautiful and historical lake of Pakistan, known as Namal Lake is also not exempted from bacterial contamination. Water quality analysis f the lake shows positive results with high concentration of *faecal coliforms* and *halophiles.* The analysis of the samples from the lake suggest the *faecal coliforms* range of 199CFU/10ml in sample NLWS4 to 349CFU/10ml in sample of NLWS2 and *Halophiles* in the range of 99CFU/10ml in sample NLWS2 to 323CFU/10ml in sample NLWS1. These levels are over the permissible WHO standards for water quality and the water is therefore not suitable for human use (Table 8).

Discussion

The range of *faecal coliforms* and *Halophiles* were high in each sample from initial to final point. *Faecal coliforms* showed elevated values from source 199CFU/10ml to 349CFU/10ml, while *Halophiles* recorded shared the range 99CFU/10ml to 366CFU/10ml in all selected lakes and well water. According to WHO standards for drinking water to be safe a 100 ml sample of water should not contain any *coliforms* bacteria. Results revealed that the quantity of *faecal coliforms* is very high in selected water sources and is above the standards of WHO. The possible cause for this contamination could be unsustainable human interference like irrigation, household use, laundry, septic tanks and direct effluents from settlements. Livestock have been noticed grazing, walking and drinking around the lakes. Farmers consolidate their livestock dung in the vicinity of lakes, where when the rain water directly flows in to the lakes, which appear like a major source of providing a growing medium for *coliforms* bacteria. Another possible cause is the intensive use of pesticides, insecticides and fertilisers in the agricultural crops around the lakes. These may be washed and the nutrient contained may provide a growing medium to bacteria.

In the light of results, the presence of high numbers of *faecal coliforms* and *Halophiles* in selected water resources indicates that the water of these lakes are not suitable for drinking purposes and other non-consumptive uses as per WHO recommendations. High rate of *Halophiles* bacteria in the wetlands is not suitable for fish as it cause de-oxygenation in water. During the field investigation in June; a large number of dead fish were recorded in the Khabeki Lake. Later on our hypothetical statement was proved correct by Punjab Fisheries Department; they claim that the mass mortality of fish was caused by de-oxygenation of the lake water. The Punjab Fisheries Department further added to their report that

blooms of red algae which caused de-oxygenation in lake water and eventually oxygen deficiency occurred, which led epidemic.

Conclusion

The *faecal coliforms* bacterial contamination level of Salt Range Lakes and well water resources is above the WHO limits for drinking purposes and thus the water from these sources is not safe for human consumption.

Implications of the Study

A spectrum of hypothetical and practical aspects regarding pathogens have been discussed which eventually goes to recommendations for taking sustainable measures in order to make the water sources safe. First, there exists a gap which needs to be filled by understanding of ecological long-term integrity of pathogens, their indicators, and consequences in linear connection to lake and ground water functions. Secondly, a comprehensive strategy should be formed to monitor and record microbial pathogens, their status, mode of action and related waterborne diseases. Proper livestock manure management is required to minimise the contact of rain or irrigation water with the manure to avoid loss of the manure slurry into surface and ground water resources. This could significantly reduce *faecal* contamination of water resources in Pakistan.

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