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Unveiling the Dynamics of *Cryptosporidium* in Urban Surface Water: A Quantitative Microbial Risk Assessment and Insights into Climatic and Seasonal Influences

Hazrat Bilal [1,*], Xiaowen Li [1,*], Muhammad Shahid Iqbal [2], Roberto Xavier Supe Tulcan [3] and Madan Thapa Chhetri [4]

[1] State Key Joint Laboratory of Environmental Simulation and Pollution Control, School of Environment, Beijing Normal University, No. 19, Xinjiekouwai Street, Beijing 100875, China
[2] International Water Management Institute (IWMI) Multan, Multan 53700, Pakistan
[3] Laboratory for Earth Surface Processes, Ministry of Education, College of Urban and Environmental Sciences, Peking University, Beijing 100871, China
[4] Department of Geosciences, Florida Atlantic University, Boca Raton, FL 33431, USA
* Correspondence: hbilal2022@fau.edu (H.B.); lixw@bnu.edu.cn (X.L.)

Abstract: In response to global urbanization and economic development, urban surface water pollution has become a universal challenge and particularly affects densely populated megacities, and Dhaka is no exception. The discharge of 98% of untreated domestic sewage and massive volumes of industrial wastewater from over 7000 industries escalate surface water crises. This study investigates microbial and fecal contamination with particular emphasis on *Cryptosporidium* in surface water, known for causing waterborne diseases, such as cryptosporidiosis. Findings reveal high *Cryptosporidium* oocyst concentrations and fecal contamination in various water bodies in Dhaka City. Among the investigated water bodies, the Buriganga River exhibits the highest *Cryptosporidium* oocyst concentration (46%), while the Balu River, Turag River, Shitalakkhya River, Dhanmondi Lake, Gulshan Lake, Banani Lake, Ramna Lake, and Crescent Lake also present high levels of oocyst concentrations ranging from 21–40%. This study also calculated infection risks and found that the infection risk of swimming is highest during the wet season and is $(3.9 \pm 2.2$ (95% CI: 3.0–5.0)) $\times 10^{-2}$ per swimming event, whereas it is approximately $(2.4 \pm 1.9$ (95% CI: 1.6–3.3)) $\times 10^{-2}$ during the dry season. Annual diving risks are approximately $(1.2 \pm 0.6$ (95% CI: 0.9–1.4)) $\times 10^{-2}$, indicating considerably high risks. Most of the sampling sites generally show significantly higher risks than other study areas like the Mymensingh and Kushtia Districts. In light of these results, we strongly recommend immediate measures to address water quality issues and mitigate the risks associated with *Cryptosporidium* contamination in Dhaka's surface water.

Keywords: water quality assessment; public health; ecological risk assessment; health risk assessment; *Cryptosporidium*

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1. Introduction

Water is a major transmission source of various parasites, and *Cryptosporidium* stands out as a prevalent protozoa responsible for intestinal infections in children and adults worldwide [1]. *Cryptosporidium* is pervasive in aquatic environments, ranging from untreated water sources to wastewater and even treated water [2]. Disease transmission typically occurs through contaminated water and food consumption, with water acting as an important vector for large-scale epidemics that propagate waterborne infections [3]. *Cryptosporidium* is the second most common cause of severe gastroenteritis in children, trailing only behind rotavirus. It contributes to a considerable number of deaths among children under the age of five worldwide and causes an estimated 2.55 million deaths annually [4]. While infections in healthy individuals often manifest with mild symptoms or

none at all, those in individuals with compromised immune systems, particularly in those with HIV infections, may present as severe, life-threatening diarrhea and cause mortality rates of up to 70% [5].

While most diarrhea cases can be prevented through improved sanitation and the provision of clean drinking water, developed countries still grapple with waterborne disease outbreaks despite the remarkable investments in sanitation infrastructure and water quality regulations. In 2019, Europe reported 10,739 confirmed cases of *Cryptosporidium*, highlighting persistent challenges [6]. *Cryptosporidium* oocysts in drinking water can survive a wide range of temperatures, but survival can be significantly prolonged at cooler temperatures [7,8], thus posing a challenge to conventional treatment methods to clear these resilient parasites from surface water [9]. Common disinfectants, such as chlorine at regular concentrations, prove ineffective against *Cryptosporidium* oocysts [10]. Consequently, even after treatment, *Cryptosporidium* may persist in water resources and pose a risk of spreading in water resources, particularly in urban settings [11].

In recent times, the river systems in Bangladesh have witnessed increased pollution attributed to rapid population growth, unregulated development along riverbanks, urbanization, unplanned industrial expansion, and agricultural activities. In addition to contamination from industrial sources, surface water experiences widespread pollution from human feces due to generally inadequate sanitation practices [12]. Buriganga River, one of the country's most polluted waterways, grapples with severe pollution issues from multiple sources. Chemical waste from industries, medical facilities, household garbage, sewage, plastics, deceased animals, and occasional oil spills from boats and other river transport systems all contribute to its contamination. Additionally, Dhaka City discharges over 4500 tons of solid waste into the Buriganga River daily [13]. The most common ailments reported are as follows: diarrhea (56.5%), skin diseases (31.1%), cholera (11.1%), and malaria (2.2%). Hence, diarrhea and skin diseases are the prevailing diseases in the Buriganga River area [14]. Various factors have deteriorated the water quality of the Buriganga River, adversely affecting its aquatic ecosystems. There is significant concern regarding certain key water parameters, including pH, BOD, DO, and chloride levels, as they consistently do not meet the environmental standards established by the Department of Environment, Bangladesh [15]. In recent years, the Buriganga River has faced an alarming crisis, escalating its status to Bangladesh's most polluted river [14]. The primary culprits behind the pollution of the Buriganga River are anthropogenic factors, including the rapid urbanization of Dhaka City, industrial revolution, population density, and climate change effects. The convergence of these elements has rendered the Buriganga River more polluted than many other rivers in the region [16]. The present study, founded on recent data and references, aims to elucidate the complex interplay of factors leading to water pollution in Bangladesh's river systems. Its overarching aim is to raise awareness, inform policymakers, and guide potential interventions for sustainable water management and pollution control measures in the region.

2. Material and Methods

2.1. Study Area

Dhaka city is centrally located in Bangladesh at a latitude of 23°42′ N and a longitude of 90°22′ E on flat, low-lying land near sea level [17]. The city, bounded by the Buriganga, Shitalakshya, Turag, and Balu rivers and Tongi Khal, is divided into two municipalities: Dhaka South City Corporation with 75 wards and Dhaka North City Corporation with 54 wards [18]. According to population statistics from 2021, Dhaka's urban areas have an estimated population of 21.5 million, indicating a high population density of 47,400 residents/km^2. The city has a humid tropical climate with an average temperature of 26.1 °C [19]. Its central and southern parts are extensively urbanized, whereas low-lying areas characterize its periphery. Its climatic conditions result in three distinct seasons: winter (November–February), premonsoon (March–May), and monsoon (June–October).

This study focuses mainly on the premonsoon season, characterized by light rain and extreme heat with maximum air temperatures reaching 40 °C.

2.2. Sample Collection

A total of 179 water samples were systematically collected from the locations mentioned above (Figure 1). The samples were meticulously categorized based on their sources. Sterilized plastic containers were used for collection, with each container uniquely identified by a key number to ensure the integrity of the samples. Pertinent details, including date, area, and type, were diligently recorded and associated with the corresponding key number for comprehensive documentation.

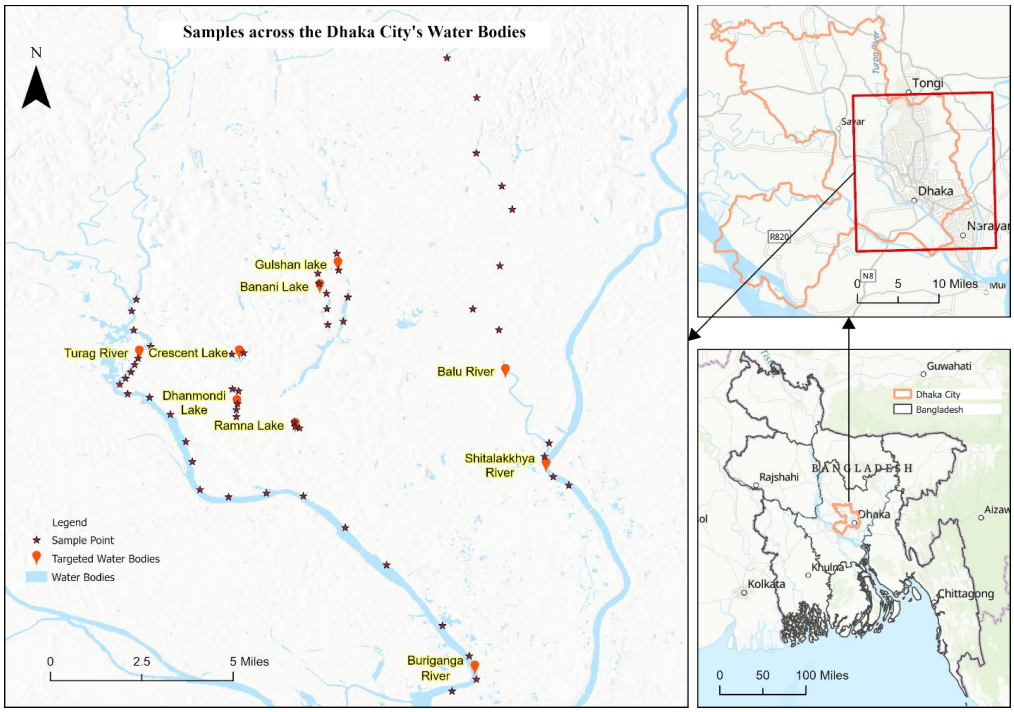

Figure 1. Targeted water bodies and sampling points in Dhaka City.

2.3. Sample Processing

All the samples were handled and transported to the microbiology and public health lab at Khulna University, according to established protocols of the U.S. EPA. The time lapse between sample collection and filtration can impact results due to potential sample degradation. Therefore, sample elution was commenced within 48 h of collection. To preserve water samples containing *Cryptosporidium* oocysts, we followed the established protocols (U.S. EPA Method 1622) by promptly chilling all samples. Samples collected earlier in the day were pre-cooled in a cooler to maintain temperatures between 1 °C and 10 °C.

Conversely, samples collected later were chilled overnight in a refrigerator to minimize ice melting during transportation [20]. The water samples collected underwent filtration using Whatman filter paper (size 42). The resulting filtrate containing residual contaminants, including parasites, was then pipetted into small 50 mL bottles. They were subsequently transferred into 10 mL tubes and centrifuged at 800 rpm for 15 min. After centrifugation, the supernatant was discarded, and the resulting pellets were carefully moved to new Eppendorf tubes, which underwent a second round of centrifugation at an increased speed of 14,000 rpm for 10 min. Following this, the upper layer was removed, and a 250 µL pellet was selected from the remaining residue for DNA extraction, following the manufacturer's protocol as outlined in the manual.

2.4. DNA Extraction

DNA extraction was performed using the deoxyribonucleic acid (DNA) Zol (Trizol) method with minor adjustments. Initially, 200 µL of the samples were mixed with 250 µL of DNA Zol, followed by thorough vortexing and incubation at room temperature. After 5 min, 150 µL of isopropanol was added to the mixture, which was then centrifuged at 7000 rpm for 10 min. The resulting DNA pellet at the bottom was carefully collected. Subsequently, the pellet was treated with 125 µL of DNA Zol and centrifuged again at 7000 rpm for 5 min. The supernatant was discarded, and 150 µL of 70% ethanol was added to the nucleic acid-containing pellet. Following another centrifugation at 7000 rpm for 5 min, the supernatant was removed, and the DNA washing process was repeated. The tubes were left upright for 10 min to allow for drying. Finally, the pellet was dissolved in 40 µL of distilled water and incubated at 55 °C for 10 min on a hotplate. The resulting samples were stored safely at −4 °C for subsequent analysis.

2.5. DNA Amplification

The samples underwent polymerase chain reaction (PCR) amplification to detect the 18S rRNA gene of *Cryptosporidium parvum*. The primers utilized in this investigation were Forward CSF AGTGCTTAAAGCAGGCAACTG and Reverse CSF CGTTAACGGAATTAACCAGAC [21]. PCR was executed using a thermal cycler and Taq DNA polymerase enzyme sourced from Fermentas, Waltham, MA, USA. Each PCR reaction mixture comprised 5 µL of extracted DNA and ten picomoles of both forward and reverse primers (see Table S1 for details). The reaction mixture was composed of 2.2 µL of Taq buffer, 2.4 µL of $MgCl_2$, 1 µL of dNTPs, 1 µL each of forward and reverse primers, 7 µL of distilled water, 0.5 µL of Taq DNA polymerase, and 5 µL of extracted DNA. PCR cycling conditions included a denaturation temperature of 94 °C, an annealing temperature of 65 °C, and an elongation temperature of 72 °C.

2.6. Gel Electrophoresis

For gel electrophoresis, agarose gel preparation involved dissolving 2 g of agarose in 100 mL of 0.5× TBE buffer, which was then heated, cooled to 45 °C, and mixed with 5 µL of ethidium bromide (1 µg/L) before being poured into a gel mold. Sample wells were created using appropriately positioned combs, and after gel solidification, the combs were removed. The gel was placed in a gel tank containing buffer, and for each sample, 20 µL of PCR product was mixed with 2 µL of loading dye. Subsequently, 12 µL of this mixture was pipetted into the gel wells. Gel electrophoresis was conducted with the gel tank oriented for DNA movement from negative to positive charges, running for 25 min at a voltage of 120 V and a current of 500 A. Post-run, the gel was examined under a UV transilluminator, and the specific amplified DNA products were identified by observing 556 bp bands for *Cryptosporidium*, aligned with a 100 bp DNA ladder (Fermentas, 68789 Leon-Rot, Germany) used as a size marker.

2.7. Prevalence Ratio

The prevalence ratio (*PR*) was calculated using the following formula:

$$PR = \left(\frac{Total\ No\ of\ water\ samples}{No\ of\ positive\ samples} \right) \times 100 \quad (1)$$

This isolated equation represents the calculation method for the prevalence ratio.

2.8. Statistical Analysis

The data obtained was carefully recorded and organized into Excel spreadsheets for analysis. Following data entry, statistical analysis was performed using one-way ANOVA to assess the significance of any observed differences. In this analysis, a significance level of

$p < 0.05$ was utilized to determine the presence of statistically significant results, indicating differences between the groups being compared.

2.9. Assessment of Human Health Risks

Although humans can be exposed to contaminants through various means, this study specifically focuses on exposure to *Cryptosporidium* through swimming and diving activities in the studied river over two sampling seasons. Similar studies have been conducted by other researchers [22,23] owing to the popularity of these recreational activities and the likelihood of water intake during swimming events. On average, individuals may ingest up to 37 mL of water per instance [24]. In Zhejiang Province, China, 10.5–21.6% of the population swims in rivers [25]. Given Bangladesh's similar climate and behavior, swimming was deemed an appropriate exposure pathway for risk assessment.

2.10. Dose–Response Assessment

The risk of infection was estimated by using the widely accepted exponential dose–response model with the following equation:

$$P_{inf} = 1 - e^{-rCV}, \quad (2)$$

where P_{inf} is the probability of infection by swimming or diving exposure, r indicates the probability (0.09) that *Cryptosporidium* can reach the target organ to cause an infection [26], C is the measured concentration of *Cryptosporidium* in water samples (n/L), and V is the water intake (L). Water intake in swimming events follows a gamma distribution (*Gamma* [0.50,45], Mean = 22.5) [24], whereas that in diving events follows a logistic distribution (Logistic [5.2702,1.5149], Mean = 5.27) [23,27].

The annual risk associated with exposure to *Cryptosporidium* can be calculated as

$$P_{infyear} = 1 - \left(1 - P_{inf}\right)^{n}, \quad (3)$$

where $P_{infyear}$ is the probability of yearly infection through swimming or diving exposure, and n is the number of exposures per year.

Finally, given that the main symptom of the diseases caused by *Cryptosporidium* is diarrhea, the likelihood of illness in healthy consumers who are infected with *Cryptosporidium* is represented by the product of $P_{infyear}$ and the illness probability of *Cryptosporidium*. The illness probability follows a β probability density distribution (β [α = 10, β = 10]) [28].

3. Results and Discussion

3.1. Land Use/Land Cover of the Watershed Area

The following comprehensive overview of land types and their distribution provides valuable insights into the land cover composition of the specified area. The concentrations and persistence of *Cryptosporidium* in water bodies are subject to various factors, including seasonality, rainfall, pollution events, geological attributes, and land use practices [29]. A land cover map was generated using the European Space Agency world cover map with a horizontal resolution of 10 m [30]. This map (Figure 2) presents a detailed breakdown of land types and their corresponding percentages within the specified region. Tree cover dominates the landscape, accounting for a substantial (29%) percentage of the study area and highlighting the considerable presence of forests or wooded areas. Shrubland constitutes a minimal 0% of the study area, indicating sparse coverage by shrubs. Grassland, representing areas characterized by grassy vegetation, occupies 1.5% of the study area. Cropland, which is used for agricultural purposes, covers a substantial portion of the study area (43%), underlining the importance of agricultural activities in the region. Built-up areas, reflecting urban or developed spaces, account for 17% of the study area. Bare or sparsely vegetated areas, indicating regions with limited plant cover, constitute 5.1% of the study area. Water bodies, including rivers or lakes, contribute to 4.6% of the landscape.

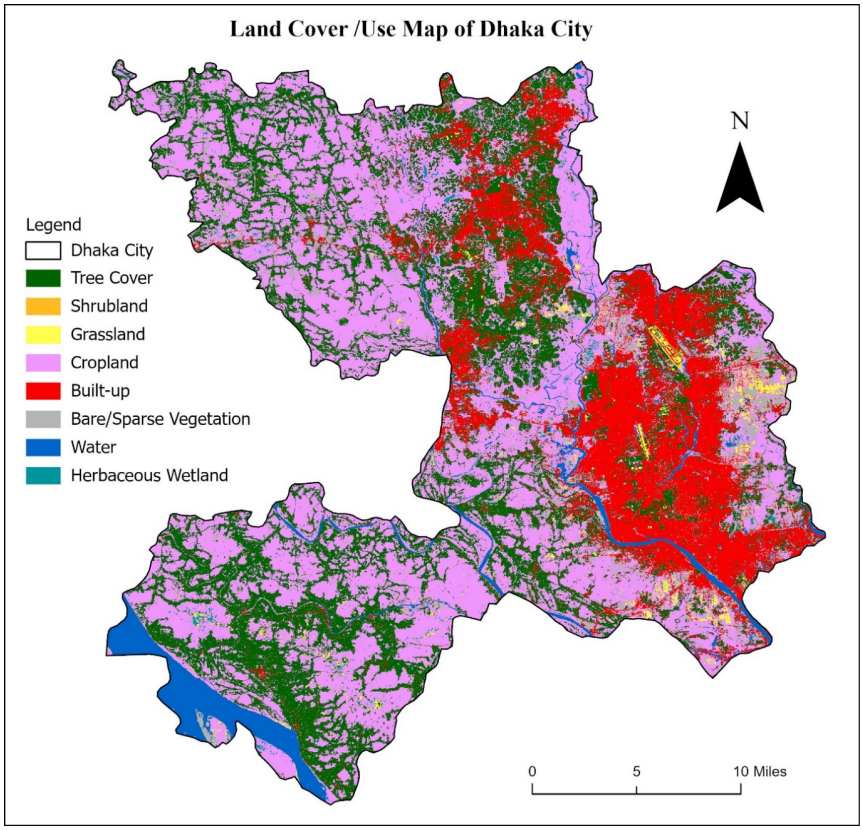

Figure 2. Land use and cover classification map of Dhaka City.

Herbaceous wetlands, which feature specific types of wetland vegetation, represent a small fraction of the study area (0.5%). Agricultural land, occupying most of the land in Figure 2, is one of the substantial factors in *Cryptosporidium* prevalence in the study area, as agricultural land is a major source of pollution for rivers and streams, ranking as the second and third potential sources of contamination for surface water like wetlands, lakes, and rivers, respectively [31]. Manure application on agricultural lands has been associated with the transmission of *Cryptosporidium* parasites to humans. Previous studies have reported a strong correlation between these intestinal parasites and the application of wastewater and animal/human excreta in agricultural production [32]. Similarly, the urban area is Dhaka's second major land use type, playing a vital role in *Cryptosporidium* prevalence due to the lack of proper sanitation and treatment mechanisms for urban wastewater. Hence, oocysts find their way into water sources, propelled by runoff from agricultural land and urban areas during flooding in monsoon seasons. The concentration of *Cryptosporidium* oocysts have been shown to increase due to runoff and overflow, and these oocysts are washed from nearby agricultural lands and households to the water bodies [33]. A comprehensive understanding of land types aids in identifying specific risk factors and can guide the formulation of targeted interventions for mitigating the fecal contamination of surface water. It thus contributes valuable insights for establishing effective water resource management strategies.

3.2. Observed Physicochemical Water Quality

Increased population density and rising pollution levels have led to considerable disruptions in the biological and physicochemical qualities of urban surface water. The water in nearly all the lakes in Dhaka City has taken on a green hue, which indicates excessive nutrient loads and the deposition of waste materials from local households and industries. Most targeted sampled locations exhibit a deep green color, raising concerns about the potential development of algal blooms [34]. Moreover, water quality parameters, such as

biochemical oxygen demand, electric conductivity, dissolved oxygen, and heavy metal concentrations, have surpassed established irrigation and drinking water standards [35]. Table 1 provides a comprehensive overview of the water quality parameters of the Crescent, Dhanmondi, Banani, Gulshan, and Ramna Lakes compared to the Bangladesh Environmental Conservation Rules of 1997 (BS ECR 97) and World Health Organization (WHO) guidelines from 1993. The pH levels of the water bodies vary: Crescent Lake exhibits a slightly alkaline value of 8.4, whereas Ramna Lake leans toward the acidic side with a pH of 6.6. Conductivity, a measure of water's ability to conduct electric currents, shows considerable disparities among the lakes, with Gulshan Lake having the highest value of 778 mS among the investigated lakes. DO concentrations fluctuate across the lakes, with Dhanmondi Lake registering the highest DO of 5.56 mg/L among the studied lakes.

Table 1. Physicochemical water quality parameters and standard limits.

Water Quality Parameters	Crescent Lake	Dhanmondi Lake	Banani Lake	Gulshan Lake	Ramna Lake	BS (ECR 97)	WHO (1993)
pH	8.3	7.6	7.4	7.8	6.638	6.5–8.5	6.5–8.5
Conductivity (mS)	419	284	262	778	266	-	-
DO * (mg/L)	5.4	5.6	3.6	3.5	4.4	4	4
Iron (<mg/L)	0.3	0.2	0.5	0.5	0.3	0.3–1.0	0.3
Temperature (°C)	24	24	25	25	26	20–30	-
Color (Pt-Co)	30	23	35	34	27	15	15
Alkalinity (mg/L)	157	117	261	255	96	200	200
CO_2 ** (mg/L)	10	12	30	23	24	15	15
Salinity	0.2	0.1	0.2	0.3	0.1	0	0
Turbidity (JTU)	16	12	18	19	13	10	5
BOD5 *** (mg/L)	17	21	23	27	25	2	-

Note: * Dissolved oxygen, ** dissolved carbon dioxide, *** biological oxygen demand.

Iron levels remain within permissible limits in accordance with guidelines, although variations are observed. Biological oxygen demand (BOD5) levels, representing the amount of DO consumed by microorganisms while decomposing organic matter over five days, vary among the lakes, as shown in the provided Table S2. Dhanmondi Lake has the highest BOD5 of 21.3 mg/L, indicating a high organic pollution level among the lakes. Crescent Lake has the next-highest BOD5 of 17.3 mg/L.

Meanwhile, the Banani, Gulshan, and Ramna Lakes show BOD5 values of 23.3–27 mg/L. These BOD5 values suggest varying degrees of organic pollution and microbial activity in the lakes, with high values indicating high oxygen demand due to the increased decomposition of organic substances. Alkalinity, carbon dioxide, salinity, turbidity, and BOD exhibit diverse patterns, reflecting the complex interplay among natural and anthropogenic factors that influence the water quality of the above lakes. These data serve as a crucial foundation for assessing the environmental health of water bodies in Dhaka City and formulating targeted measures for their preservation and remediation.

Ref. [34] reported that arsenic, nickel, cadmium, and lead concentrations in these water resources exceed the standard values for irrigation, fisheries, and manufacturing use. Additionally, an unpleasant odor has become perceptible around the lakes due to excessive eutrophication, contributing to water quality deterioration.

3.3. Cryptosporidium Prevalence

Cryptosporidium oocysts, indicating fecal contamination, are prevalent in almost all selected water bodies in Dhaka City. Their prevalence rates are 36%, 40%, 40%, 26%, 33%, 21%, 27%, and 28% in the Balu River, Turag River, Shitalakkhya River, Dhanmondi

Lake, Gulshan Lake, Banani Lake, Ramna Lake, and Crescent Lake, respectively (Figure 3). Among the investigated water bodies (Figure 4), the Buriganga River has the highest concentration of *Cryptosporidium* oocysts (46%). This finding underscores the concern about the level of waterborne parasites in urban water systems and the urgent need for comprehensive water quality management and sanitation measures. The heightened presence of *Cryptosporidium*, particularly in the Buriganga River, raises critical public health concerns, necessitating targeted interventions to mitigate the risk of waterborne diseases and ensure the safety of water resources in Dhaka City.

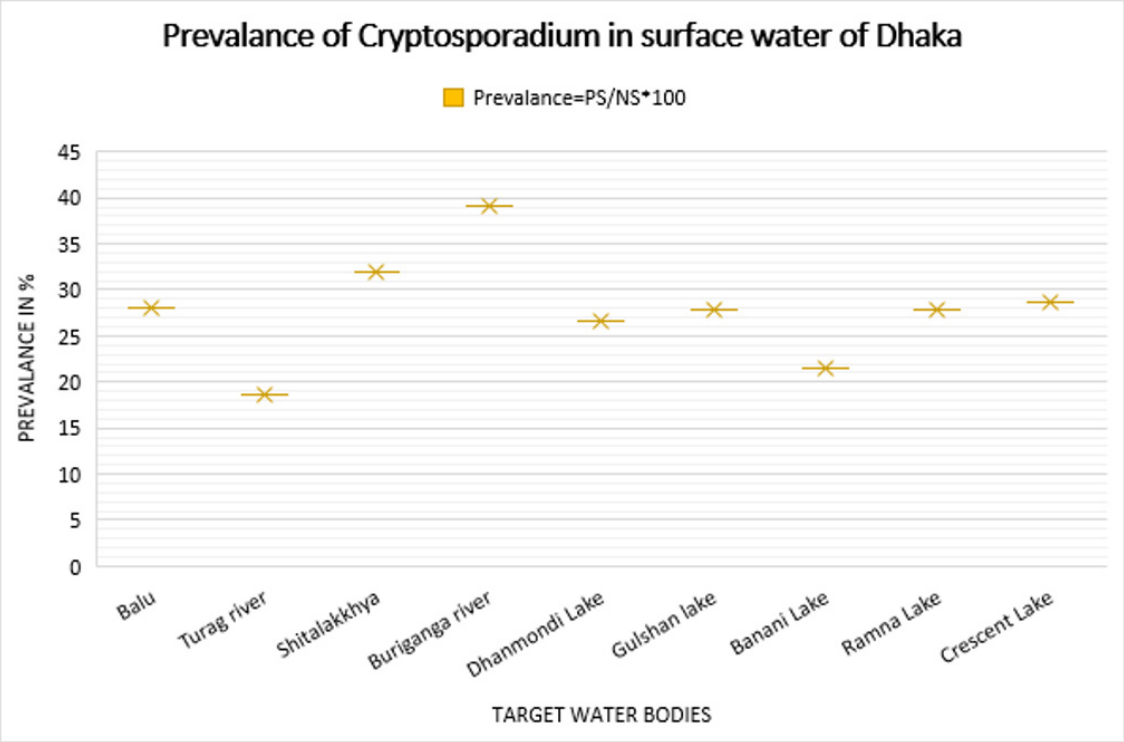

Figure 3. Prevalence of *Cryptosporidium* oocytes in the water bodies of Dhaka City.

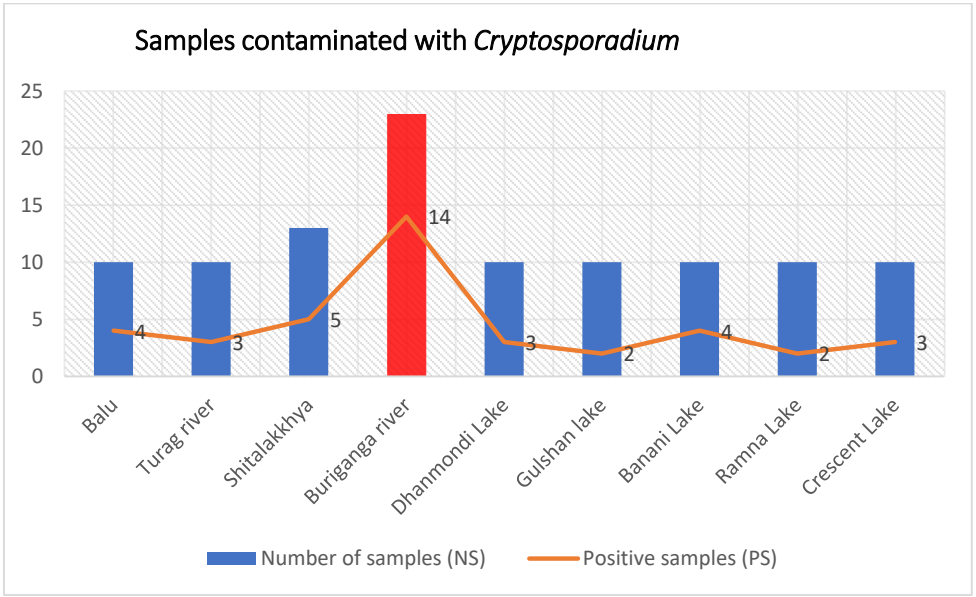

Figure 4. Samples contaminated with *Cryptosporidium* oocytes.

3.4. Potential Health Risks of Cryptosporidium

The infection risk of swimming is highest during the wet season. It is (4 ± 2 (95% CI: 3.0–5.0)) × 10^{-2} per swimming event, whereas it is approximately (2.4 ± 2 (95% CI: 1.6–3.3)) × 10^{-2} during the dry season (Figure 5a). Annual diving risks are approximately (1.2 ± 0.6 (95% CI: 0.9–1.4)) × 10^{-2}, as shown by the mean data from all sites and seasons (Figure 5b). In general, most of the sampling sites in this work exhibit significantly higher risks than other study areas, such as the Three Gorges Reservoir (1.4 × 10^{-3}) [23] or Pardo River (5.4 × 10^{-4}–2.8 × 10^{-4}) [22] but have risks comparable to those of other areas in Bangladesh [36]. Furthermore, the risk of swimming and diving activities across the sampling sites is, on average, over 100 times higher than the acceptable risk level for drinking water of 1 × 10^{-4} set by the U.S. EPA (1989). The high concentration of *Cryptosporidium* directly affects risk probability; other studies that found low *Cryptosporidium* concentrations, which were 50-fold higher than the U.S. EPA threshold, also highlighted the risks of swimming in contaminated waters [37,38]. The daily probability of contracting infections in adults and eventually children due to high exposure frequency is also high considering the elevated number of oocysts (Table 2).

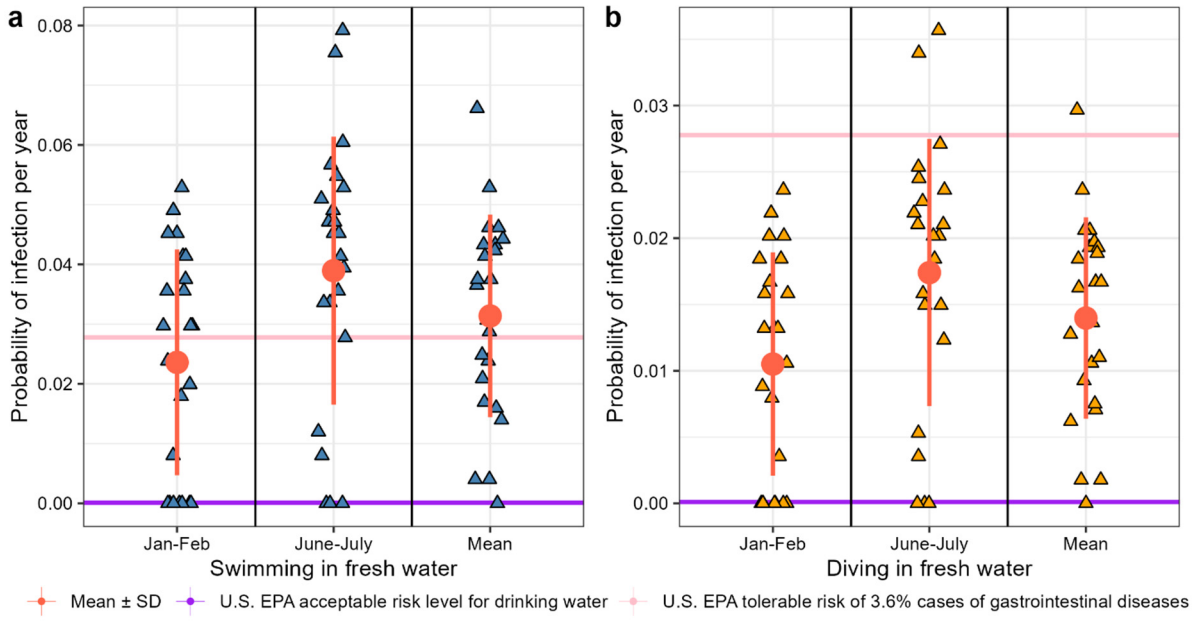

Figure 5. Scatter plot showing the probabilities of infection in adults through exposure to *Cryptosporidium* via swimming (**a**) and diving (**b**) events in the selected sampling sites along the Buriganga River. Probabilities were given over the two sampling periods and the annual average alongside guidelines for health protection. The mean ± standard deviation for each subgroup is given as a point range.

Table 2. *Cryptosporidium* concentrations and the estimated probabilities (mean values ± standard deviation) of infection and contracting diseases caused by exposure during recreational activities in sites containing residual oocyst contamination. All given values exceeded the U.S. EPA threshold of 1 × 10^{-4}.

		Probability of Infection per Day (Time)		**Probability of Infection per Year**		**Probability of Illness per Year**	
Sampling	***Cryptosporidium* Oocysts/10 L**	**Swimming**	**Diving**	**Swimming**	**Diving**	**Swimming**	**Diving**
January–February	11.96 ± 9.61	1.2 ± 1.0 × 10^{-3}	5.3 ± 4.3 × 10^{-4}	2.4 ± 1.9 × 10^{-2}	1.1 ± 8.4 × 10^{-3}	1.2 ± 1.0 × 10^{-2}	6.2 ± 4.9 × 10^{-3}
June–July	19.87 ± 11.56	2.0 ± 1.2 × 10^{-3}	8.8 ± 5.1 × 10^{-4}	3.9 ± 2.2 × 10^{-2}	1.7 ± 1.0 × 10^{-2}	2.0 ± 1.2 × 10^{-2}	1.0 ± 0.6 × 10^{-2}
Mean	15.91 ± 8.69	1.6 ± 0.9 × 10^{-3}	7.1 ± 3.9 × 10^{-4}	3.1 ± 1.7 × 10^{-2}	1.4 ± 0.8 × 10^{-2}	1.6 ± 0.9 × 10^{-2}	8.2 ± 4.5 × 10^{-3}

3.5. Transmission and Epidemiology

Cryptosporidial infections begin when individuals ingest sporulated oocysts at an infectious dose that typically ranges from approximately 10 to 100 oocysts [39]. These oocysts then undergo excystation within the host's gastrointestinal tract, releasing four infective sporozoites, which invade host epithelial cells and undergo successive rounds of asexual and sexual reproduction [40]. The sporozoites further mature into trophozoites, which initiate asexual proliferation through merogony, forming meronts. Meronts develop six or eight nuclei, each integrated into a merozoite, allowing them to infect other host cells and undergo recycling as meronts or merozoites or commence the sexual cycle. In the sexual cycle, merozoites transform into either macrogamonts or microgametes. The fertilization of microgametes and macrogamonts produces zygotes, which subsequently undergo sporogony, generating either thin- or thick-walled oocysts that each contain four sporozoites. Thick-walled oocysts are excreted in feces, facilitating transmission to different hosts. By contrast, thin-walled oocysts lead to autoinfection within the same host, initiating a new cycle of infection [41]. Notably, thick-walled oocysts exhibit remarkable environmental resilience, remaining viable in the environment for extended periods and displaying high resistance to the chlorine disinfection of drinking water [42].

3.6. Influence of Climate, Seasonality, and Floods

Cryptosporidiosis exhibits distinct seasonal patterns influenced by several factors, including agricultural cycles, human waste production, climate patterns, and local rainfall activity [43,44]. These factors affect the prevalence of *Cryptosporidium* infections, with precipitation and human/agricultural waste production being their primary drivers [45]. Studies that incorporated wet periods into their sampling designs have shown higher detection rates than those that did not. These wet periods correspond to phases of increased precipitation, leading to high overland flow, subsurface infiltration, and the potential remobilization of *Cryptosporidium* oocysts as contamination pathways (Table 2). Climate change projections indicate heavy and persistent rainfall that will likely result in increased recharge and overland flow rates, especially in areas with dynamic flows and shallow water tables [46]. Studies have shown that *Cryptosporidium* oocysts are extensively released from soil-applied manure following intense rainfall [47]. However, the complex effects of climate change can affect oocyst release, survival, and transport differently, with marked regional variations [44]. Temperature and solar radiation play crucial roles in oocyst survival, with low temperatures below 15 °C favoring survival and high temperatures, increased UV radiation, and desiccation leading to degradation [48]. This situation might explain the high prevalence of *Cryptosporidium* in temperate and cold regions and could also be influenced by increasing livestock numbers and shifting rainfall patterns [43]. This study found a notable disparity in *Cryptosporidium* contamination levels between the wet (June–July) and dry (January–February) seasons (Figure 6). The findings indicate that the prevalence of *Cryptosporidium* is higher during the wet season than during the dry season, suggesting a heightened risk of waterborne contamination during periods of increased rainfall and environmental moisture. The elevated contamination levels in the wet season may be attributed to various factors, such as runoff from contaminated land, the increased transport of pathogens through surface water, and a conducive environment for the persistence and transmission of *Cryptosporidium* oocysts. Understanding this seasonal variation is pivotal for implementing targeted interventions and improved water management practices during the wet season to mitigate the risk of *Cryptosporidium* contamination, safeguarding public health, and ensuring the safety of water resources.

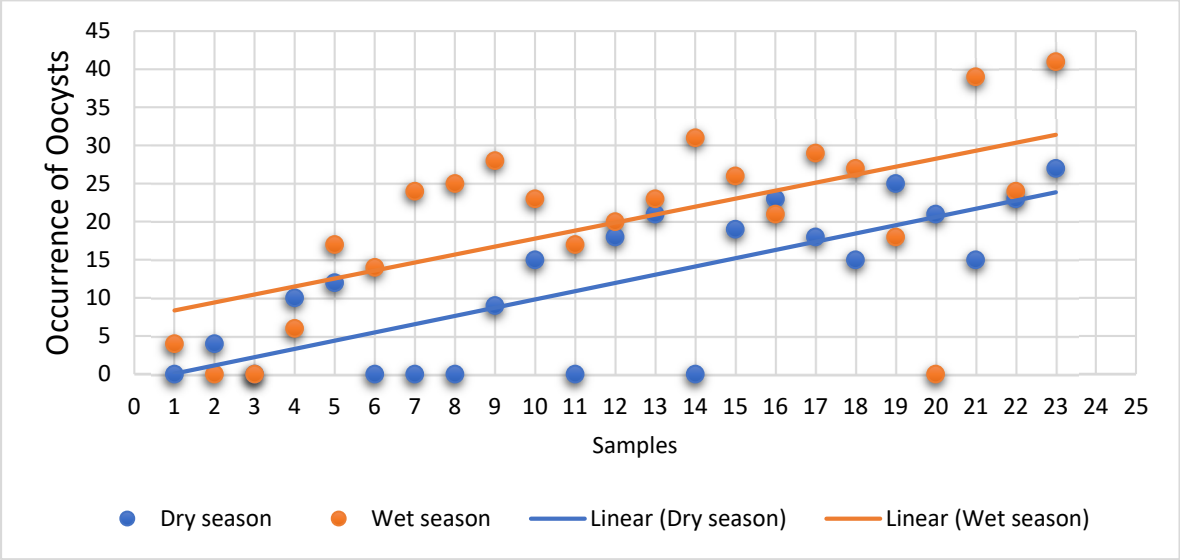

Figure 6. Seasonal variation in the prevalence of *Cryptosporidium* oocysts in the Buriganga River.

Flood susceptibility risk maps prepared using geographic information systems and multi-criteria analysis have been shown to closely resemble historical flood ranges [49]. These maps are crucial for determining evacuation routes in highly flood-prone areas [50]. Additionally, flood risk maps have been utilized to identify hotspots that are highly susceptible to flooding and potential sources of pollutant contaminants [51]. Figure 7 illustrates the flood susceptibility categories and their corresponding percentages of the total area in acres. Regions classified with low flood susceptibility constitute 18% of the total area, covering 887,757.3 acres. Regions with moderate flood susceptibility account for 31% of the total area and are equivalent to 1,516,912 acres.

Meanwhile, regions with high flood susceptibility constitute 33% of the total area and span 1,623,604 acres. Finally, the exceptionally high flood susceptibility category accounts for 18.85% of the area, representing 935,947 acres. This breakdown provides a clear overview of flood susceptibility distribution, aiding the formulation of targeted strategies for flood risk management and mitigation in different zones based on flood susceptibility levels. Based on the results, we can say that floods significantly influence *Cryptosporidium* contamination in surface water. During flood occurrences, runoff from agricultural lands, urban areas, and sewage systems acts as conduits for *Cryptosporidium* oocysts to infiltrate rivers and water bodies. In Dhaka, Bangladesh, where cropland/agricultural land covers 43% and built-up areas/urban areas occupy 17% of the total land as depicted in Figure 2, these land use and cover characteristics markedly contribute to *Cryptosporidium* contamination in river water, as the increase in occurrence of *Cryptosporidium* seen during the monsoon season, as illustrated in the flood events in Figure 6, is propelled by monsoon rains and inadequate drainage systems, leading to the overflow of sewage and wastewater from residential and industrial zones, along with runoff from agricultural land, thereby transporting untreated human and animal waste laden with *Cryptosporidium* oocysts into rivers such as the Buriganga, Turag, and Balu. Moreover, flooding inundates low-lying areas and informal settlements, exacerbating sanitation issues and directly discharging fecal matter into river water. Consequently, the interaction between land use, flooding, and deficient infrastructure significantly contributes to the proliferation of *Cryptosporidium* contamination in Dhaka's river waterways.

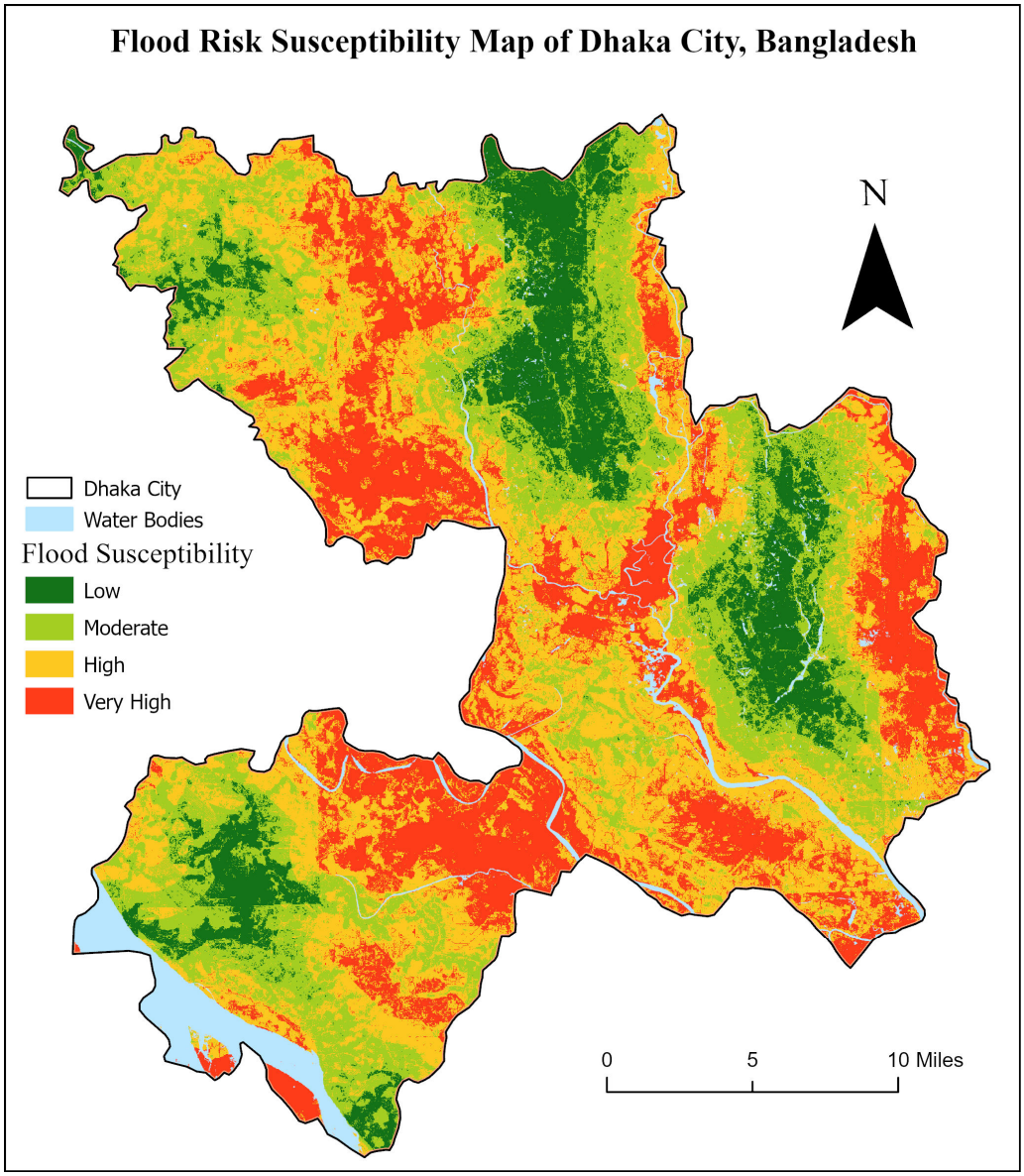

Figure 7. Flood risk map indicating highly susceptible areas in Dhaka City.

Dhaka City's high flood risk, as delineated by the flood risk map, has important implications for water quality and fecal contamination. During flooding, the inundation of water bodies can lead to mixing sewage and industrial pollutants, compromising water quality. Increased water levels may overwhelm sanitation systems, resulting in the discharge of untreated wastewater into rivers and lakes. This contamination pathway severely threatens public health, elevating the risk of waterborne diseases due to fecal contaminants. Moreover, flooded areas may experience a breakdown in sanitation infrastructure, further exacerbating the potential for the fecal contamination of water sources. A considerable proportion of households in Dhaka are highly vulnerable to flooding [14], with 63% and 20% of slum and non-slum households falling in this category. A total of 63% of slum inhabitants are highly vulnerable, whereas 78% of non-slum habitats are in the low-vulnerability category [14]. Addressing flood-related effects on water quality is imperative for safeguarding the health and well-being of Dhaka City's residents and necessitates integrated strategies for flood management and water sanitation.

3.7. Sources of Surface Water Pollution in Dhaka City

Lakes serve as critical water sources and play vital ecological roles. However, their water quality faces considerable degradation due to various human activities, including agriculture, urbanization, local industries, and refreshment activities. The primary contributor to this pollution is the improper discharge of pollutants into water bodies [52]. Sources of contamination are classified into two primary categories: point sources and nonpoint sources [53] (Table 3). Point sources are relatively identifiable, quantifiable, and manageable, encompassing discharges from industries, households, and wastewater treatment plants [54]. By contrast, nonpoint sources of contamination are elusive and challenging to control, with key contributors being agricultural practices, such as livestock manure application, nitrogenous fertilizer use, and soil nitrogen mineralization [52].

Table 3. Observed and reported sources of surface water pollution in Dhaka City.

Pollutant Categories	Point Sources	Nonpoint Sources
Heavy metal	Industrial effluents, waste from the pharmaceutical industry and hospitals, and effluents from thermal power plants	Herbicides, insecticides, pesticide runoff, and metal smelting and refining
Nutrients	Effluents from the effluent treatment plants of industries	Agricultural waste and agricultural runoff
Organic chemicals	Paper mill effluents and municipal solid and liquid waste	Emission of domestic waste and agrochemical and farm runoff
Sedimentation	Drainage from construction sites (area < 20,000 m^2)	Drainage from construction sites (area > 20,000 m^2)
Pathogens	Animal excreta, untreated wastewater, and municipal solid waste	Wastes emitted from households and farms and leachate from sanitation systems

Sewage, solid waste, and industrial discharge are primary contributors to surface water pollution in the urban regions of Bangladesh. According to findings from a 2009 study conducted by the Bangladesh Centre for Advanced Studies, around 40% of the industries examined had operational effluent treatment plants (ETPs), while 10% were in the process of implementing ETPs. The study also noted that the remaining half of the industries lacked ETP facilities [55]. Industries without ETPs or with incomplete ETPs discharge a considerable volume of untreated or inadequately treated solid and liquid waste directly or indirectly into nearby water bodies. Based on the overall discharge volume into water systems, the primary source of pollution is the pulp and paper sector (47%), succeeded by the pharmaceutical industry (16%), metal manufacturing (14%), food processing (12%), agrochemical production (7%), synthetic chemical manufacturing (1%), and other industrial sectors (3%) [56]. The unregulated urban expansion and industrial development in Dhaka City have resulted in significant pollution of its surface water reservoirs. In the last twenty years, an industrial hub has arisen in Kaliakoir Upazila, Gazipur District, situated approximately 25 km northeast of Dhaka City, lacking adequate planning [53]. Industries within this vicinity discharge approximately 30 billion liters of liquid waste annually into adjacent water bodies, such as Ratanpur Khal and Mokosh Beel [57]. The lack of treatment facilities in nearly 80% of industries leads to the discharge of untreated or inadequately treated toxic effluents, worsening water quality [58]. In Dhaka City, surface water bodies like lakes, canals, and ponds show elevated levels of BOD, COD, and pathogens, suggesting the discharge of poorly treated or untreated industrial effluents and waste into waterways. Additionally, despite city corporations collecting around 50% of municipal waste, a significant portion of the collected garbage ends up being dumped into surface water bodies, worsening the degradation of water quality [53].

4. Conclusions

This study addressed urban river pollution in Dhaka, Bangladesh, a highly populated megacity with significant challenges in meeting the UN's 2030 sustainable development goals. Dhaka's urban rivers are polluted due to a population of 21 million people in 1464 km^2. Over 7000 companies release 98% of the untreated residential sewage along with substantial industrial effluents. This study specifically investigated parasitic contamination and focused on *Cryptosporidium*, a waterborne pathogen causing cryptosporidiosis. The findings of this work reveal troubling concentrations of *Cryptosporidium* oocysts, indicating fecal contamination. Among the investigated water bodies, the Buriganga River exhibits the highest concentration of *Cryptosporidium* oocysts, and Balu River, Turag River, Shitalakkhya River, Dhanmondi Lake, Gulshan Lake, Banani Lake, Ramna Lake, and Crescent Lake show varying degrees of contamination. This study calculated infection risks and found that the infection risk of swimming is highest during the wet season and is $(4 \pm 2$ (95% CI: 3.0–5.0)) $\times 10^{-2}$ per swimming event, whereas it is approximately $(2.4 \pm 2$ (95% CI: 1.6–3.3)) $\times 10^{-2}$ during the dry season (Figure 5a). Annual diving risks are approximately $(1.2 \pm 0.6$ (95% CI: 0.9–1.4)) $\times 10^{-2}$, indicating considerably high risks. These findings underscore the critical need for additional investigations in the Mymensingh and Kushtia Districts to evaluate the prevalence of *Cryptosporidium* in human and animal populations. Therefore, conducting further monitoring campaigns and exploring remediation alternatives should be considered to safeguard the livelihoods of nearby residents. Additionally, a comprehensive exposure frequency and duration analysis will further improve the current estimates' reliability. Some families may use water for other purposes, including drinking, tooth brushing, food preparation, and dishwashing, potentially exposing them to higher risks. Such investigations can provide essential data for understanding the transmission dynamics of *Cryptosporidium* and guide effective disease control and prevention measures. As Dhaka navigates the path toward sustainable development, prioritizing water quality management emerges as a vital component for achieving a healthy and sustainable urban environment.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w16101352/s1, Table S1: Primer set used for Cryptosporidium (Elsafi et al., 2013) [21]; Table S2: Characteristics of prominent water bodies sampled in Dhaka City.

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