



## RESEARCH PAPER

## OPEN ACCESS

## Bacteriological analysis of drinking water sources in CKNP region of Gilgit –Baltistan, Pakistan

Maisoor Ahmed Nafees<sup>1\*</sup>, Khalil Ahmed<sup>1</sup>, Shamsher Ali<sup>2</sup>, Rehmat Karim<sup>2</sup>, Tika Khan<sup>2</sup>

<sup>1</sup>*Department of Biological Sciences, Karakoram International University (KIU) Gilgit-Baltistan, Pakistan*

<sup>2</sup>*Integrated Mountain Area Research Center (IMARC), KIU, Gilgit-Baltistan, Pakistan*

**Key words:** Karakoram, National Park, CKNP, *E. coli*, Water quality, Bacteria, water sources, glaciers, Enterococci, *Salmonella*.

<http://dx.doi.org/10.12692/ijb/5.4.54-59>

Article published on August 18, 2014

### Abstract

The study was focused on the Bacteriological characteristics of drinking water within the selected villages fall in Central Karakoram National Park (CKNP) Gilgit-Baltistan, Pakistan. Different water sources were taken in to account including streams, springs, rivers and other storage structures viz; wells and Tanks. A total of 51 water samples were collected from 17 different localities and transported to lab in battery operated portable refrigerators. All necessary data including coordinates was mustered. Sources were examined with a strategy to sample from source mid and last points. The idea was to evaluate the stage and subsequent level of contamination. The level of contamination was ranged from 0-160 CFU for *E-coli*, 0-85 for *Enterococci* and total bacterial count ranged from 2-180 CFU, whereas no any *Salmonella* species was detected in one liter. The well and surface water was highly contaminated and the spring sources were less or no contaminated depending upon protected and open sources respectively. While the contamination level showed a culminated trend from mid to last usage points.

\* **Corresponding Author:** Maisoor Ahmed Nafees ✉ [maisoor.nafees@kiu.edu.pk](mailto:maisoor.nafees@kiu.edu.pk)

## Introduction

Bacteriological contamination in potable water is not only the result of natural factors. It has been aggravated by anthropogenic activities especially in the developing countries. Water of good drinking quality with balanced minerals is of prime importance to carry on normal human physiological activities. The sporadic eruption of water borne diseases has coerced the experts to assess the relevant sources for the responsible pathogens and water quality indicators. Contaminated drinking water contributed to a number of health issues in developing countries like more than one billion cases of diarrhea that occur annually (WHO 2013). Access to safe water is a basic human need and, therefore, a basic human right. Contaminated water deteriorates both the physical and social health of all peoples. According to WHO, more than 80% of diseases in the world are attributed to unsafe drinking water or to inadequate sanitation practices (Petersen, 2003; WHO 2003). Globally, 1.1 billion people rely on unsafe drinking water sources from lakes, rivers, and open wells (WHO, 2000).

According to world health organization (WHO), there were estimated 4 billion cases of diarrhea and 2.2 million deaths. The research area is also a home to mighty glaciers which are deemed to be the water towers and are largest, next to polar region. The Central Karakoram National Park represents the largest source of fresh water in Pakistan and it harbors the one of the largest mountain glacier systems in the world. (Siachin 75 km, Baltoro 57km, Hisper-Biafo 122 km). These glaciers are said to be the life-blood of Pakistan and all originating within Pakistan boundaries and feeding the mighty Indus and its tributaries. They are the key source of drinking water, domestic use, irrigation, wetlands and hydro power generation. (Ahmed and Joyia 2003).

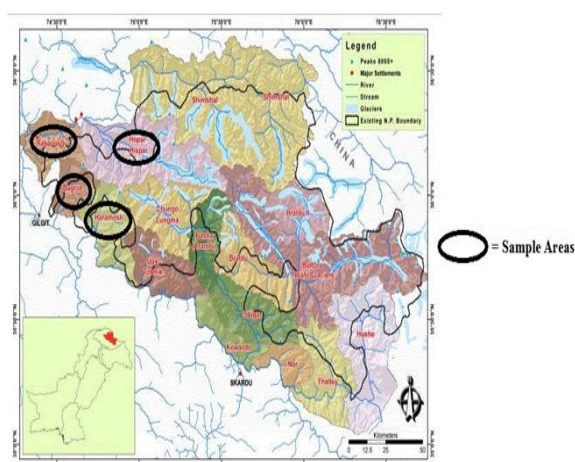
The role of sanitation and safe water in maintaining health has been recognized for centuries. The provision of water, sanitation and good hygiene services is vital for the protection and development of human resources (Fewtrell *et al.*, 2004).

Therefore, it has become necessary to monitor water quality to observe the demand and pollution level of water sources. Several water analyses have been regularly conducted by different scientific groups across the country. The present work is a primary attempt to examine the water quality of various potable water resources within the buffer zone of CKNP. The main focus is to collect the information from the selected areas within CKNP, that how the water resources are being utilized and what is the microbiological picture of drinking water.

## Material and methods

### Research site

Water Samples were collected from various localities within CKNP, including Sikandarabad, Minapin, Pisan (Rakaposhi area, Nagar). Hoper valley Nagar, Bagrote and Haramosh valleys, Gilgit. Figure 2 shows the sampling sites in the central Karakoram National Park. Table 1 shows the points/sources from where samples were collected within above mentioned localities.



**Fig. 1.** Map of CKNP (Central Karakoram National Park) Gilgit-Baltistan, Pakistan.

### Sample collection

Total 51 water samples were collected in from July 2013 to November 2013 from different localities with diverse type of water sources having distinct ecology and topography. All the samples were collected in 1000 ml pre sterilized Glass bottles and were transported in battery operated refrigerators to Water Quality Lab Karakoram International University (KIU), Gilgit, Pakistan and processed immediately.

All necessary details were mustered in a pre-designed data sheet including the GPS values in order to have a record for monitoring purposes and to develop a permanent system of surveillance. Sampling was done in closer conformity with general guide to the sampling techniques and recommendations for preservation and handling of samples, (ISO 5667-1, 5667-2)

#### Microbiological parameters

The collected water samples were analyzed for various microbiological (Bacteriological) parameters. The procedure for analysis was followed as per standard methods for the examination of water and waste water (APHA, 2000; AWWA, 2000). The parameters analyzed were *E.coli*, *intestinal Enterococci*, Total Bacterial Count and *Salmonella*.

The membrane filtration (MF) technique, (ISO 7704) was used to filter 100 ml of volume from water samples to ascertain *E.coli*, *Intestinal Enterococci* and *Salmonella*. The Chromogenic (EC X-GLUC Agar) was used for the detection of *Escherichia coli* (ISO 9308-1). The determination of *Enterococci* was carried out by filtering 100 ml of water from respective samples and subsequent culturing on Slanetz and Bartley agar (ISO 7899-2). The conformity test for *Enterococci* was done using Bile Esculin Azide Agar (BEA). For detection of *Salmonella* in 1 liter Hektoen Enteric Agar, Rappaport vasillaidis Broth were used after passing the filtered paper in to enrichment media containing buffered peptone (ISO 6579-1981). Total Bacterial

count (Heterotrophic Plate Count, APHA 9215-B) was revealed through pour plate technique by pouring 1ml sample in to Petri dishes and adding yeast extract agar as a growth medium accordingly. Control Petri plates for all parameters were also kept in incubation in order to avoid any contamination chances in medium. All the bacterial cultures were aided by incubating the relevant Petri dishes to required growth temperatures and their number was counted on total Colony forming units (CFU) basis.

#### Results and discussion

Table 1 shows the result of all the samples collected from various localities having three different type of sources. In the history of water quality testing labs in Gilgit Baltistan, Chromogenic (EC X-GLUC Agar) was used for the first time as medium to culture *Escherichia coli*. Which is said to be the most efficient selective media for the isolation of said water quality indicator (ISO 16649-2,2001). Moreover, the source mid and last point assessment differentiates the current study with similar previous approaches in the area (Ahmed and Shakoori, 2002). The *E.coli* percentage was at higher end ranging from 1 to 160 CFU in mid and last usage points as compare to the sources. Springs with protected structures were least contaminated in Bagrote Valley as compare to the protected spring sources in Hoper valley. The water quality condition of wells as a storage structure was worst, feeding water from streams and allied irrigation channels made it more vulnerable to Bacteria. The average count within the samples collected from wells was 125 CFU (Table 2).

**Table 1.** Bacteriological Parameters In Drinking Water Collected From Different Localities/Sources Of Central Karakoram National Park (CKNP), Gilgit-Baltistan, Pakistan.

| Site ID | Site Name                | Water Class | <i>E.Coli</i> /<br>100ml | <i>Enterococci</i> /<br>100ml | Total Bacterial<br>Count | <i>Salmonella</i> in 1<br>Liter |
|---------|--------------------------|-------------|--------------------------|-------------------------------|--------------------------|---------------------------------|
| SIK1    | Sikandarabad Ithen       | stream      | 25                       | 16                            | 40                       | Absent                          |
| SIK2    | Sikandarabad Khantip     | well        | 60                       | 80                            | 120                      | Absent                          |
| SIK3    | Sikandarabad Tehsil Area | well        | 120                      | 85                            | 130                      | Absent                          |
| HAK1    | Hakalshal, Hoper         | spring      | 2                        | Absent                        | 16                       | Absent                          |
| HAK2    | Hakalshal Mid tape,      | spring      | Absent                   | Absent                        | 10                       | Absent                          |
| HAK3    | Hakalshal Last Tape      | spring      | 15                       | 4                             | 25                       | Absent                          |
| BRO1    | Broshal Tank, Hoper      | spring      | 49                       | 7                             | 120                      | Absent                          |
| BRO2    | Broshal Doulat House     | spring      | 79                       | 19                            | 121                      | Absent                          |
| BRO3    | Broshal School Tap       | spring      | 21                       | 11                            | 65                       | Absent                          |

|      |                           |        |        |        |        |        |
|------|---------------------------|--------|--------|--------|--------|--------|
| RAT1 | Ratal Tank, Hoper         | spring | 3      | 6      | 44     | Absent |
| RAT2 | Ratal Ghulam House        | spring | Absent | 4      | 25     | Absent |
| RAT3 | Ratal Roadside Tap        | spring |        | 3      | 21     | Absent |
| PIS1 | Sholay,Pisan              | spring | 4      | Absent | 65     | Absent |
| PIS2 | Ismail House,Pisan        | spring | 1      | 3      | 16     | Absent |
| PIS3 | Dainy,Pisan               | spring | Absent | 2      | 6      | Absent |
| MIN1 | Sholay,Minapin            | spring | 6      | 6      | 42     | Absent |
| MIN2 | Dasguni,Minapin           | spring | 2      | 15     | 85     | Absent |
| MIN3 | kot masjid,Minapin        | spring | 1      | 12     | 14     | Absent |
| SAN1 | Sanikar Tank              | spring | Absent | Absent | Absent | Absent |
| SAN2 | Sanikar Mid Public Tape   | spring | Absent | 4      | 4      | Absent |
| SAN3 | Sanikar Lower Tape        | spring | Absent | Absent | 1      | Absent |
| HOP1 | Hohey Tank                | spring | Absent | Absent | 1      | Absent |
| HOP2 | Hohey,Ihsan Ali House     | spring | Absent | 4      | 2      | Absent |
| HOP3 | Hohey,Ghulam Raza House   | spring | Absent | 1      | 1      | Absent |
| DAT1 | Datuchi Tank              | spring | Absent | Absent | 4      | Absent |
| DAT2 | Datuchi, Ali Ghulam House | spring | Absent | Absent | 3      | Absent |
| DAT3 | Datuchi,public Tape       | spring | Absent | Absent | 5      | Absent |
| FAR1 | Farfu Tank                | spring | Absent | Absent | 4      | Absent |
| FAR2 | Farfu Azhar House         | spring | Absent | Absent | Absent | Absent |
| FAR3 | Farfu lower Tap           | spring | Absent | Absent | 2      | Absent |
| BUL1 | Bulchi Tank               | spring | Absent | Absent | 4      | Absent |
| BUL2 | Bulchi,M.Hussain House    | spring | Absent | Absent | 1      | Absent |
| BUL3 | Bulchi,Adil House         | spring | Absent | Absent | 5      | Absent |
| CHI1 | Chira Tank                | spring | Absent | 7      | 1      | Absent |
| CHI2 | Chira,Ashur Ali House     | spring | 2      | Absent | 4      | Absent |
| CHI3 | Chira,Mehboob House       | spring | 1      | 3      | 2      | Absent |
| BRU1 | Brumday Source            | stream | 75     | 38     | 80     | Absent |
| BRU2 | Brumday Mid               | stream | 27     | 25     | 40     | Absent |
| BRU3 | Brumday Last              | stream | 130    | 35     | 40     | Absent |
| SHA1 | Shatot Source             | stream | 10     | 17     | 5      | Absent |
| SHA2 | Shatot Mid                | stream | 93     | 35     | 48     | Absent |
| SHA3 | Shatot last               | stream | 27     | 24     | 24     | Absent |
| HUR1 | Hurban Source             | stream | 100    | 40     | 45     | Absent |
| HUR2 | Hurban Mid                | stream | 155    | 60     | 44     | Absent |
| HUR3 | Hurban last               | stream | 160    | 20     | 140    | Absent |
| SAS1 | Sassi Source              | stream | 65     | 26     | 30     | Absent |
| SAS2 | Sassi Mid                 | stream | 6      | 13     | 35     | Absent |
| SAS3 | Sassi last                | stream | 34     | 24     | 100    | Absent |
| HAN1 | Hanuchal Source           | stream | 3      | Absent | 80     | Absent |
| HAN2 | Hanuchal Mid              | stream | 50     | Absent | 100    | Absent |
| HAN3 | Hanuchal last             | stream | 50     | 19     | 180    | Absent |

### Statistical analysis

Statistical analysis shows that there is a significant relationship between mid and last point contamination with type of distribution medium. Fecal contamination at the points of corrosion in Galvanized Iron (GI) pipes and presence of livestock along the sources, further deteriorated the drinking water quality. Majority of water samples except the sources of Bagrote Valley were higher than the permissible limit predetermined by WHO for *E-coli* and total bacterial count in drinking/surface water.

The contamination level was quite alarming in streams and wells specially *E. coli* and *Enterococci* in the study area. Moreover, there is significant relationship among the level of contamination with respect to the water class, where the stream water and wells have not shown a vivid difference in terms of *Enterococci* contamination (table 2).

Mean Contamination results on Table No -1 reveal that there is no any significance difference among the *E. coli*, *Enterococci* and Total bacterial Count (TBC)

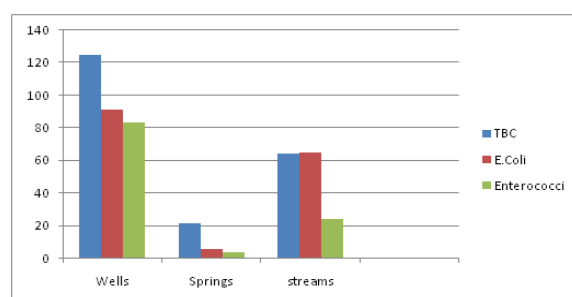
with respect to sample points (Source, Mid and Last). But the occurrence of infestation is vivid in sources with different origin, type of source and the protection and accessibility to the drinking water

source. Some other factors including anthropogenic interaction and deterioration of medium can be more influential in the presence of mentioned parameters.

**Table 2.** Variation in bacteriological parameters in relation to water class.

| Water Class | Total Bacterial Count      | <i>E.Coli</i> /100ml       | <i>Enterococci</i> /100ml  |
|-------------|----------------------------|----------------------------|----------------------------|
| Stream      | 64.44 ± 6.55 <sup>a</sup>  | 63.12 ± 7.27 <sup>a</sup>  | 24.48 ± 2.161 <sup>a</sup> |
| Well        | 125.00 ± 2.78 <sup>b</sup> | 91.33 ± 12.86 <sup>a</sup> | 83.83 ± 0.98 <sup>b</sup>  |
| Spring      | 21.74 ± 3.31 <sup>c</sup>  | 5.76 ± 1.61 <sup>b</sup>   | 3.41 ± 0.48 <sup>c</sup>   |
| LSD         | 0.00                       | 0.01                       | 0.00                       |

Means (SE) values followed by same letter (s) across columns are not significantly different with respect to water class at  $P \leq 0.05$ .



**Fig. 2.** Mean Bacteriological contamination level (CFU) at various sources within CKNP.

#### *Factors influential in water contamination and management*

The poorly managed resources and improper distribution of drinking water from the source to last usage point is a key factor in determining the overall microbial quality of water. The farming practices in tandem with anthropogenic activities from March to October, and open grazing practices during autumn and winter has culminated the contamination of sources. Proper protection and surveillance of sources and the distribution medium is utmost necessary to get the desired quality of drinking water in home taps. The streams and other forms of surface water which feeds wells are used for irrigation purposes too. The seclusion of the two distinctive systems is indispensable to minimize the risk of water borne diseases which are common in the study area. Some better sources (springs) have been abandoned due to myths depicting ill effects of its usage other than biological and chemical contamination. Proper counseling and awareness among local populace is

inevitable to keep them on better drinking water sources

#### **Acknowledgements**

Study is part of PhD research and was possible due to generous financial grant from Italian government and the government of Pakistan through SEED project in collaboration of Karakoram International University, Gilgit-Baltistan. The author is highly indebted to the supervision of Dr. Andrea Lami, Paleo- Limnologist, Institute of Ecosystem Studies, Council of National Research, Italy. We are also thankful for CKNP management team for their ongoing support during field visits.

#### **References**

- Ahmed K, Shakoori AR.** 2002. *Vibrio cholerae* E1 Tor,Ogawa 01, as the main etiological agent of the two major outbreaks of gastroenteritis in the Northern Areas of Pakistan. Pakistan Journal of Zoology **34**(1), 73-80 p.
- Ahmed S, Joyia F.** 2003. Northern Areas Strategy for sustainable Development., pp.67. Retrieved from on July 23, 2014.  
[http://cmsdata.iucn.org/downloads/nassd\\_strategy.pdf](http://cmsdata.iucn.org/downloads/nassd_strategy.pdf)
- APHA.** 2000. American Public Health Association, 1994. Standard Methods for the Examination of Water and Waste water 20th Edition. Part 9000, P:

9-40. Retrieved from on August 3, 2014.

globallast.imo.org/monograph%2015%20RandD%20Symposium.pdf

**AWWA.** 2000. American Water Works Association 2000, National water-quality Assessment programme U.S. Department of the Interior, U.S. Geological Survey Ground Water and Drinking Water.

**Fewtrell L, Colford J.** 2004. Water, Sanitation and Hygiene: Interventions and Diarrhea a Systematic Review and Meta-analysis. The International Bank for Reconstruction and Development / World Bank. Retrieved from on July 12, 2014.

**ISO.** 1999. International Organization for Standardization, 1999 (7899-2) Water Quality Detection and Enumeration of Intestinal *Enterococci*, Part2: retrieved from on July 11, 2014.

**ISO.** 2000. International Organization for Standardization (7704) Evaluation of membrane used for microbiological analysis. Retrieved from <http://iso.org/iso/home/store/catalogue>.on August 4,2014

**ISO.** 2000a. International Organization for Standardization. (9301-1) Water Quality - Detection and Enumeration of *Escherichia coli* and *coliform* Bacteria-Part1

**Petersen PE.** 2003. The World Oral Health Report 2003: continuous improvement of oral health in the 21st century—the approach of the WHO Global Oral Health Programme. Community Dentistry and oral epidemiology **31(s1)**, 3-24.

**Raza H, Ahmed K, Ali I, Abbas I, Rasmussen ZA.** 1998. Case control study of etiology of Diarrhea in Oshikhandass, Northern Areas, Pakistan. Journal of Pediatric Gastroenterology and Nutrition **27(2)**, 263 p.

**Vega M, Pardo R, Borrado E, Debi L.** 1998. Effects of seasonal and polluting effects in quality of River water by exploratory data analysis. Water resources Journal **32(12)**, 3581-592.

**WHO.** 2002. World Health Organization. Global Water Supply and Sanitation Assessment Report.

**WHO. World Health organization.** 2000. Monitoring Bathing Waters - A Practical Guide to the Design and Implementation of Assessments and Monitoring Programmes. 106-157 p.

**WHO.** 2003. World Health Organization. Press release sg/sm/8707 obv/348. 'water-related diseases responsible for 80 per cent of all illnesses, deaths. In developing world', says secretary-general in environment day message. Retrieved from on august 7, 2014.