Physico-chemical and microbial assessment of drinking water sources across world's largest glacial deposits in Karakoram Mountain Ranges, Pakistan

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Abstract: Karakoram Mountain Ranges, Pakistan harbor largest glacial deposits in the world outside poles. They are key source of life and livelihoods of more than 15 million people living downstream. Water samples tested for physico-chemical and microbial health revealed severe bacterial contamination. A total Bacterial count, occurrence of *E. coli* and *Enterococci* in alpine origin sources indicate their vulnerability and undesirable human interferences into fragile mountain ecosystems making them unsafe for consumption. However, chemical ingredients found within the specified water quality limitation. Water sources need proper attention to mitigate frequency and prevalence of water borne diseases in the area.

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1. Introduction

In the last few decades global population has bulged so the deterioration in climate changes. This has increase pressure on natural resources in general and water availability in particular **(Cropper and Griffiths 1994)**. More than eighty countries of the world making almost half of the total global population are suffering from acute shortage of water have entered into a crisis situation **(Birks 1977)**. This has led to a dramatic increase in cost of water infrastructure **(Rosegrant et al. 2002)**. Water, both in the rivers and underground has got polluted **(Goel 2006)**. Over one billion people do not access safe drinking water, three billion lack sanitation facility and eighty percent (80%) of infectious diseases are waterborne killing 2.2 million every year **(Petersen 2003; World Bank 1999)**.

The Central Karakoram National Park (CKNP) within the Karakoram Mountain Ranges, Pakistan holds the major source of fresh water in the country. Out of largest glacial and river systems outside poles Baltoro is the remarkable feeding some 30 tributaries which constitute a total surface area of 1,219 sq. km. These glaciers are life-blood for Pakistan nurturing the mighty Indus and its offshoots. They meet the needs for drinking, domestic use, irrigation, wetlands and industrial use and electricity **(Acreman 2001).**

Water supply contaminations with sewage and human excreta poses maximum risk to public health **(Mara and Cairncross 1989)**. However, water quality testing enables to avoid such pollutions **(Hrudey et al. 2003)**. 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)**. Globally, 1.1 billion people rely on unsafe drinking water sources from lakes, rivers, and open wells **(WHO, 2000)**.

Mountain communities living close to these perennial mighty glacial masses depend for their subsistence agriculture, drinking, household use and power generation. It is important to assess water quality to know ranges of human interventions and their impact on the lives of rural communities. Present study was carried out for the first time in history from the area. It was aimed at providing scientific insights of water quality, its fitness for consumption, level of contamination, nature of contamination, polluting agents and potential mitigation measures. Furthermore, findings will help water analysis and monitoring schemes to improve overall water quality management and protection of important water bodies and ecosystems in CKNP and beyond.

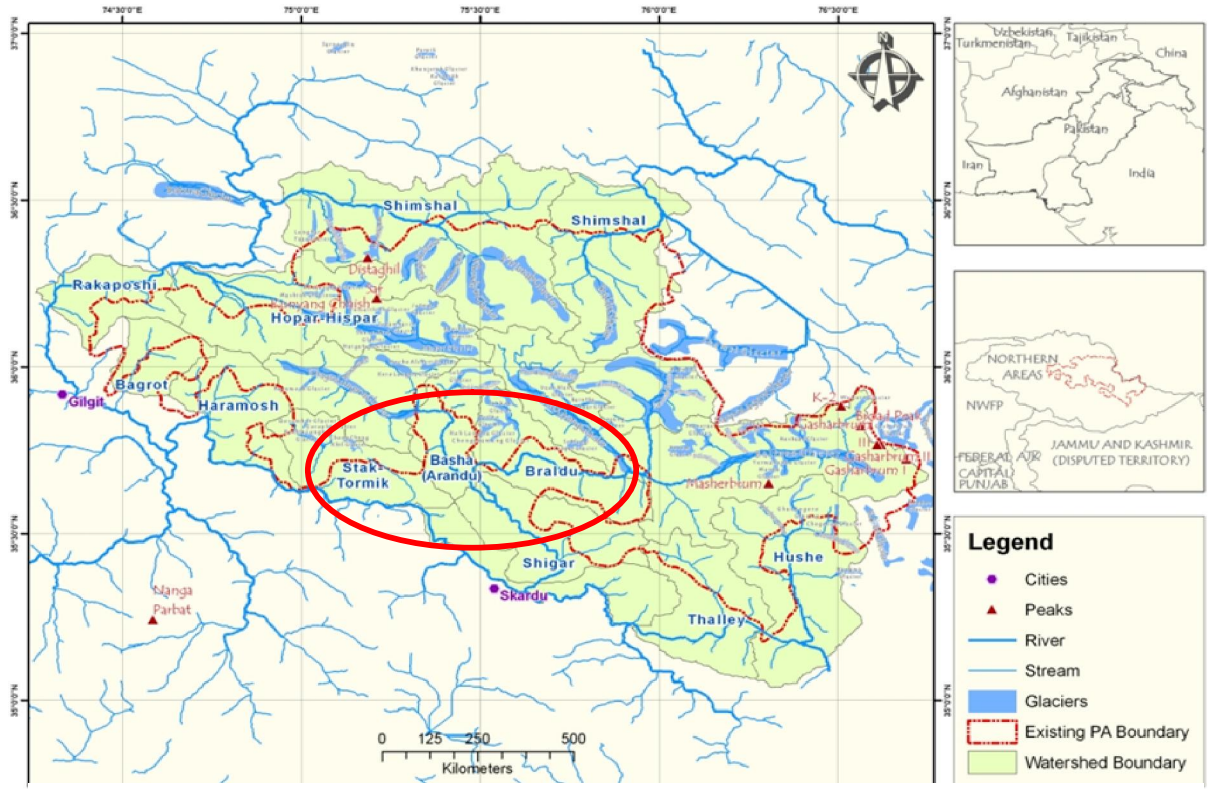

Figure: Map of study area showing sampling sites

2. Materials And Methods

Sampling

A total of 60 samples were collected from Basha and Baraldu valleys in Central Karakoram National Park **(See figure)**. Collection sites were divided into three categories i.e. source, mid and last. HDPE pre sterilized bottles of 500 ml and 1000 ml were used for sample collection. Ten (n=10) samples were collected from each sites with slight variation in distances including source, middle points and last consumption points. 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)**. Samples were transported in battery operated refrigerators to the 'Water Quality Lab', Karakoram International University (KIU) and processed immediately. All ecological attributes were taken into account with GPS coordinates for future monitoring and to develop a permanent system of surveillance. Water sampling and subsequent analysis campaign continued between June 2012 and November 2014.

Bacteriological Analysis

Following the standard procedure for examination of water and waste water, samples were analyzed for various microbiological (Bacteriological) parameters **(APHA and AWWA 2000)**. The parameters analyzed were *E. coli, Enterococci* (intestinal), Total Bacterial Count and *Salmonella.* The membrane filtration technique (ISO 7704) was used to filter 100 ml water samples to ascertain *E. coli, Enterococci and Salmonella*. The Chromogenic (EC X-GLUC Agar) was used for the detection of *E. coli* (ISO 9308-1). The conformity test was performed with triptic soya agar. The culture and isolation of *Enterococci* was carried out by filtration of 100 ml of water subsequent culturing on Slanetz and Bartley agar (ISO 7899-2). The conformity test for *Enterococci* was done using Bile Esculin Azide agar. 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)**. Oxoid *salmonella* testing kits were used pathogenic conformation. Total Bacterial count **(Heterotrophic Plate Count, APHA 9215-B)** was processed through pour plate technique by pouring 1 ml sample into Petri dishes and adding yeast extract agar as a growth medium accordingly. 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.

Physico-chemical investigation

The pH of the each sample was determined using

a pH meter **(Adwa, AD 1020)** equipped with a temperature probe (Degree of accuracy 0.01). Turbidity was determined with Turbidimeter (Velp Scientifica, Model TB- 1). Nephalometric Turbidity Units (NTU) were taken into consideration. Similarly, conductivity meter (model AD 3000, Adwa) used to determine electrical conductivity and TDS. In accordance with the ISO standards reference solutions were used for calibration of equipment and accuracy of readings (0.01).

Spectrophotometry

Spectrophotometer **(Techcomp, UV, dual beam, Model UV2300 II)** was used for assessment of the fallowing parameters;

Total Nitrogen

The sum of nitrate nitrogen, nitrite nitrogen, ammonium nitrogen and organically bonded nitrogen, each determined separately. Ammonium, nitrite and organic nitrogen are oxidised to nitrate using potassium peroxodisulphate in boric acid-sodium hydroxide buffer. The oxidation of the nitrogen compounds is performed in an autoclave at 120 °C, resulting in a pH change of the buffer from 9.7 to 5.0. The resulting nitrate was determined by spectrophotometry at 220 nm **(APHA, AWWA, WEF, 1998; Valderrama, 1981)**.

Ammonium

Pipetted 25 ml of the sample into the 50 ml polypropylene Erlenmeyer flask with screw cap and added respective reagents. Three blanks samples were prepared per batch, using 25 mL of ultra-pure water in place of the test sample. The absorbance was set at 690nm **(Fresenius et al. 1988; Grasshoff and Johannsen 1972).**

Total Phosphorous

The spectrophotometric determination was performed at the wavelength of 890 nm with 5 cm cuvettes optical path and the spectrophotometer was zeroed by using de-ionised water without reagents. This procedure details the determination of total phosphorus in natural freshwater, surface water, and drinking water, in the form of ortho-phosphate and organic phosphorus compounds capable of conversion to ortho-phosphate under the oxidative digestion procedure described **(Tartari and Mosello 1997; Valderrama 1981)**.

Table 1: Population of *E.coli*, Enterococci, TBC in three sampling-points of study area valley. Means followed by different letter(s) in the same column are significantly different from one another at LSD ≤ 0.05

Valleys	Points	E. coli	Enterococci	TBC
Barlado	Source	38.083A	17.16A	146.17A
	Mid	24.25AB	22.25A	120.94B
	Last	19.41B	18.08A	116.75B
	LSD	15.564	6.7571	22.957
Basha	Source	18.14A	16.57A	261.14A
	Mid	12.57AB	11.85B	121.95A
	Last	9.57B	11.42B	121.29A
	LSD	7.2328	4.0505	222.76

Reactive Orthophospahte

After mixing reagents with each 25 ml sample and preparing three blanks and repeatability the spectrophotometric determination was performed at the wavelength of 890 nm with 5 cm cuvettes optical path and the spectrophotometer is zeroed by using de-ionised water without reagents **(Tartari and Mosello 1997; Valderrama 1981)**.

Ion Chromatography

Through ion chromatography **(Dionex ICS 30000)** fallowing Anions and Cations have been ascertained. The said analysis has been carried out at Chemistry Lab, Institute of Ecosystem Studies, Council of National Research (CNR), Verabania, Italy. **Anions**: chlorides, nitrates, sulfates; (ISO 10304-1. Water quality - Determination of dissolved fluoride, chloride, nitrite, orthophosphate, bromide, nitrate and sulphate ions, using liquid chromatography of ions. Part. 1: method for water with low contamination). **Cations**: sodium, potassium, magnesium and calcium. (ISO 14911 Water quality - determination of dissolved Na+, NH4+, K+,, Ca2+, Mg2+,, and using ion chromatography - method for water and waste water).

3. Results And Discussion

Bacteriology

Results for E. coli, Enterococci and TBE from the valleys showed some declining trend from source to last point **(table 1).**

Physical parameters

Turbidity was recorded higher in some samples **(table 2)**; however other indicators taken into account did not show any alarming situation in the study area. Some points exhibited little higher TDS while rest of locations remained in the range of given standards.

Chemistry

Details are expressed in table 3 and 4 given below showed a normal ranges of the chemical

ingredients tested from all points. Both valleys (table 3, Basha valley; table 4, Baraldu valley) vary slightly from one to another in some cases; however there was no any major point of concern or deviation from the international acceptable standards.

Table 2: Physical Parameters of Source Mid and Last Points (Means followed by different letter(s) in the same column are significantly different from one another at LSD ≤ 0.05)

Valleys	Points	Temp	pH	Turbidity	Conductiv	TDS
Braldo	Source	1.56B	6.96A	7.92B	320.00A	170.75A
	Mid	1.73AB	6.97A	16.303AB	335.83A	165.92A
	Last	1.81A	7.03A	23.58A	307.67A	164.25A
	LSD	0.2442	0.0921	11.378	33.443	9.4311
Basha	Source	1.85B	7.01A	8.69A	229.39AB	161.37B
	Mid	1.92A	6.98A	7.14A	260.61A	187.21AB
	Last	1.85B	6.95A	8.95A	213.13B	212.46A
	LSD	0.0646	0.0949	2.1658	31.389	39.706

4. Discussion

Keeping in view WHO guidelines, water used for drinking purposes was unsafe. Contamination level was alarming in streams and surface water as compare to the spring sources. Our results were in conformation with Chapman et al. **(1971)**, who disapproved McCarisson myth of increasing water contamination increases risk of goiter. Similarly, bacteriological investigations are in closer agreement with the studies of Craun et al 2003, where the *E.coli* has been termed as the organism of choice for monitoring and surveillance programs in addition to a disinfection indicator.

The physico-chemical parameters however haven't shown any unusual trend, in most of the samples ph level was ranging from 6.5 to 7.5 which are acceptable and the ratio of total dissolved solids and conductivity was within the limit. Average turbidity of the samples was higher. Apart from the aesthetic value of water, presence of certain silt and sand particles may pose threat of gastric disorders and precursor behind kidney stone. The ratio of patients suffering from renal infections is considerable in the region.

Current research findings have encouraged stakeholders to establish a permanent water quality monitoring system to ensure provision of safe drinking water within CKNP of Karakoram Mountain Ranges, Pakistan. Further, the type and status of drinking water distribution system can be rectified if the source of contamination is along the medium from Head to tail. The identification of an alternative source (spring) and its possible use for the local inhabitants is possible with addition of allied sources in to the database, especially in the villages where stream water is the prime source of drinking.

The population residing within CKNP especially the areas which remain aloof from the primary and secondary health care facilities during prolonged winter may become the chronic patients carrying gastrointestinal infestations. There is a dire need to address the personal hygiene as well as the pattern of open defection near the sources which may lead to a worst case scenario in shape of mild to severe and chronic ailments.

Table 4: Chemical Parameters Source Mid and Last Points Braldo Valley (Means followed by different letter(s) in the same column are significantly different from one another at LSD ≤ 0.05)

Points	T.Alk	Cl	SO_4	$N\text{-}NO_3$	$N\text{-}NH_4$	Ca	Mg	Na	K	RP	TP	TN	SI	F
Source	1.24A	0.67A	80.47A	274.25A	5.33A	52.75A	29.84A	3.82A	16.18A	2.41A	4.66A	0.33A	4.50A	0.47A
Mid	1.21AB	0.63B	75.87B	272.83A	4.91A	50.57B	28.18AB	3.60AB	15.69A	2.54A	4.79A	0.33A	4.37A	0.45B
Last	1.19B	0.60C	74.16B	270.33A	4.70A	50.57B	27.87B	3.35B	15.11B	2.41A	4.75A	0.32A	4.13A	0.44C
LSD	0.03	0.02	3.06	9.17	0.76	1.95	1.91	0.43	0.56	0.33	0.69	0.01	0.49	0.01

Table 3: Chemical Parameters Source, Mid and Last Points Bhasha Valley (Means followed by different letter(s) in the same column are significantly different from one another at LSD ≤ 0.05)

Points	T.Alk	Cl	SO_4	$N\text{-}NO_3$	$N\text{-}NH_4$	Ca	Mg	Na	K	RP	TP	TN	SI	F
Source	1.27A	0.27B	32.81A	511.43A	6.71A	30.94A	5.61A	1.62A	4.30A	6.57A	12.85A	0.65A	2.34A	0.36A
Mid	1.32A	0.38A	34.31A	542.00A	5.714A	1.61A	5.94A	1.71A	4.24A	7.00A	11.71A	0.62B	2.15B	0.31A
Last	1.32A	0.32AB	33.92A	492.14A	5.14A	31.30A	5.81A	1.81A	4.20A	6.2A	11.14A	0.63AB	2.16B	0.29A
LSD	0.07	0.09	1.66	55.48	1.59	4.59	0.82	0.26	0.91	1.43	1.72	0.02	0.13	0.09

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