

Kathmandu University Journal of Science, Engineering and Technology



Suspected faecal contamination of Phewa Lake: Spatial patterns and effects of rainfall

Richard G. Storey^a, Ananta Dhakal^b, and Subodh Sharma^{*a}

^a Aquatic Ecology Centre, School of Science, Kathmandu University, Dhulikhel, Kavre, Nepal. ^bGandaki Province Academy of Science and Technology, Pokhara, Kaski, Nepal.

Abstract

Phewa Lake was once popular for swimming, but most local people now perceive it to be unsafe due to urban development and beliefs about sewage contamination. Nevertheless some people, especially children, still swim or bathe in Phewa's waters. Our objectives were to determine whether swimmers are at risk of waterborne disease, how the risk varies around the lake, and how it varies with rainfall. We measured concentrations of faecal indicator bacteria (thermotolerant coliforms) in lake water samples from five points along the urbanized eastern shore, from the mainly agricultural northern shore, the mainly forested southern shore, and the middle of the lake. We also sampled two urban streams. We sampled on ten occasions from October 2021 to January 2023, including monsoon, post-monsoon and winter. During dry periods concentrations of thermotolerant coliforms were low (median 3-60 cfu/100 mL) in all lake samples and very high (median 3000-45,000 cfu/100 mL) in the urban streams. After rain, concentrations in lake samples near the urban area rose to median 400-2000 cfu/100 mL, exceeding USA guideline values. Concentrations were generally higher on the urbanized side of the lake than on the mainly agricultural and forested sides, and generally increased along the presumed flow path towards the lake outlet. We recommend further investigations to determine the sources of contamination. We also recommend developing guideline faecal indicator bacteria concentrations for recreational waters in Nepal, with regular monitoring and public signage to protect people from illness at popular swimming sites.

Keywords: Faecal indicator bacteria; Monitoring; Recreational waters; Waterborne disease.

1. Introduction

In Nepal waterborne diseases account for 15% of all illness and 8% of total deaths [1], making water quality a major public health concern for the country [2]. To date, most attention has been focused on drinking water [3, 4, 5]. However globally, direct contact with contaminated water via swimming and bathing is a common pathway for various waterborne diseases [6, 7]. The most common diseases are gastrointestinal, followed by skin, ear-related, respiratory and eye-related diseases. Most recreational waterborne diseases are caused by faecal contamination [7].

Faecal contamination may introduce a wide variety of pathogenic (disease-causing) micro-organisms into natural waterbodies [7]. Because it is impractical to test for all possible pathogens, testing typically focuses on faecal indicator bacteria (FIBs). These bacteria may not themselves cause disease but are known to originate in the guts of warm-blooded animals, and therefore indicate that other potentially pathogenic microorganisms are likely to be present [8]. Numerous studies [9, 10, 11] have shown that concentrations of FIBs correspond to the risk of infection from common pathogens, and therefore governments set standards for FIB concentrations that correspond to tolerable vs. intolerable infection risks [12]. Thermotolerant coliforms are a group of bacteria that are almost exclusively found in the guts of warm-blooded animals, and therefore have been used as FIBs in many countries [8, 9, 13].

Pokhara City (estimated population 511,000 in 2025) [14] is situated on the eastern shore of Phewa Lake. Situated in a picturesque mountain valley and close to well-known hiking trails, Phewa Lake

is a popular recreational location for local residents, national and international tourists. Many of Pokhara's long-term residents report that they used to swim regularly in Phewa Lake but would not do so now because of perceived water pollution from nearby homes, farms, hotels and businesses. Perceptions are based on rumours of illegal sewage discharges to the lakes, as well as on Pokhara City's lack of reticulated sewage system and the abundance of dogs, cows, buffalos and other animals that roam the streets. Nevertheless, boating and fishing on the lake remain popular, and some people dive into the water while boating. More frequently, children can be seen swimming and adults bathing close to the shore. It appears that local residents and visitors have differing perceptions or tolerances for the risk of illness from contact with Phewa Lake water. However, to our knowledge there are no data that can support an objective estimate of this risk.

The overall aim of this study was to assess the risk of infection by waterborne diseases from swimming or bathing in Phewa Lake. Secondary aims were to determine the spatial patterns of disease-causing micro-organisms in relation to popular swimming places and urban development, and to assess temporal patterns, especially in relation to rainfall. Our purpose was to provide local authorities and the public with information about the risks of swimming and bathing at different places and times in Phewa Lake, and with preliminary data that may indicate sources of faecal contamination so that contamination can be managed, and Phewa Lake made safe for swimming again.

 $^{^*}$ Corresponding author. Email: subodh.sharma@ku.edu.np

R. G. Storey et al.

2. Methods

2.1. Study area

Phewa lake is a sub-tropical lake located at the western end of the Pokhara Valley in the mid-hills region of Nepal. It has a maximum depth of about 22 m, an area of 4.33 km2 and catchment area of 122.53 km2 [15].

Phewa Lake experiences a monsoonal climate. Lake surface water temperatures typically vary from 15-18 °C in January to 28-30 °C in summer (June-August) [16]. The annual rainfall at nearby Pokhara Airport is typically between 3000 and 5000 (average 4050) mm [17], of which 80% falls between June and September and almost no rain falls between November and February. The high rainfall results in a high flushing rate, with the lake volume replaced at least 10 times per year, mainly during the 4-month monsoon season.

Most water enters the lake via the Harpan River at the western end and leaves the lake through a dam at the eastern end (see Fig. 1). Pokhara City (estimated population: 511,000 in 2025) [14], located on the relatively flat eastern shore of the lake, has a dense commercial/entertainment area extending to near the lake shore. In contrast, the hilly southern side is covered mostly in forest and on the hilly western and northern sides the land is mostly in agriculture. On the north side a number of houses and hotels are located close to the lake shore, whereas on the southern side only one or two hotels are close to the shore (see Fig. 1).

2.2. Study sites

Two sets of study sites were sampled. The first set, called Lakeside-Damside, included five "urban lake" sites (labelled UL1-5) around the lake shore adjacent to the entertainment areas known as Lakeside and Damside. Also in this set were two sites in small urban streams (labelled US1-2) and one reference site in the middle of the lake (labelled ML). The second set, called Whole Lake, included sites around the whole perimeter of the lake: three of the urban sites (UL1, 3 and 5), three "agricultural lake" sites (AL1-3) along the northern lake shore adjacent to mostly agricultural land, one "forested lake" site (FL) near the forested southern shore, and one "agricultural river" site (AR) in the mouth of the Harpan River which drains a largely agricultural area. This set also included the mid-lake reference site (ML).

2.3. Sampling

Water samples were taken on a total of ten occasions over 15 months, from October 2021 to December 2021 and from August 2022 to January 2023. This period included monsoon, postmonsoon and winter seasons. The Lakeside-Damside set of sites was sampled seven times from October 2021 to January 2023 (see Table 1). Four of these sampling occasions were during settled weather with no rainfall in the previous five days, while three sampling occasions followed heavy rain, with 80-300 mm of rainfall in the five days before sampling. The Whole Lake set of sites was sampled three times from September to October 2022, all during wet weather with 45-270 mm of rainfall in the previous five days (see Table 1).

On each occasion, water samples were taken between 9 am and 11 am. The samples were collected in sterile 250 mL, plastic bottles and were immediately placed in a dark insulated bag containing ice for transportation. On return to the laboratory (a dedicated room in the first author's house), they were refrigerated and processed within eight hours of collection.

Water temperature and electrical conductivity were measured at the time of sampling to within ± 0.8 °C and $\pm 2\%$, respectively, using a Lutron WA2015 multimeter. Turbidity was measured (to

within $\pm 10\%$) in water samples immediately on return to the laboratory using a Thermo Scientific Aquafast II Orion AQ2010 benchtop meter.

2.4. Bacteriological analysis

Concentrations of thermotolerant coliforms (TtCs) in the water samples were determined using a standard membrane filtration technique. The samples were brought to room temperature then filtered using cellulose nitrate filters (47 mm diameter, 0.45 m pore size). The volume of sample filtered was adjusted between 1 and 100 mL, depending on the expected concentration of TtCs, in order to achieve a countable number of colony-forming units (cfu). TtCs on the filter membranes were cultured using a Wagtech Palintest (Potatest +) kit. In this method, each filter membrane was placed on an absorbent pad soaked in M-Lauryl Sulfate broth in a sterile aluminium petri dish. The petri dishes were stacked, resuscitated for two to three hours at room temperature then incubated in the Wagtech incubator for 24 hours at 44 °C. After incubation, yellow "droplets" > 1 mm diameter on the membrane were counted as thermotolerant colony forming units. With every set of field samples, two laboratory blanks of sterile water were processed, following the same steps as field samples, to ensure that contamination was not affecting results.

2.5. Statistical analysis

Summary data (medians and percentiles) were calculated, and correlations plotted, using Microsoft Excel 2019 (version: 2103). Boxplots were plotted using RStudio 23.09.1 statistical software.

3. Results

Rainfall in the five days before sampling, water temperatures, conductivity and turbidity on the different sampling dates are shown in Table 1.

Concentrations of TtCs varied widely among the different sites and the different sampling occasions. The lowest concentrations were always found in the middle of the lake (see Fig. 2). Mid-lake concentrations varied from a median of 3 cfu/100mL during dry weather to a median of 17 cfu/100 mL after heavy rain. The highest concentrations were always found in the urban streams (see Fig. 2). US1 varied from a median of 3125 cfu/100 mL during dry weather to more than 11,000 cfu/100 mL after rain, while US2 (Phirke Khola) had a median concentration above 40,000 cfu/100 mL with or without rainfall. Generally, concentrations of TtCs increased along the lakeshore from UL1 to UL5. This is probably the main flow direction in the lake, hence the TtC concentration appeared to be increasing as water flowed past the commercial/entertainment area between Lakeside and Damside. During dry weather, TtC concentrations in all samples at all of the lake sites (except for one occasion at UL5 Damside) were below the USEPA guideline values for recreational water quality (geometric mean of 126 cfu E. coli/100 mL, single sample maximum value of 235 cfu E. coli/100 mL; equivalent to 160 and 300 cfu thermotolerant coliforms/100 mL according to Hachich et al. 2012).

In the Whole Lake sample set, TtC concentrations were lower on the western and northern sides of the lake than beside Lakeside and Damside, however, at the Harpan River mouth concentrations were similar to those at Lakeside (see Fig. 3). On the forested southern shore we recorded a very high concentration (too many to count) on one occasion, but low concentrations of 1-2 cfu/100 mL (similar to mid-lake concentrations) on the other two occasions.

Rainfall had a strong, though variable, influence on TtC concentrations (see Fig. 4). During dry weather, TtC concentrations were always relatively low. At some lake sites (e.g. ML and UL4) there was a progressive increase in TtC concentration with increasing

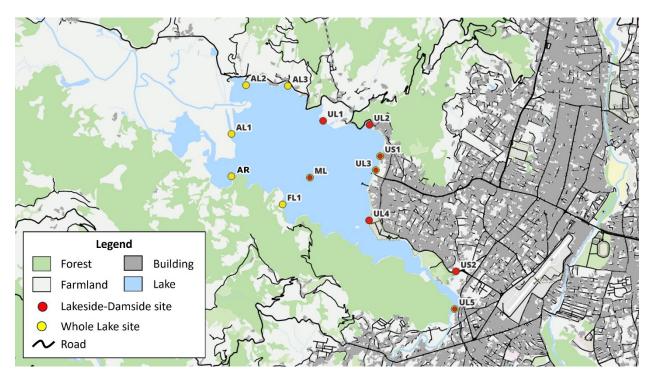


Figure 1: Map of Phewa lake and surrounding showing location of sampling sites. Site name abbreviations comprise adjacent land use (U= urban, A= agricultural, F = forest) and waterbody type (L= lake, R= river, S= stream). ML= mid-lake.

Table 1: Sampling dates, rainfall in the five days before sampling, and water quality at the time of sampling for the two sets of sites. Water quality data are given as the range between lowest and highest values among the lake sites (urban stream sites were excluded as their values were very different from lake sites).

Data set	Date	Rainfall (mm)	Water temperature (°C)	Conductivity (≠Scm-1)	Turbidity (NTU)
Lakeside-Damside	9-Oct-21	223	27.0-27.5	46-69	no data
	3-Nov-21	0	23.0	45-81	no data
	1-Dec-21	0	20.9-21.3	51-98	0.9-5.1
	18-Aug-22	83	29.0-29.5	35-50	6.6-14.6
	1-Sep-22	342	24.1-24.7	37-60	18.7-53.0
	20-Oct-22	0	23.4-23.8	38-65	8.2-13.3
	18-Jan-23	0	15.8-16.2	66-99	3.1-11.2
Whole lake	20-Sep-22	44.5	26.4-26.7	27-46	5.9-30.1
	26-Sep-22	74.3	26.5-28.0	30-50	5.4-35.2
	9-Oct-22	270.8	23.8-26.4	26-70	11.4-28.1

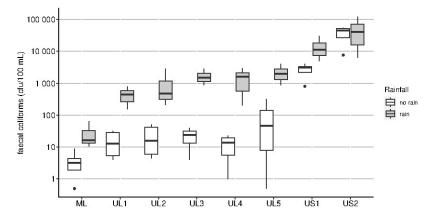


Figure 2: Concentrations of thermotolerant coliforms in Lakeside-Damside sites during dry weather and following rain. Site name abbreviations comprise adjacent land use (U= urban) and waterbody type (L= lake, S= stream). ML= mid-lake. Each box shows the median (thick line), upper and lower quartiles (top and bottom of box) and highest/lowest values (extent of whiskers). Outliers (>1.5 times the interquartile range) are shown by dots.

R. G. Storey et al.

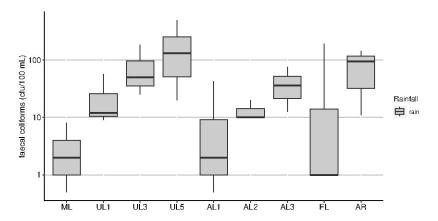


Figure 3: Concentrations of thermotolerant coliforms in Whole Lake sites. Site name abbreviations comprise adjacent land use (U= urban, A= agricultural, F = forest) and waterbody type (L= lake, R= river, S= stream). ML= mid-lake. Each box shows the median (thick line), upper and lower quartiles (top and bottom of box) and highest/lowest values (extent of whiskers). Outliers (>1.5 times the interquartile range) are shown by dots.

rainfall, whereas at others, maximum concentration was observed at moderate rainfall, and higher rainfall was associated with lower concentrations. The greatest increases occurred at UL2-UL5, nearest the main commercial/entertainment area at Lakeside, while smaller increases occurred in the mid-lake reference site and at UL1. The urban streams showed a smaller increase than the lake sites after rain, however their TtC concentrations were always extremely high. After heavy rain, all the lake sites except the mid-lake site were three to 12 times higher than the USEPA guideline value for recreational water quality.

Turbidity was strongly correlated with TtC concentrations at some sites but not at others. Pearson correlations were, in descending order, R=0.95 (UL4), 0.92 (UL5), 0.86 (UL2), 0.65(ML), 0.22 (UL1) and 0.14 (UL3). Generally, sites showing a strong correlation between rainfall and TtC concentration also showed a strong correlation between turbidity and TtC concentration.

4. Discussion

Our results indicate that at all times of year thermotolerant coliforms (TtCs) are present in all parts of the lake. During periods without rainfall the concentrations of thermotolerant coliforms near the entertainment area at Lakeside and Damside were within the safe limit recommended by USEPA for swimming and bathing (corresponding to an illness rate of eight cases per 1000 swimmers) [12]. However, after rainfall, samples near the entertainment area of Lakeside-Damside exceeded the USEPA guideline by a significant margin. This indicates that people swimming or bathing near Lakeside or Damside after rain have a significant risk of developing gastro-intestinal illness. Near-shore areas on the predominantly agricultural northern and western sides of the lake generally had lower coliform concentrations than the urban side, and (on the three dates when we sampled) remained within USEPA guideline values for swimming and bathing. However the Harpan River, which drains a largely agricultural catchment, had higher concentrations, approaching those of the urban sites.

The presence of TtCs in all samples suggests that Phewa Lake is being contaminated with faecal material from humans and/or human-associated animals at all times of year. This is not proven conclusively by our data, as some TtCs can occur naturally in the environment [8, 12]. Also, TtCs can come from wild birds and mammals as well as humans and domestic animals, and further, bacteria from past contamination events can be stored in lake sediments and resuspended by waves and wind [18, 19]. However, the fact that we found one very high coliform concentration near a hotel on the forested southern shore of the lake suggests that hotels (and

probably also residences) discharge faecal material directly into the lake from time to time. Also, TtC concentrations in the two urban streams were always high, regardless of rainfall, indicating constant discharge of faecal material from hotels or residences in the Lakeside area.

TtC concentrations in lake samples were much higher after rain than during dry weather. This is likely because rainfall mobilizes animal faeces deposited on roads, and/or causes sewage overflows from hotels, restaurants, other businesses or homes. At some sites, however, TtC concentrations were lower after very heavy rainfall than after moderate rainfall. This is likely because bacteria becomes diluted by large volumes of water run-off or overflows during heavy rain.

Following rain, coliform concentrations generally increased along the lakeshore from UL1 to UL5, indicating that the lake water is gradually picking up TtCs as it moves past Lakeside towards Damside. Flow paths from the urban area to the lake could be via surface runoff groundwater, or both. Our data cannot conclusively show whether the main source is road runoff or sewage overflows, but patterns among the sampling sites and over time can give an indication. Occasional high coliform concentrations at one sampling point compared to others could suggest a discharge near that point, whereas a gradual increase between UL1 and UL5 would suggest road runoff, or multiple discharges, are more likely to be the main source. In our data, we typically found a gradual increase, suggesting diffuse sources such as road run-off, or multiple sources. However, UL3 (Hallan Chowk) sometimes had higher concentrations than UL2 and UL4, suggesting a nearby point discharge also contributes bacteria. This could be the inflow at US1 or another unknown source. The fact that these occasional high concentrations were not related to high rainfall also suggests a point discharge.

The correlation between TtC concentration and turbidity has practical value. Although turbidity was not be a precise predictor of TtC concentration, this study showed that it can be used as a visual indicator of an increased risk of illness from invisible bacteria.

5. Recommendations for future research and policy

Bacteria in stormwater runoff are most likely to be from animal (mainly dog, cow and buffalo) droppings on streets, whereas sewage bacteria are of human origin. Microbial source tracking (MST) can discriminate between bacteria of human and nonhuman origin [20], and we recommend using MST to determine the main source of faecal bacteria in Phewa Lake. If sewage discharges and overflows are a significant source of faecal contamination, we recommend stricter enforcement of laws preventing such

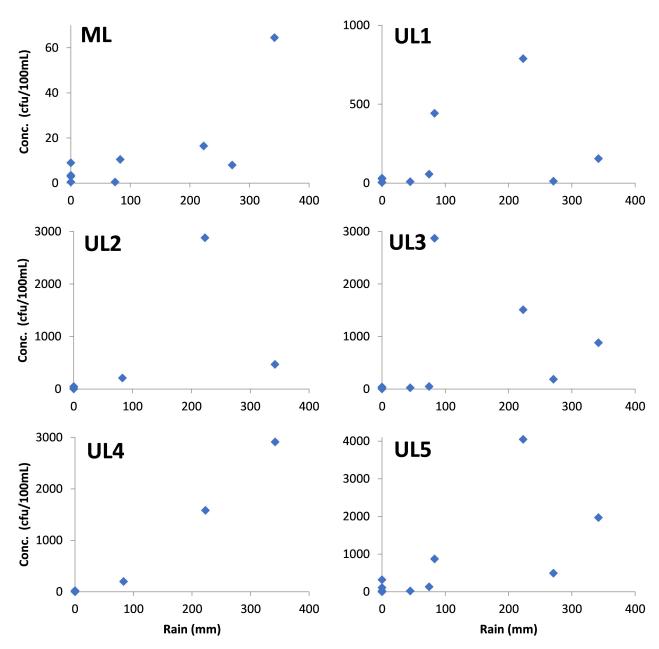


Figure 4: Concentration of thermotolerant coliforms vs. rainfall at the urban lake sites (UL1-5) and the mid-lake reference site (ML). Note the different y-axis scales on different subplots.

R. G. Storey et al.

discharges. If animal droppings on streets are shown to be a significant source of faecal contamination of Phewa Lake, we recommend new regulations to keep dogs, cows and buffalo away from streets that are connected to Phewa Lake via stormwater pipes and channels.

We also recommend regular monitoring of faecal indicator bacteria at popular swimming and bathing areas (up to weekly during the peak swimming season), along with signage to alert the public when concentrations of indicator bacteria exceed safety standards. Monitoring should target Escherichia coli or intestinal enterococci rather than TtCs, as per current international best practice [13, 6], as these indicators are found almost exclusively in human and animal faeces [8] and correlations with infections rates are established[10, 6].

Nepal has safety standards for drinking water but not for contact recreation in freshwaters. Given the number of people who swim and bathe in rivers and lakes across Nepal, and the multiple potential sources of faecal contamination, we recommend that the Government develop such standards, following the WHO (2021) guidelines [6], to manage and reduce risks of developing gastrointestinal illness from swimming and bathing.

We also recommend further research to determine how long after a rainfall event the water is likely to be safe for swimming again. Reduction in bacteria levels after a rain event depends on factors such as sunlight, water turbidity, temperature, pH and the concentration of grazing animals in the water [21, 22], thus a locally-adapted model incorporating these factors is required for accurate predictions.

6. Conclusion

Our results show that there are measurable concentrations of thermotolerant coliforms at all times in Phewa Lake, and indicate that after rain there is a significant risk of gastrointestinal illness from swimming and bathing. These results highlight the need for regular monitoring of faecal indicator bacteria and better management of stormwater and/or sewage in areas adjacent to Phewa Lake. With improved management, Phewa Lake could be made safe for swimming again.

References

- [1] Warner N R, Levy J, Harpp K & Farruggia F, Drinking water quality in Nepal's Kathmandu Valley: A survey and assessment of selected controlling site characteristics, 16(2) (2008) 321–334. ISSN 1435-0157. https://doi.org/10.1007/s10040-007-0238-1.
- [2] Acharya K, Khanal S, Pantha K, Amatya N, Davenport R J & Werner D, A comparative assessment of conventional and molecular methods, including MinION nanopore sequencing, for surveying water quality, 9(1) (2019) 15726. ISSN 2045-2322. https://doi.org/10.1038/s41598-019-51997-x.
- [3] Rai S K, Ono K, Yanagida J I, Ishiyama-Imura S, Kurokawa M & Rai C K, A large-scale study of bacterial contamination of drinking water and its public health impact in Nepal, 14(3) (2012) 234.
- [4] Magar A T, Khakurel M, Pandey S L, Subedi K, Manandhar U K, Karanjit S & Paudyal R, Comparative microbiological assessment of drinking water collected from different areas of Kathmandu Valley, 6 (2019) 39–43. ISSN 2382-5499. https://doi.org/10.3126/tujm.v6i0.26577.
- [5] Tiwari S, Timalsina B, Sitaula S, Awasthi M P, Mahat A & Pant R R, Evaluating the major sources of surface water quality

- in Pokhara Metropolitan City, Gandaki Province, Nepal, 29 (2023) 29–38. ISSN 2382-5200. https://doi.org/10.3 126/hebids.v9i1.59584.
- [6] World Health Organization. Guidelines on recreational water quality. Volume 1: Coastal and fresh waters. World Health Organization, Geneva (2021).
- [7] Adhikary R K, Mahfuj M S E, Starrs D, Croke B, Glass K & Lal A, Risk of human illness from recreational exposure to microbial pathogens in freshwater bodies: A systematic review, 14(2) (2022) 325–343. ISSN 2451-9685. https://doi.org/10.1007/s12403-021-00447-z.
- [8] Hachich E M, Di Bari M, Christ A P G, Lamparelli C C, Ramos S S & Sato M I Z, Comparison of thermotolerant coliforms and Escherichia coli densities in freshwater bodies, 43(2) (2012) 675–681. ISSN 1517-8382. https://doi.org/10.1590/s1517-83822012000200032.
- [9] Tallon P, Magajna B, Lofranco C & Leung K T, Microbial indicators of faecal contamination in water: A current perspective, 166(1-4) (2005) 139-166. ISSN 1573-2932. https://doi.org/10.1007/s11270-005-7905-4.
- [10] Wiedenmann A, Krüger P, Dietz K, López-Pila J M, Szewzyk R & Botzenhart K, A randomized controlled trial assessing infectious disease risks from bathing in fresh recreational waters in relation to the concentration of Escherichia coli, Intestinal Enterococci, Clostridium perfringens, and Somatic Coliphages, 114(2) (2006) 228–236. ISSN 1552-9924. https://doi.org/10.1289/ehp.8115.
- [11] Prüss A, Review of epidemiological studies on health effects from exposure to recreational water, 27(1) (1998) 1–9. ISSN 1464-3685. https://doi.org/10.1093/ije/27.1.1.
- [12] United States Environmental Protection Agency. Implementation guidance for ambient water quality criteria for bacteria. Tech. rep., U.S. Environmental Protection Agency, Washington, DC (2002). Guidance Document.
- [13] Mansilha C R, Coelho C A, Heitor A M, Amado J, Martins J P & Gameiro P, Bathing waters: New directive, new standards, new quality approach, 58(10) (2009) 1562–1565. ISSN 0025-326X. https://doi.org/10.1016/j.marpolbul.2009.03.018.
- [14] World Population Review. World population review Data on global demographics. URL https://worldpopulationreview.com/.
- [15] Rowland F E, North R L, McEachern P, Obrecht D V, Gurung T B, Jones S B & Jones J R, Phytoplankton nutrient deficiencies vary with season in sub-tropical lakes of Nepal, 833(1) (2019) 157–172. ISSN 1573-5117. https://doi.org/10.1007/s10750-019-3897-8.
- [16] Rai A K, Limnological characteristics of subtropical Lakes Phewa, Begnas, and Rupa in Pokhara Valley, Nepal, 1(1) 33– 46. ISSN 1439-863X. https://doi.org/10.1007/s10201 0070027.
- [17] Husen M A, Storey R G & Gurung T B, Water quality patterns, trends and variability over 17+ years in Phewa Lake, Nepal, 28(1) (2023) e12426. ISSN 1440-1770. https://doi.org/10.1111/lre.12426.

- [18] Haller L, Poté J, Loizeau J L & Wildi W, Distribution and survival of faecal indicator bacteria in the sediments of the Bay of Vidy, Lake Geneva, Switzerland, 9(3) (2009) 540–547. ISSN 1470-160X. https://doi.org/10.1016/j.ecolind.2008.08.001.
- [19] Gregory L F, Gitter A, Muela S & Wagner K L, Should contact recreation water quality standards be consistent across hydrological extremes?, 166(1) (2019) 12–23. ISSN 1936-704X. https://doi.org/10.1111/j.1936-704x.2019.03298.x.
- [20] Korajkic A, McMinn B R & Harwood V J, Relationships between microbial indicators and pathogens in recreational water set-

- tings, 15(12) (2018) 2842. ISSN 1660-4601. https://doi.org/10.3390/ijerph15122842.
- [21] Wilkinson J, Jenkins A, Wyer M & Kay D, Modelling faecal coliform concentrations in streams, Water Res., 29(3) (1995) 847. URL https://api.semanticscholar.org/CorpusID: 129140440.
- [22] Deller S, Mascher F, Platzer S, Reinthaler F F & Marth E, Effect of solar radiation on survival of indicator bacteria in bathing waters, 14(3) (2006) 133–137. ISSN 1803-1048. https://doi.org/10.21101/cejph.a3380.