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Article

The Spatiotemporal Distribution of Two Bacterial Indexes in a Small Tibetan Plateau Watershed

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Abstract: Microbial contamination is now more common than chemical contamination in Tibet, and water-borne microbes can cause a number of diseases that threaten public health. Thus, in order to clarify the spatiotemporal distribution of bacteria in small watersheds for which there is no data in Tibet, we set up four sampling points along an upstream-downstream transect of the Xincang River Basin. We collected 239 water samples in 2014 and 2015, and evaluated their total constituent numbers of bacteria (TB) and coliforms (TC). The results of this study show that the microbial contamination of the Xincang River Basin is mild-to-moderate in terms of TB and TC contents, and that these concentrations vary significantly in different seasons. Results show that in summer TB and TC concentrations in the downstream section of this river were highest and that microbial contamination was most serious. Data also demonstrate that precipitation is the most important factor underlying increases in TB and TC concentrations during the summer months; both these variables are significantly correlated with precipitation, while animal husbandry and domestic sewage are the main sources of microbial contamination overall. The results of this study are likely to reflect the basic characteristics of small watersheds for which there is no data to some extent, and are thus of significant practical importance for protecting their ecological environments and promoting sustainable development.

Keywords: microbial contamination; bacteria; contamination sources; small watershed; Tibetan Plateau

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1. Introduction

Water is the source of life, the key to production and the foundation of ecology. This natural resource is both irreplaceable and a key resource for countries, related to economic, ecological, and even national security. However, as economies develop and urban construction and population levels accelerate, surface waters have become more-and-more polluted by irrational discharges of municipal waste, domestic sewage, industrial and hospital wastewater, and human and animal manure which all degrade water quality. Thus, shortages in water resources as well as pollution, ecological deterioration, flooding, and droughts all pose serious threats to global economic development, the health of populations, the available environment for human inhabitation, and national security. Numerous rivers and lakes around the world have been polluted and have suffered ecological degradation as a result of rapid local economic development; relevant examples of rivers include the Delaware in the United States [1], the Thames in the UK [2], and the Rhine in Germany [3], as well as Lake Biwa in Japan [4]. A number of studies have also indicated that the water quality of 40% of rivers and estuaries do not meet environmental standards because of pathogenic microorganisms [5]. Water polluted by pathogenic microbes can also lead to a variety of water-borne infectious diseases, including diarrhea, nausea, gastroenteritis, and typhoid dysentery, all of which are significant threats to human health, especially in children and people with weak immune systems [6-13]. Bacterial pollution can also affect the quality of recreational water [5]; microbial contamination has led, for example, to a number of water-borne infectious diseases in the Mekong River [9].

Long-term systematic research on the microorganisms present in Chinese rivers and lakes has generally been limited, and just a handful of reports have appeared. The results of one survey carried out over more than a year revealed rather serious microbial contamination in the Wenyu River, Beijing, including significant organic pollution and eutrophication [14]. Similarly, the overall water quality in the reservoir area of the Three Gorges Dam was mainly at Grade ◎ of the Water Quality Standard for the Surface Water Environment (GB / 3838-2002), although taking the index of fecal coliform bacteria into account, the quality of this water is classified as Grade ◎ or sub-Grade ◎ overall [15]. Further work has shown spatiotemporal variability in the distribution of pathogens in the Haihe River Basin as microbial pollution is significantly correlated with population density, urbanization rate, and the proportion of tertiary industry contributing to local gross domestic product [16].

The water resources of Tibet are very rich and diverse; the water resources of this region are ranked first within China and account for 15.8% of the national total. The per capita water resources of Tibet are also the highest in China, even though water per square land kilometer in this region is just 40% of the national average, indicating an uneven distribution [17]. At the same time, precipitation in Tibet is also extremely irregular throughout the year, as more than 80% occurs between June and September. According to a surface water report for Tibet published in 2012, the quality of this resource is good; 98.96% of the total rivers evaluated reached, or exceeded, Grade ◎ surface water environmental quality [18]. Water in most parts of Tibet meets Chinese national standards as well as World Health Organization international standards [19] as the major rivers and lakes in this region remain in good condition [20]. The population of this region is also scattered in agricultural and pastoral areas because of complex local physical and geographical conditions. The distribution of the Tibetan population is also significantly related to rivers, as 80.46% of people live less than 10 km away from a water course [21]. Thus, the main sources of drinking water in this region are river and groundwaters that have not been specially purified or disinfected; as just sedimentation and simple filtration are applied, the quality of river water directly influences the quality of drinking water and has health implications for local people in river basins where data has not been collected. Few studies have been carried out to date on the levels of microbial contamination in typical small Tibetan rivers.

The aims of this study were therefore to: (1) Identify the overall level of microbial contamination within the Xincang River Basin; (2) Evaluate the spatiotemporal characteristics of changes in total bacteria (TB) and coliform (TC) levels, and; (3) Analyze the main driving forces and factors affecting changes in TB and TC levels within the watershed, including precipitation, animal husbandry, and domestic sewage. Water samples were collected during wet and dry seasons along an upstream-to-downstream river basin transect; we evaluated both TB and TC levels in the laboratory, and locally-investigated human and animal activities. The results of this study are likely to provide important baseline data for local environmental protection as well as public health management in these small Tibetan Plateau river basins.

2. Materials and Methods

2.1. Study Area

The research reported in this paper was conducted within the Xincang River Basin on the Tibetan Plateau. This River Basin is located 6.2 km from Dazi County and 35.8 km from the city of Lhasa (Figure 1) and encompasses latitudes between 29.48 degrees and 29.68 degrees, as well as longitudes between 91.24 degrees and 91.50 degrees. The area of this basin is 137.8 km^2, and its highest and lowest elevations are 5,539 m and 3,662 m, respectively.

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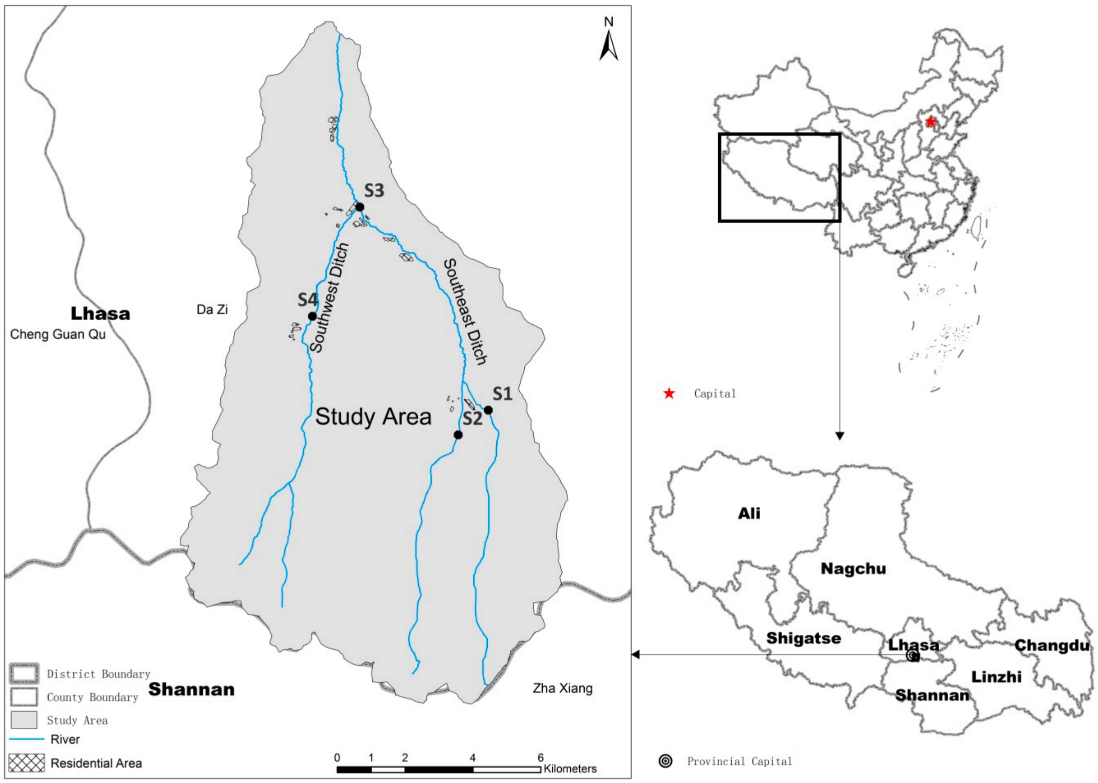

Figure 1. Map to show the location of our study area and sampling sites (S).

The Xincang River has two major tributaries, referred to as the Southeast and Southwest ditches, which both run from south-to-north through the village of Xincang. This village contains eight resident groups, and comprised a 2015 population of 1,540 people in 346 households. This corresponds to an average of 4.4 people per household. In the downstream (northern) section of the river basin, at elevations between 3,750 m and 3,800 m, arable land is the main land use type, while at elevations between 3,930 m and 4,200 m, in the midstream section, the main land use types are grassland and arable land. In contrast, in the upstream (southern) section of the area at elevations more than 4,500 m, the main land use type is grassland. This land is only suitable for animal husbandry and there is no industrial activity at these high elevations. Although the rainy season in Tibet extends between June and September, this season only lasts between July and August in the Xincang River Basin. It nevertheless rains enough throughout this period to generate runoff onto the land; this runoff can transport large volumes of sediment, household waste, and even human and animal excrement into the river.

Two modes of production are seen within the Xincang River Basin, planting and animal husbandry, corresponding with typical activities within Tibet in general. Statistics show that 85.3% of the Tibetan population inhabits an area between 3,500 m and 4,100 m above sea level [21], a range that encompasses the altitude of the Xincang River Basin. A number of small watersheds like the Xincang River Basin are known throughout Tibet and the population of this region is distributed significantly alongside rivers; farmers and herdsmen live on plains, and obtain their drinking water from the local river. However, few studies have been carried out on these small watersheds because of a lack of data, even though such rivers share a number of characteristics with one another, including the Xincang River. We chose the Xincang River Basin as our study area because it is close to the Lhasa National Ecological Research Station, Institute of Geographic Sciences and Natural Resources Research (IGSNRR), China Academy of Sciences (CAS), and so basic data such as temperature and precipitation can be obtained. The results we present for the Xincang River Basin are likely to reflect, to a certain extent, the basic circumstances of other small watersheds for which data are not available. Our results therefore provide a key reference for similar future work, especially the protection of local drinking water safety.

2.2. Sampling Locations and Data Collection

In order to generate information about the spatial change characteristics of microbes and the effects of human activities on Xincang River Basin water quality while taking the distribution of residents and river trends into account, we set up four sampling sites throughout the basin (Figure 1). Thus, site 1 and site 2 are located in the Southeastern Ditch, upstream of the residential area, an area within the upper reaches of Xincang River Basin. In contrast, site 3 is located in the residential area downstream, while site 4 is located to the north of the settlement in the Southwestern Ditch, in the middle of the basin. Basic information about these four sampling sites is provided in Table 1; their locations are shown in Figure 1.

Table 1. Sampling sites.

Site	Longitude (E)	Latitude (N)	Altitude (m)	Description
S1	91.402284	29.565959	4,196	Upstream
S2	91.396532	29.566618	4,182.	Upstream
S3	91.356450	29.604769	3,916	Midstream
S4	91.364425	29.619121	3,820	Downstream

We collected samples between July 2014 and April 2015, encompassing both wet and dry periods in Tibet. We recovered a total of 239 water samples from our four points. The collected water samples were protected from direct sunlight exposure and tested the bacteria within four hours.

2.3. Microbiological Assays

2.3.1. Bacterial Indicators

Microbiological indicators provide the main basis for assessing surface water microbial contamination. However, because of low concentrations of pathogenic microorganisms and testing difficulties, we used a detection indicator bacterial series to evaluate both microbial contamination status and water safety. In this context, the most commonly used indicators internationally [22, 23] are values for TB, TC, fecal coliforms [9], *Escherichia coli*, and fecal streptococci. At present, however, the index system used to evaluate Chinese river water quality is based mainly on conventional physical and chemical indicators and little consideration is currently afforded to microbiological evaluation. The *Environmental Quality Standards for Surface Water* (GB / 3838-2002), for example, only take FC concentrations into consideration [24] which means that microbial contamination levels in Chinese rivers currently remain unclear and these indexes are not very useful to the management and sustainable development of water courses.

Related research on microbial indicators in surface water has shown that TB values are useful for evaluating the degree of sample microbiological contamination; thus, a higher standard plate-count of bacteria implies a greater degree of microbial contamination and a higher probability that pathogenic microorganisms are present [25]. Similarly, the use of TC values is also common to analyses of this type; as the largest number of bacteria is found in the intestines of humans and other warm-blooded animals, TC values can be detected from feces. As TC values are also correlated to a certain extent with water pathogens, this indicator is also a valuable indicator of the presence of water-borne bacteria. Indeed, in 1914, the United States Department of Public Health used TC values as a microbial indicator of the presence of manure pollution in drinking water; this indicator is now used commonly to evaluate levels of water pollution, used routinely for water quality monitoring in China. Thus, as people in both agricultural and pastoral areas of Tibet routinely obtain their

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drinking water directly from untreated rivers around their dwellings, we chose to mainly monitor TB and TC levels.

2.3.2. Detection Method

We compared the accuracy and convenience of various detection methods [26] and chose to use the standard plate counting approach to evaluate TB levels, expressed as colony forming units (CFU) per milliliter of water (CFU/ml). We also detected TC levels using the enzyme substrate method, expressing this concentration as the most probable number (MPN) in 100 ml water. All our tests were carried out in accordance with the *Standard Examination Methods for Drinking Water* (GB / T5750-2006) [27], and all water samples were tested at the Center for Disease Control and Prevention within the Tibet Autonomous Region.

We applied current Chinese water quality standards [28-31] in this study and our microbiological evaluation criteria are listed in Table 2. These standards suggest that when the TB concentration in water is less than 1,000 CFU/ml, microbial contamination is considered not serious. However, when the TC level is less than 100 MPN/ml, microbial contamination is mild-to-moderate and water can be drunk if disinfection measures are applied. Only in cases where TC levels are undetectable can water be drunk directly as it meets Chinese *Standards for Drinking Water Quality* (GB / T5749-2006).

2.4. Data Sources

We obtained Digital Elevation Model data from the Data Center for Resources and Environmental Sciences, CAS, and generated land use data via visual interpretation from Landsat remote sensing images. Climate data, including precipitation and temperature, was provided by the Lhasa National Ecological Research Station, IGSNRR, CAS.

2.5. Statistical Analyses

We recorded and sorted all data for analyses, computed the minimum, maximum, median, mean, and standard deviations (SD) of TB and TC values, and simulated the relationship between month and qualified rate of microbiological indexes in the Xincang River Basin using Microsoft Excel 2010. Statistical analyses were performed using the software SPSS 20.0 (IBM Corp., Armonk, NY, USA); we used Kruskal-Wallis tests to analyze differences in TC and TB concentrations at different locations and between seasons, assuming a significant difference is a P value was between 0.01 and 0.05. We assume a high degree of significance if a P value is less than 0.01, and assessed relationships between microorganisms and precipitation, as well as between microbiological contamination condition and main sources, using Pearson correlation coefficients [32]. Several figures in this paper were generated using the software OriginPro 2017 (OriginLab Corp., Northampton, USA).

Table 2. Criteria used to evaluate level of microbial contamination in the Xincang River Basin.

Bacteria	**TB (CFU/ml)**				**TC (MPN/100ml)**		
Value	TB ≤ 10^2	10^2 < TB ≤ 10^5	10^5 < TB ≤ 10^6	TB > 10^6	Not detected (ND)	TC ≤ 10^3	TC > 10^3
Evaluation result	Oligosaprobic zone	β-mesosaprobic zone	α-mesosaprobic zone	Polysaprobic zone	Drinking water standard	First rank drinking water source	Second rank drinking water source
Degree of Contamination	Mild	Moderate	Moderate	Serious	Mild	Moderate	Serious

Notes: Pollution zone divisions based on *Pollution Control Microbial Ecology (2005)* and *The Water and Wastewater Monitoring Analysis Method (1997)*. In *Standards for Drinking Water Quality* (GB / T5749-2006), TB must not exceed 100 CFU/ml and TC must not be detectable in a 100 mL sample if water is to be considered safe to drink without treatment.

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3. Results

3.1. TB and TC Concentrations

We counted microbiological indicators in our water samples using the software Microsoft Excel 2010 (Table 3). Results show that the TB concentration in the Xincang River Basin varied greatly, ranging between undetectable levels and 840 CFU/ml (mean: 83 CFU/ml; median: 35 CFU/ml; SD: 129.42).

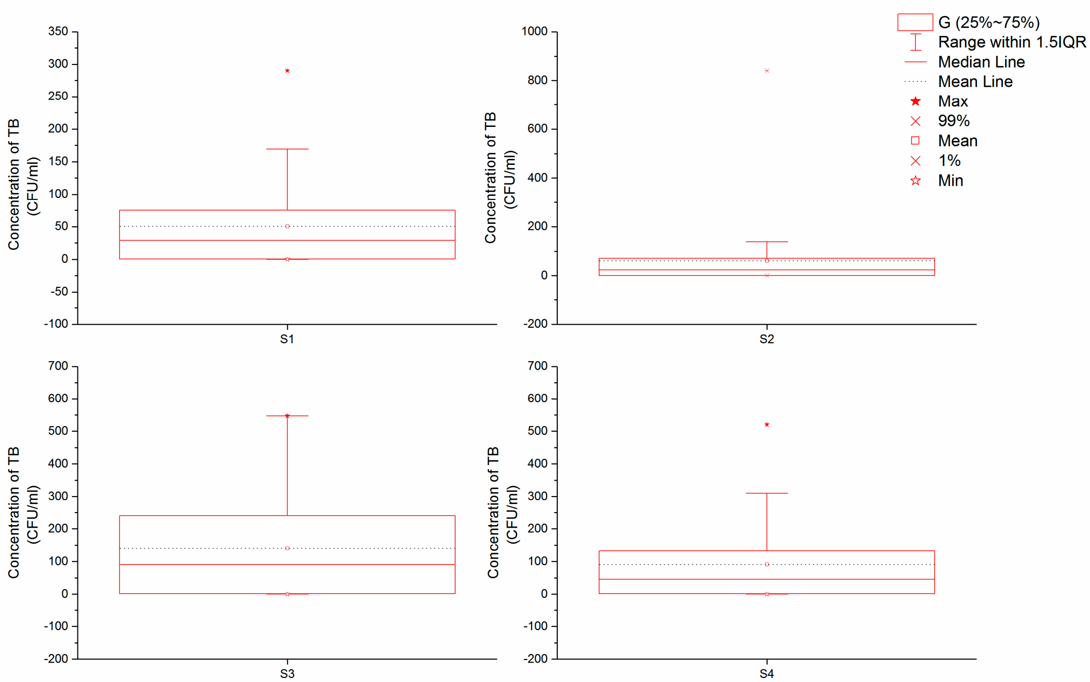
Although the maximum recorded TC level in water samples from the Xincang River Basin was 500 MPN/100 ml, sometimes this index was not detectable (mean: 300 MPN/100 ml; median: 235 MPN/100 ml; SD: 1.93).

Table 3. Descriptive bacterial contamination statistics for the Xincang River Basin.

Bacteria	N	Mean	Max	Median	Min	SD
TB (CFU/ml)	239	83	840	35	ND	129.42
TC (MPN/100 ml)	239	300	500	235	ND	1.93

Our results reveal significant differences in TB and TC concentrations between sampling points (Figure 2a, b); the maximum TB concentration we recorded within the Xincang River Basin was 840 CFU/ml at S3. Indeed, across all four sampling points, the mean concentration of TB at S3 was the highest, 135 CFU/ml, while at S1 and S2, this concentration was less that 100 CFU/ml in most (more than 75%) samples (recorded means at S1 and S2 were 50 CFU/ml and 61 CFU/ml, respectively). Similarly, although the concentration of TB in some water samples from S4 was greater than 100 CFU/ml, both the mean and median were less than 100 CFU/ml, higher than values recorded at S1 and S2, but lower than those from S3.

The data presented in Figure 2b show that the mean concentrations of TC at S1 and S2 approached 280 MPN/100 ml, while the mean at S4 was a little higher, about 300 MPN/100 ml. In contrast, the mean TC concentration at S3 was 331 MPN/100 ml, the highest recorded at our four sampling points.



(a)

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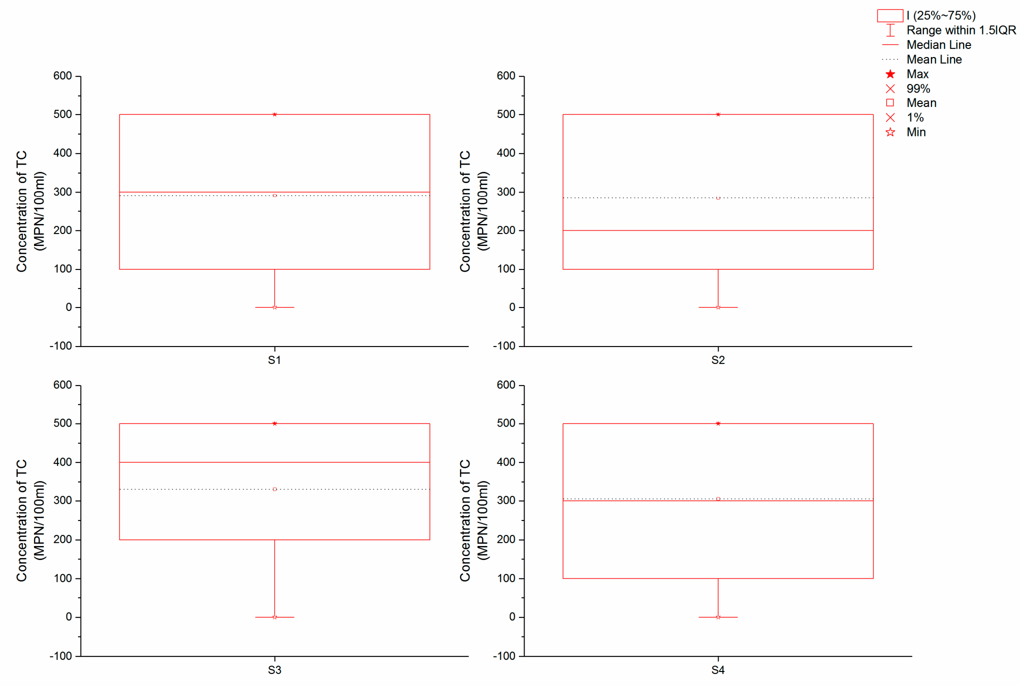

(b)

Figure 2. Results from sampling sites. (a) TB concentrations; (b) TC concentrations.

3.2. Qualified Microbiological Index Rate

Because local people on the Tibetan Plateau always use surface water or groundwater adjacent to their dwellings as drinking water sources, the Xincang River Basin is the main water source for the village of Xincang. Applying the *Standards for Drinking Water Quality* (GB / T5749-2006), we determined microbial contamination in this river, noting that when TB is less than 100 CFU/ml and TC is undetectable in 100 ml water samples, the sample is qualified at the point of microbial index. We therefore applied a rate equivalent to the proportion of microbial qualified water samples versus the total number of samples. Our data show that of 239 water samples we collected from Xincang River Basin, 31 meet both TB and TC requirements and thus the qualified microbiological index rate was 12.97%.

Results also show that qualified microbiological index rates differ at different sampling points within the Xincang River Basin; thus, the rate recorded at S2, 23.73%, was the highest, while those recorded at S1 and S4 were 11.86% and 10.17% respectively, and that at S3 was the lowest, just 5.08%. The S3 qualified rate was less than a quarter that at S2 and just half the rate recorded at S1 and S4.

Comparing the qualified rates of water samples from different seasons, our data show that the proportion of microbes in water samples varies in a 'V-shape' throughout the year (Figure 3). The qualified microbiological index rate began to decrease from the spring onwards, reaching its lowest rate in the summer, before starting to rise again in the autumn to another relatively high winter level.

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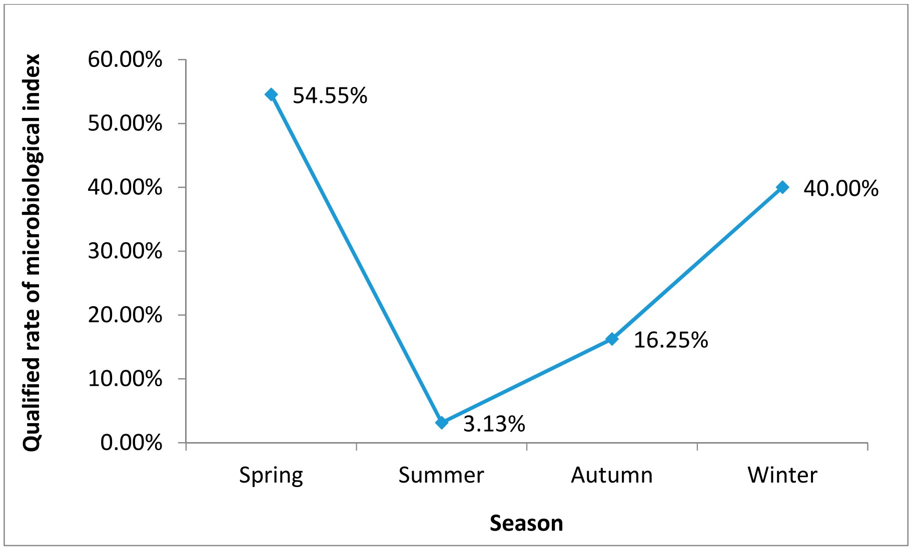

Figure 3. Annual changes in the qualified microbiological index rate.

3.3. Correlations between Bacterial Levels and Rainfall

Rainfall varies significantly throughout the year in the Xincang River Basin, with precipitation often close to zero during low flow periods between November and March. Daily precipitation changes and TB values between July 20th, 2014, and July 31st, 2014 (Figure 4) show that changes in these two indexes mirror one another; peak precipitation in this period was on July 24th, 2014, when the value for TB was also maximal. Similarly, precipitation on July 26th, 2014 was zero and TB was also decreased accordingly.

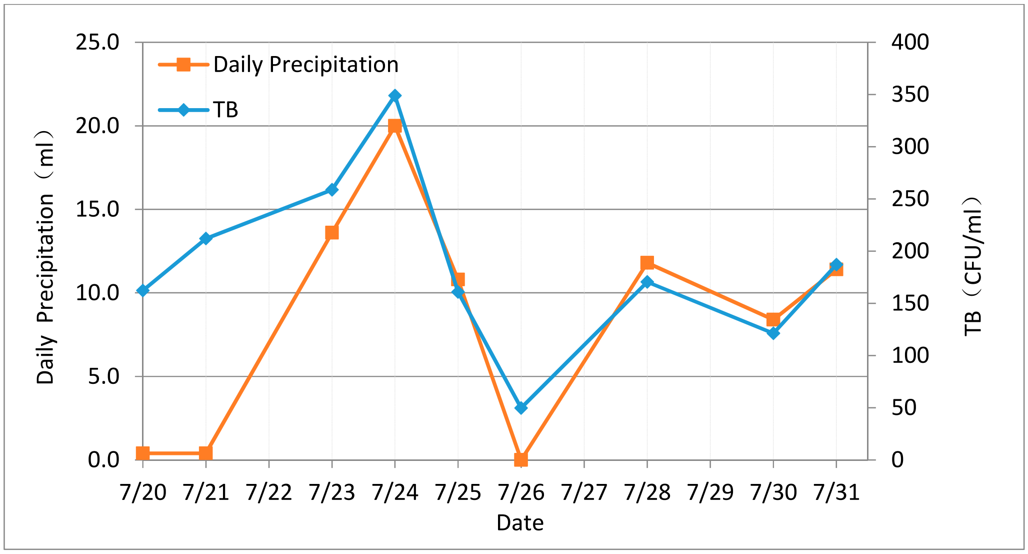

Figure 4. Changes in daily precipitation and TC from July 20th, 2014, to July 31st, 2014.

Statistical data show that changes of precipitation in annual in Tibet conform to an inverted 'V-shape', precipitation is the most in summer, but the proportion of microbes in water samples varies in a 'V-shape', just on the contrary. Thus, in order to further evaluate the relationship between rainfall and bacterial levels, we analyzed the statistical correlations between monthly precipitation, TB, and TC using the software SPSS 20.0. Results show that both TB and TC are significantly positively correlated with precipitation (**Table 4**); indeed, TB is significantly correlated with precipitation at the 0.05 probability level (two-tailed) and with a correlation coefficient of 0.789.

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Similarly, the correlation between TC and precipitation is at the 0.01 probability level (two-tailed) and has a correlation coefficient of 0.876. Data also show that there is a significant correlation between TB and TC at the 0.01 probability level (two-tailed) with a correlation coefficient of 0.886.

Table 4. Correlation analysis results between precipitation, TB, and TC.

		Precipitation	TB (CFU/ml)	TC (MPN/ml)
Precipitation	Pearson correlation	1	0.789*	0.876**
	Sig. (two-tailed)		0.035	0.010
	N	7	7	7
TB (CFU/ml)	Pearson correlation	0.789*	1	0.886**
	Sig. (two-tailed)	0.035		0.008
	N	7	7	7
TC (MPN/ml)	Pearson correlation	0.876**	0.886**	1
	Sig. (two-tailed)	0.010	0.008	
	N	7	7	7

* Correlation is significant at the 0.05 probability level (two-tailed).

** Correlation is significant at the 0.01 probability level (two-tailed).

Data show that the concentration of bacteria fluctuates based on the amount of precipitation. Thus, during periods of abundant precipitation, the volume of bacteria in the watershed increased causing a concomitant increase in microbial contamination.

4. Discussion

4.1. Overall Microbial Contamination

Applying relevant Chinese water quality standards, we utilized the microbiological evaluation criteria shown in Table 2. Data show that because the maximum TB and TC concentrations recorded in the Xincang River Basin were 840 CFU/ml and 500 MPN/100 ml, respectively (Table 1), microbial contamination of this watershed is not serious. At the same time, however, the level of microbial contamination at each sampling point was different; data show that both the mean and median of TB at S1 and S2 were nearly 50 CFU/ml, while at S4 these values were a bit higher than S1 and S2 but less than 100 CFU/ml. These data suggest that S1, S2, and S4 are all oligo-contaminated, the microbiological condition that can be considered mild or moderate. In contrast, because the mean TB concentration at S3 was more than 100 CFU /ml, this site can be classified within the β -mesosaprobic zone. The mean TC concentration at S3 was highest, followed by S4; this implies that microbiological contamination at S3 is more serious than at S4 although both levels are still moderate. In contrast, contamination at S1 and S2 is much lighter at just mild levels.

4.2. Spatiotemporal Characteristics of TB and TC Distribution

We chose Spring (March, April, and May), Summer (June, July, and August), Autumn (September, October, and November), and Winter (December, January, and February) as the four time periods used in this study to analyze the bacterial characteristics of the Xincang River Basin. Upstream, midstream, and downstream were used as spatial scales, and we applied Kruskal-Wallis tests to determine microbe spatiotemporal variability at the three locations across the four seasons. The results of these tests reveal highly significant differences in the concentrations of TB and TC between different seasons ($P = 0.000$, less than 0.05). The spatial distribution of TB between different locations was also significant ($P = 0.006$), while that of TC was not ($P = 0.238$).

Comparison of TB and TC concentrations (Figure 5) shows that downstream microbial contamination is significantly more serious than in either upstream or midstream sections. Results show that concentrations of TB and TC downstream were 148 CFU/ml and 333 MPN/100 ml, respectively, while in the midstream section, these concentrations were 83 CFU/ml and 297

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MPN/100 ml, respectively. Microbial contamination upstream was slight, with TB and TC concentrations at 56 CFU/ml and 288 MPN/100 ml, respectively, lower than in either downstream or midstream sections. Previous studies have shown that the levels of river microbial contamination are related to local land use, population density, and economic development [16]. More residential areas and thus higher population levels are located in the downstream sections of the Xincang River Basin, resulting in much larger volumes of rural and domestic sewage discharge and rendering microbial contamination much more serious. Because of a higher population density, it is also important to protect public health and safety within the downstream section of this river basin; thus, studies that deal with the microbial contamination of rivers are of paramount importance to their protection, sustainable development, and the maintainance of local public health.

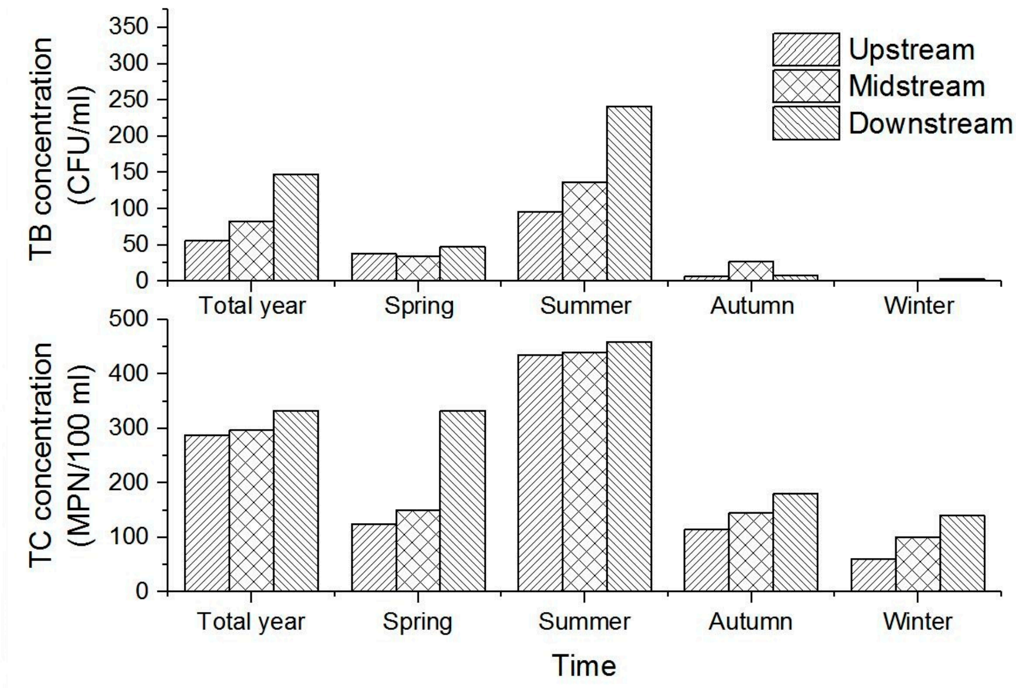

Figure 5. TB and TC concentrations between different seasons and locations.

Our data show that the qualified microbiological index rate at different locations between seasons was highest in the spring, irrespective of river section (Figure 6). Second highest levels were seen in the winter and then the autumn, with the lowest rate recorded in the summer. Within the same season, the upstream qualified rate was the highest while the downstream rate was the lowest.

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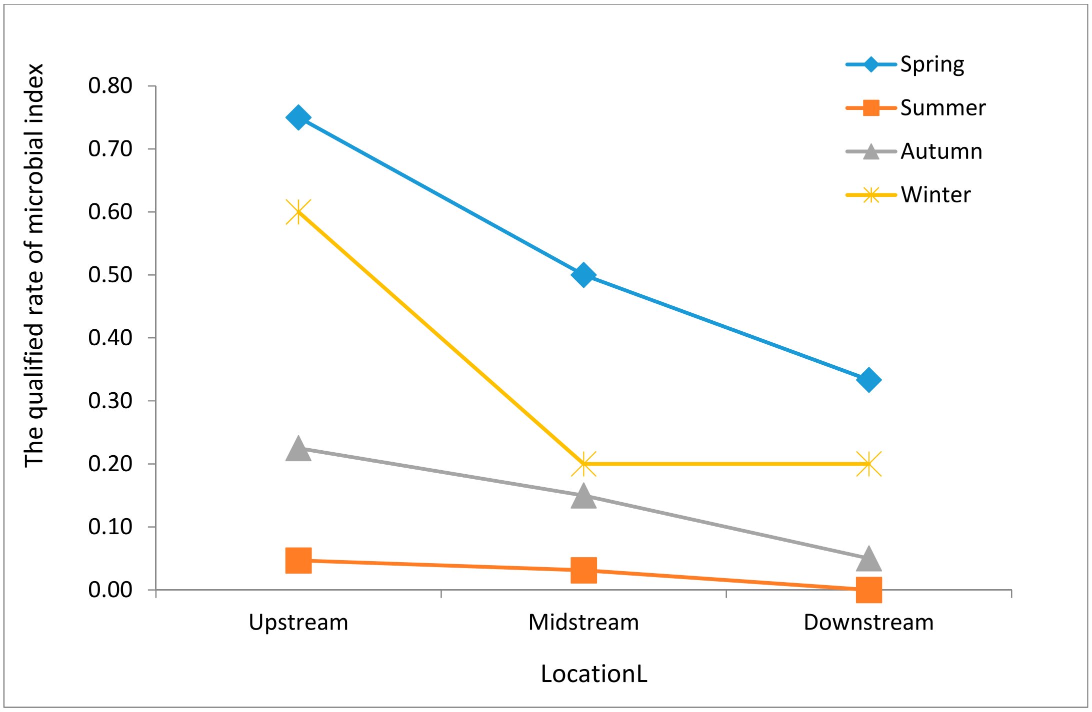

Figure 6. Water qualified rate values for different river sections.

The data presented in Figures 5 and 6 reveals a significant difference in the temporal distribution of bacteria. Sampling results show that microbial contamination was most serious during the summer when the qualified microbiological index rate was also lowest, and mean concentrations of TB and TC were 142 CFU/ml and 442 MPN/100 ml, respectively. The vast majority of precipitation on the Tibetan Plateau (90%) is concentrated in the summer (June, July, and August) and so surface runoff is common at this time. This runoff scours surface dust, humus, animal feces, and other pollutants into the river, resulting in excessive TB and TC values [33]. It can therefore be concluded that non-point source pollution carried by surface runoff exerts a significant effect on the bacterial levels of water resources [34-36]. As a result, TB and TC levels are higher in summer than in other seasons, while the qualified rate of water samples is correspondingly lower. Farmers and herdsmen also discharge more domestic sewage into the river during summer, while some local residents wash their clothes in the water. The river water temperature in summer is also higher than in other seasons; a relatively higher temperature is more conducive to the survival and reproduction of microorganisms. It is therefore straightforward to conclude that the summer microbial concentration of the Xincang River Basin is generally high, and contamination is more serious.

4.3. Sources of Microbial Contamination

Previous work has shown that microbiological contamination is dispersed, sporadic, and influenced by a range of interacting environmental factors including the physical characteristics of a watershed, climatic conditions, and agricultural management practices [37]. The influence of human activities on the water quality of the Tibetan Plateau is mostly manifest by production process and day-to-day life; human activities which affect the water quality of the Xincang River Basin mainly include animal husbandry and domestic sewage.

4.3.1. Rural Domestic Sewage

Because most rural counties in China lack a sewage pipe network and treatment systems, domestic sewage is discharged at will, often into the local river where it seriously affects water quality [38]. Although rural domestic sewage mainly includes kitchen waste as well as water from washing and flushing [39], in these areas of Tibet the toilets comprise dry pail latrines [40] and are relatively simple. Nevertheless, waste from these simple dry pail latrines, as well as kitchen sewage, and washing water pollute rivers via groundwater seepage and surface runoff.

Household surveys and calculations show that each person within the Xincang River Basin produces 35.36 L of domestic wastewater daily. Thus, combining the population, we calculated daily volumes of upstream, midstream, and downstream wastewater to be 5.5 m^3, 4 m^3, and 44.93 m^3, respectively [41]. In addition to domestic sewage discharge, laundry washing directly in the river as well as the disposal of household garbage also affects river water quality. We performed a further analysis to assess the relationship between amount of domestic sewage and the qualified microbial index rate using Pearson's correlation coefficient. These results show that sewage discharge is significantly correlated with the qualified microbial index rate. The Pearson correlation coefficient in this case is -0.847; this indicates that a larger sewage discharge will cause more serious microbial pollution.

4.3.2. Animal Husbandry

Human and animal manure is the main source of TC [42, 43]. Surface runoff from abundant rainfall carries livestock effluent into rivers and causes the TC concentration to exceed safety standards. As the livestock within the Xincang River Basin are mostly free-range outdoors, they drink directly from river and defecate everywhere throughout the basin. Although local residents do collect yak feces as fuel, more than one-third of this excrement is nevertheless abandoned on riverbanks and in grasslands.

According to the excretion coefficient recommended by the State Environmental Protection Administration, the volume of fecal emissions downstream is highest, reaching 27,581 tons, followed by upstream (16,443 tons) and midstream (9,016 tons)[41]. Thus, the qualified TC rate in the downstream river section was lowest, just 6%, while rates in midstream and upstream sections were 7% and 11%, respectively. The qualified TC rate trend recorded within the Xincang River Basin is consistent with fecal discharge; livestock manure is the main TC source in areas where grazing intensity is greatest and thus microbial contamination is more serious.

Fences are not always present in the rural areas of Tibet to isolate livestock from drinking water sources. This means that animals are able to drink directly from sources also used by humans, further increasing the risk of contamination.

5. Conclusions

The results of this study reveal several basic changes in microbe levels between different seasons and locations in a typical small Tibetan watershed, the Xincang River Basin. We show that both TB and TC values within a typical small watershed exhibit obvious spatiotemporal distribution characteristics; in summer, the mean values for both these indexes are much higher than in other seasons, while the qualified microbiological index rate of water samples was much lower at this time than during the winter and spring. Our data show that the qualified rate of water microbes changed over the year according to a 'V-shaped' pattern. Results also reveal significant differences in TB, TC, and qualified microbial rates between upstream, midstream, and downstream river sections. Values for TB and TC were lowest upstream, followed by midstream, and greatest downstream, while the opposite relationship is seen in the qualified microbiological rate. Our data also reveal that TB and TC values are significantly correlated with precipitation, with correlation coefficients of 0.789 and 0.876, respectively; this means that precipitation is a key factor leading to microbial changes in the river. Finally, we show that animal husbandry and domestic sewage constitute the main sources of microbial contamination within the Xincang River Basin.

The local government has recently taken microbial contamination into consideration when developing a rural drinking water safety project, selecting sources as far upstream as possible away from human and animal activities. Although a step in the right direction, this local drinking water safety initiative is not sufficient; if possible, water source protection areas should be established within the Xincang River Basin, and fences should be installed to prevent livestock contamination. In addition, microbial pollution monitoring of rivers and groundwater should be enhanced in local areas and a risk warning system put in place to ensure drinking water safety for residents, to maintain the ecological health of the river, and to promote local sustainable development.

Because of the limitations of this study, detailed quantitative work on the degree of influence of precipitation and the mechanisms by which it affects TB and TC was not carried out; this would be an important direction for further research in this small river basin. It is also very important to carry out further analyses on the natural and social factors influencing the spatiotemporal distribution of bacteria in small watersheds across Tibet in order to enhance their protection. Other factors in addition to precipitation can also influence bacteria in river water, including temperature, solar radiation, livestock management practices, and seasonal patterns of recreational water use [44, 45]. Additional comprehensive and accurate research is necessary in these areas where limited data has so far been collected.

There are a large number of watersheds like the Xincang River Basin on the Tibetan Plateau that have similar natural and socioeconomic characteristics. Thus, the results of this study can be considered to some extent indicative of the basic characteristics of other small watersheds where limited data has been collected. The results of this study are therefore of practical significance in protecting the ecological environment of typical small watersheds, achieving local sustainable development, guaranteeing drinking water safety, and enhancing human health in the agricultural and pastoral areas of Tibet.

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