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Drinking Water Quality Characterization and Heavy Metal Analysis in Springs of Dewan Gorah, District Palandri, Azad Jammu and Kashmir, Pakistan¤

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Abstract: In this study, water quality and microbial characterizations of fresh water springs of union council Dewangorah, Palandri, AJK have been studied. Sampling was done from January to October 2017, from fifteen different locations of study area, which are a drinking water source for natives of the area. Water samples were collected in sterilized glass bottles for physicochemical and microbial analysis and in clean plastic bottles for heavy metals analysis from various locations. Various physiochemical parameters including pH, Total dissolved solids (TDS), Conductivity, Chlorides (Cl^-), Fluorides (F^-), Chemical Oxygen Demand (COD), hardness and alkalinity were measured by standard methods. Heavy metals concentrations including Arsenic (As), Chromium (Cr), Copper (Cu), Iron (Fe), Lead (Pb) and Nickel (Ni) were evaluated using atomic absorption spectroscopy. It was found that concentration of Pb and Cr were above permissible limits of WHO at all locations. Standard plate count (SPC) was used to determine total viable count and total coliform counts in water samples. Various bacterial colonies were isolated from water samples and characterized by performing different biochemical tests including Gram staining, Catalase, Oxidase and Citrate utilization tests. Results showed that various physicochemical parameter such as pH, TDS, Cl^-, F^-, COD and heavy metals including As, Cu, Fe were within World Health Organization (WHO) standards. While values for conductivity of few samples were above permissible limits of WHO. The calculations for human health risk showed that heavy metals at present levels are not posing a threat to the population. Microbial analysis showed that water samples contain different pathogenic bacteria like *E. coli, Salmolella* spp., *shigella* spp etc. and are not fit for drinking purposes. The quality of drinking water of Dewangorah must be monitored by regulatory authorities and further research is required to identify the sources of water contamination and its mitigation.

Keywords: Springs, water quality, microbial identification, heavy metals, health risks, palandri, AJK

Introduction

Water quality is a major concern worldwide due to presence of microbial and toxic pollutants in water sources. Anthropogenic sources are major contributors of impurities in water. Deficiency of resources and lack of awareness are the major causes of water pollution issues in developing countries including Pakistan (Ahmed et al., 2010). A majority of the population in northern part of AJK relies upon fresh water springs of drinking water source. Contamination of fresh water springs is an important concern for the health of consumers as most of the springs in villages are unprotected and prone to contamination due to agricultural, animal farming and solid waste dumping in nearby areas. Physicochemical and microbiological analyses of water are two important parameters for monitoring of water quality (Ghumman, 2011). Microbial communities play an important role in the aquatic environmental conditions as they can cause different diseases as pathogenic bacteria (Janjua et al., 2009). Springs also supply irrigation water to the adjacent agricultural areas and are subjected to the pollution by a number of sources. Different diseases like cholera, dysentery, typhoid fever and other gastrointestinal tract infections are caused by poor microbial quality of water. High concentration of heavy metals in water cause toxic effects on consumers including cancer and neurotoxic disorders. The possible sources of heavy metals in springs are attributed to natural weathering process of rocks as they are natural components of the earth's crust. Proper management of springs are required if full benefits are to be gained and maintained in the near future**.**

The study area comprised of springs from union council Dewangorah in district Palandri, AJK. Along the selected sites are residential area, hospital and agriculture sites. It is located at latitude 33° 42′ 54″ N, longitude 73° 41′ 9″ E and features a subtropical highland climate due to an elevation of 1638 m above mean sea level (Khan et al., 2014). The area is at a distance of 80 km from Rawalpindi and Islamabad, Pakistan. Weather is quite erratic. Village has mild to warm temperatures durin g spring and autumn, humid temperatures during summer and cold to snowy during winter. The maximum temperature is up to 38 °C during the mid-summer months. During winter months it is recorded to be minimum of -1 °C. Most rainfall occurs during the monsoon season i.e. July to September. Their domestic water source is only springs and they also use it for the irrigation of agricultural areas surrounding the village

Various studies have been conducted on water quality in different areas of Pakistan but little information is available on the study area selected in the present work

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(Ali et al., 2016; Khan et al., 2015; Muhammad et al., 2011). A study performed on physicochemical parameters of drinking water quality in Muzaffarabad, AJK concluded that the physicochemical parameters of water samples from river Jehlum were found fit for drinking and irrigation purposes (Sarwar et al., 2007). A study for the risk assessment of heavy metals and physicochemical parameters in drinking water samples of Kohat showed that the concentration of all the selected trace metals except for Ni, were within WHO limits (Khan et al. 2012). A study was carried out in Peshawar city to determine the water quality in water distribution network (Amin et al., 2012). The study included physicochemical and microbiological parameters of water quality and observed microbial contamination in various water samples of study area. Hashmi et al., (2012) carried out monitoring of drinking water quality in Rawalpindi/ Islamabad area and found little or no chlorine residual concentration and microbial contamination in water distribution network. Therefore, in this research work an attempt has been made to assess the water quality of springs that are a source of drinking and irrigation water for the residents of Dewangorah, Palandri, AJK and risk assessment analysis for heavy metals found in the drinking water source.

Materials and Methods

Sample Collection and Physicochemical Analysis

Water samples were collected from fifteen (15) different points of village Dewan for physicochemical and microbiological analysis according to standard protocols (Fig. 1). The samples were collected on bimonthly basis from January to October, 2017 in 100 ml sterilized glass bottles according to standard methods (APHA, 2012). Temperature, pH, EC and TDS were measured onsite by pH meter (Hanna pH meter sensION 1) and conductivity meter respectively. COD was measured using water analyzer (HS-1800). Total Alkalinity as mg of $CaCO_3$ was determined by acid titration method. Chloride and hardness values were measured by volumetric analysis of water samples (APHA, 2005).

Microbiological Analysis

Microbial analysis of the water samples was carried out within 24 hours of sample collection. The washed petri plates were sterilized by autoclaving (121 °C, 15 psi) and oven dried (171 °C) for 60 minutes. The nutrient agar and selective agars including Eosine methylene Blue agar (EMB), Mackonkey agar and Salmonella Shigella agar (SS) (Oxoid^{TM}) were prepared and poured into sterile petri plates according to manufacturer's instructions. The water samples were spread on agar plates after performing sterility test of the media for 24 hours. Standard plate counts (SPC) were determined for all the spread water samples. Furthermore, individual isolated colonies were streaked repeatedly on sterile agar containing petri plates to isolate the pure cultures of bacteria. Wherever required, serial dilutions of the water samples were also prepared to have countable range of bacterial colonies. For identification of bacteria, various characteristics including colony morphology, Gram reaction, citrate utilization, catalase, and oxidase tests were performed (Bergey's Manual of Determinative Bacteriology, 1994). The isolated and purified

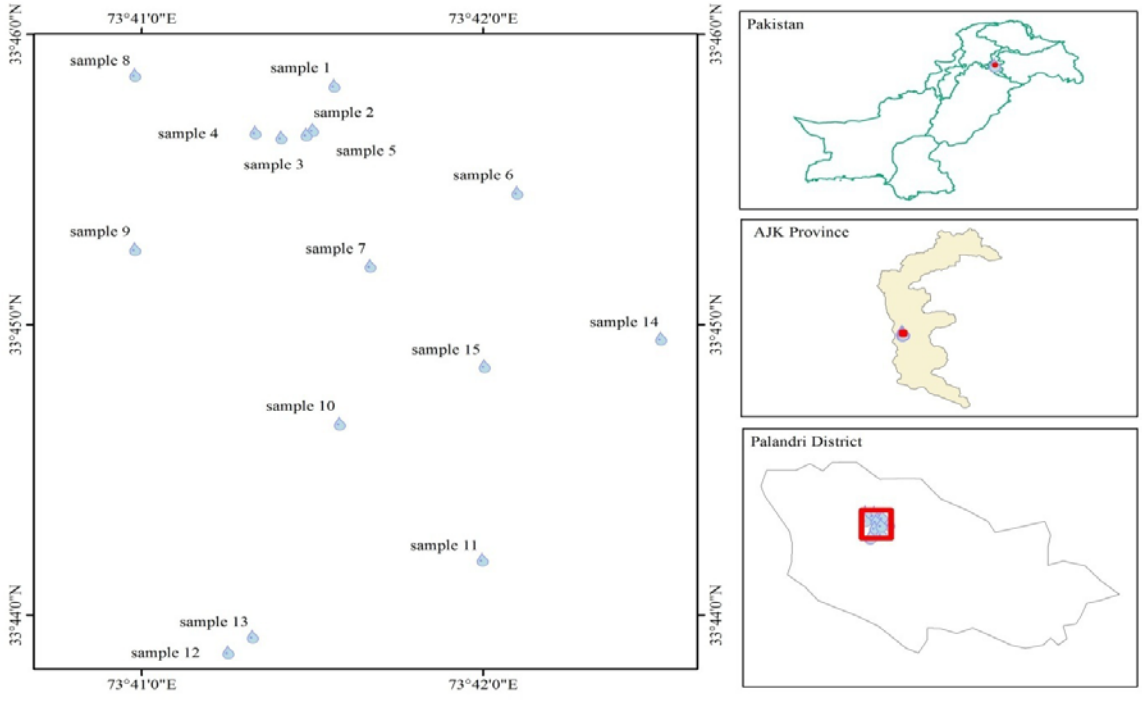

Fig. 1 Map showing the location of study area and sampling points.

microbial cultures were preserved in 15% glycerol stock solution according to standard methods.

Heavy Metals Analysis

For heavy metals analysis, 100 ml of water sample was taken in clean plastic bottles rinsed with distilled water. The samples were kept overnight after adding 5ml HNO_3. Later, the samples were filtered and stored in dark and dry place until analysis (EPA, 1995). The analysis of heavy metals (Cu, Cr, Ni, Pb, and Fe) was carried out by using flame atomic absorption spectrophotometer (Perkin Elmer AAS 700). Each sample was analyzed in duplicate. Standard solution of all the heavy elements was prepared from a stock standard solution of 1000 mg/l for the calibration of the atomic absorption spectrometer. Arsenic analysis was performed on site by Arsenic kit (Hanna, 2100).

Assessment of Human Health Risk

Target hazard quotient (THQ) was determined to assess the health risk to humans exposed to the heavy metals concentrations determined during the study. THQ is the estimate of the level of risk (non-carcinogenic) due to pollutant exposure. Hazard index (HI) is the sum of hazard quotients. All the assessment was carried out as per USEPA Region III Risk-Based Concentration Table (USEPA 2011; Muhammad et al. 2011).

Following equations were used to calculate the human health risk:

$$THQ = \frac{EF \times ED \times IR \times Cf \times CM}{WAB \times ATn \times RfD} \times 10^{-3}$$

$$HI = THQ(Pb) + THQ(Cu) + THQ(Cr) + THQ(Fe) + THQ(Ni)$$

Results and Discussion

Physicochemical Parameters of Water Samples

The results of various physicochemical parameters determined for water samples are given in Table 1. The pH values ranged between 5.9–6.7 in water samples. The highest value of pH was recorded in sample 2 (pH = 6.7), the lowest value was recorded in sample 6 (pH = 5.9), the water samples No.1, 2, 8 and 10 showed pH values within the permissible limits of WHO *i-e.*, (6.5-8.5). Various other studies show similar ranges of pH value in drinking water sources (Saeed and Hashmi, 2014). TDS is an important parameter of water quality as its high values indicate high mineral content in water. Permissible limit for TDS in drinking water is 500 mg/l. The concentration of TDS in various samples was found between 240 to 450 mg/l. Electrical conductivity is a measure of electrolytes in water containing mineral salts. According to WHO standards, its prescribed limit is 400 mg/l (WHO 2011). The results showed that EC value ranged between 47 mg/l to 97 mg/l in various water samples.

Table 1. Results of the physicochemical analysis of water samples.

Sample No.	pH	Cl^- (mg/l)	Na^+ (mg/l)	TDS (mg/l)	EC (µS/cm)	COD (mg/l)	Fluoride (mg/l)	Alkalinity (mg/l)	Hardness (mg/l)
1	6.6	10.6	17.5	340	51	62.38	0.7	42	252
2	6.7	12.9	21.4	240	61	64.07	1.5	34	220
3	6.3	17.7	29.7	420	64	73.56	1.4	20.7	202
4	6.1	10.6	17.5	320	47	68.83	1.1	38	123
5	6.4	9.4	15.5	340	51	69.86	1.2	78	160
6	5.9	8.2	13.6	360	55	63.06	1.5	54	174
7	6.1	9.4	15.5	350	53	72	0.1	128	210
8	6.5	15.3	25.3	280	69	76.64	0.9	45	125
9	6.2	15.3	25.3	380	57	62.66	0.8	78	154
10	6.5	21.2	35.07	390	81	66.04	0.9	98	172
11	6.2	22.4	37	350	97	66.52	0.5	132	158
12	6.4	16.5	27.2	390	87	70.45	1.3	94	78
13	6.3	13	21.43	330	86	69.19	0.4	27	189
14	6.0	11.2	15.7	450	92	72.59	0.9	34	108
15	6.3	13.5	17.3	340	77	69.06	1.5	23	89

Table 2. Microbial counts in water samples (CFU/ml).

Sample No.	Total counts	*Salmonella and Shigella*	*E. coli*	Heterotrophic bacteria
	*TNTC	70	2.31×10^2	TNTC
	TNTC	44	28	99
	2.60×10^2	0	1.25×10^2	70
	32	0	0	0
	1.29×10^2	6	4	89
	TNTC	1.75×10^2	89	TNTC
	89	51	62	25
	44	6	0	28
	TNTC	TNTC	TNTC	TNTC
0	TNTC	TNTC	TNTC	TNTC
1	70	5	41	30
2	2.78×10^2	6	2.68×10^2	1.52×10^2
3	TNTC	2.37×10^2	TNTC	TNTC
4	30	11	13	0
5	1.43×10^2	5	1	68

*TNTC=Too numerous to count

In the study area, the chloride concentration ranged from 8.2-22.4 mg/l in water samples which is well within permissible limits (WHO 2011). Sample 6 showed chloride concentration of 8.2mg/l whereas sample 11 showed highest chloride concentration of 22.4 mg/l. The concentration of sodium ions ranged from 13.6 to 37 mg/l with the highest concentration in sample 11, which are high as compared to permissible limit of WHO standard *i.e.*20 mg/l. Out of 15 samples, seven samples showed Na^+ concentration within permissible limits (WHO, 2011). Alkalinity and hardness of all water samples were found within permissible limits of WHO.

COD in water samples indicates the amount of dissolved oxidizable organic matter including the non-biodegradable content present in it (APHA, 2012). The values of COD were found in the range of 62.38 mg/l to 76.64 mg/l in the present study. Analysis of water samples have shown fluoride concentration ranging between 0.1-1.5 mg/l in various water samples. The

values were within permissible limits of WHO. Although fluoride is beneficial to human health at low concentrations (0.7–1.2 mg/l) as it provides protection against dental caries, whereas at concentrations exceeding 4 mg/l, it causes fluorosis and affects teeth and bones (US-EPA, 2011).

Table 3. Biochemical tests performed on microbes

Isolates	**Gram staining**	**Catalase test**	**Oxidase test**	**Citrate utilization test**
1	Positive	Negative	Negative	Positive
2	Positive	Negative	Negative	Positive
3	Negative	Positive	Positive	Negative
4	Negative	Positive	Negative	Positive
5	Negative	Positive	Positive	Negative

Microbiological Quality of Water Samples

Table 2 shows the viable counts (CFU/ml) for various bacteria found in water samples. Four different microbiological media were used for enumeration and identification of bacterial species. The microbial

counts showed variation in different water samples ranging from 0 CFU/ml - too numerous to count (TNTC) number of colonies.

CFU/ml counts of bacteria were higher in nutrient agar medium followed by Macconkey agar. However, for salmonella- shigella (SS medium) comparatively low CFU count was recorded as nutrient agar is a general media for the growth of bacteria. On nutrient agar medium, TNTC count was recorded for other water samples including S-1, S-2, S-6, S-9, S-10 and S-13. Similarly, S-9, S-1, S-10, S-6, S-13, also showed TNTC count of bacteria on Macconkey medium as well as showing the presence of indicator microorganisms *E. coli*. In case of EMB and SS medium, TNTC was recorded for S-3, S-9, S-10 S-13 and S-9, S-10 samples respectively. According to WHO guidelines, there should be no *E. coli* / 100 ml for safe drinking water; whereas 0-10 *E. coli* /100 ml are acceptable limits recommended for developing countries (WHO, 2011).

Table 4. Colony morphology of isolated microbes.

Isolates	Shape	Elevation	Margin	Surface	Texture	Color
1	Round/ circular	Flat	Entire	Smooth	Dry	Dark grey
2	Round/ irregular	Flat	Filiform	Wavy	Dry	Light yellow
3	Irregular	Convex	Lobate	Smooth	Dry	Light brown
4	Filamentous	Raised	Undulate	Wavy	Dry	Light brown

Table 5. Concentrations of heavy metals in water samples

Sr. No.	**Cr (mg/l)**	**Ni (mg/l)**	**Pb (mg/l)**	**Fe (mg/l)**	**Cu (mg/l)**	**As (mg/l)**
1	0.15	ND	0.15	ND	0.18	0
2	0.26	ND	0.22	ND	0.15	0
3	0.25	0.17	0.21	0.64	0.05	0
4	0.2	0.15	0.25	0.01	0.09	0
5	0.36	ND	0.14	0.38	0.09	0
6	0.2	0.13	0.18	0.51	0.02	0
7	0.21	ND	0.21	0.01	0.16	0
8	0.29	ND	0.24	0.30	0.12	0
9	0.19	ND	0.09	0.20	0.21	0
10	0.24	0.13	0.17	0.46	0.04	0
11	0.26	ND	0.08	0.40	0.02	0
12	0.15	0.11	0.23	0.52	0.05	0
13	0.17	ND	0.16	0.61	0.10	0
14	0.18	ND	0.14	0.19	0.01	0
15	0.01	ND	0.18	0.62	0.02	0

Table 6. Health risk assessment of heavy metals in water samples

Metals	THQ
Pb	$2.5X10^{-3}$
Cu	$1.71X10^{-0.4}$
Cr	$1.98X10^{-2}$
Fe	$1.31X10^{-4}$
Ni	$6.8X10^{-4}$
Hazard index (HI)	$4.02X10^{-2}$

E. coli is an indicator microorganism for fecal contamination in water samples and the presence of *E. coli* positive results shows that other pathogenic microorganisms may also be inhabiting the water reservoir or source. These results are further supported by positive results of *salmonella* and *shigella* at few locations in the study area as these microbes are pathogenic and are considered responsible for different disease outbreaks including typhoid, dysentery and other disorders in human beings (Newton et al., 2013). It signifies the fact that regular monitoring of microbial quality of drinking water is vital to avoid health risks from residing in nearby areas. Results reported by Zhang et al. (2014) also depicted microbial diversity in water reservoir based upon various characteristics of bacteria.

The results of various biochemical tests performed on isolated bacteria are given in Table 3. The age of microbial culture may result in false reaction in gram staining and may not give reliable results. Therefore, all the biochemical tests were performed on freshly grown cultures. Similarly, gram staining was carried out on 24 hours grown culture, which categorized bacteria in to two main groups, i.e., gram positive having purple staining and gram negative having pink staining. Isolates from samples 10 and 12 showed gram-negative isolates while the remaining isolates were gram positive. Gram positive bacteria usually include *Bacillus, Listeria, Staphylococcus, Streptococcus, Enterococcus and Clostridium* species of bacteria. Gram negative, including the cyanobacteria, spirochetes, and green sulfur bacteria, and most Proteobacteria. Sample 10 and 12 bacterial isolates were positive for catalase test and rest of isolates were negative. Positive bacteria for catalase activity normally include, *Staphylococcus, Bacillus, Micrococcus, Enterobacteriacae, Vibrio cholerae* and catalase negative bacteria include *Streptococcus and Clostridium.* Microscopic analysis was carried out to study the morphology of microbes isolated from different water samples (Table 4). Few colonies of microbes were irregular and circular in shape, having flat, convex and raised elevated surfaces. Margins were entire, filiform, lobate and undulated type. The bacterial colonies were dark gray, light yellow and light brown in color. Their surfaces were wavy, smooth, and dry in texture on the media plates.

Bacterial isolates from sample 10 were found positive for oxidase test and other were found negative. Dark purple color was observed on all microbial colonies, which indicates positive result for gram positive bacterial presence. Bacterial species having positive oxidase test which includes *Pseudomonas spp, Aeromonas spp, Vibrio spp, Alcaligenes spp, Neisseria spp. Haemophilus spp. Enterbacteriaceae* family includes various pathogens, such as *Escherichia, Salmonella, Vibrio, Helicobacter, Yersinia,* and many other distinguished genera. It also includes free-living bacterial species carrying out nitrogen fixation (Zhang et al., 2014). They show positive results for oxidase test which depicted by results of this study as most of the isolates showed positive oxidase test in our results.

Positive Catalase test is also a characteristic of *Enterobacteriaceae* family as catalase enzyme is a characteristic of most cytochrome containing aerobic and facultative anaerobic bacteria. The end product in aerobic metabolism is hydrogen peroxide and found to be lethal if accumulated in bacterial cells (Claiborne, 1985). A study by Saeed and Hashmi (2014) has reported the presence of *Proteobacteria* species in water samples of Rawal lake. Almost similar species of bacteria are reported there in springs based upon their morphological and biochemical characteristics.

Concentration of Heavy Metals

The heavy metals including Arsenic (As), Chromium (Cr), Copper (Cu), Iron (Fe), Lead (Pb) and Nickel (Ni) were determined at fifteen various locations of study area (Table 5). The concentrations of Pb in various water samples ranged between 0.09- 0.25 mg/l therefore, Pb was found to be in higher concentrations than WHO permissible limits (0.05) at all the locations of study area. Cr concentration was ranged from 0.01 to 0.36 mg/l which exceeded WHO permissible limit (0.05 mg/l) except for sample 15 which showed a value of 0.01mg/l. Similarly, the concentration of Cu was found within permissible limits at all locations ranging between 0.01 to 0.21 mg/l. The concentration of Fe was found ranging from not detected (ND) to 0.64 mg/l at sampling point No.5 and lies within WHO standard limits of 1.0 mg/l (WHO 2011). Ni concentration ranged from ND to 0.17 mg/l; which falls within the permissible limits of WHO (0.2 mg/l). The concentration of Pb exceeded the permissible limit of WHO followed by concentrations of Cr at various locations. Cu plays important role in biological activities but its higher concentrations tend to accumulate in liver and brain (Zhang et al., 2015). Whereas, Pb is reported to be a neurotoxicant which causes brain damage. It also accumulates in the human body via water uptake (Khan et al., 2015). The organisms are at a risk of adverse effects of water contaminants due to heavy metals contamination due to water consumption.

Based upon the concentration of heavy metals in water samples, human health risk was estimated by calculating target hazard quotient (THQ) (Table 6). It is the estimate of the level of risk (non-carcinogenic) due to pollutant exposure. Hazard index (HI) was also calculated, that is the sum of hazard quotients for all the heavy metals analyzed in this study. According to US-EPA, human health risk assessment is a process to estimate the nature and probability of adverse health effects in humans exposed to chemicals in contaminated environmental media, now or in the future. Risk assessment for heavy metals is estimated using parameters introduced by USEPA including THQ and HI (USEPA, 2011). THQ is a dimensionless quantity and is a ratio of concentration of heavy metal content in the water. THQ should be less than 1,

otherwise it indicates potential non-carcinogenic risks to the exposed human population. According to Khan et al. (2015), THQ reflects the level of concern due to contaminant exposure. In the present study Pb, Ni, Cr, Fe and Cu showed THQ values <1; whereas the HI is found to be $4.02X10^{-2}$.

Conclusion

The present study depicts the water quality characterization of springs in a village of district Palandri with most of the physicochemical parameters well within the standard drinking water quality given by WHO. Heavy metals analysis and assessment of health risk to population has shown that there is no significant risk to human health at the present levels. But the concentration of Pb and Cr in all collected samples was found higher than permissible limits and needs continuous monitoring. The overall microbial-ogical characterization indicated that bacterial species most prevalently present in water samples belong to *Enterobacteriaceae* and *Staphylococcus* family. It signifies the presence of pathogenic bacteria in water samples and the need of continuous monitoring and source identification of contaminants in this area.

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