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Research article

The Relationship between Hydro-Climatic Variables and *E. coli* Concentrations in Surface and Drinking Water of the Kabul River Basin in Pakistan

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Abstract: Microbial water contamination is a risk for human health, as it causes waterborne diseases like diarrhea. *E. coli* is a faecal indicator microorganism. Climate variables, such as temperature and precipitation, influence *E. coli* concentrations in surface and drinking water resources. We measure and statistically analyse *E. coli* concentrations in drinking and surface water in the Kabul River Basin. *E. coli* concentrations are very high in the basin. Drinking and bathing water standards are violated. Water temperature, surface air temperature, discharge and precipitation were positively correlated with *E. coli* concentrations. Precipitation induced runoff transports of *E. coli* from agricultural lands to Kabul River and high temperature coincides with high precipitation and discharge. A linear regression model was developed to assess the net effect of the climate variables on *E. coli* concentrations. We found that climate variables accounted for more than half of the observed variation in *E. coli* concentrations in surface ($R^2 = 0.61$) and drinking water ($R^2 = 0.55$). This study indicates that increased precipitation together with higher surface air temperature, as expected in this region with climate change, were significantly correlated with increased *E. coli* concentrations in the future. Waterborne pathogens are expected to respond similarly to hydro-climatic changes, indicating that disease outbreaks could well become more frequent and severe.

Keywords: Kabul River; water quality; climate change; *E. coli*; regression analysis

1. Introduction

Diarrhea, including infectious (bacteria, parasites, and viruses) and non-infectious (food intolerances or intestinal diseases) diarrhea, remains a major public health problem around the world [1,2]. People affected by diarrheal disease are those with the low hygienic facilities. Children below the age of five, primarily in Asian and African countries are the most affected due to waterborne diseases [3]. About 2.3 billion people are suffering from waterborne diseases worldwide [1] and annually an estimated 0.7 million deaths are due to diarrhea [4]. In the Kabul River Basin in the North-West of Pakistan, where this study is conducted, approximately 7% of deaths, including children and adults, is attributable to waterborne diseases, such as diarrhea [5].

The prevalence of diarrheal disease may be affected by climate change and its associated factors. For instance, more diarrheal cases were recorded after floods due to the contamination of food and drinking water [6] and more floods are expected with climate change in the future [7,8]. Several studies have focused on the impact of climate-change on diarrhea [9]. Such studies are, however, difficult due to under-reporting and non-registration in hospitals of these cases and the many confounding variables [10,11]. The disease risk due to waterborne pathogens is related to the concentration of waterborne pathogens in surface and drinking water [5,12]. People are exposed to pathogens by drinking contaminated water, using it for recreation or eating vegetables irrigated or washed with contaminated water [2,13].

Pathogen concentration in surface waters and, drinking water are influenced by changes in hydro-climatic variables, such as water temperature, surface air temperature, precipitation and discharge. Increased surface air and water temperature, as expected in the future, may increase the inactivation and therefore reduce the concentration of pathogens in the surface water [14,15]. Increased precipitation may decrease the surface water concentration due to dilution [16], while decreased precipitation may increase the surface water concentration, because a larger percentage of the discharge originates from the more constant input from point sources [17]. Extreme precipitation events are expected to increase the concentration of pathogens in the surface water, as manure and sewage applied to the agricultural land is taken into the river with the overland flow [18], and because of sewer overflows [19] and re-suspension from sediments [20]. The pathways through which changes in hydro-climatic variables influence waterborne pathogen concentrations in surface water are relatively well understood, but the net effect is poorly quantified. [21].

The main objective of this study was to evaluate *E. coli* concentrations and analyse the influence of the hydro-climatic variables water temperature, surface air temperature, precipitation and discharge on surface and drinking water *E. coli* concentrations in the Kabul River Basin in Khyber Pakhtunkhwa Province, Pakistan. We focus on *E. coli* rather than pathogens, because sampling of pathogens is expensive. Although, pathogens and *E. coli* are not necessarily correlated, presence of *E. coli* indicates faecal pollution. *E. coli* has been extensively used as an indicator bacterium for faecal pollution of water sources [22]. We hypothesise that pathogens and *E. coli* have a similar response to changes in hydro-climatic variables.

This study seizes the opportunity to assess the impacts of flooding and other hydro-climatic variables on *E. coli* concentrations in a region that floods every year. The resulting empirical correlation and general linear model will help to assess potential future threats due to climate change in this region and other developing regions that are prone to flooding. We have described the study area, sampling procedure and the statistical analysis (Section 2) and then statistically relate the measured *E. coli* concentrations to the hydro-climatic variables (Section 3).

2. Material and Methods

2.1. Study Area Description and Sampling Locations

The study area is the Kabul River Basin in the Khyber Pakhtunkhwa Province (KPK in Pakistan) situated east of Warsak dam. Nine sampling sites in the river and five sampling sites of drinking water sources from Nowshera city were selected (Figure 1). These sites were generally selected near, in between, and after the river passes through big cities to allow for different point and non-point pollution inputs. After one year of sampling, four river sampling points were excluded, because the *E. coli* concentrations among the points were highly correlated and time and money were better spent otherwise (Table 1).

Table 1. Overview of the surface and drinking water sampling sites selected for this study, their location, data availability, water use and main contamination source.

No.	Location	Data availability	Predominant water use	Main contamination source
Surface water samples				
1	Warsak (Reservoir)	2 years	Water storage, drinking, power generation, and irrigation.	Non-point
2	Shabqadar (North branch)	1 year	Irrigation and recreation	Non-point
3	Sardaryab (Middle branch)	1 year	Irrigation and recreation	Non-point
4	Shah Alam (South branch)	2 years	Irrigation	Point (Raw-sewage from Peshawar)
5	River Khyali (Swat river near Charsadda)	2 years	Irrigation	Point (Raw- sewage from Charsadda)
6	M T Pull (Junction Swat and Kabul Rivers)	2 years	Irrigation	Non-point
7	Amangarh (Junction three branches)	1 year	Irrigation	Point (Animal sheds)
8	Nowshera (Inside city)	2 years	Recreation	Point (Raw-sewage from Nowshera)
9	Hakeem Abad (East of Nowshera)	1 year	Irrigation and recreation	Non-point
Drinking water samples				
10	NCT (Tube Well)	2 Years	Drinking and other domestic use	n.a.
11	NSB (Hand Pump)	2 Years	Drinking	n.a.
12	HKTW (Tube Well)	2 Years	Drinking and other domestic use	n.a.
13	AGO (Dug Well)	2 Years	Drinking and other domestic use	n.a.
14	Boys College (Dug Well)	2 Years	Drinking and other domestic use	n.a.

Kabul River is a 700 kilometres long river that starts in the Sanglakh Range of the Hindu-Kush mountains of Afghanistan and ends in the Indus River near Attock, Pakistan [23,24]. The main tributaries of Kabul River are the Logar, Panjshir, Kunar, Gharband, Bara and Swat Rivers (see Figure 1b). After the Afghanistan-Pakistan border, Warsak dam is situated (site 1 in Figure 1). From the Warsak reservoir irrigation canals run through the heavily cultivated areas towards Peshawar and return to the river at Shah Alam (site 4 in Figure 1). After Warsak dam, Kabul River is divided into three tributaries: Sardaryab (site 2 in Figure 1), Shabqadar (site 3 in Figure 1) and Shah Alam (site 4 in Figure 1). The Swat River (called Khyali (site 5 in Figure 1) near Charsadda) runs from the mountains in the north of the study area into Kabul River at MT Pull (site 6 in Figure **1**ure 1). All these river branches join again near Amangarh (site 7 in Figure 1), the river crosses farming areas and small towns before it reaches Nowshera (site 8 in Figure 1). Nowshera is divided in two parts by the river and suffers the most from floods. After Nowshera, Kabul River passes Hakeem Abad (site 9 in Figure 1) before entering into the Indus River at Attock. Kabul River water is used for drinking, irrigation, power generation and recreational purposes at different points (see Table 1).

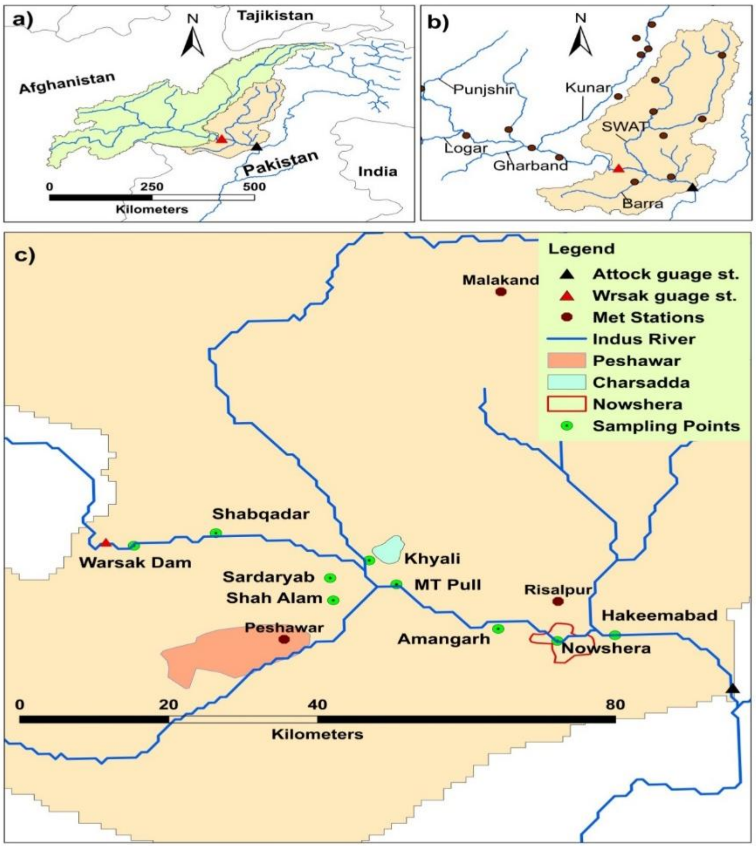

Figure 1. Study area map of the lower Kabul River Basin: (a) shows the full basin along with neighboring countries. (b) Kabul basin indicating the main branches (indicating names) and meteorological stations, (c) shows the downstream area of Kabul Basin with urban areas of Peshawar, Charsadda, and Nowshera (highlighted). Green points correspond to the sampling points in Table 1, while the red and black triangles are the guage station where discharge is measured. At black tiangle Kabul River discharges into the Indus River.

Kabul River crosses two major climatic belts. Its upper stream has a continental warm-summer climate with a mean summer temperature of approximately 25 ℃ and a mean winter temperature below 0 ℃ [25]. In the down-stream area in Pakistan, Kabul River crosses a region with a dry desert climate, with maximum daily temperatures in early summer that often exceed 42 ℃ and mean monthly temperatures in winter that measure 10 ℃ [23,26,27]. High temperature in summer causes snow melt on the mountain slopes. With increasing temperatures, more and more snow melts quickly and the increased runoff increases the discharge of Kabul River and thus causes floods in May and June. Annual precipitation in the full basin is less than 500 mm, although precipitation is higher on the mountain slopes around its headwaters. Extreme precipitation in the monsoon season causes flooding in late July or early August every year.

Peshawar, Charsadda, and Nowshera are the main cities in this basin. The population in the two latter cities (in total 3.14 million) is at high risk of frequent flooding and waterborne diseases associated with the floods [28]. *E. coli* enters the surface water with the sewer effluent, with manure from the animal sheds and with precipitation that has run off the agricultural lands. Upstream from Warsak dam, sewage from the main cities Jalalabad, Qandahar and smaller settlements like Torkham, Landi Kotal, Jamroad, and Sparesung entered into Kabul River. Manure and sometimes raw sewage are applied to the agricultural lands along the river as fertilizer [29]. In the current study area, downstream of Warsak dam, sewage from Charsadda, Nowshera and from the big city of Peshawar (3.13 million inhabitants) and from smaller settlements is collected in sewers and directly enters Kabul River without treatment. Peshawar used to have a waste water treatment plant, but this has not functioned since the 2010 floods. Agriculture (crops like wheat, corn, sugarcane, barley and vegetables like tomato, spinach, okra) is practiced on the banks of the river outside the cities and more inland, and manure and sometimes untreated sewage are applied to these lands as fertilizers. Additionally, near Amangarh raw manure from animal sheds is entered into the river directly.

Kabul River recharges the groundwater. Due to extensive pumping of groundwater and low river flow, the water table in the basin declines very rapidly. Therefore, groundwater may be recharged with contaminated river water and this is a problem for drinking water supplies [30]. The drinking water sampling locations are all located in the city of Nowshera, as Nowshera is expected to be mostly influenced by the flood. The selected sampling points are a tube well (NCT), hand pump (NSB), the Hakeem Abad tube well (HKTW), a dug well (AGO) and a dug well at the Boys College. Both HKTW and Boys College are quite close (100 meters) to the river banks, while the others are located further away from the river. During flooding the HKTW and Boys College are submerged in the Kabul River water. Water from these sources is mainly used for drinking, bathing and other domestic use. People usually consume water from the wells and pump directly without any treatment, such as water boiling, chlorination or filtration. AGO is an exception, as recently water filters have been installed.

2.2. Water Sampling

Water samples were collected biweekly for 30 months (April 2013–September 2015) from the nine sampling locations along Kabul River and the five drinking water sources from the city of Nowshera. Surface water samples were collected in sterilized plastic containers from three points in the river; both banks and the middle of the river. All three samples were then mixed to take one composite sample, which is seen as a good representation of the full width of the river at each location. Water temperature was measured on the spot. For microbial analysis the samples were transferred to sterile plastic bottles. Drinking water samples were collected in separate clean sterilized plastic bottles. Water collection from tube wells and hand pumps was allowed to run for 5 minutes before filling the bottles and then the water flow was reduced to enable filling of the bottle without splashing. Gases from the bottles were expelled by filling up, then emptying over the source, and refilling in the same manner. Water collection from the open well is different from the water collection of tap sources. From the open well we fill the bucket and dip the pre-sterilized bottle at 7 to 9 cm depth for 5 minutes to fill and expel out all air. Upon completion of samples collection, surface and drinking water samples were kept in a cold box and transferred to the laboratory for microbial analysis within 8 hours of sampling.

2.3. Microbial Analysis (E. coli)

All samples were analysed for *E. coli* at the Nuclear Institute of Food and Agriculture, Peshawar. The Most Probable Number (MPN) technique was used to determine the *E. coli* concentration of the water samples in colony forming unit (cfu)/100 mL [31]. To prepare, all the glass wares were sterilized in a hot air sterilizer at 160 ℃ for 2 hours. After cooling at room temperature these were used for the analysis. For total coliform counts a series of five fermentation tubes of Mackonky broth (Merck) were inoculated with appropriate volumes of ten-fold dilutions of water samples, and incubated at 37 ℃ for 24 h. Gas-positive tubes were considered positive for the presence of total coliform [32]. Gas-positive Mackonky broth tubes were subjected to further analysis for the confirmation of *E. coli*. The tubes were incubated at 44.5 ℃ for 24 hours [33,34]. Each positive test-tube was also exposed to a hand-held long-wavelength (366 nm) ultraviolet light lamp (Merck). Fluorescence in the tube denoted the presence of *E. coli* [34]. Concentrations of *E. coli* in water samples were recorded using an MPN table [35].

2.4. Hydrological and Metrological Data

Figure 1 shows the sampling locations, discharge gauge and meteorological stations. Water temperature was measured at the sampling site when the water samples were collected. Daily maximum, and minimum surface air temperature, and precipitation were obtained from the metrological stations situated in the studied area. Daily discharge (Kabul River) at Warsak dam and Attock stations were obtained from Water and Power Development Authority Pakistan. Discharge and precipitation were both used as variables in this study. Although precipitation influences discharge, in this case precipitation is seen as an indicator of runoff nearby, while the discharge also takes account of upstream areas. Such approach is commonly described in the literature [15,36]. We also include both water temperature and mean surface air temperature (minimum + maximum/2) to get an understanding of their relation with *E. coli* concentration.

2.5. Statistical Analysis

All the data were statistically analysed using the SPSS 22.0 computer package. *E. coli* and discharge data were log-normally distributed, so we always use the $\log_{10}$ transformed data for both variables. Average surface air temperature was used for all the measurement sites normally distributed, while water temperature followed a bimodal distribution and this is also seen in other studies [37,38]. Precipitation was gamma distributed. Descriptive statistics of the data were studied. Then standard Pearson correlations between *E. coli* concentration, water temperature, surface air temperature, and Kabul River discharge were calculated. Spearman's rank correlation analysis between *E. coli* concentration and precipitation was performed. We studied whether precipitation summed over between two and seven days obtained better correlation than daily precipitation. A longer time span could be expected to have a better correlation as precipitation may take a while before it runs off into the river, but after some time the water is out of the system and correlation may decrease. Summation of precipitation with comparative means was done in other studies as well [15,39-42]. We found different optimum summation times (including the sampling day) for the different sampling sites to have the highest correlation with the concentration of *E. coli*. We use the optimum for each sampling site.

We studied the observed difference in *E. coli* concentration in surface and drinking water by fitting the data to a general linear model to evaluate the comparative contributions of hydro-climatic variables (independent variables) to the observed variations in *E. coli* concentrations (dependent variable). The model is explained in detail in the results section. All hydro-climatic variables are added to the model one by one and the significance of their contribution to the results is evaluated. We also test whether or not interaction effects should be included in the model and we test the independent variables for collinearity. The coefficient of determination (R^2), determines how well the model explains the observed variation in *E. coli* concentrations. All statistical tests were analysed for significance at the 95% confidence level ($p < 0.05$).

3. Results

3.1. Correlations

All samples were positive for *E. coli*, with very high concentrations compared to drinking water guidelines (<1 cfu/100 mL for low risk drinking water (WHO 2011), see Figure 2). The surface water samples showed higher *E. coli* concentration than the drinking water samples (Table 2 and Figure 3); this water is grossly polluted, according to pollution categories from the World Health Organisation [WHO, 43]. *E. coli* concentrations in surface water range from 820 to 160 000 cfu/100 mL and in drinking water from 11 to 540 cfu/100 mL. The mean *E. coli* concentrations per sampling site ranged from $119–8.4 \times 10^4$ cfu/100 mL subjected to different sampling sites, and the median values were 40–92 000 cfu/100 mL, including both surface and drinking water samples. The sampling sites have comparable *E. coli* concentrations. Warsak and Khyali seem to have the comparatively lowest concentrations. Afterwards sewage from Peshawar and Charsadda is added to the river. However, concentrations at Warsak are already very high. Concentrations are highest at Shah Alam, which is located just after the sewage of Peshawar has entered the river, and Nowshera, where the sampling site was located just after the sewage from Nowshera enters Kabul River. AGO has the lowest values among the drinking water sources. That may be due to the filter installed at the site. Nevertheless, concentrations are still very high. The biweekly water temperature ranged from 9.9 to 29.1 °C. Surface air temperature ranged from 5.5 to 47 °C and mean precipitation over the full 30 months from 2109 to 3333 mm. Peshawar is the hottest and the driest, while Malakand is the coldest and the wettest of the meteorological stations. Discharge ranges from 2.2 to 3.8 $\log_{10}$ m^3/s and discharge at Warsak is lower than discharge at Attock (See Figure 1), which is understandable as River SWAT and the irrigation canal enter Kabul River within the study area. The seasonal pattern is comparable for all variables, with high concentrations, temperature, precipitation and discharge in the months June to August.

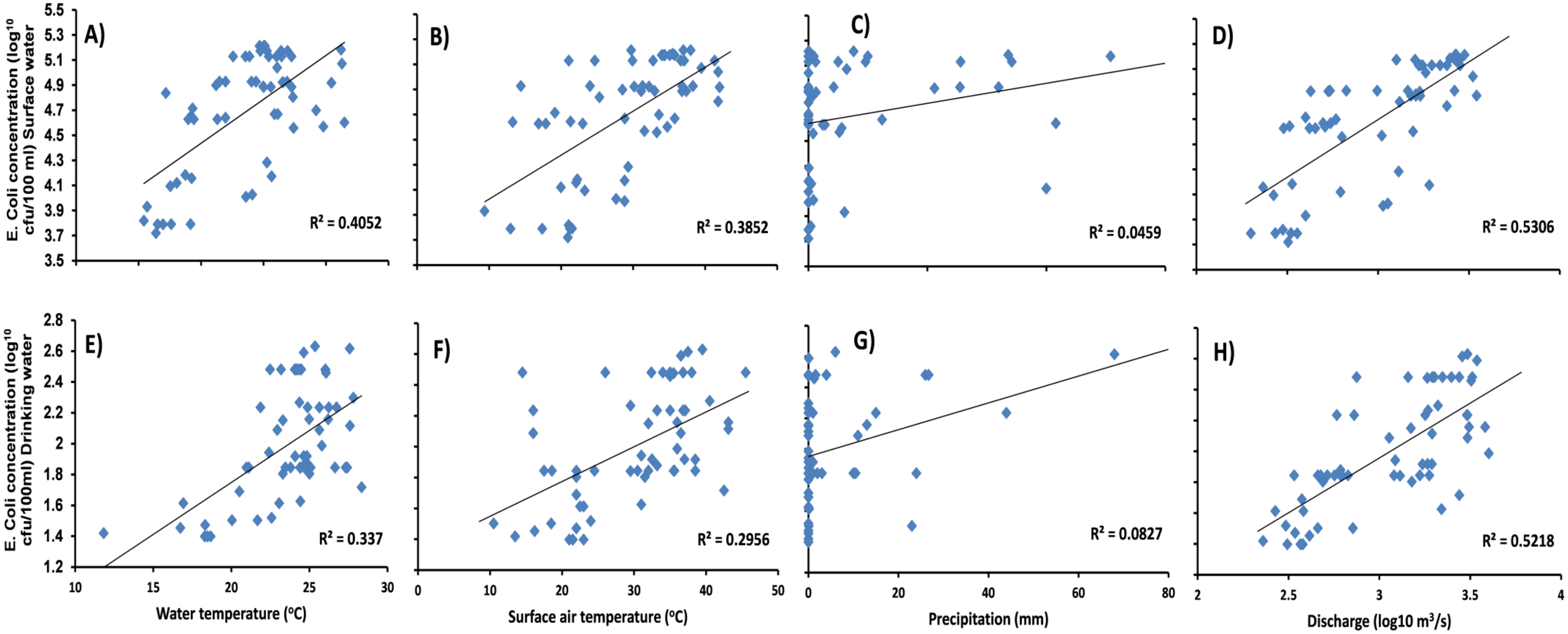

Figure 2. Correlations between the *E. coli* concentration (log cfu/100 mL) averaged over all (Nine in the first year and five in the second year) Surface water (A-D) and five drinking water (E-H) sampling locations and the hydro-climatic variables water temperature (℃) (averaged over all corresponding sampling locations) (A and E) surface air temperature (℃) averaged over the meteorological station (B) and measured at Risalpur (F) total precipitation (mm) summed over the meteorological stations (C) and measured at Risalpur (G) Kabul River discharge (log m^3/s) averaged over Warsak and Attock (D) and at Attock (H).

Table 2. Summary of biweekly *E. coli* concentrations and water temperature data. The Sampling period is March 2013–September 2015.

Measurement site	Latitude	Longitude	Water temper0ature(°C)			*E.coli* concentration $\log_{10}$ (cell/unit)				N
			Mean	Min	Max	Mean	Median	Min	Max	
Warsak	34.16	71.35	18.1	9.9	26.0	4.7	4.7	2.9	5.2	60
Shabqadar	34.10	71.33	18.4	10.2	26.8	4.7	4.5	3.7	5.2	25
Sardaryab	34.13	71.69	18.7	10.4	27.1	4.7	4.5	3.7	5.2	
ShahAlam	34.08	71.64	18.8	10.5	26.6	4.9	5.0	3.7	5.2	
Khyali	34.14	71.70	17.3	9.9	25.8	4.7	4.5	3.7	5.2	
MT Pull	34.09	71.74	18.6	10.3	26.0	4.9	4.9	3.7	5.2	
Amangarh	34.08	71.92	18.8	10.7	26.9	4.7	4.7	3.7	5.2	25
Nowshera	34.00	71.97	19.9	10.5	27.1	4.9	5.0	3.7	5.2	25
Hakeem Abad	34.01	72.04	19.4	11.2	27.8	4.8	4.7	4.0	5.2	60
NCT	34.00	71.59	23.9	10.9	28.4	2.2	2.0	1.0	2.7	60
NSB	33.59	71.59	24.1	11.3	29.1	2.2	2.1	1.4	2.7	60
AGO	34.00	71.56	23.6	12.3	28.6	2.1	1.6	1.2	2.5	60
HKTW	34.00	71.55	22.7	12.1	28.1	2.1	1.8	1.3	2.7	60
Boys College	34.00	71.59	24.0	12.5	28.5	2.1	1.7	1.1	2.7	60

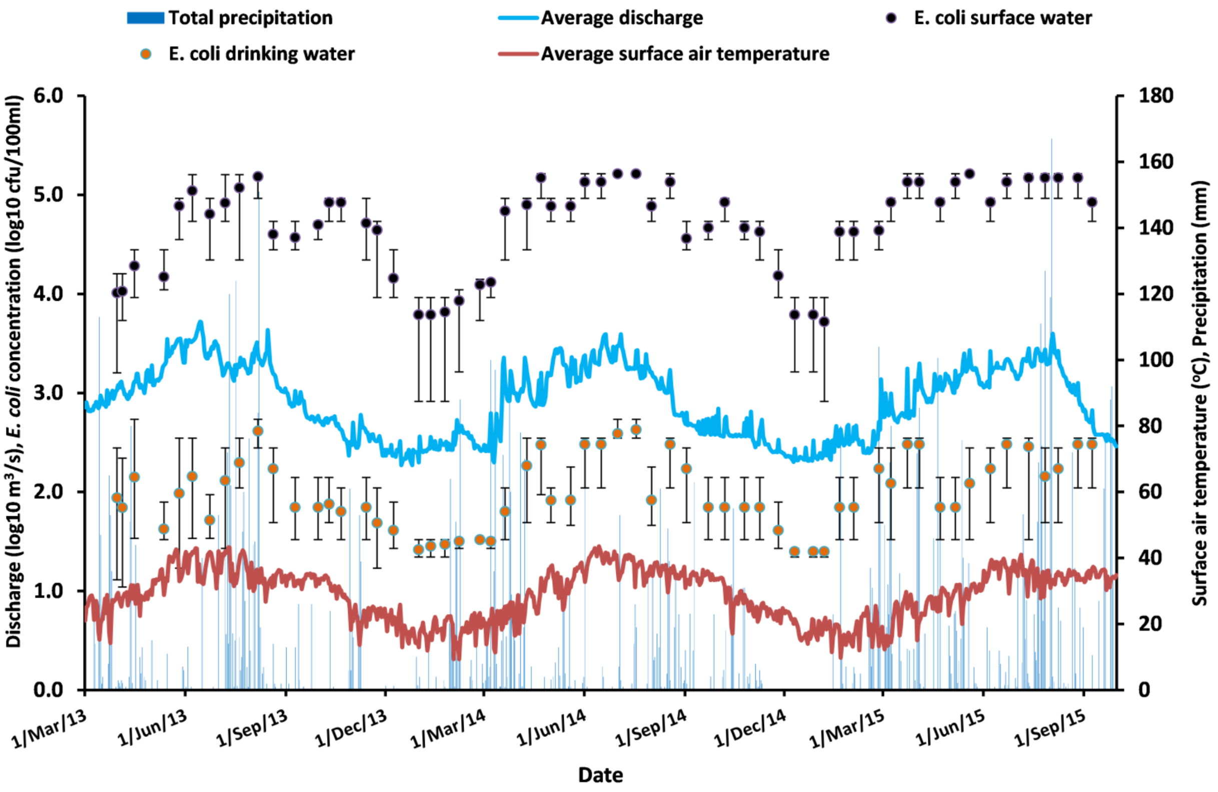

Figure 3. *E. coli* concentration of both drinking water and surface water sampling sites (bullet is mean, whiskers represent the spread in concentrations over the sampling sites), mean surface air temperature and total precipitation over the three meteorological stations, and mean discharge over the two discharge measurement points.

Summary of daily surface air temperature, total and maximum daily precipitation, days with precipitation, and discharge of Kabul River over the period April 2013–September 2015 are discussed in Table 3. Correlation analysis showed that *E. coli* concentrations in surface water correlate significantly positively with the hydro-climatic variables water temperature, surface air temperature, precipitation and discharge for most of the sampling sites (Table 4).The mean of the correlations over the 9 sampling sites are equal for water temperature, surface air temperature and discharge ($R^2 = 0.64$), although differences between the sites exist. Shah Alam often has the lowest correlation. This sampling site is strongly influenced by direct inputs from Peshawar and this may influence the relation with hydro-climate variables. For precipitation only significant correlations were found for sites that have 2.5 years of data ($n = 60$). The average correlation over the significant sites ($R^2 = 0.33$) is much lower than for the other hydro-climatic variables. The correlations between the average *E. coli* concentrations over the sampling sites and average water temperature, surface air temperature, discharge and total precipitation are similar to the average correlations over the sampling sites, except for the correlation with discharge that is higher (0.728). For average *E. coli* concentrations discharge is the most important variable.

The *E. coli* concentration of drinking water samples were also significantly positively correlated with hydro-climatic variables. The mean of the correlation coefficients over the drinking water sites were lower than the mean of the correlation coefficients over the surface water sites. However, correlations between average *E. coli* concentrations in drinking water and hydro-climatic variables were only marginally different from the correlations between average *E. coli* concentrations in surface water and hydro-climatic variables. The correlation between average *E. coli* concentrations in drinking water and average discharge was the highest at 0.723. Although correlation does, of course, not imply causation, such a high correlation would indicate that discharge strongly influences the *E. coli* concentrations in drinking water. In particular during floods that makes sense, as some of the drinking water sources can be submerged in the flood water. Together with high temperatures that could cause growth in standing water in the tropics, these high correlations could be explained.

Table 3. Summary of daily surface air temperature, total and maximum daily precipitation, days with precipitation, and discharge of Kabul River over the period April 2013–September 2015.

	Mean surface air temperature			Precipitation		
Meteorological stations	Mean	Min	Max	Total over sampling period	Max daily	Days with precipitation
Peshawar	30.5	11.3	47.0	2422	110	252
Malakand	26.6	5.5	41.5	3333	137	296
Risalpur	29.6	8.5	45.9	2109	80	226
	Discharge ($\log_{10}$ m^3/sec)					
Discharge stations	Mean	Min	Max			
Warsak	3.0	2.2	3.7			
Attock	3.1	2.3	3.8			

Figure 2 graphically presents the correlation between *E. coli* concentration of surface water samples with water temperature (both averaged over all sampling sites), surface air temperature averaged over the meteorological stations, total precipitation on the sampling day summed over the meteorological stations, and Kabul River discharge, averaged over the two discharge measurement locations. Correlations are high, in particular for discharge (R^2 is 0.53 for surface and 0.52 for drinking water samples). The correlation with precipitation is lower than expected (R^2 equals 0.046 for surface and 0.083 for drinking water samples). The low R^2 is due to high concentrations at days without precipitation.

Table 4. Correlations for each Sampling site and for Average *E. coli* and corresponding hydro-climatic variables. For precipitation the sum of days that had the highest correlation was chosen (see footnote). Correlations are statistically significant ($p < 0.01$) unless otherwise indicated. For the average surface water correlation averages over the meteorological and discharge measurement stations were used. Precipitation was summed over 2 days, unless indicated otherwise.

Sampling site	Water temperature	Surface air temperature	Precipitation	Discharge
Warsak♦Δ	0.675	0.607	0.332	0.727
Shabqadar♦Δ	0.672	0.730	−0.085**	0.553
Sardaryab♦Δ	0.625	0.677	−0.133**	0.533
ShahAlam♦Δ	0.538	0.548	0.283*	0.683
Khyali●Δ	0.580	0.595	0.371	0.691
MT Pull●Δ	0.678	0.578	0.344	0.695
Amangarh▲∞	0.644	0.713	−0.043**$^{\&}$	0.596
Nowshera▲∞	0.634	0.582	0.306*	0.719
Hakeem Abad▲∞	0.666	0.695	0.003**$^{\&}$	0.569
Average surface water	0.637	0.620	0.348	0.728
NCT▲∞	0.462	0.438	0.228**$^{\&}$	0.468
NSB▲∞	0.541	0.380	0.432$^{\&}$	0.508
AGO▲∞	0.325*	0.508	0.202**$^{\&\&}$	0.615
HKTW▲∞	0.427	0.391	0.291*$^{\&}$	0.612
Boys College▲∞	0.366	0.406	0.299*$^{\&\&\&}$	0.607
Average drinking water ▲∞	0.580	0.544	0.309*	0.723

**—Correlation is not significant.

*—Correlation is significant at the 0.05 level.

♦—Precipitation and surface air temperature measured at meteorological station Peshawar.

●—Precipitation and surface air temperature measured at meteorological station Malakand

▲—Precipitation and surface air temperature measured at meteorological station Risalpur

Δ—Discharge measured at Warsak dam station

∞—Discharge measured at Attock station

$^{\&}$—Precipitation on the sampling day is used $^{\&\&}$. Precipitation sum of 5 days is used $^{\&\&\&}$. Precipitation sum of 6 days is used

3.2. Model

A general linear model was applied to assess the impact of the hydro-climatic variables on *E. coli* concentrations. The multi-variable general linear model has the following form:

$$\log(Y) = \beta_0 + \beta_1 T + \beta_2 p + \beta_3 \log(D) + \beta_4 T * \log(D) + \varepsilon \qquad \text{Equation 1}$$

where Y represents the dependent variable (*E. coli* concentration), β_i are constants, T is the temperature in °C; this could be either water or surface air temperature, p is the total precipitation summed over a different number of days (see Table 4) to the sampling day in mm, D is the Kabul River discharge in m^3/s, T ^{x}log (D) represents the interaction effect of water or surface air temperature and Kabul River discharge, while ε is the residual error. Not all hydro-climatic variables have been included for all sampling sites (see Table 5). Water temperature and surface air temperature have not been added to the model together, because they are highly correlated ($R^2 = 0.65$ for average surface air temperature and water temperature averaged over the surface water sampling sites). Discharge and water temperature are also correlated ($R^2 = 0.42$ for average discharge and water temperature averaged over the surface water sampling sites), but no collinearity existed for these variables and they add conceptually different processes to the regression model. For averaged surface and drinking water samples the fitted model explained a large proportion of the variation in *E. coli* concentration in Kabul River. The resulting model had a coefficient of determination (R^2), adjusted for degrees of freedom, of 0.61 for average surface water and 0.55 for average drinking water sources.

Table 5. β estimates and R^2 for the models for all sampling locations in Kabul River Basin. ⅄Sign indicate statistically significant ($p < 0.05$) contribution of the variables. Each sampling site is linked to the same meteorological and discharge station as explained in Table 4. Water temperature is used in the model unless indicated otherwise. Precipitation is summed over 2 days (sampling day and day preceding the sampling day), unless indicated otherwise.

Sampling site	β0	β1	β2	β3	β4	R2
Warsak	−6.093⅄	0.465⅄	0.005	3.373⅄	−0.145⅄	0.66
Shabqadar	3.351⅄	0.038*⅄				0.51
Sardaryab	3.376⅄	0.038*⅄				0.43
ShahAlam	−1.995	0.256⅄		2.288⅄	−0.085⅄	0.50
Khyali	−0.560	0.205	0.004	1.562⅄	−0.059	0.55
MT Pull	−1.800	0.274⅄	0.002	2.023⅄	−0.082⅄	0.58
Amangarh	3.379⅄	0.038*⅄	0.008♦			0.52
Nowshera	−1.333	0.215⅄	0.004	1.909⅄	−0.065⅄	0.59
Hakeem Abad	3.496⅄	0.035*⅄	0.007♦			0.49
Average surface water	−1.888	0.259⅄	0.001	2.076⅄	−0.079⅄	0.61
NCT	1.181⅄	0.025*⅄	0.008♦⅄			0.26
NSB	−0.006	0.050⅄	0.010⅄	0.254		0.41
AGO	0.207	0.016*⅄	0.005Δ⅄	0.371⅄		0.42
HKTW	−0.351		0.007⅄	0.708⅄		0.40
Boys College	−0.352		0.006	0.713⅄		0.38
Average drinking water	−2.174	0.100	0.005⅄	1.288	−0.030	0.55

*—Surface air temperature is used instead of water temperature

♦—Precipitation is summed over 3 days

Δ—Precipitation is summed over 5 days

β-estimates of all independent variables and their coefficient of determination (R^2) for all the sampling sites and have been listed in Table 5. For the surface water sites that had 60 biweekly samples, the best-fit models included the variables water temperature, precipitation (except Shah Alam), discharge and the interaction effect of water temperature and discharge. Not all variables had a significant contribution, but inclusion did improve the model fit. For the sites that had only 25 biweekly samples, surface air temperature significantly contributed to the model and in some cases also precipitation was included. The sign of all beta values is positive, except for the interaction effect. The sign for that is negative in all cases. The intercept is sometimes positive and in other cases negative. In all cases, the beta value for precipitation is very low. One milimeter increase in precipitation, while the other variables stay the same, increases the *E. coli* concentration by 0.001–0.008 $\log_{10}$ cfu/100 mL. Surface air temperature has a more or less equal influence for the four sites with 25 biweekly samples. There one degree increase in temperature increases the *E. coli* concentration by 0.035–0.038 $\log_{10}$ cfu/100 mL. For the other sites, the influence of water temperature is a factor 10 higher, from 0.215–0.465 $\log_{10}$ cfu/100 mL for each degree increase in water temperature. Also the impact of discharge is large. An increase of one $\log_{10}$ m^3/s increases the *E. coli* concentration by 1.56–3.37 $\log_{10}$ cfu/100 mL. We can put this into perspective for MT Pull, for example, a site with average beta values for water temperature and discharge. Here a difference of 15.7 degrees in water temperature between minimum and maximum (see Table 5) would result in a change of 4.1 $\log_{10}$ cfu/100 mL, while a difference of 1.5 $\log_{10}$ m^3/s in discharge between minimum and maximum would also result in a change of 0.41 $\log_{10}$ cfu/100 mL. The adjusted R^2 ranges from 0.43 to 0.66.

The models are much more variable for drinking water. The adjusted R^2 is lower than for surface water sites. It ranges from 0.26 to 0.42. For all sites, except Boys College, precipitation significantly influences the *E. coli* concentrations. For some temperature (water or surface air) contributes significantly, while for others temperature is not included in the model. For others discharge contributes significantly, while for others discharge is not included in the model. The interaction effect is never included in the model, except for the average model. The beta value for precipitation is similar, if not slightly higher, than for surface water samples, while the beta value for temperature and discharge are a factor 10 lower than for the surface water samples. The two sites that are submerged in water when the river floods, HKTW and Boys College, have a very similar model. The model includes precipitation and discharge, and discharge has an understandable much higher impact on these two drinking water sites than on the other drinking water sites.

4. Discussion

We measured *E. coli* concentrations in surface water of Kabul River and drinking water in the city of Nowshera through which Kabul River runs. Concentrations in surface water are very high (2.9–5.2 $\log_{10}$ cfu/100 mL); the river is grossly polluted. Concentrations in Kabul River are higher than found in earlier studies in Bangladesh (2.9–3.4, [44]), China (1.8–3.4, [45]), Southeast Asia (2.8–4.3,[46]), and Côte d'Ivoire (2.55–3.47, [47]). The reason for that is that the river basin is densely populated by people, who nearly all are connected to a sewer, but where the waste water treatment systems have been broken by a large flood in 2010. In addition to people, the basin is also densely populated by livestock, including cattle, buffaloes, sheep and goats. The concentrations in Kabul River do compare to other studies, such as for Ghana (0–9.0, [48]), for Santa Cruz watershed in Arizona (5.15–5.73, [49]), for Indiana lakes and streams (5.20–5.90, [50]). While at Mekong River basin the concentrations are less than Kabul River (2.0–3.1, [51]). Concentrations in drinking waters are also very high (1–2.7, $\log_{10}$ cfu/100 mL). The water found at all of the drinking water sites is unsuitable for drinking and should at least be boiled before consumption. The concentrations found compare to concentrations found in other studies such as, (2.30–2.91, $\log_{10}$ cfu/100 mL [52]).

E. coli concentrations in surface water were highly correlated with discharge, and in most cases also with precipitation. The reasons for higher *E. coli* concentrations in wet weather are increased runoff of agricultural lands and urban areas, leakage from manure storage (diffuse sources) and re-suspension of sediments in the river [21]. Precipitation is linked to runoff in the study area, while discharge is linked to runoff upstream. Precipitation is in most cases summed over several days, which mimics the time it takes for runoff to reach the river. Positive correlations between *E. coli* concentrations and precipitation and discharge were also observed in other studies [53-58]. The positive correlations indicate that during wet weather diffuse sources are relatively more important than in dry weather situations.

Also for drinking water sites *E. coli* concentrations were positively correlated with discharge and precipitation. In Kabul River Basin the groundwater is quickly replenished by river water [59]. This means that the same mechanism could be in place for drinking water as for surface water. During flooding the drinking water sites could also be directly influenced by flood water. That is in particular the case for the sites that are submerged in the river during floods (HKTW and Boys College). High correlation between *E. coli* concentrations and drinking water from bore holes and tube wells and precipitation and discharge were also found in other studies [52,60]. The reasons they provided included improper wall construction around the well, or improper coverage. Moreover, toilets might be built close to the wells that lead to leaching in situations of high precipitation.

E. coli concentrations in surface and drinking water were also positively correlated with average water and surface air temperature. These positive correlations can be explained by coinciding high temperature with high precipitation and discharge (see Figure 2). The observed positive correlation with water temperature does, therefore, not mean that increased temperature lead to the increased *E. coli* concentration through bacterial growth. Positive correlation of the *E. coli* concentration with average water and surface air temperature were in line with other studies [61-63]. Several authors also provide the coinciding high temperature, precipitation and discharge as main reason for the positive correlation [54,64] while others link it to bacterial growth [65-67]. We have not found evidence in the literature of bacterial growth in flowing water. Kabul River is a reasonably large river (mean discharge 1000 m^3/s) with a short residence time (less than a day in the study area) [68]. Growth in such a river is unlikely. A positive correlation between *E. coli* concentration and drinking water could be related to bacterial growth in wells and in-line with [52]). However, discharge and precipitation also influence the drinking water sites in a similar way as the surface water sites, which could indicate that the coinciding high temperature, precipitation and discharge are also the main reason for a high correlation with temperature for the drinking water sites.

Our general linear model explains a large part of the variation in *E. coli* concentration. The R^2 values for the models for average surface and drinking water *E. coli* concentrations were 0.61 and 0.55 respectively. This means that hydro-climatic variables are very important determiners of the microbial water quality of Kabul River and the drinking water sites near this river. Of course, also other variables will be important, such as sewage inputs, livestock numbers, manure management, land use, and the mechanisms that determine the transfer of water from Kabul River to the drinking water sites. The R^2 found in our study for surface water sources is high compared to other studies. In other studies R^2 often ranges between 0.1 and 0.5 [15,55,69]. Kay, Wyer [70] do find similar values ($R^2 = 0.49–0.68$) for the River Ribble drainage basin in the UK by including similar climatic and environmental variables. For drinking water sources Long, Lloyd [71] conducted the study in South China and find $R^2 = 0.29$ for *E. coli*.

E. coli is an indicator faecal contamination of surface or drinking water. Although the presence of high concentrations of *E. coli* indicate the likely presence of more harmful pathogens, *E. coli* is mostly not directly correlated to pathogens [72]. Water use is also an important factor to include in health risk assessments. Bathing and irrigations are important uses of the Kabul River water that could pose health risk to the population. The sampled drinking water is used for drinking, but its treatment afterwards (possibly boiling) will influence the risk. This makes it difficult to directly conclude on the importance of the observed concentrations and relation with hydro-climatic variables for the health risk in Kabul River Basin. For that, pathogen data for the basin would be required, although this is expensive [73]. However, as the *E. coli* concentrations, also in the drinking water sources, are very high, it is safe to say that the population in the basin will be at risk of waterborne pathogens and resulting faecal-oral diseases. The hydro-climatic variables, to which the *E. coli* concentrations in the Kabul River Basin are strongly related, are expected to change in the future for the basin, with increased temperature, precipitation and discharge [74] Such changes will further increase the *E. coli* and pathogen concentrations in the surface and drinking water sites and also likely increases the health risk for the population in the basin.

5. Conclusions

Based-on analysis of biweekly *E. coli* samples for nine surface and five drinking water sources over a period of 30 months (April 2013–September 2015), water and surface air temperature, precipitation and discharge of Kabul River in the North West of Pakistan, we conclude the following:

- All of the *E. coli* surface water samples exceeded USEPA bathing water quality standards. Therefore, the Kabul River is not suitable for swimming or bathing. Similarly, all of the *E. coli* drinking water samples exceeded the WHO standards for drinking water quality (<1/100 mL). Therefore, drinking water of Nowshera is not suitable for drinking without additional treatment.
- Temperature, precipitation and river discharge were found to correlate positively with *E. coli* concentrations at most of the sampling sites.
- Our linear regression models for average surface and drinking water explain 61% and 55% of the variability in the observations respectively. The variables water temperature, precipitation, discharge and an interaction factor of temperature and discharge were included in the model.

Although *E. coli* concentrations are not necessarily correlated to waterborne pathogen concentrations, the high concentrations do indicate likely presence of waterborne pathogens and a resulting health risk for the population. Based on our analysis, we can conclude that expected increases in temperature, precipitation and discharge likely increase the *E. coli* concentrations further. This likely also increases the health risk for the population in the Kabul River Basin.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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