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Fecal Contamination and High Nutrient Levels Pollute the Watersheds of Wujiang, China

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Abstract: Freshwaters in China are affected by point and non-point sources of pollution. The Wujiang District (Suzhou City, China) has a long history of canals, rivers, and lakes that are currently facing various water quality issues. In this study, the water quality of four rivers and a lake in Wujiang was assessed to quantify pollution and explore its causes. Seventy-five monthly samples were collected from these water bodies (five locations/samples per area) from August to October 2020 and were compared with nine control samples collected from a water protection area. Fifteen physicochemical, microbiological, and molecular–microbiological parameters were analyzed, including nutrients, total and fecal coliforms, and fecal markers. Significant monthly variation was observed for most parameters at all areas. Total phosphorus, phosphates, total nitrogen, ammonium–nitrogen, and fecal coliforms mostly exceeded the acceptable limits set by the Chinese Ministry of Environmental Protection. The LiPuDang Lake and the WuFangGang River were the most degraded areas. The studied parameters were correlated with urban, agricultural, industrial, and other major land use patterns. The results suggest that fecal contamination and nutrients, associated with certain land use practices, are the primary pollution factors in the Wujiang District. Detailed water quality monitoring and targeted management strategies are necessary to control pollution in Wujiang's watersheds.

Keywords: water quality; nutrients; fecal contaminations; lakes and rivers; land use; Wujiang district

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1. Introduction

Freshwater pollution from natural processes and human activities is a major threat for aquatic ecosystems and human health [1]. Freshwater quality assessments are routinely carried out worldwide to ensure clean water for humans (drinking water supply and other domestic uses) and aquatic organisms [2]. Identifying sources of pollution and applying appropriate management strategies is crucial to minimize potential water-quality induced risks for public health [3]. Pollutants affecting freshwater quality are introduced into freshwaters by point and non-point sources [4]. Point sources are clearly identifiable, e.g., urban sewage or industrial wastewater discharges. In contrast, non-point sources cannot be easily located, e.g., storm-water runoff and sewage overflows, or runoffs from urban or agricultural land uses [5]. Runoffs from these sources may lead to excessive nutrient concentrations, and leaks of toxic chemicals and pathogenic microorganisms. Thus, accurate freshwater monitoring is required to facilitate appropriate management strategies identifying pollution and potential outbreaks of waterborne diseases [6]. In addition, freshwater quality assessments enable the identification of key factors that cause spatio-temporal water quality variation, facilitating the improvement and ultimate optimization of a watershed's freshwater quality [7].

Watersheds are important for biodiversity and also for providing habitat for aquatic species, many of them with high social and economic values [8]. The structure and connectivity of watersheds have been proven to be highly influenced by the local hydrological cycle and biogeochemical processes in the watershed [9]. Additionally, watersheds act like sponges for cities, reducing the risks associated with flooding [10]. Watersheds and urban water systems in China face various environmental and human health challenges. Freshwaters, such as lakes, rivers, and canals, are affected by point and non-point pollution sources. Wujiang is located at the Taihu Lake basin, bordering the Jiangsu and Zhejiang provinces [11], and belongs to the Yangtze River Delta, which is the core urban area in China with large density of population, high levels of urbanization, and rapid economic development [12]. Due to the rapid urbanization of the region, freshwater systems are affected by various sources such as domestic sewage, industrial wastewater, and agricultural runoff, which result in poor water quality and ecosystem health [6,13]. Pollutants in urban freshwater systems depend not only on the level of urbanization, but also on the land use and management strategies such as policies for restructuring and upgrading industries and commercial businesses [14]. The complex interactions between natural processes and anthropogenic activities make the tracing of pollutants difficult, compromising the restoration of polluted waterbodies [13]. Therefore, identifying the sources of pollution and assessing the extent of water pollution in Wujiang's waterbodies are crucial steps to develop appropriate management strategies to minimize potential public health risks [7]. Apart from traditional physicochemical analyses, microbiological parameters such as fecal coliform (or thermotolerant coliform) counts can be used as indicators of fecal contaminations [15]. However, the detection and quantification of fecal coliforms alone does not indicate the source of fecal contamination, which is important for applying appropriate public health measures [16]. Microbial source tracking (MST) can provide information on the host-associated (human, animal, or avian) fecal contaminations in an affected environment [4].

In Wujiang, dynamic freshwater monitoring methods have not been established; therefore, manual monitoring and analysis of water quality are required for further improvement and the sustainable management of Wujiang's waterbodies. Yet, detailed analyses of the water quality in rivers and lakes across Wujiang and assessments of sources of fecal contamination are limited. Vadde et al. [6] applied a combination of physicochemical, microbiological, and statistical methods to evaluate the pollution level in Tiaoxi River, a major inflow river of Taihu Lake, and identified locations of increased pollution risk. Kogure et al. [11] assessed the water status of Taipu River in Wujiang using chemical analysis and bioassays and compared their results. Further analysis is required to assess water pollution, especially in low flow rates caused by the region's flat relief.

The purpose of this study was to assess the water quality of a lake (LiPuDang, LPD) and four rivers (XiDaGang, XDG; WuFangGang, WFG; DeDeTang, DDT; ZiXingTang, ZXT) of the Wujiang District, Suzhou, China. LPD is an important source for agriculture and aquaculture, while all four rivers are water sources for agriculture, large and small scale animal farms, and industrial processing units. All these activities deteriorate the watershed's water quality. The ZXT River connects Jiangsu and Zhejiang provinces, and is a part of the inter-provincial shipping routes. It also flows through the towns of Taoyuan and Wuzhen, which are world-famous historical towns with high touristic value [17]. To assess the water quality of these waterbodies, we used a comprehensive combination of monthly physicochemical, microbiological, molecular and land use analyses, aiming to suggest targeted measures for the sustainable management of Wujiang's freshwater resources, which could be also applied in other regions with similar characteristics.

2. Materials and Methods

2.1. Study Area and Sampling Locations

The study was carried out in the Wujiang District, Suzhou, China. Water samples were collected from four rivers namely the XiDaGang River (XDG), the WuFangGang River

(WFG), the DaDeTang River (DDT), the ZiXingTag River (ZXT) and one lake, the LiPuDang Lake (LPD) (Figure 1). At each river or the lake, monthly water samples were collected from five locations from August to October 2020. Water samples were also collected from three locations in the water protection area of the Taihu Lake and used as control samples (Figure 1; Table S1) resulting in a total of 3 months × 5 locations × 5 areas + 3 months × 3 control locations = 84 samples.

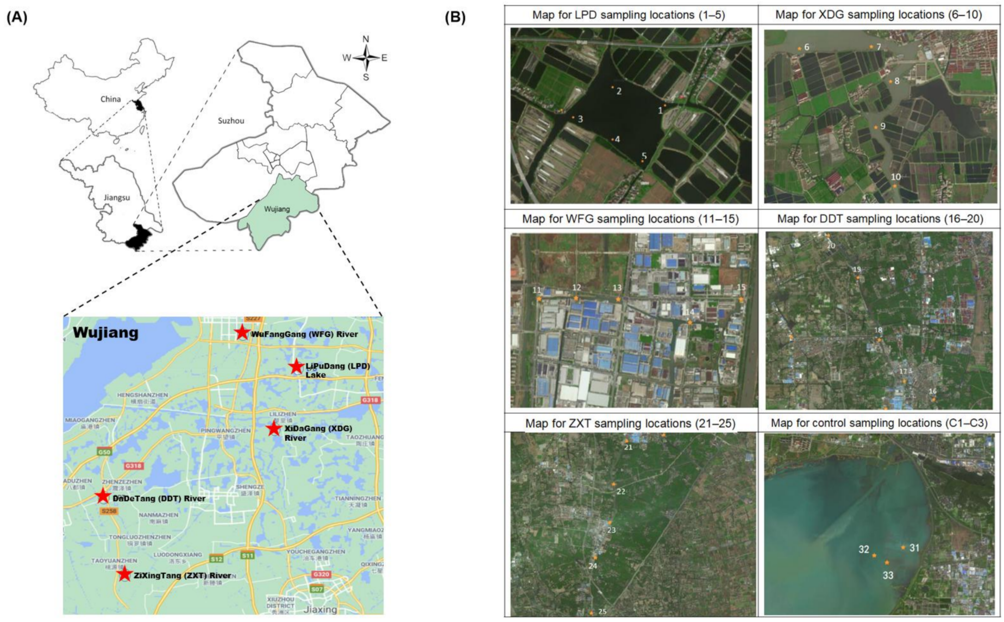


Figure 1. Maps of study areas in Wujiang District, Suzhou, China (**A**); Maps of sampling locations in the lake and rivers in Wujiang District (**B**).

2.2. Land Use Analysis

Land use maps were prepared by using the ArcGIS 10.3 and ArcGIS Pro Geographical Information Systems (Environmental Systems Research Institute Inc, Redlands, CA, USA). On the basis of Google Earth China Service Map of Suzhou city, we created one layer of buffer zone with radius of 1000 m around all sampling locations of the four rivers and a lake. By referencing the official land use maps of Wujiang District of Suzhou as well as Google maps covering the sample areas, we digitized the detailed land use types within these buffer zones in accordance with the National Code for Classification of Urban Land Use and Planning Standards of Development Land (GB50137-200), and calculated the land use composition within the areas of buffer zones.

2.3. Field Sampling and Sample Processing

The surface water samples were collected in sterile 5 L polypropylene containers for molecular microbiological analyses and kept at ambient temperature until they were brought to the laboratory. The samples for nutrients and the microbiological parameters were transported to the laboratory in ice. The parameters including air temperature, water temperature, pH and conductivity were measured on site. The water samples for physicochemical and microbiological analyses were processed within 8 h after collection. The samples for molecular microbiological analyses were filtered through 0.45 µm pore size mixed cellulose ester (MCE) membrane filters (Millipore, UK) in triplicate to collect the microorganisms for DNA extraction. The filters were stored at −25 °C prior to extraction.

2.4. Physicochemical Analyses

The following physicochemical analyses of water were carried out in this study. Air temperature (AT), water temperature (WT), electrical conductivity (EC), and pH were measured onsite using a portable EC/TDS TEMP Waterproof Combo Meter (C-100, HM

Digital Inc, Culver City, CA, USA) and a portable pH meter, respectively. Total nitrogen (TN), total phosphorous (TP), nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), ammonium nitrogen (NH_4-N), phosphate (PO_4-P), total organic carbon (TOC), dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), and potassium permanganate ($KMnO_4$) were measured by chemical testing methods following the China National Standards (Table S2). In addition, chlorophyll *a* (Chl *a*) was measured using 90% acetone by spectrophotometry, following the methods of the American Public Health Association [18].

2.5. Microbiological Analyses

The total coliforms (TC) and fecal coliforms (FC) or thermotolerant coliforms counts were determined to assess the microbiological quality of the water as reported in our previous studies [6,19]. In brief, 100 µL samples of serially diluted water samples were plated on Harlequin^{TM} *E. coli*/coliform medium (LabM, Heywood, UK) [20], and the plates were incubated at 37 °C for 24 h. The *E. coli* and coliforms were counted to determine the average number of total colony forming units (cfu) per mL of water. The FC in the water samples were carried out by the membrane filtration method suggested by American Public Health Association [18]. After dilution, 2 mL of water samples were filtered through Isopore^{TM} 0.45 µm polycarbonate membrane filters (Millipore, Cork, Ireland), after which the membrane filters were placed on mFC agar medium (HopeBio, Qingdao, China). The agar plates were incubated in water bath at 44.5 °C for 24 h, and the colonies with blue shades were counted to determine the number of FC colony forming units (cfu) per 100 mL.

2.6. Quantification of Host-Specific Fecal Markers

The Genomic DNA was extracted from membrane filters using the PowerSoil DNA isolation kit (Mo Bio, Carlsbad, CA, USA) using the manufacturers protocol. The extracted DNA was quantified using NanoDrop ND 2000C spectrophotometer (Thermo Scientific, Marietta, OH, USA) and stored at −25 °C until they were used for quantitative PCR (qPCR). The qPCR assays were performed to assess the universal (BacUni) and human (HF183 Taqman), avian (AV4143), and swine (Pig-2-Bac) [21–24] associated fecal markers. All the qPCR reactions were run in triplicates, and the final reaction volume used was 20 µL. The primers and probes, the methods used to determine the accuracy and efficiency of the standard curves, are described in Vadde et al. [16]. The qPCR standard curve for each essay was generated using a seven-point 10-fold serial diluted plasmid DNA. Samples collected at three time points (August, September, and October 2020) from each location were used to assess the detection frequency and the abundance of host-specific fecal markers.

2.7. Statistical Analyses

Statistical analyses were applied in R.v4.0.3 [25]. A one-way analysis of variance (ANOVA) was used to analyze potential differences between samples or between sampling periods (months) based on each sampled parameter. A two-way ANOVA was performed to determine whether the interactions of study area and month (time) had significant influences on each physicochemical and microbiological parameter. The correlation between physicochemical parameters, microbiological parameters, and land use percentages was analyzed using Spearman's non-parametric rank correlation test. Samples collected from five study areas in three months were pooled for this analysis. A principal component analysis (PCA) was applied to find the most influential factors of the dataset and reduce the complexity of a data dimensions with a minimum loss of the actual information [26]. The PCA was carried out using physicochemical, microbiological, and molecular data (host-specific fecal markers), except for DO and TC, for which data were not available from control locations on one occasion, and therefore, we removed these data from the PCA. The data were log-transformed and all analyses were applied in R v4.03. The Kaiser–Meyer–Olkin (KMO) and Bartlett's tests were applied on the data set prior to the PCA to ensure that PCA results well represent the effects of factors.

3. Results and Discussions

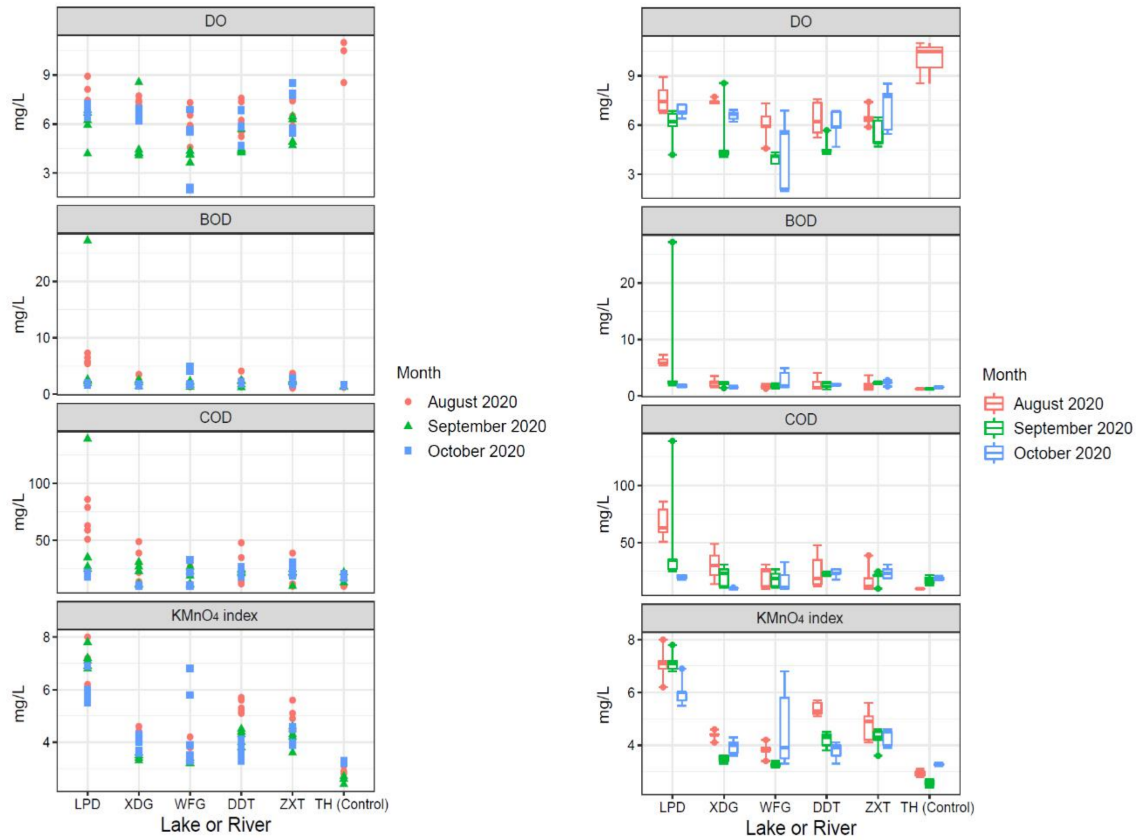
3.1. Physicochemical Results and Variation

Air temperature (AT) varied significantly between months at all sampling areas ($p < 0.01$; Tables S3–S8). Warmer temperatures were observed in August (31–35.8 °C) compared to September (24.4–35.0 °C) and October (20.7–24.4 °C). AT did not vary between sampling locations at each area. However, significant AT differences ($p < 0.001$) were observed between the sampling areas (Table S9). WT varied significantly between the months and the sampling areas ($p < 0.001$; Tables S3–S9). Warmer WT were observed in August (31–35.0 °C) compared to September (24.4–27.8 °C) and October (20.4–24.5 °C). The WT varied significantly between months sampled and the sampling areas; however, the values did not vary significantly between sampling locations in each river/lake (Tables S3–S7). The variations in temperatures between sampling areas in the same month might be caused by varied time on the sampling day.

The pH values ranged from 8.14 to 8.94 in LPD lake, and the values did not show any significant difference between the months sampled or between locations (Table S3). The pH values observed for the LPD lake were higher than those of values observed for the rivers, XDG (7.7–8.24), WFG (7.49–7.96), DDT (7.2–7.36), and ZXT (7.2–7.7) (Tables S4–S7). The pH varied significantly between the months and the sampling areas (Table S9). However, the values observed in all the sampling locations were within the guideline value (pH 6–9) of Ministry of Environmental Protection (MEP) of China for Surface water Class III. As reported in a previous study [27], the natural water bodies require a pH range of 6.5–8 to support the aquatic life.

EC varied significantly between months ($p < 0.01$; Tables S3–S7) at LPD, ranging from 371 to 429 µS/cm, and XDG, ranging from 305 to 430 µS/cm, but no significant monthly variation was observed ($p > 0.05$) for WFG (260–464 µS/cm) or DDT (310–521 µS/cm and ZXT (288–626 µS/cm). EC varied significantly between sampling areas and the months ($p < 0.001$); however, the interaction between both were insignificant ($p > 0.05$, Table S9). All EC values were below the pollution risk limits (1000 µS/cm) set by the international standards [27] and fell within the common range reported for the freshwater environments (10 to 1000 µS/cm) [28]. The conductivity in streams and rivers is affected by factors such as the presence of clay soils, bedrocks, and inorganic dissolved solids. The chloride, nitrate, and phosphate ions from the sewage or wastewater discharge were reported to increase the conductivity in water [29].

The DO, BOD, COD, and $KMnO_4$ values highly varied between the sampling areas. DO, COD, and $KMnO_4$ also changed with the month of sampling. Significant interactions between months and areas were observed in COD ($p < 0.05$) and $KMnO_4$ ($p < 0.001$) (Figure 2 and Table S9). No significant interactions were observed between five sampling locations in any of the rivers or lake ($p > 0.05$, Tables S3–S7). DO levels varied significantly between months in LPD, XDG, and DDT ($p < 0.05$, Tables S3, S4 and S6). Among the samples collected in three months, DO levels did not meet the MEP guideline values (≥5 mg/L) in at least one of the samples from LPD (4.19–8.92 mg/L, Table S3), XDG (4.07–8.55 mg/L, Table S4), and ZXT (4.69–8.51, Table S7). In WFG, DO ranged from 3.63 to 7.31 mg/L, with the average DO levels in September and October lower than the MEP guideline value (Table S5). In DDT, DO ranged from 4.26 to 7.58 mg/L. The average DO level in DDT in September was lower than the MEP guideline value (Table S6). The DO is the amount of oxygen that is present in water. Low levels of oxygen (hypoxia) or no oxygen levels (anoxia) can occur when excess organic materials, such as large algal blooms, are decomposed by microorganisms.



(**A**) (**B**)

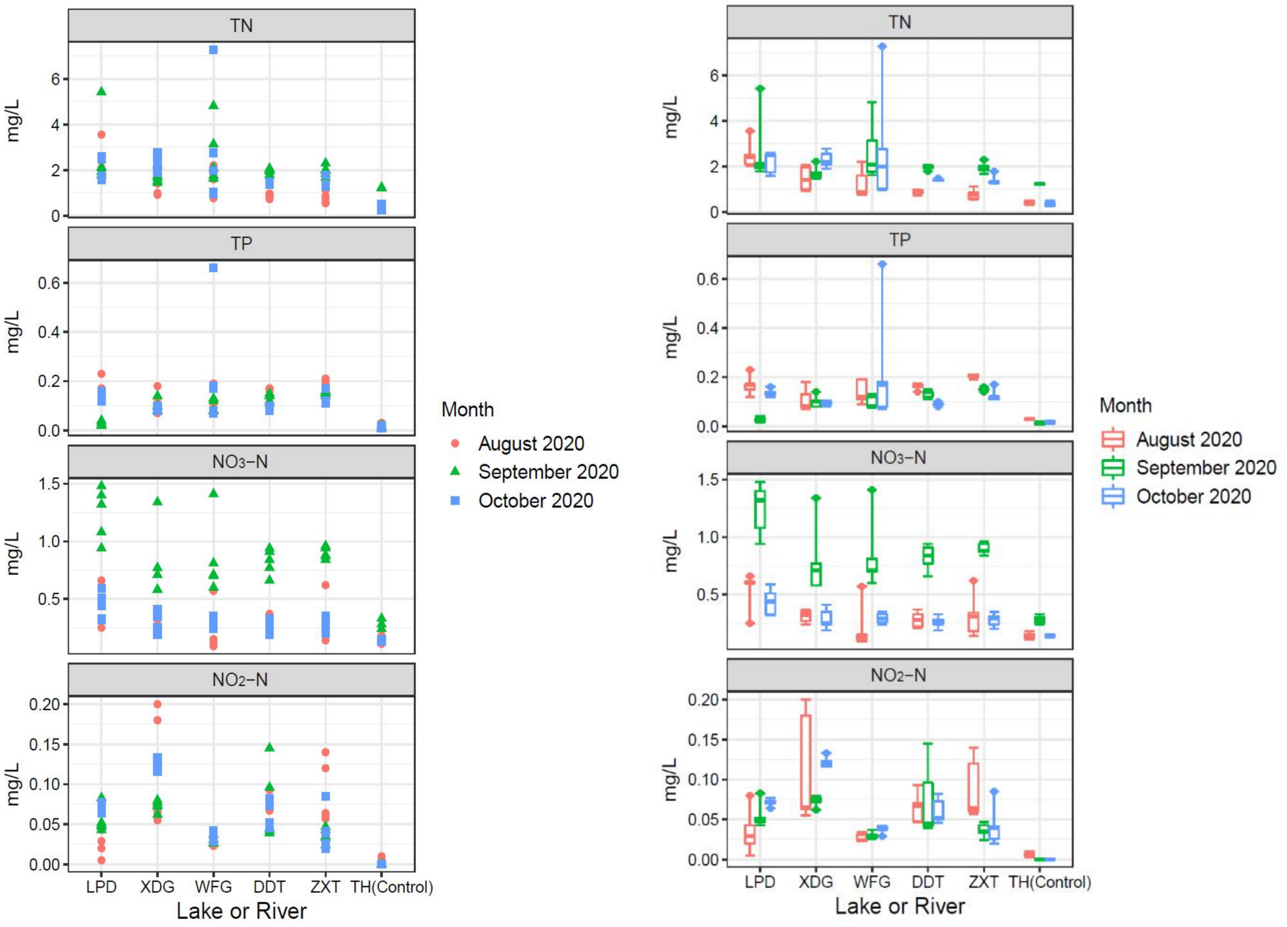
Figure 2. The variation in dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), and potassium permanganate ($KMnO_4$) values observed in different sampling locations and months. The individual value for each parameter (**A**) and boxplots (**B**) with minimum and maximum values (whiskers), median value (line within each box), and quartile interval (box) are shown.

BOD_5 varied significantly between sampling locations only in XDG and ZXT. No significant variations between months were observed for this parameter. BOD_5 levels were high in LPD (ranged from 1.6 to 27.2 mg/L), especially in August and September, when the average levels were above the acceptable limit set by MEP (≤4 mg/L, Table S3). Some sampling locations showed extremely high levels of BOD_5 up to 27.2 mg/L. The mean BOD_5 levels were in acceptable limit in all four rivers, namely XDG (1.4–3.5 mg/L), WFG (1.3–4.9 mg/L), DDT (1.2–4.1 mg/L), and ZXT (1.1–3.7 mg/L). Only a few samples had exceeded BOD_5 levels. Similar to BOD, the COD levels were also extremely high in LPD lake (10–49 mg/L) in August and September as compared to the MEP guidelines (≤20 mg/L). In all of the sampled rivers, exceeded COD levels were observed in at least two out of the three months. In terms of $KMnO_4$, significant variations between months sampled were observed in LPD ($p < 0.01$), XDG ($p < 0.001$), and DDT ($p < 0.001$). The average levels in LPD (5.5–8 mg/L) were higher than the MEP guideline value (≤20 mg/L) in August and September. The mean $KMnO_4$ levels were in the acceptable limit in all the rivers including XDG (2.2–4.6 mg/L), WFG (3.2–6.8 mg/L), DDT (3.3–5.7 mg/L), and ZXT (3.6–5.6 mg/L). In the aquatic systems, the oxygen demands results from the oxidation of organic matter or the chemical reactions. BOD_5 measures the oxygen consumed by the microorganisms while breaking down the organic matter in a five day period, whereas COD measures the amount of oxygen required for oxidation of total organic matter in a given sample [30], and $KMNO_4$ is used as an oxidizing agent in the COD measurement. These measures indicate the organic matter input in the water body, for which the primary sources include industrial wastewater and domestic sewage.

In general, varied TN levels were associated with both study areas and the months of sampling ($p < 0.01$ for both, Table S9). No significant interactions were observed between areas and months in terms of their relationships with TN ($p > 0.05$). For an individual lake or river, the TN values varied between the sampling locations and the values were higher than the MEP acceptable limit ($\leq$1 mg/L) in all the rivers/lake and nearly in all the months sampled (Figure 3, Tables S3–S7). The values were in the range for LPD (1.58–3.56 mg/L), XDG (0.92–2.78 mg/L), WFG (0.77–7.28 mg/L), DDT (0.73–2.07 mg/L), and ZXT (0.55–1.97 mg/L) during the sampling period, and the values significantly varied between months in three rivers (XDG, DDT, and ZXT). In general, the sources for high levels of TN in water bodies include runoff from agriculture, animal manure, discharge from wastewater treatment plants, and sewer leakage [31]. The NO_3-N values varied significantly with study areas and months ($p < 0.001$), with no interactions in between the two factors ($p > 0.05$, Table S9). NO_2-N varied with study areas ($p < 0.001$) but not with months sampled ($p > 0.05$); however, the interaction between NO_2-N and the study areas was dependent on the months (Table S9). The NO_3-N and NO_2-N levels were within the MEP acceptable limits ($\leq$10 mg/L and $\leq$0.15 mg/L, respectively) in all the locations, and significant monthly variations in both parameters were observed in LPD, WFG, and ZXT (Figure 3, Tables S3–S7). NO_3-N varied significantly with months in XDG ($p < 0.01$) and DDT ($p < 0.001$), while NO_2-N varied with sampling locations in DDT ($p < 0.01$). Furthermore, NH_4-N changed significantly with different areas ($p < 0.001$) and months ($p < 0.05$), but the interactions were not interdependent ($p > 0.05$, Table S9). The NH_4-N levels were within acceptable limit ($\leq$1 mg/L) in LPD and DDT; whereas, in the other three rivers, the values were outside the acceptable limit at least in two months during the sampling period (Figure 4, Tables S3–S7). Particularly in WFG, the NH_4-N levels were extremely high (0.54–6.82 mg/L) in October, which indicates the high pollution, particularly fresh wastewater entry into the river. All the above parameters were either in the range of the acceptable limit, or well below the acceptable limit or not detectable (ND) in the control locations (Figure 3 and Table S8).

TP varied significantly between months ($p < 0.05$) but not study areas ($p > 0.05$, Table S9). The interaction between TP and months was dependent on areas ($p < 0.05$, Table S9). PO_4-P, on the other hand, varied with both areas and months ($p < 0.05$ for both), and the interactions were significantly dependent on each other ($p < 0.01$, Tables S3–S7). Within each study area, no significant variation was observed in either TP or PO_4-P between sampling locations. Nonetheless, significant changes in TP between months were observed in LPD, DDT, and ZXT ($p < 0.001$ for all), and significant changes in PO_4-P between months were observed in LPD ($p < 0.001$) and DDT ($p < 0.01$). The TP (Figure 3) and PO_4-P (Figure 4) levels were higher than acceptable limits ($\leq$0.2 mg/L and $\leq$0.02 mg/L, respectively) at least in two months in LPD (TP ranged 0.02–0.23 mg/L, PO_4-P ranged ND-0.18 mg/L). Among all the study areas, PO_4-P levels were above the acceptable limit in XDG (0.06–0.15 mg/), WFG (0.06–0.53 mg/L), and DDT (0.06–0.15 mg/L) (Tables S4–S6). Previous studies showed that run-off from fertilized cropland, animal manure, and domestic sewage entry are likely causes for the increased level of TP in aquatic systems [6,24].

The TOC and the Chl *a* levels (Figure 4) varied significantly with both study areas and months ($p < 0.001$, Table S9). There were also significant interactions between the two relationships ($p < 0.001$). Within study areas, TOC varied significantly between sampling months (Tables S3–S7) in LPD (ranged 6.4–10.5 mg/L, $p < 0.001$), XDG (3.9–6.6 mg/L, $p < 0.001$), WFG (3.6–6.1 mg/L, $p < 0.001$), and ZXT (4.1–5.2 mg/L, $p < 0.05$). TOC also changed significantly with sampling locations in DDT (4.3–5.2 mg/L, $p < 0.05$) and ZXT ($p < 0.05$). Similar to other parameters including COD, BOD, and $KMnO_4$, TOC levels were higher in LPD compared to all four rivers. The Chl *a* level was extremely high in LPD lake (103.9–502.2 ug/L, Table S3) as compared to the four rivers (ranged from 3.46 to 63.2 ug/L, Tables S4–S7). In LPD and XDG, significant monthly variation in Chl *a* was observed (Figure 4, Tables S3 and S4) and the values correlate well with the temperature and nutrients (Figure 8). The high Chl *a*, indicate the high algal or phytoplankton growth in the lake/rivers. Warmer temperature and high amount of TP and other nutrients can contribute to the high algal growth [22,25].

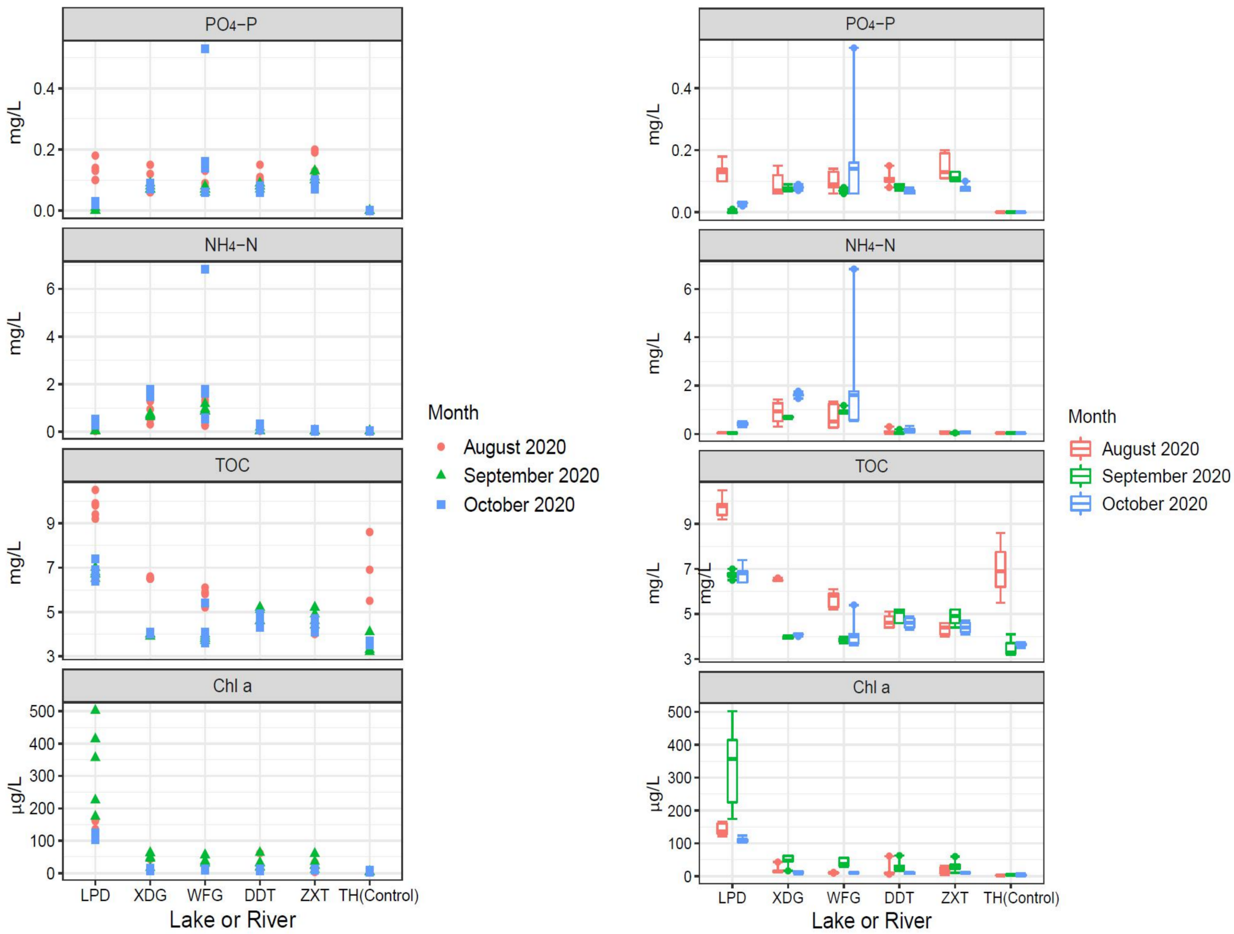


(**A**) (**B**)

Figure 3. The variation in total nitrogen (TN), total phosphorus (TP), nitrate-N (NO_3-N), and nitrite-N (NO_2-N) values observed in different sampling locations and months. The individual value for each parameter (**A**) and boxplots (**B**) with minimum and maximum values (whiskers), median value (line within each box), and quartile interval (box) are shown.

3.2. Microbiological Results and Variation

TC varied significantly between months (Tables S3–S7) at LPD ($p < 0.01$), ranging from 1.48 to 3.36 log cfu/mL, and at WFG ($p < 0.05$), ranging from 1.78 to 3.74 CFU/mL (Figure 5). No significant monthly variation was observed ($p > 0.05$) for DDT (1.18–3.60 log CFU/mL), ZXT (0.0–3.30 log CFU/mL), and XDG (0.00–2.71). TC varied significantly between sampling locations at XDG ($p < 0.05$) but not at LPD, WFG, DDT, and ZXT. Significant TC differences ($p < 0.001$) were observed among the sampling areas; however, the monthly variations and the interactions between both months and sampling areas were insignificant (Table S9). TC are common environmental microorganisms found in soils, water, plant materials, effluents, and also in the feces of warm blooded animals. Therefore, detection of only TC does not indicate the fecal contaminations; hence, FC or *E.coli* are used as indicators of fecal contaminations [32,33].



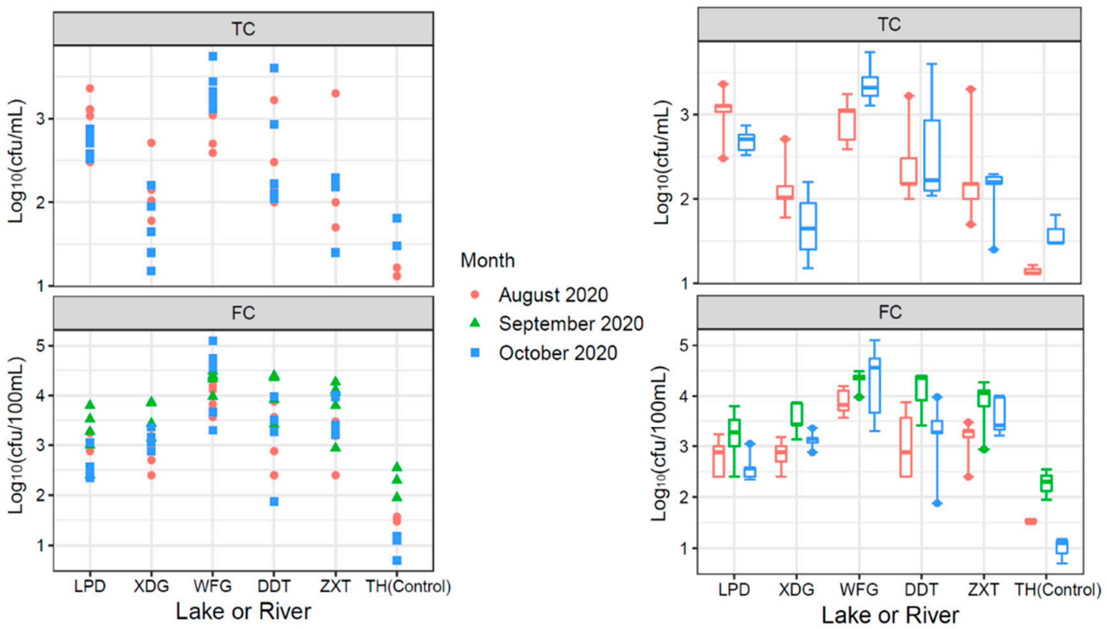
(**A**) (**B**)

Figure 4. The variation in phosphate (PO_4-P), ammonia-N (NH_4-N), total organic carbon (TOC), and Chlorophyll *a* (Chl *a*) values observed in different sampling locations and months. The individual value for each parameter (**A**) and boxplots (**B**) with minimum and maximum values (whiskers), median value (line within each box), and quartile interval (box) are shown.

The FC values varied significantly with the study areas ($p < 0.001$), and also sampling time ($p < 0.001$). No significant interactions were observed between months and areas (Table S9). The FC counts were in the range of 2.40–3.24 (LPD), 2.40–3.87 (XDG), 3.57–5.10 (WFG), 1.88–4.40 (DDT), and 2.40–4.27 (ZXT) log cfu/100 mL (Figure 5, Tables S3–S7). Significant changes between months were observed in XDG ($p < 0.01$) and DDT ($p < 0.05$). Notably high levels of FC counts were observed in WFG and DDT as compared to other sampling locations and control (Table S8). FC counts are used as guidelines for microbial water quality to assess fecal contamination. Although FC does not always correlate with the presence of pathogenic bacteria, a high concentration of FC indicates the impaired water quality and increased health risk associated with the presence of pathogenic microorganisms [34].

3.3. Molecular Microbiological Results and Variation

Fecal contamination in water systems is one of the major sources of pollution and exceeded nutrients. High concentrations of fecal pollution increase the probability of the existence of pathogenic bacteria. In all the four rivers (XDG, WFG, DDT, and ZXT) and the lake (LPD) investigated in this study, the FC was found exceeding the MEP of China class III standard in the water samples collected in at least one out of three months sampled. Class III standard refers to surface water in natural reserve areas centralized for source of drinking water, but mainly applicable for industrial and agricultural water. Therefore, it is particularly important to trace the host-specific source of the fecal pollution and take actions to prevent further damage to the water quality. Conventionally, FC and fecal indicator bacteria (FIB) are used as indicators; however, they were reported to grow and reproduce in non-fecal environments [35]. As a result, it was suggested that instead of using FC or FIB as the standards for fecal contamination, host-specific microbial source tracking (MST) genetic markers could be used. MST markers have considerably better specificity and sensitivity compared to traditional microbiological methods. Furthermore, MST markers can be host-specific, which means they indicate the abundance of fecal contamination from particular sources [36]. Quantification of these genetic markers now plays an important role in water pollution studies and environmental management. Studies have been conducted worldwide in different types of water bodies (rivers, lakes, and marine environments) using various host-specific markers to quantify the fecal contaminations. The *Bacteroides* 16S rDNA proved to be effective markers to quantify the host-specific fecal contaminations [6,21,22,24,37–41]. In our previous studies, we have successfully applied host-specific gene markers and high throughput sequencing methods to assess fecal contaminations in Taihu watershed [16,39].



(A) (B)

Figure 5. The variation in the total coliform (TC) and fecal coliform (FC) values observed in different sampling locations and months. The individual value for each parameter (**A**) and boxplots (**B**) with minimum and maximum values (whiskers), median value (line within each box), and quartile interval (box) are shown.

In the current study, we have successfully performed assays for four MST markers, targeting universal (BacUni) [21], human-associated (HF183 Taq) [18], avian associated (AV4143) [20], and swine-associated (Pig-2-Bac) *Bacteroidales* [19] markers. Both BacUni and HF183 Taq fecal markers were detected in high numbers at all sampling locations in rivers and lake, indicating that fecal contamination was high at all sampling locations investigated (Figure 6). BacUni and HF183 markers varied significantly ($p < 0.001$) between the sampling areas and the months sampled. The abundance of BacUni and HF183 showed the same trend, while AV4143 and Pig-2-Bac were negative in most of the samples and the levels were below the detectable limit (data not shown). The results indicated that human feces may be the main component of total fecal pollution in the investigated watersheds. There is a large number of possible causes of high fecal marker levels, for example, non-standardized or self-constructed domestic water drainage pipes, direct discharge of domestic wastewater, effluents from sewage treatment plants, industrial discharges, and non-point source pollution from farmlands [42]. As Wujiang has many rivers, canals, and lakes, the fecal pollution may flow from other rivers or lakes when precipitation is high or water levels are high [43].

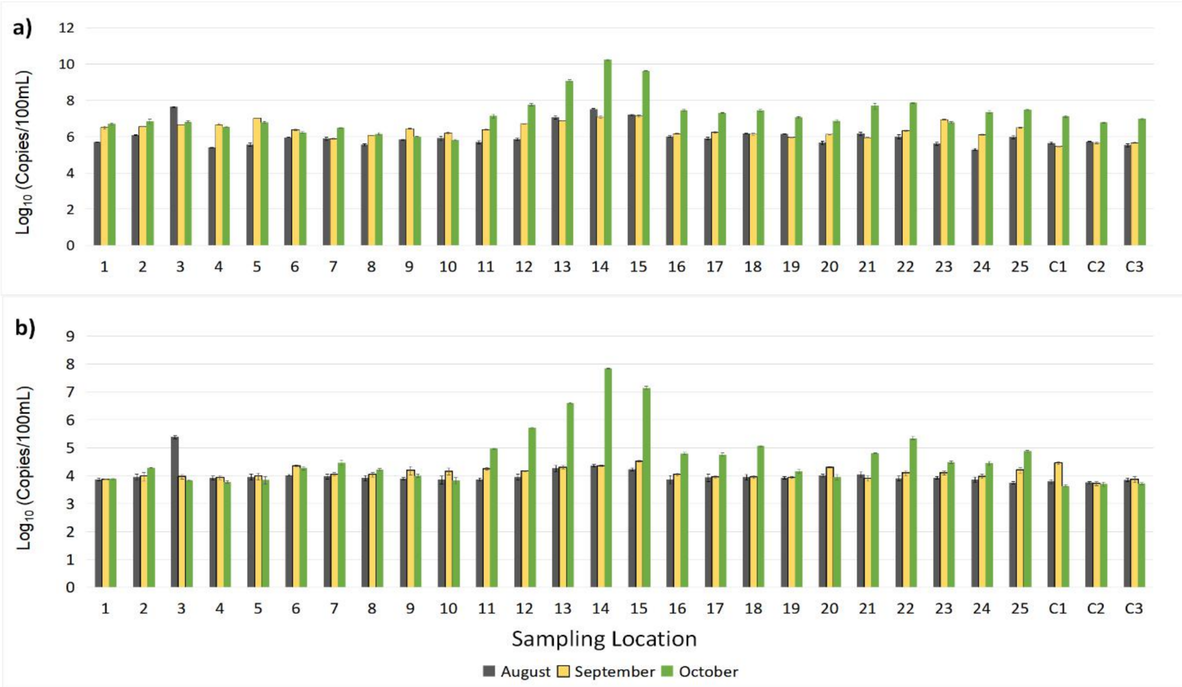


Figure 6. Variations of host-specific fecal MST markers in different locations. (**a**) BacUni. (**b**) HF183. Sampling locations 1–5, LPD lake; 6–10, XDG river; 11–15, WFG river; 16–20, DDT river; 21–25, ZXT river; C1-C3, Control locations.

Compared with our previous study at Tiaoxi River, the universal *Bacteroidales,* BacUni marker in current 25 sampling locations was slightly higher than the previous results, while the human-specific HF183 marker quantified was similar to the previous test results [16]. In October, both markers were detected in significantly high levels in WFG (sampling locations 11–15) as compared to other two months studied. During the sampling, significant bad odors was also observed at WFG (especially at sampling location 14), and we found out that some of the nearby factories were reopened for operation recently and the waste was directly discharged into the river. Combining the observations and land use patterns, the industries might be the major source for changes in the water quality and also fecal contaminations. On the other hand, it was not clear why one of the sampling locations in LPD (location 3) had high abundance of fecal markers in August. However, the surrounding land use (a large number of farmlands, a higher proportion of low-density residential areas than other nearby sampling sites) suggest that increase in the proportion of human-specific fecal pollution in the samples in that particular month in addition to industrial production, high temperature, and possible non-standardized domestic water drainage in the nearby villages in the same month may be one of the influencing factors. In addition, in October, there was also an increase in universal and human-associated fecal markers in all sampling sites of ZXT. Direct discharge of wastewater from a factory was observed near the sampling site 21, which did not occur in the first two months. Since it is not clear whether the effluent meets the standards, the pollution source of the site cannot be directly identified as factory discharge. However, the results were correlated with the physicochemical and microbiological properties. A large amount of black and oily matter also appeared on water surface at sampling location 21, resulting in the continuous occurrence of oily substances in the water surface between the sampling locations 21 and 22. This phenomenon happened at the same time with elevated levels of physicochemical parameters and might be related to the sudden rise of fecal pollution in ZXT.

3.4. Relationship between Water Quality and Land Use

The land use pattern of each sampling location in 1000 m radius is shown in Figure 7 and the details of each land use classification are shown in Table S10. The surrounding environment of each sampling areas is shown in Figures S1–S5. In LPD lake, the river and lake were the major land use (>50%), and the lake is surrounded by a significant proportion of agricultural land (11–13%) and low-density residential land (5.7% to 8%) (Figure 7). Many aquaculture farms and small-scale private animal farms were located around this lake. The canals/small rivers connected to this lake also appeared to be highly polluted. It is possible that pollution from aquaculture farms, small rivers, and private animal farms enter the lake, which causes the pollution to this lake.

The dominant land uses around XDG River are river/lake (43.32–58.77%), agricultural land (19.58–26.67%), followed by low density residential land (5.01–16.68%) (Figure 7). A few percentages of Class A/B/C industrial land and municipal utilities were also found to be present around this river. The main causes for pollution in this river could be run-off from the agricultural land and discharges from the residential areas, industries, and municipal utilities.

The land use patterns around WFG River are mainly Class A industrial land (38.7–59.3%), followed by public green land (12.66–32.32%) and road (11.06–12.75%) (Figure 7). A large part of the medium-density residential land (2.17–5.58%) and municipal utilities (0.02–2.69%) are also distributed around this river. Compared with other rivers and lakes, the surroundings of WFG are more urbanized. In October, the discharge of wastewater observed from factory during sampling, combined with the dramatic increase of the pollution indices in this month, indicate that the exceeding pollutant sources may came from improper discharge of raw sewage/wastewater, industrial emissions, and a small amount of domestic sewage, since there were limited places available for residence or livestock.

The main land types around the DDT River are agricultural land (8.04~22.92%), public green land (36.71~50.55%), low-density residential land (7.27~11.25%), class B industrial land (0.49~16.81%) and rivers and lakes (6.25~12.07%). As shown in Figure 7, land use composition of DDT is complex compared to other rivers and lakes. DDT had lower DO; it may be due to the increase of residential land and industrial land. In this case, industrial and residential discharge may reduce the oxygen dissolved in the water. In addition, agricultural land contributes a lot to DDT land use. Therefore, agriculture runoff may lead to the increased phosphorus content and FC in water sample observed in this river. Therefore, agricultural runoff and industrial and residential discharge were probably the main causes of pollution in this river.

The main land use near ZXT river is public green land (6.47~26.1%), agricultural land (8.04~22.92%), and low-density residential land (7.27~11.25%). Therefore, the main sources for increased nutrients such as TP and NH_4-N may be from the fertilization of lawns, farm runoff, animal feces, and domestic sewage near the river that can led to eutrophication of the water bodies.

3.5. Correlation Between Parameters

Spearman's correlation analysis show significant correlations among the physicochemical and microbiological parameters and certain land use patterns (Figure 8; Figures S6–S8). EC was positively correlated with nitrogen-related pollution (TN, NO_3-N), percentages of class B industrial land, municipal utilities, and low-density residential land. It was negatively correlated with percentages of class A industrial land and road. DO was found negatively correlated with a number of the pollution factors including nitrogen-related parameters (TN, NH_4-N, NO_3-N) and fecal markers (FC, BacUni, HF183). High percentages of low-density residential land, river, and lake were found positively related with high DO. The parameters KMnO4, TOC, COD, and BOD were positively correlated with each other.

This group of parameters represented reductive pollutions and organic matter in water. There was a trend that these parameters increased with AT, WT, pH, and also percentages of river and lake around the sampling locations.

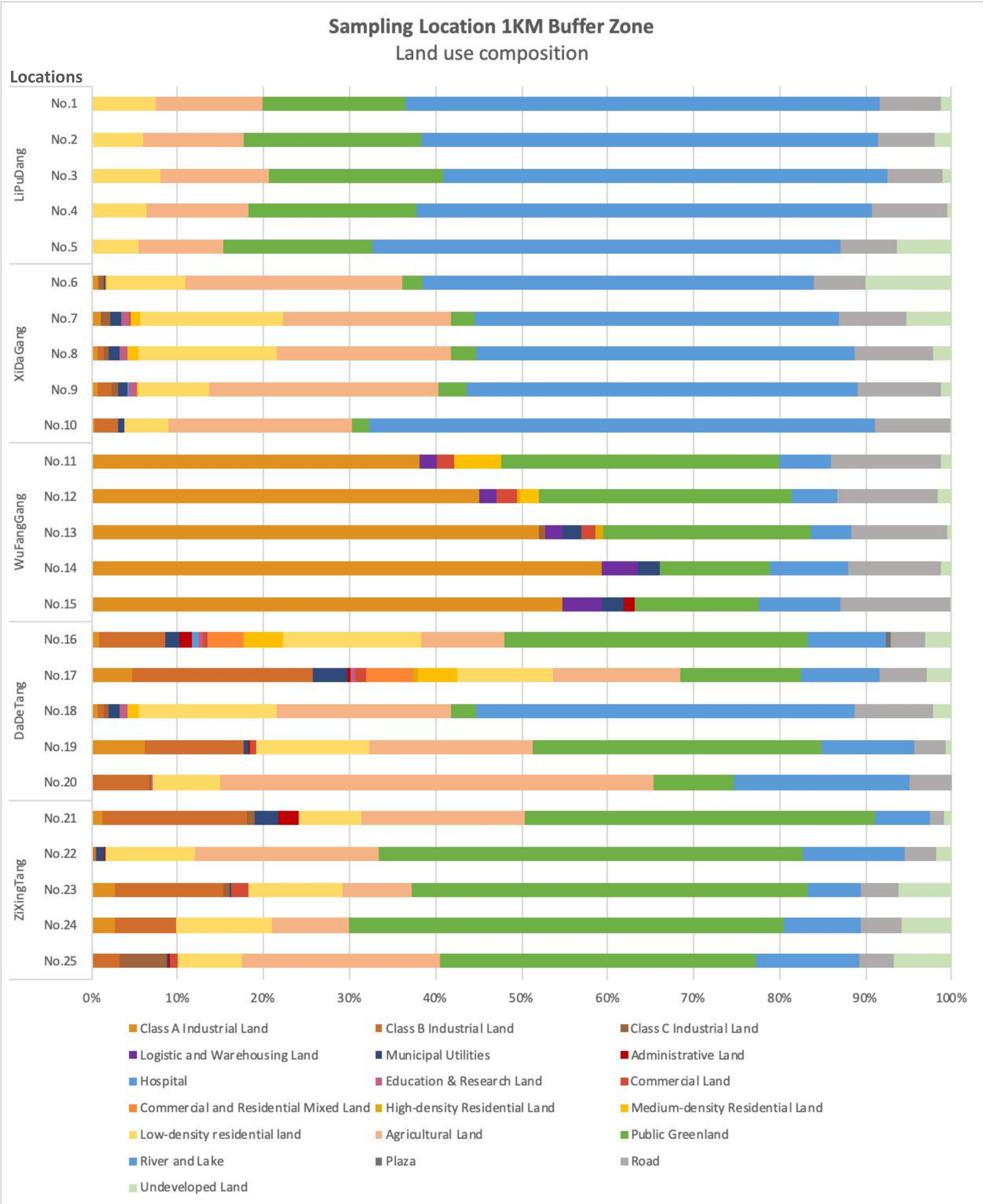


Figure 7. Land use pattern of 1 Km buffer zone around 25 sampling locations. Different colors indicate corresponding land use classifications. Location number 1–5, LPD lake; 6–10, XDG river; 11–15, WFG river; 16–20, DDT river; 21–25, ZXT river.

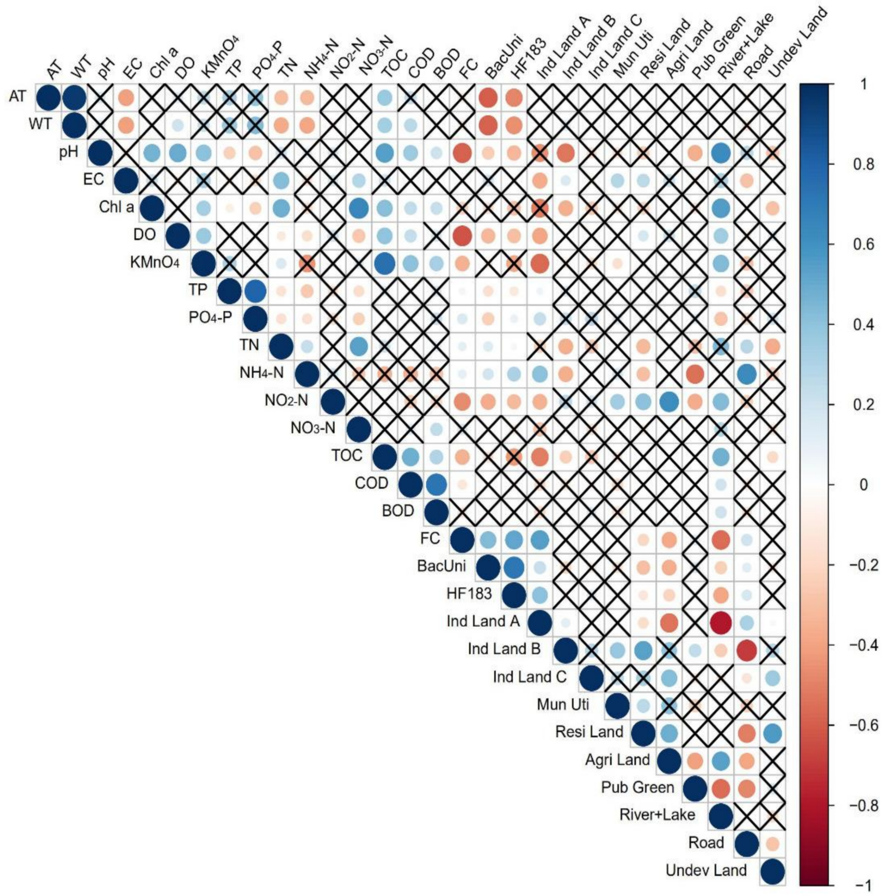


Figure 8. Spearman's correlation map of the physicochemical, microbiological, and land use pattern percentages. Each correlation was presented by one colored circle in the figure. The blue color indicated positive correlations, while the red color indicated negative correlations. Depth of the colors and sizes of the circles indicated strengths of the correlations. Non-significant ($p > 0.05$) correlations were presented by a cross on the circle. The control samples were not included. TC was excluded due to unavailability of the September data. (Ind Land A, B, C—Class A, B, C Industrial Land, respectively; Mun Uti—Municipal Utilities; Resi Land—Residential Land; Agri Land—Agricultural Land; Pub Green—Public Green Land; Undev Land—Undeveloped Land).

TN and NH_4-N were found negatively correlated with temperatures. TN was positively correlated with NH_4-N and NO_3-N, which might be the two main sources of TN in the water samples. TN and NH_4-N were both negatively correlated with class B industrial land and low-density residential land, while both of them were positively correlated with percentages of road. NH_4-N positively correlated with class A industrial land. NO_2-N, on the other hand, was positively correlated with municipal utilities, low-density residential land, agricultural land, and river and lake. TP and PO_4-P was strongly and significantly correlated, indicating phosphate could be a main source of TP. In the current study, TP and PO_4-P were negatively correlated with pH, Chl *a*, TN, NH_4-N, NO_2-N, and percentages of river and lake. TP and PO_4-P were found positively correlated with Class A industrial land. Chl *a* is the indicator for algal growth in the watersheds. Chl *a* levels were positively correlated with reductive pollutions ($KMnO_4$, COD, BOD), nitrogen-related pollution (TN, NO_3-N), and organic matters (TOC) in water. High Chl *a* was also found related to high river and lake percentage around the sampling locations. Class B industrial land and undeveloped land were negatively correlated with Chl *a*.

Fecal contaminations were indicated by parameters including FC, BacUni, and HF183. According to the correlation analysis, firstly there was a strong correlation among these three measurements. In addition, these parameters were negatively correlated with pH, DO, and NO_2-N. They were also positively correlated with TN and NH_4-N. Class A industrial land and road were the land use pattern that might be related to this type of pollution. Low-density residential land, agricultural land, and river and lake were found to be negatively correlated with fecal markers. It is important to note that the correlations described above are an interpretation of the results from the correlation analysis. The positive or negative correlation does not lead directly to cause and effect relationships. The positively correlated land use and water quality does not mean that the land use caused those pollution parameters; therefore, the correlation results should be used with caution.

In general, we observed high percentages of river and lakes correlated with high organic matter (TOC, COD, BOD, Chl *a*, $KMnO_4$), high NO_2-N, but also high DO, and they correlated negatively with fecal contamination. Road percentages positively correlated with TN, NH_4-N, and also fecal contamination factors. The municipal, low-density residential and agricultural lands positively correlated with NO_2-N but negatively with fecal contamination. As study areas are located in rural and semi-rural areas of Wujiang District, the majority of low-density residential land is villages. It is different from low-density residential areas in urban-settings that are well-designed, fully paved, high-quality single-detached housing neighborhoods. Villages are mostly un-paved and scattered in the surrounding agricultural land, and these lands have very few human uses. Additionally, with their permeable surface, the pollutants are possibly absorbed by the soil and underground water. In contrast, the roads are all paved and impermeable, and the run-offs along the roads are generally considered primary sources of pollution to the surface water; therefore, positive correlation between roads and fecal contamination factors was observed in this study.

Class A industrial land correlated positively with PO_4-P, NH_4-N, and fecal contamination. Although this class was described as non-pollution industry, improper treatments of industrial water and direct discharge of domestic water might also reduce water quality. Class B industry correlated positively with EC and negatively with some physicochemical parameters (TN, NH_4-N, TOC, Chl *a*). Weak correlation was observed with Class C industries, and these types of industries occupied small percentages around the sampling locations. The industrial lands (Class A, B, C) in China are classified in accordance to the level of disturbance to the surrounding living conditions (residential and public facilities) rather than to the level of pollution of surface water. Class A industrial land generally includes industries such as retail, financial businesses, publishing, and high-tech industries with relative higher number of working populations. In this regard, although Class A industrial land by definition is supposed to be "non-pollution" industries, it is among the most heavily used land areas, which also require careful treatment of industrial and domestic water discharge.

3.6. Multi-factor Analysis—Principal Component Analysis (PCA)

The KMO test shows the distribution of a data set, and it should show values higher than 0.5, otherwise the data set is not considered as suitable for PCA analysis [44]. Bartlett's test shows whether the parameters in a data set are inter-dependent. A data set should be inter-independent ($p < 0.05$) if the data set is to be used in a PCA [44]. In the current study, the KMO analysis showed a result of 0.6386, while the Bartlett's test resulted in $p < 2.2 \times 10^{-16} < 0.05$. These tests are indicating that the PCA test would be a suitable testing method for the current data set, reducing redundant affects by correlated parameters and for analyzing the main influencing parameters.

As shown in Table 1, the PCA resulted in four principal components, which account for 83.27% of the total variations. According to Liu et al. [45], the loadings of each variable could be classified as strong (>0.75), moderate (0.75–0.5), and weak (0.5–0.3). In the analysis of the present study, no strong loading was observed. The moderate and weak loadings observed are shown in bold in Table 1. The first component (PC1) was responsible for 45.69% of the total variations observed, and it was composed of moderate loadings of data for fecal coliform (FC) and the MST markers, BacUni, and HF183 on the positive side, and also a weak positive loading for the MST marker, HF183. These results indicate that fecal contamination is the major source of variations observed in this study. The factors such as improper discharge of domestic drainage and untreated sewage might be responsible for high levels of fecal contaminations observed in the locations. The second component (PC2) was also explained by the same variables as PC1. However, two more variables, Chl *a* and PO_4-P, had negative and positive weak loadings, respectively. These results showed that except for the influences of fecal-contamination related factors, Chl *a* and PO_4-P were also the main reasons for the changes. These factors are usually related to nutrient pollution, which were probably from domestic waste and agricultural non-point source pollution [46]. Chl *a* is the main indicator of algal/phytoplankton growth and also eutrophication [47]. PO_4-P pollution are mainly from soil erosion, agriculture run-off, and municipal and industrial discharges [48]. As for the component PC3, Chl *a* had a weak positive loading, while NH_4-N had a moderate negative loading. Similarly, this component could be explained by nutrient pollution. The PC4 is a combination of fecal contaminations and the pollution by multiple nutrients. Both NH_4-N and Chl *a* had moderate negative loadings, while FC had a weak positive loading. In summary, the most influential factors are FC and universal and human-associated fecal contaminations. In addition to that, PO_4-P and NH_4-N were the most impacting nutrient parameters observed in this study. High Chl *a* was a result of various environmental factors including high nutrients, temperature, and sunlight. The overall correlation analysis showed significant correlations between Chl *a* and multiple nutrients including TN, NO_3-N, and TOC (Figure 8). However, in the special case of extremely high Chl *a* level observed in LPD, no correlation was observed between the chlorophyll level and any specific nutrients based on the overall data.

Table 1. Loadings of physicochemical and microbiological variables resulting from PCA analysis. The four principal components (PC1 to PC4) are shown, and the values in bold indicate moderate or weak loadings.

Parameters	PC1	PC2	PC3	PC4
WT	−0.011	0.007	−0.020	0.010
pH	−0.011	0.013	0.008	−0.019
EC	0.017	−0.024	0.043	−0.008
BOD	0.023	−0.043	0.180	−0.088
COD	−0.005	−0.055	0.218	−0.079
$KMnO_4$ index	0.012	−0.052	0.100	−0.062
TN	0.086	−0.086	0.071	−0.206
TP	0.129	−0.191	−0.068	0.049
NO_3-N	0.034	−0.199	0.214	−0.150
NO_2-N	−0.110	0.209	−0.172	−0.112
PO_4-P	−0.117	**0.312**	0.231	0.173
NH_4-N	0.268	−0.057	**−0.698**	**−0.509**
TOC	−0.014	−0.041	0.081	−0.069
Chl *a*	0.025	**−0.396**	**0.445**	**−0.577**
FC	**0.546**	**−0.544**	−0.034	**0.491**
BacUni	**0.591**	**0.461**	0.265	−0.182
HF183	**0.477**	**0.313**	0.079	0.034
Eigenvalue	1.631	0.640	0.394	0.308
% of variance	45.69	17.93	11.02	8.63
Cumulative % of variance	45.69	63.61	74.64	83.27

Figure 9 shows the distribution of all samples on the two-dimensional coordinate system composed of PC1 and PC2, which accounts for 63.61% of total variations. As shown in the figure, most of the factors including pH, EC, WT, TOC, COD, BOD, and $KMnO_4$ index were not the main cause of variations observed. However, TN, TP, NO_3-N, and NO_2-N had weak loadings in the variations. The parameters Chl *a* and PO_4-P were more influential on PC2-axis, with different orientations. The parameters, BacUni, HF183, FC, and NH_4-N significantly contributed to PC1. In terms of lakes and rivers, the control locations were not similar to any of the study sites. Similar distributions of nutrient contents were observed in the lake and three rivers (XDG, DDT, ZXT). Compared to the control, these water bodies were higher in most of the nutrients such as TN, TP, NH_4-N, NO_3-N, and Chl *a*, and also fecal contamination parameters like FC and host-specific MST markers. Compared with other rivers and the lake, although WFG had relatively low PO_4-P and NO_2-N, it was largely affected by high fecal contamination levels, especially in the samples collected in October 2020.

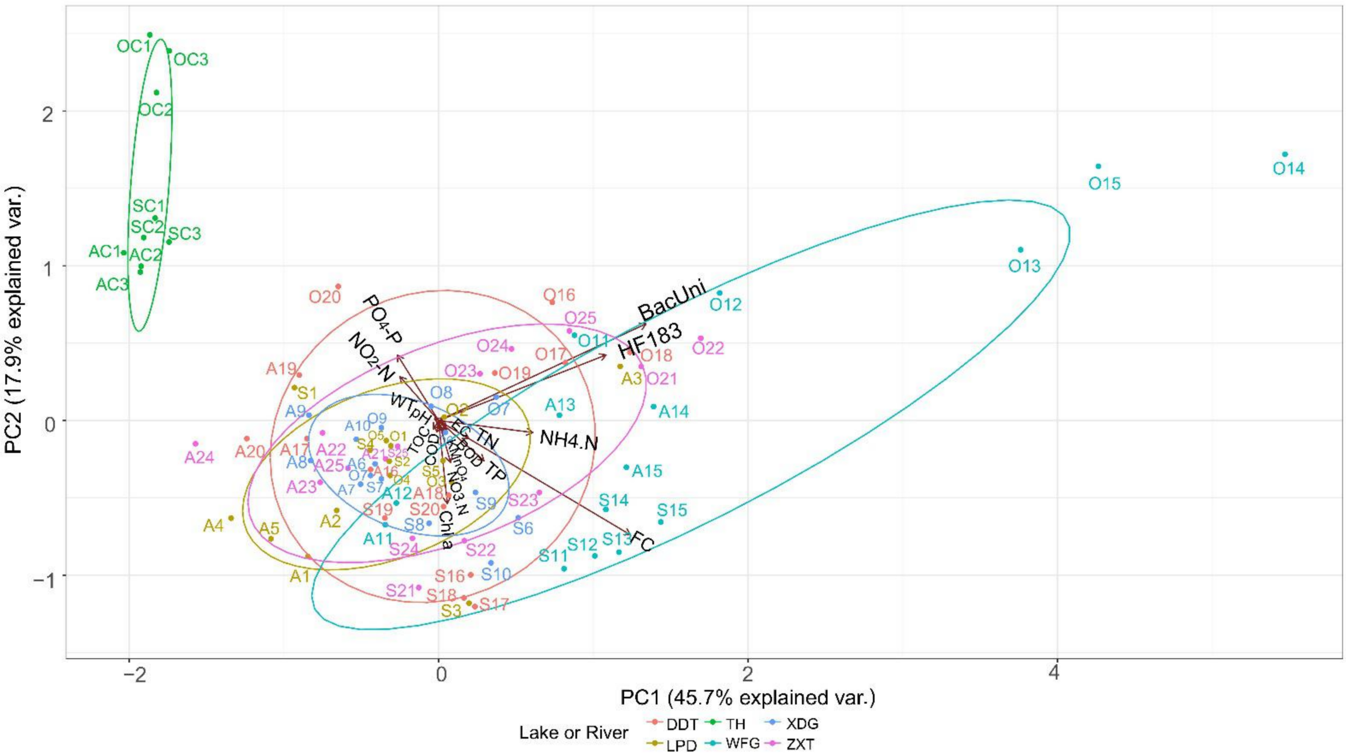


Figure 9. The principal component analysis (PCA) of physicochemical and microbiological variables observed in this study. The labels indicate both sampling locations and sampling time. The locations are indicated by the numbers (1–25 for rivers/lake, C1–C3 for control locations), while the sampling time are indicated by the first letter (A = August, S = September, O = October). Each lake or river is grouped by the circle and marked with different colors. Length of the respective arrow shows the proportion a factor explains for the total variation.

4. Conclusions

In this study, the water quality of four rivers (XDG, WFG, DDT, ZXT) and a lake (LPD) in Wujiang District was analyzed and compared with control locations and Chinese MEP guideline values. The correlations among the physicochemical, microbiological, and fecal markers and the principal components that affect the variations and the influence of all these parameters by land use pattern were also studied. The overall results indicated that several parameters, including multiple nutrients and fecal indicators, in each watershed exceeded the acceptable limit. As compared to control locations, all five study areas in Wujiang were affected by various pollution sources. The lake, LPD, and the river, WFG, were significantly affected by pollutants. The high levels of nutrients led to the overgrowth of algae in LPD, which was evident from the extremely high concentrations of Chl *a* in this lake as compared to other watersheds. In WFG, high levels of NH_4-N, FC, and both universal and human-feces specific markers were observed. The FC and BacUni and HF183 were detected in all the sampling locations; however, their abundance varied between watersheds, and high levels were evident in WFG river. The Class A industrial land and the road percentages showed positive correlation with PO_4-P, NH_4-N, and fecal contaminations. Although Class A industrial land is described as non-pollution industry, improper treatments of industrial water and direct discharge of domestic water might also reduce water quality. Moreover, identifying the type of industries located along the rivers might be helpful for the future studies. In this study, the sampling was done for three months (August to October 2020) to assess the current levels of pollution in these watersheds. However, longer-term studies including sampling at different seasons are necessary to make stronger conclusions and for better management and control of pollution in these watersheds.

Supplementary Materials: The following are available online at https://www.mdpi.com/2073-4441/13/4/457/s1: Supplementary Tables S1 to S10, Supplementary Figures S1 to S8. Table S1. Sampling locations in the rivers and the lake, including the geographic coordinates (GPS coordinates), Table S2. The methods used for measuring or testing the physicochemical parameters, Table S3. Physicochemical and microbiological characteristics of LiPuDang (LPD) with statistical analyses, Table S4. Physicochemical and microbiological characteristics of XiDaGang (XDG) with statistical analyses, Table S5. Physicochemical and microbiological characteristics of WuFangGang (WFG) with statistical analyses, Table S6. Physicochemical and microbiological characteristics of DeDeTang (DDT) with statistical analyses, Table S7. Physicochemical and microbiological characteristics of ZiXingTang (ZXT) river with statistical analyses, Table S8. Physicochemical and microbiological characteristics of samples collected from Taihu lake source water protection area (Control locations) with statistical analyses, Table S9. Two-way ANOVA results for physicochemical and microbiological parameters based on the interactions between study area and sampling time, Table S10. The specific explanations of each land use classification, Figure S1. Surrounding environment near LiPuDang (LPD) sampling locations, Figure S2. Surrounding environment near XiDaGang (XDG) sampling locations, Figure S3. Surrounding environment near WFG sampling locations, Figure S4. Surrounding environment near DaDeTang (DDT) sampling locations, Figure S5. Surrounding environment near ZiXingTang (ZXT) sampling locations, Figure S6. Spearman's correlation map of the physicochemical, microbiological and land use pattern percentages (if applicable) for (a) LPD and (b) XDG, Figure S7. Spearman's correlation map of the physicochemical, microbiological and land use pattern percentages (if applicable) for (a) WFG and (b) DDT, Figure S8. Spearman's correlation map of the physicochemical, microbiological and land use pattern percentages (if applicable) for (a) ZXT and (b) Control area.

Author Contributions: Conceptualization: R.S. and X.J.; methodology: R.S., S.L., J.L., and X.J.; field sampling: R.S., S.L., X.F., W.L., Z.G., and S.G.; formal analysis: S.L., Z.G., X.F., S.G., W.L., and J.L., investigation: R.S., S.L., and Z.G., resources: J.S. and Y.Z., data curation: R.S. and S.L., writing—original draft preparation: R.S., S.L., Z.G., X.F., and S.G., writing—review and editing: R.S. and S.L.; supervision: R.S., project administration: R.S.; funding acquisition: R.S. All authors have read and agreed to the published version of the manuscript.

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