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Critical investigation of heavy metals and microbiological analysis of surface and groundwater in Khyber Pakhtunkhwa, Pakistan

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This study aimed to analyze heavy metals and microbiological quality of ground water (GW) and surface water samples (SW) in Khyber Pakhtunkhwa, Pakistan. Different parameters including color, turbidity, electrical conductivity (EC), pH, salinity, biological oxygen demand (BOD), heavy metal ions detection, total dissolved solids (TDS) and detection of coliforms were analyzed. The obtained results were matched with World Health Organization (WHO) prescribed standards. The pH values were from 7.92 to 8.61 and 7.98 to 9.60 in GW and SW respectively. The value of TDS in GW and SW ranged from 354 to 576 ppm and 118.9 to 671.1 ppm respectively. BOD of GW was 0.10 to 0.70 ppm and in SW from 72.9 to 126.07 ppm. The Copper (Cu) concentration in GW and SW was varied from 0.0091 to 0.1066 mg/L and 0.0046 to 0.0080 mg/L, respectively. The Iron (Fe) range was -0.1673 to 0.3848 mg/L in GW, while in SW it was 0.1419 to 0.2712 mg/L. The Zinc (Zn) value ranged from 0.1177 to 0.1860 mg/L and 0.0987 to 0.1224 mg/L in GW and SW respectively. Manganese (Mn) in GW and SW ranged from 0.0551 to 0.1546 mg/L and -0.1278 to -0.1426 mg/L, respectively. MPN value was from 12 to 1600 MPN/100ml. All parameters of GW fell within the WHO desirable limits except GW2, whose pH deviated from the WHO permissible limit. While the SW was not within WHO recommended values. After practicing suitable disinfection procedures, surface water can be used for agriculture and household activities.

Keywords: Ground water, Surface water, Microbiological, Household, Most probable Number, Khyber Pakhtunkhwa

INTRODUCTION

Water is the most abundant and transparent physical substance on earth and has covered approximately 70% surface of the earth. About 1% of water is available for human usage like drinking and agriculture (Tahir et al. 2008). Water which could be used for human use should be "safe and wholesome," which means that it must be free from poisonous chemicals and pathogenic agents (Ganapathi et al. 2011). Clean and fresh water must be free of contaminants to ensure wellness (Ahmed *et al.* 2004). In past years, it has been noted that the groundwater is drastically polluted due to increased men's activities (Mishra and Bhatt, 2008). The increase in urbanization and industrialization lead to water spoilage. Many industries discharge their improperly treated wastes into rivers and canals, which have a vast amount of heavy metal ions like Pb, Cr and Fe (Ahmed and Tanko, 2000). The pollutants like viruses, bacteria, heavy metals, salts and nitrates come into the water supplies due to the improper treatment of human and livestock wastes and industrial effluents (Singh and Mosley, 2003).

Drinking water may appear colorless and odorless, but harmful pathogens can be invisible to the naked eyes (Ahmed et al*.* 2004). Bacteriological water analysis is used to find waterborne pathogens. Many diseases like diarrhea, cholera, dysentery, infectious hepatitis and typhoid fever are caused due to pollutant water (Anwar *et al.* 2004). On the report by WHO, 80% of the diseases in developing countries are caused by the biological contamination of drinking water (Wright et al. 2004). Microorganisms and waterborne pathogens enter our sources of water. There should be no microorganisms that cause diseases or any bacteria in potable water, which is representative of fecal contamination. According to WHO "Guidelines for drinking water quality," there is a minimum of 17 different bacteria that can be tracked in drinking water that can adversely affect human well-being. A reliable method of quality assessment is the detection of fecal indicator bacteria in drinking water (WHO, 1993). Existence of the fecal coliform is indicative of a health hazard that fecal contamination pose (Mcquaig et al. 2006).

According to an estimation, approximately 10 million deaths occur in under-developed and developing countries annually from waterborne infections; about half are children (Mott and Cline, 1980). In Pakistan, the problem has become myriad and studies have shown vast contamination of drinking water in big cities like Islamabad, Karachi, Lahore, and Peshawar (Anwar *et al.* 2004). Pakistan is among those countries where access to safe potable water is satisfactory. Therefore, the current study analyzes the critical investigation of heavy metals and microbiological analysis of surface and groundwater in Khyber Pakhtunkhwa, Pakistan.

MATERIALS AND METHODS

Collection of samples

Water samples were collected from 8 different locations in Khyber Pakhtunkhwa (Table 1). Ground water samples were collected from four areas (GW1 to GW4) having different sources, while surface water was collected from 4 different areas (SW1 to SW4) (Table 1).

Table 1: Detail of sample sites.

Samples	Location
GW1	Sheikh Maltoon Town
GW2	AWKUM Boys Hostel 1
GW3	Bari Pura
GW4	Hayatabad
SW1	Board Markets
SW2	Pabbi
SW3	Mardan Nowshehra Road
SW4	Momin Town

GW=Ground water, SW=Surface water

Physio-chemical analysis

Turbidity of GS and SW was measured by a Nephelometer and measured in a nephelometric unit. While other Physico-chemical parameters like pH, salinity, conductivity, total dissolved solids and biological oxygen demand were measured using standard instruments (pH Digital meter, electrical conductivity meter and HM Digital TDS meter) (APHA, 1995). Biological oxygen demand was measured by the titration method (APHA, 2005).

Heavy metal ions detection

The water samples were digested to remove the matrix, which interfered with the heavy metal by standard protocol (APHA, 1995). The digested water was taken to the PerkinElmer AAS Pin AAcle 900T atomic absorption spectrometer. The straw of the machine was dipped in the water sample.

Bacteriological analysis

Isolation, identification and confirmation of coliform

0.5ml from each sample was spread upon the MacConkey agar through a sterilized spreader (Oparaocha et al. 2010). Then MacConkey agar plates were incubated at 37 ºc for 24 hours. Lactose fermenting colonies were further processed for identification. Triple Sugar Iron Agar, Citrate and Urease tests were done to further identify LF colonies. Following 24 hours of incubation, changes in the color of media and gas production in the TSI were considered positive. For *E. coli* identification, a loop full of broth was streaked upon EMB media and the metallic sheen color was noted, which was an indication of *E. coli* (Adetunde. and Glover, 2011).

Enumeration of microorganisms

Most probable number

The MPN method detected and counted coliforms in the water (Adetunde and Glover, 2011). 10 ml of water sample was added to 5 tubes of double-strength lactose broth. A sample of 1ml was added to the next five tubes of single strength and 0.1 ml was added to the next 5 tubes of single strength. Now, these tubes were incubated for 24 hours at 37ºc. Changes in color and the gas produced in Durham tubes were considered positive. MPN values were determined by standard probability tables (Al-tomi, 2007).

RESULTS

Physio-chemical analysis

In the ground water sample, the pH value ranged from 7.92 to 8.61. Only the GW2 exceeded the WHO permissible limits i.e. 8.61 (Table 2). While the pH value of industrial wastewater ranged from 7.98 to 9.60 (Table 2). The salinity value of drinking water samples ranged from 0.482 PSU to 0.624psu. DWS3 has a high value of salinity which was 0.624 PSU (Table 2). The salinity of industrial wastewater ranges from 0.194 PSU up to 1.281 PSU (Table 2). Match factories have the highest value of salinity (1.281 PSU).

The ground water conductivity ranged from 869 to 1001 (µS/cm), while the permissible value is 1000 µS/cm. Only the GW3 sample exceeded the WHO value, 1001 µS/cm (Table 2). The conductivity of the surface water ranged from 303.4 to 2426 µS/cm (Table 2).

The value of total dissolved solids in ground water ranged from 354 ppm to 576 ppm. While the TDS value of surface samples ranged from 118.9 ppm to 671.1 ppm (Table 2).

Table 2: Physio-chemical parameters of ground and surface water samples.

Parameters	GW1	GW2	GW3	GW4	SW1	SW2	SW3	SW4	WHO
Color	CL	CL	CL	CL	C	CL	C	C	CL
Turbidity	0.2	0.1	0.3	0.25	44.34	3.54	37.2	11.83	5
pH	8.28	8.61	7.92	8.14	9.60	8.16	7.98	8.20	7-8.5
Salinity	0.482	0.520	0.624	0.528	0.643	0.728	0.194	1.281	-
Conductivity	869	904	1001	906	1210	1369	303.4	2426	1400 µS/cm
TDS	440	354	576	444	591.8	671.1	149.3	118.9	1000ppm
BOD	0.54	0.10	0.70	0.36	81	72.9	114.53	126.07	-

Cl= Colorless GW= Ground water SW = Surface water

Table 3: Concentration of heavy metal ions in ground and surface water samples.

Parameters	GW1	GW2	GW3	GW4	SW1	SW2	SW3	SW4	WHO
Copper (Cu)	0.0269	0.1066	0.0396	0.0091	0.0057	O.0046	0.0080	0.0067	2mg/L
Iron (Fe)	0.3848	-0.1673	-0.1968	0.2243	0.2065	0.1419	0.2712	0.1692	0.3mg/L
Zinc (Zn)	0.1671	0.1463	0.1177	0.1860	0.1107	0.0987	0.1212	0.1224	3.0mg/L
Manganese (Mn)	0.1546	-0.1375	0.0551	0.1378	-0.1426	-0.1410	-0.1292	-0.1278	0.05mg/L

The values of Biological Oxygen Demand in ground water ranged from 0.10 to 0.70 ppm. The values of Biological Oxygen Demand in surface water ranged from 72.9 ppm to 126.07 ppm (Table 2).

The Copper (Cu) concentration in the GW sample varied from 0.0091 to 0.1066 mg/L. While in SW, the copper concentration was from 0.0046 to 0.0080 mg/L and the WHO permissible limit is 2 mg/L (Table 3)

The Copper (Cu) concentration in the GW sample varied from 0.0091 to 0.1066 mg/L. While in SW, the copper concentration was from 0.0046 to 0.0080 mg/L and the WHO permissible limit is 2 mg/L (Table 3). The iron (Fe) range was -0.1673 to 0.3848 mg/L in GW. While SW samples have an Iron concentration from 0.1419 to 0.2712 mg/L (Table 3). The entire ground and surface water sample have the highest value than the WHO standards (0.03 mg/L). In GW samples, the Zinc (Zn) value ranged from 0.1177 to 0.1860 mg/L, while in SW samples, it ranged from 0.0987 to 0.1224 mg/L (Table 3). The Zn value for all the water samples was within the permissible limits of WHO (3.0 mg/L).

Manganese (Mn) in GW ranged from 0.0551 to 0.1546 mg/L. In SW, Mn varied from -0.1278 to -0.1426 mg/L (Table 3). Both ground and surface water samples exceeded the WHO permissible limit (0.04).

Bacteriological analysis of Ground and Surface water samples

All the ground water samples gave a negative result for the coliforms. No growth was observed on nutrient and MacConkey agar for drinking water samples. For the surface water sample, many colonies were observed on nutrients as well on MacConkey agar. For specie identification, different biochemical tests like TSI, Urease, Citrate and Indole were carried out.

All the surface water samples gave a positive result for TSI (Fig. 1).

Figure 1: Acidic Slunt and But of TSI.

SW1 and SW2 gave a negative result for citrate. No change was observed in the color of the citrate media (Fig. 2). While the SW3 and SW4 gave a positive result for citrate (Fig. 3).

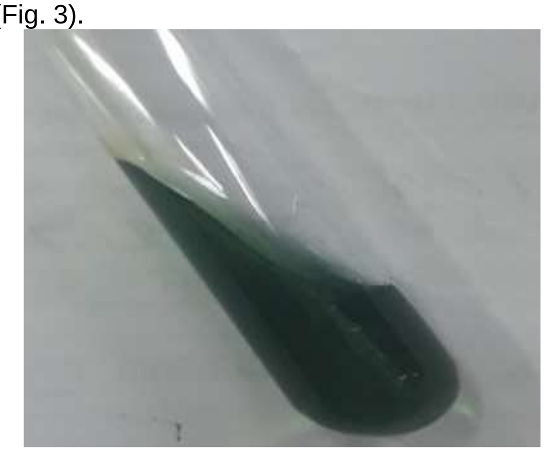

Figure 2: Shows a negative citrate test.

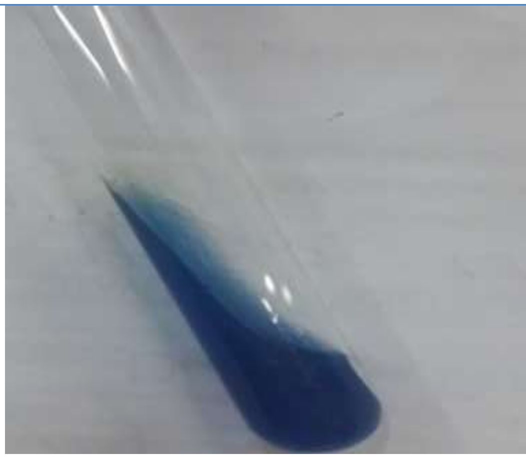

Figure 3: Shows a positive citrate test.

All samples gave a negative result for the urease test (Fig. 4). SW1 and SW2 gave positive results for the Indole test. While the SW3 and SW4 gave negative results for the Indole test (Table. 4)

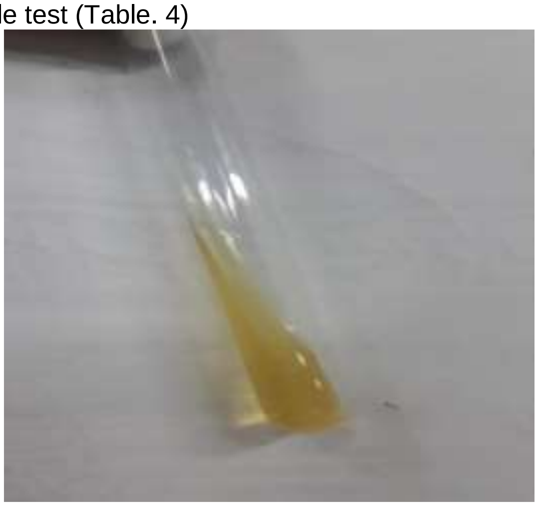

Figure 4: Shows negative results for urease.

Table 4: Analysis of coliforms through biochemical test.

Sample no	TSI	Citrate	Indole	Urease	Species
SW1	++	-	+	-	*E. coli*
SW2	++	-	+	-	*E. coli*
SW3	++	+	-	-	*Enterobacter*
SW4	++	+	-	-	*Enterobacter*

Enumeration of microorganisms

Most probable number method

The present investigation showed that in ground water samples, the MPN value was 0 MPN/100ml, while in surface water, MPN ranged from 12 to 1600 MPN/100ml (Table 5). The positive tubes were turbid and gas was present in the Durham's tubes. In SW1, there were 26 MPN/100ml while SW2 had 1600, SW3 had 900 and SW4 had 12 MPN/100ml (Table 5), which was beyond WHO permissible limit (0MPN/100ml).

Table 5: Number of positive tubes and MPN values.

S.No	10ml (Double strength)	1ml (Single strength)	0.1ml (Single strength)	MPN/100ml
SW1	4	2	1	26
SW2	5	5	4	1600
SW3	5	5	3	900
SW4	2	3	0	12

DISCUSSION

Groundwater source is considered the main water supply source for human usage in the KPK (domestic, agricultural and industrial). The Physicochemical and Microbiological assessment of water samples of KPK was used to evaluate the suitability of water for drinking, industrial and agricultural purposes. A sensitivity analysis showed that now a day's water quality deteriorated. The main factors that affect the hydrochemistry of groundwater of KPK city are wastewater and agriculture activities.

In our study, all the ground water samples were colorless because there were no microorganisms, so no pigments were formed. The water appeared colorless. Also, there were no suspended solid particles that added color to the water. The surface water samples except the SW2 were colored because of the presence of microorganisms, especially algal bloom and the presence of the different suspended particles. According to the WHO, drinking water should be colorless (Appelo and Postma, 2005).

Turbidity of ground water was within the WHO limit, which was less than 5 NTU. Similarly, all the surface samples having turbidity ranging from 3.54 to 44.34 exceeded the standard value published by WHO. Turbidity indicates the clarity of water and reveals the presence of suspended matter. The presence of suspended particles deteriorates the ecosystem of water by not allowing sunlight to reach inside. Mustafa et al. (2016) analyzed the water of the capital Islamabad and found that all the samples were under the permissible limit of WHO, which is 5 NTU.

In ground water, the pH value ranges from 7.92 to 8.61, while the standard value is 7 to 8.5 as per WHO standards. Only the GW2 exceeded the permissible limits, which are municipal supply water to hostel No1 at Abdul Wali Khan University Mardan KPK. It may be due to the corrosion of pipes or the tank's walls used for storage. While the pH value of surface water ranged from 7.98 to 9.60. Only the SW1 sample had a pH of 9.6, exceeding the standard value of pH. This was because the SW1 sample was taken from the Marble factory and marble stones have calcium carbonate, which affects the pH of water. Sadaf et al. (2014) conducted a study on the physicochemical characteristics of drinking water in Baluchistan and found that pH ranged from 6 to 9, which was higher than the WHO standards.

The salinity values of all the water samples were within the permissible limit of the WHO, so the ground water is fit for use. It will not show any adverse effects related to high salinity. The conductivity of water samples was within the permissible limit; only the SW4 exceeded the limit. The conductivity was increased by the suspended particles present in the water. The conductivity of water is its capacity to pass electric current. Electrical conductivity determines the amount of total dissolved salts (TDS) (Dahiya and Kaur, 1999). Sehar et al*.* (2011) conducted research and analyzed drinking water; the EC value recorded ranged from 385 mho/cm to 2390 mho/cm.

In this study, only the GW3 (576), SW1 (591.8) and SW2 (671.1) exceeded the value of TDS. TDS measures dissolved mineral contents in water. High total dissolved solid values make the water unusable for human utilization and agriculture usage purposes (Lehigh University, 2012). It gives an irksome taste to water and adds an evacuant effect (APHA, 1995). TDS is considered a secondary standard for drinking water. High TDS reduces the purity of water which reduces photosynthesis in aquatic plants. It also leads to an increase in temperature, which threatens many marine organisms' lives. Research carried out by Sehar et al. (2011) at Rawalpindi noted that the TDS value of drinking water was from 395 to 1400 mg/L.

In the present study, all the ground water samples were within the permissible limit of the biological oxygen demand. The hazardous level of dissolved oxygen to humans in the water was below 3 mg/L, while the permissible limit is (3 to 5 mg/L). Low dissolved oxygen content is because of the decomposition of organic substances, which relates to a high biological load in the water. But all the surface water in our study exceeded the permissible level, so they are not fit for domestic and agricultural purposes.

In this study, all the water samples have Cu levels below the WHO permissible limit of 2mg/L. In GW and SW, the Manganese concentration was higher than the WHO standard, which is 0.5 mg/L. A low level of Iron concentration was recorded in all samples than the permissible limit by WHO, which is 0.3 mg/L. Only the GW1 was a little deviating, having a value of iron 0.3848 mg/L. Zinc concentration in the drinking and industrial wastewater samples was within the permissible limit of WHO, which is 5 mg/L. The zinc concentration in tap water was higher due to the leaching of zinc from piping and fittings (Cohen et al. 1960). In a Finnish survey, in 67% of public water supplies, the zinc concentration in water samples was below 0.2 mg/L; much higher concentrations were found in tap water, the highest being 1.1 mg/L (Hiisvirta et al.1986). Yasin et al. (2015) analyzed drinking water samples for heavy metal analysis and revealed that only zinc and lead concentrations exceeded the normal values recommended by WHO.

In GW the MPN value was 0 MPN/100ml, which means that all the drinking water samples were free of any pathogenic bacteria which can cause diarrhea and other infections in humans. These samples were potable and can also be used for other human uses. Farooq et al. (2008) analyzed total coliforms in the Rawalpindi district and found that overall, the MPN value was less than 1.1 MPN/100ml, but at station # 3, it was found to be 23.0 MPN/100ml. The number of coliforms counted by the MPN method in SW was hazardous because these numbers are very high and can cause many infections. MPN ranged from 12 to 1600/100ml, and the WHO permitted value is 0/100ml.

In Pakistan, the most serious problems are bacteriological contamination of drinking water, leading to waterborne infections (Morris and Levin, 1994). Industrial samples analysis confirmed the presence of *E. coli* and other coliforms. Therefore, these samples were all unsatisfactory for human consumption, aquatic life, and agriculture purposes. If used for agricultural purposes, this industrial water can cause infection in humans and can cause diseases to plants and other crops.

CONCLUSION

This study analyzed ground water and surface water samples for the physical, chemical including heavy metal, and microbiological parameters. For all the GW samples, the physical, chemical and microbiological parameters were found within the recommended WHO guidelines for drinking water except for GW2, whose pH deviated from the WHO permissible limit. Almost all drinking water samples were safe for human consumption and other domestic purposes. The SW samples deviated from the physical, chemical and microbiological WHO guidelines. MPN showed that the SW was highly contaminated by microorganisms and hence industrial wastewater was unsafe for domestic and agricultural purposes. After practicing suitable disinfection procedures, SW can be used for agriculture and household activities.

CONFLICT OF INTEREST

All the authors have no conflict of interest in the present study.

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AUTHOR CONTRIBUTIONS

MK Conceptualized, Investigated, performed all experiments and also wrote the manuscript. KR, SYS and KM helped in performing some experiments and funding acquisition. IUH, SU and MK contributed in software, contributed in data collection and validation. All authors read and approved the final version.

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