UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

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Monitoring the seasonal dynamics of the microbial load on the Machángara River

.

Alison Briggette Cabrera Ontaneda

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Alison Briggette Cabrera Ontaneda

Nombre del profesor, Título académico **Antonio Machado, Ph.D. en Ing. Biomédica**

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Nombres y apellidos:	Alison Briggette Cabrera Ontaneda
Código:	00211957
Cédula de identidad:	1150317038
Lugar y fecha:	Quito, 20 de diciembre de 2023

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RESUMEN

La contaminación de las aguas superficiales es un problema mundial que afecta a los países en desarrollo que carecen de infraestructuras adecuadas de tratamiento de aguas residuales. Lo que supone riesgos significativos tanto para su economía como para la salud pública. El objetivo de este estudio fue examinar la variabilidad espacio-temporal de la carga microbiana e identificar patógenos de interés sanitario en el río Machángara, en Quito (Ecuador), durante las estaciones lluviosa, de transición y seca. *Escherichia coli* y los coliformes totales se cuantificaron utilizando medios de cultivo cromogénicos, mientras que para la identificación de organismos se empleó la reacción en cadena de la polimerasa (PCR). Los microorganismos objetivo abarcaban diversos patotipos de *E. coli*, como enterohemorrágico (EHEC), enteroinvasivo (EIEC), enteropatogénico (EPEC) y enteroagregativo (EAEC), así como *Cryptosporidium* y *Giardia* spp, *Helicobacter pylori*, especies de *Campylobacter* (*C. jejuni, C. upsaliensis* y *C. coli*), *Mycobacterium tuberculosis* y *Mycobacterium leprae*. Los resultados revelaron que los niveles de *E. coli* y coliformes superaban las normas internacionales (Directrices de la Unión Europea, Estados Unidos de América y Brasil). En particular, el estudio identificó los cuatro patotipos de *E. coli* (EHEC, EIEC, EPEC y EAEC), *Cryptosporidium* y *Giardia* spp., *H. pylori, C. jejuni, C. upsaliensis* y *M. tuberculosis. Cryptosporidium* spp., EPEC, *H. pylori* y *C. upsaliensis* estuvieron presentes de forma esporádica, mientras que el resto de patógenos mostraron una contaminación persistente. Este estudio proporciona información valiosa sobre la dinámica microbiana del río Machángara, subrayando su profunda contaminación y enfatizando la urgente necesidad de estrategias de mitigación para facilitar su remediación.

Palabras clave: Río Machángara, dinámica microbiana, *Escherichia coli*, coliformes, parásitos, estacional.

ABSTRACT

Surface water pollution is a global issue, impacting developing countries that lack adequate wastewater treatment infrastructure. This poses significant risks to both its economy and public health. The objective of this study was to examine the spatio-temporal variability of the microbial load and identify health-concern pathogens in Machángara River in Quito (Ecuador); across the rainy, transitional, and dry seasons. *Escherichia coli* and total coliforms were quantified using chromogenic culture media, while the identification of organisms employed polymerase chain reaction (PCR). The targeted microorganisms encompassed various *E. coli* pathotypes, such as enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteropathogenic (EPEC), and enteroaggregative (EAEC), as well as *Cryptosporidium* and *Giardia* spp., *Helicobacter pylori*, *Campylobacte*r species (*C. jejuni, C. upsaliensis,* and *C. coli*), *Mycobacterium tuberculosis*, and *Mycobacterium leprae*. The findings revealed that levels of *E. coli* and coliforms surpassed international standards (Guidelines of the European Union, United States of America, and Brazil). Notably, the study identified all four *E. coli* pathotypes (EHEC, EIEC, EPEC, and EAEC), *Cryptosporidium* and *Giardia* spp., *H. pylori, C. jejuni, C. upsaliensis,* and *M. tuberculosis*. *Cryptosporidium* spp., EPEC, *H. pylori,* and *C. upsaliensis* were sporadically detected, whereas the remaining pathogens were exhibited as persistent contamination. This study provides valuable insights into the microbial dynamics of the Machángara River, underscoring the profound contamination of the river and emphasizing the urgent need for mitigation strategies to facilitate its remediation.

Keywords: Machángara River, microbial dynamics, *Escherichia coli*, coliforms, parasites, seasonal.

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1. INTRODUCTION

1.1 Global water pollution

One of the most critical challenges facing humanity in the contemporary era is related to issues concerning both the quantity and quality of water. Most human activities utilizing water result in the generation of wastewater, thereby contributing to environmental problems (Environment and Natural Resources Department, 2022). Water, as a vitally important resource, has faced increasing constraints in recent years. The exponential growth of the global population has led to a heightened demand for water, exacerbating the volume of wastewater and its associated pollution (Schwarzenbach, Egli, Hofstetter, von Gunten, & Wehrli, 2010). This rising crisis has culminated in a global crisis, particularly impacting developing countries with less robust health infrastructures.

Nowadays, low- and middle-income countries treat only 28% and 8%, respectively, of municipal and industrial wastewater before discharge (Environment and Natural Resources Department, 2022). Shockingly, on a global scale, approximately 80% of wastewater is released into the environment without undergoing adequate treatment (United Nations, 2017). This pollution predicament is poised to worsen in the future due to climate change, resulting in elevated temperatures and the intensification of water cycles (United Nations, 2017). These changes will have far-reaching repercussions, negatively impacting the economies and productivity of countries. Furthermore, this environmental challenge extends beyond economic concerns, affecting ecosystems and humans. The increased exposure to pathogenic microorganisms or chemicals occurs through various pathways, including the food chain and recreational sources such as contaminated surface waters (Magana-Arachchi & Wanigatunge, 2020). Addressing this multifaceted problem requires concerted global efforts to develop sustainable water management practices by improving wastewater treatment infrastructure, and mitigating the adverse effects of climate change on water resources.

1.2 Pichincha province

The province of Pichincha, situated in Ecuador, spans an approximate area of 9,466 km² and boasts an elevation of 2,816 meters above sea level. Pichincha possesses diverse climates including humid tropical, humid and dry mesothermal, paramo, and frigid, evidencing a temperature fluctuation between 8 to 24 ºC. An approximate population of 3,228,233 resides nowadays in Pichincha province, which is positioned within the Guayllabamba River basin (Prefectura de Pichincha, 2021) and features numerous rivers as extensive water networks, such as Monjas, Chiche, San Pedro, Machángara, and others located in the capital, Quito (EPMAPS, 2014).

1.3 Machángara River

The city of Quito grapples with pollution issues similar to those previously described. Currently, Quito treats only 3% of the wastewater discharged before entering rivers and streams (EPMAPS, 2020). The Machángara River is a crucial water source for Quito and encapsulates the gravity of the anthropogenic contamination. Machángara River originates from the Atacazo hill and crosses Quito reaching the Cumbayá area and eventually converging with the San Pedro River to form the Guayllabamba River (Chuqui, 2021). The Machángara River possesses favorable features, such as a steep gradient, turbulence, and efficient oxygenation, facilitating a self-purification mechanism and spanning approximately 22.5 km with an average flow of 4 m³/s and a daily organic matter load of around 52 tons (Reinoso C, 2015). Despite all these advantages, the river confronts a formidable pollution challenge upon entering the urban environment. Upon reaching the city, the Machángara River bears the brunt of a substantial pollution load receiving a staggering 76% of wastewater discharges (Reinoso C, 2015). This pollution burden becomes especially pronounced during the dry season when the river flow primarily consists of untreated sewage (Huang et al., 2016). This stark reality underscores the severe repercussions of the environmental crisis facing the region.

1.4 Analysis of microbial load in rivers

One of the most significant risks associated with water source contamination is the proliferation of microorganisms and the subsequent transmission of infectious diseases (Rodríguez-Miranda, García-Ubaque, & García-Ubaque, 2017). The role of water in spreading infectious diseases is a global leading cause of outbreaks in 132 countries (World Health Organization., 2003). In Latin America, water sanitation issues contribute to over 4,000 premature deaths, primarily linked to increased exposure to bacteria, protozoa, and viruses, elevating the risk of infection through the fecal-oral route of transmission (Pan American Health Organization, 2016). Numerous pathogens have been reported in studies, such as *Escherichia coli* pathotypes *Cryptosporidium* spp., *Giardia intestinalis, Legionella* and *Salmonella* species, *Vibrio cholerae*, and new emerging agents like *Helicobacter* species (Magana-Arachchi & Wanigatunge, 2020). Microbial monitoring in water sources is crucial for the Public Health system. The assessment of water quality and pollution commonly evaluates certain types of microorganisms such as *E. coli* and total coliforms (a group of Gram-negative oxidase negative, non-spore-forming, aerobic, or facultative anaerobic rod-shaped bacteria usually from Enterobacteriaceae family), being universally used as an indicator of contamination as well (Schwarzenbach et al., 2010). Thus, high levels of *E. coli* in water usually provide clear evidence of fecal contamination, while high levels of coliforms (without high levels of *E. coli*) are likely associated with other contamination sources (such as soil or vegetation among others) providing a warning of more serious contamination(s). Nonetheless, the absence of coliform bacteria and *E. coli* does not necessarily imply that the water is unlikely to be contaminated because other bacteria and pathogens could be present and more resistant to disinfection (Brandt, Johnson, Elphinston, & Ratnayaka, 2017).

In the case of the Machángara River, the escalating pollution issue had been previously reported and characterized by its multiple microbial proliferation. Preliminary analyses in this

watercourse revealed significant concentrations of *Escherichia coli* and total coliforms exceeding international standards. Moreover, various health-significant microorganisms have been documented, including *E. coli* pathotypes (enteroaggregative *E. coli* and enteroinvasive *E. coli*), *Cryptosporidium* and *Giardia* parasites, and several bacterial general such as *Legionella, Pseudomonas, Salmonella,* and *Shigella* (Borja-Serrano et al., 2020; Vinueza et al., 2021) Despite these alarming findings, there is a conspicuous absence of longitudinal studies and this type of evaluation is essential for a comprehensive assessment of the dynamic microbial load within this river. Therefore, the primary objective of this study is to examine the spatio-temporal variability of microbial load in the Machángara River, with a specific focus on health-relevant microorganisms. The present study was conducted at three designated sampling points (a control point before Quito and two contaminated points within Quito) during three seasons (dry, transitional, and rainy) to provide a nuanced understanding of river microbial dynamics over time and space. This study evaluated the current contamination and contributed to building a foundation for informed decision-making and effective strategies to combat water pollution and Public Health risks.

2. METHODS

This study was conducted within a research group and shares methods with the research work titled ‘‘Monitoring Microbial Load in the Seasonal Dynamics of the San Pedro River’’, performed by María Paula Yepez del Pozo Tobar.

2.1 Sample site and collection

Water samples were collected from three sampling points across Machángara River located in the province of Pichincha, Ecuador (**Figure 1**). Samples from points 1 and 2 were taken from urban sites with high proximity to population and several contamination effluents while point 0 located outside of Quito was used as control. Samples were collected between November 2022 and July 2023 on two different dates during each of the three seasons, more exactly, rainy, transitional, and dry seasons (**Table 1**).

Most of the microbial evaluation is similar to previous studies (Borja-Serrano et al., 2020; Vinueza et al., 2021) that also evaluated the microbial load in rivers of Ecuador. Briefly, samples were collected by duplicated in glass jars of 800 mL capacity each, previously sterilized by autoclaving at 121ºC. Samples were collected by immersing the bottles in superficial water (0.3 m depth). To preserve the samples, they were transported in a cooler at 4ºC with ice packs to the Microbiology Institute at the Universidad San Francisco de Quito (IM-USFQ).

2.2 Sample preparation for microbiological analysis

All samples were filtered using a vacuum pump under aseptic conditions (Chemical Duty Pump, Millipore, Merck, Burlington, MA, USA) through a 0.45 µm nitrocellulose membrane (Millipore, Merck, Burlington, MA, USA). For highly contaminated points (M1 and M2) up to 2 membranes were used to avoid malfunction of the filtration step, while for the control points (M0) a single membrane was used. The subsequent procedures were adapted

from previous studies (Borja-Serrano et al., 2020; Vinueza et al., 2021). Once at least 100 mL of water was filtered, the membrane was removed with sterile tweezers and placed in a Falcon tube with 20 mL of sterile distilled water. For resuspending the particles and microorganisms, the Falcon tube was vortexed for 10-15 minutes at maximum speed. The membrane was then removed, and the Falcon tubes were centrifuged at 7000 rpm for 15 minutes. The supernatant was discarded, and the pellet was suspended in 2 ml of sterile distilled water. Each sample was divided into 3 aliquots of 500 µL. One aliquot for the extraction of bacterial DNA, another aliquot for microbial growth cultures, and an extra aliquot for sample storage.

2.3 Cultivation of *Escherichia coli* and coliforms

For the total count of *Escherichia coli* and coliforms, serial dilutions from one of the aliquots were cultivated in Chromocult Agar culture medium (Merck; Biolab, Wadeville, Gauteng, South Africa) by the 3-drop culture technique according to previous protocols (Borja-Serrano et al., 2020; Herigstad, Hamilton, & Heersink, 2001; Naghili et al., 2013; Vinueza et al., 2021). Briefly, a volume of 10 µL of each sample and their serial 10-fold dilutions (from 10^{-1} until 10^{-4}) was deposited horizontally on the upper edge of the media petri dish in triplicate (so-called 3-drop culture). The petri dish was then turned upside down, allowing each sample to drop down without touching the bottom edge or joining each other. Finally, they were incubated at 37°C for 24-48 hours and all colony-forming units (CFU) of each sample were enumerated taking into account their respective serial dilutions.

2.4 DNA extraction procedure

DNA extraction from samples was performed following the manual of the commercial PowerSoil DNA Pro Kit (Qiagen, Venlo, Netherlands) following the manufacturer's instructions. As previously stated, this commercial kit used PowerBead tubes, which contained a buffer that dispersed the soil particles, dissolved humic acids, and protected nucleic acids from degradation (Borja-Serrano et al., 2020). Finally, the DNA solution was stored at -20 ºC

for further PCR.

2.5 Molecular identification of pathogen genera

As the DNA of the samples was extracted, specific primers were employed for the determination of pathogen genera and/or species by PCR (polymerase chain reaction) (**Table 2**). The PCR Matermix consisted of a final volume of 15 µL, containing 3 µL of 1X Green GoTaq Flexi buffer (Promega, Madison, USA), 0.9-3 µL of 2.0 Mm $MgCl_2$ (Promega, Madison, USA), 0.3-1 µL of 0.20 Mm dNTPs Mix (Promega, Madison, USA), 0.45-1 µL of 0.5 Mm of each PCR primer; 0.08-0.15 µL of 0.5 U GoTaq Flexi DNA polymerase (Promega, Madison, USA), 1-2 µL of template DNA, and the remaining volume of DNA-free water. The PCR was performed in a thermocycler (Bio-Rad Laboratories Inc.). Positive and negative controls were used for the identification of the pathogenic species, the positive controls were provided by the IM-USFQ. The PCR products were visualized using electrophoresis with 1.5-2% agarose gel and staining with SYBR Safe.

3. RESULTS

3.1 *Escherichia coli* and total coliform counts Machángara River

The count of *E. coli* and total coliforms in the Machángara River was analyzed in three different collection points during three seasons (**Table 1**). As shown in **Table 3**, the average amount of *Escherichia coli* and total coliforms in all samples of Machángara River exceeds the limits allowed by the European Union guidelines (European Union Law, 2006), the United States of America standard values of the Recreational Water Quality Criteria (EPA, 2012), and the Brazilian guidelines for bathing waters according to the CONAMA Resolution No. 274 of November 29, 2000 (Ambiente, 2001), excepting for the *E. coli* count at point M0 during the rainy season.

As shown in **Figure 2**, the levels of *E. coli* and total coliforms demonstrated the same trend of results, where M1 and M2 points showed superior microbial levels when compared to the M0 control point. During the rainy season, the sampling point with the highest amount of *E. coli* and total coliforms was the M2 point with 9×10^7 CFU per 100 mL and 2.42×10^8 CFU per 100 mL, respectively. While during the transitional season the sampling collection point M1 contained the highest amount of *E. coli* and total coliforms, more exactly 4×10^8 and 7.5×10^8 CFU per 100 mL, respectively. Finally, in the dry season, the sampling point M1 had a concentration of *E. coli* CFU and total coliforms of 5.83×10^8 and 1.13×10^9 CFU per 100 mL, respectively. Therefore, the highest concentrations of *E. coli* and coliforms were detected in the dry season and the lowest were detected in the rainy season, as expected. Furthermore, the M0 control point remained with the lowest values of microbial load in all seasons. It is important to mention that the M1 point evidenced the highest count values for *E. coli* and total coliforms in the transition and dry seasons showing slightly higher microbial loads when compared to the M2 point. Nonetheless, the M2 point demonstrated the highest microbial count values when compared to the M1 point in the rainy season.

3.2 Molecular identification of pathogens on superficial waters of Machángara River

Further molecular characterization was performed by polymerase chain reaction (PCR) to evaluate the presence of clinically relevant primary and opportunistic pathogens in our group set of samples (**Table 4**). Several pathogens were identified on superficial waters of the Machángara River, more exactly *E. coli* pathotypes (EAEC, EHEC, EPEC, EIEC), *Cryptosporidium* and *Giardia* spp., *Mycobacterium tuberculosis, Helicobacter pylori, Campylobacter jejuni*, and *C. upsaliensis. E. coli* pathotypes and *Giardia* spp. were identified at M1 and M2 points in all three seasons, except EPEC which was found only in the dry season at the same points. At the same collection points, *C. jejuni* was detected in both rainy and dry seasons, while *C. upsaliensis* and *Cryptosporidium* spp. were only identified in transitional and dry seasons, respectively. Meanwhile, *Helicobacter pylori* was only detected in the transitional season at the M1 point. Finally, *Mycobacterium tuberculosis* was identified in all samples over time. It is important to mention that *Mycobacterium leprae* and *C. coli* were not identified at Machángara River in the present study.

4. DISCUSSION

In Ecuador, the current state of pollution, primarily caused by agricultural, untreated domestic, and industrial wastes, significantly impacts the major water resources (Borja-Serrano et al., 2020; Vinueza et al., 2021). Consequently, water bodies face heightened contamination fostering the proliferation of opportunistic and primary pathogens. The Machángara River, receiving 76% of wastewater discharges from the city (Reinoso C, 2015), stands out for its high microbial load in the region. Moreover, the values of *E. coli* and total coliform surpassed the maximum limit according to international legislation (**Table 3**).

As illustrated in **Figure 2**, a recurring pattern emerges where points M1 and M2 consistently exhibited the highest values of *E. coli* and total coliform counts throughout all seasons. This context could be partially explained due to certain key industries in Quito, such as food production, chemicals, and manufacturing, as major environmental impactors as previously postulated (Reinoso C, 2015). These industries were located at M1 and M2 points, where M1 point is identified as the focal industrial area of contamination and also includes nearby agricultural regions. These contamination sources contribute to the sustained elevation in microbial counts over time. Contrastingly, the M0 point demonstrated the lowest values for *E. coli* (0-2×10^4 CFU/100 mL) and total coliforms (5×10^3 - 3×10^4 CFU/100 mL). This could be attributable to rural anthropogenic activities (local agricultural and untreated domestic wastewater) due to its substantial distance from urban areas. Furthermore, **Figure 2** showed an increase in the *E. coli* and total coliform loads as the rainy season ended and the dry season started. As expected, the increased microbial concentrations can be explained due to the reduced water volume in the river.

In addition, the present results were in agreement with previous studies realized at same river (Borja-Serrano et al., 2020; Vinueza et al., 2021), revealing elevated values that surpass

the same international guidelines, more exactly *E. coli* concentration ranges of 1.17-9.18×10^2 and 2.25×10^4 CFU/100 mL and coliforms concentration ranges of 3.95-5.15×10^4 and 3.25×10^4 CFU/100 mL. Likewise, similar values were quantified in other global studies. In Ghana, (Apau et al., 2022) a study reported the highest levels of *E coli* and coliforms (9.29×10^6 and 9.1×10^{11} CFU/100 mL, respectively). Meanwhile, studies in India and Indonesia evidenced lower levels of *E coli* (5.8×10^4 and 7.9×10^5 CFU/100 mL, respectively) and coliforms (1.2×10^5 and 3.5×10^5 CFU/100 mL, respectively) (Mariya, Kumar, Masood, & Kumar, 2019; Puspitasari & Hadi, 2022). Although most of these studies reported lower microbial loads, these regions also reported troubles in their inefficient wastewater treatment plant programs.

Regarding the microorganisms detected in the present study (**Table 4**), the determination of certain *E. coli* pathotypes was also assessed, more exactly enteroaggregative *E. coli* (EAEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), and enteroinvasive *E. coli* (EIEC). Most of them remained consistently present in points M1 and M2 throughout the seasons, except for EPEC appearing exclusively in the dry season. Although *E. coli* stands as a crucial parameter for monitoring water quality and is widely employed as an indicator of fecal contamination (Farfán-García, Ariza-Rojas, Vargas-Cárdenas, & Vargas-Remolina, 2016), little is still known about the persistency of *E. coli* pathotypes in most worldwide rivers. In Ecuador, previous reports underscored the predominance of EHEC, EIEC, and EAEC pathotypes in this river (Borja-Serrano et al., 2020; Vinueza et al., 2021). However, the present work was able to confirm its persistence among different seasons. In addition, studies in Brazil and Mexico identified EPEC and EAEC in the Beberibe and San Pedro Rivers (Freitas, Paiva, Carvalho Filho, Cabral, & Rocha, 2015; Ramírez Castillo, Avelar González, Garneau, Márquez Díaz, et al., 2013). In Italy, a study identified EHEC and EAEC in the Reno River (Ferronato et al., 2013). The significance of these organisms lies in the presence of pathogenic strains impacting public health leading to illnesses such as diarrhea, urinary

infection, sepsis, meningitis, and even death (Restrepo-Álvarez, Bernal, Ascuntar-Tello, & Jaimes, 2019). Several primary pathogenicity mechanisms have been reported on these *E. coli* pathotypes allowing their success in population dissemination, even in aquatic environments, and host immune responses (Cangui-Panchi et al., 2023). Some examples of these pathogenicity mechanisms include host epithelial adhesion among EAEC and EPEC (Farfán-García et al., 2016), enterotoxins production such as STX (Shiga) in EHEC, and even immune invasion strategies of the epithelium in EIEC (Alarcón Ruth & Li Jessica, 2007).

Moreover, *Cryptosporidium* and *Giardia* parasites were also detected at points M1 and M2 being responsible for gastrointestinal illness. Their presence is usually associated with the proximity to pollution sources, particularly sewage and animal waste discharge (Li et al., 2019). Recently, reports on the Sacramento and San Joaquin Rivers in California have found that extreme weather events, such as heavy rainfall, can negatively impact oocyst concentrations by dilution (Li et al., 2019), which could explain the absence of *Cryptosporidium* spp. in the remaining seasons. In opposition, *Giardia* spp. was consistently present in M1 and M2 during all seasons. The viability of *Giardia* cysts in the environment usually increases at low or moderate temperatures (Wilkes et al., 2011). This could explain their persistent presence over time in the typical temperate climate on the river in the central Andean region. The present study confirmed the preliminary results of previous cross-sectional studies realized at the same river (Borja-Serrano et al., 2020; Vinueza et al., 2021), evidencing a higher prevalence of *Giardia* over *Cryptosporidium* in rivers of Pichincha.

Finally, other pathogens were also identified in the present study, more exactly, *Helicobacter pylori*, *Mycobacterium tuberculosis*, *M. leprae*, *Campylobacter jejuni*, and *C. upsaliensis*. *H. pylori* is associated with gastric infections and peptic ulcers (Machado, Zamora-Mendoza, Alexis, & Álvarez-Suarez, 2023) and was only detected in the M1 point in the

transitional season. Although the definitive infection mechanism of this pathogen is not fully known, water sources have been implicated as a risk factor for transmission with a higher prevalence in developing countries (Salih, 2009). However, animal waste disposal and sewage discharge are issues recognized at M1 point and constitute factors associated with the transmission of *H. pylori* infection (Bellack, Koehoorn, Macnab, & Morshed, 2006).

Regarding *M. tuberculosis*, its detection in environmental samples has not received as much attention. However, evidence suggests that water may be an important vector of transmission of tuberculosis (Mtetwa, Amoah, Kumari, Bux, & Reddy, 2022). Preliminary studies in Tanzania revealed its relation to animal waste disposal, encompassing a broad spectrum including urine, feces, oronasal mucus, and even secretions (Jaramillo Tobón, 2011). Thus, the presence of animals and even humans at all three points (M0, M1, and M2) could explain its incidence over time. On the other hand, *M. leprae* was absent in all locations over time, which could be potentially associated with the low presence of reservoirs (Tió-Coma et al., 2019), and the currently low incidence of clinical cases in Ecuador (Moncada Diego, 2019). Lastly, *C. jejuni* and *C. upsaliensis* species were detected in two and one of the three seasons, respectively, at M1 and M2 points. *C. jejuni* is considered a primary cause of bacterial gastroenteritis and it is frequently detected in surface water being associated with wastewater discharge (Pitkänen, 2013). This species exhibits mechanisms to respond to environmental stress, surviving in various environments, such as aquatic environments at low temperatures (Nilsson, 2018). Hence, its higher incidence is understandable. On the other hand, the usual reservoirs of *C. upsaliensis* are limited to domestic animals (Igwaran & Okoh, 2019), so its prevalence in the environment is usually lower. In recent years, reports have emerged that recognized them as an emerging human enteropathogenic microorganism (Igwaran & Okoh, 2019). Therefore, its presence is also a risk factor to be considered in further studies.

5. CONCLUSIONS

The substantial wastewater discharge and numerous contamination sources close to the Machángara River demonstrated a consistently high microbial load in M1 and M2 points throughout the seasons (rainy, transitional, and dry). The high counts of *E. coli* and coliforms exceeded not only the international legislation but also surpassed the contaminations reported in the literature. Peak levels were particularly observed during the dry season, as expected, correlating with increased organism concentrations attributed to reduced water dilution. Furthermore, it is important to mention that the M0 point exhibited the lowest count values of *E. coli* and coliforms. Similarly, nearly all the pathogens analyzed in this study remained absent at this location. Results at M0 point suggest that the water resource, in its natural state, does not pose a microbial load of concern. However, M1 and M2 points within Quito confirmed numerous sources of contamination leading to these microbial augmentations and risking public health among the population.

Likewise, various primary pathogens of health concern were identified, including *E. coli* pathotypes (EHEC, EIEC, EPEC, and EAEC), *Cryptosporidium* and *Giardia* spp., *H. pylori, C. jejuni*, *C. upsaliensis,* and *M. tuberculosis,* in M1 and M2 points. However, it is important to note that *M. tuberculosis* was also detected in the M0 point. Moreover, some of these organisms exhibited dynamic population changes according to the season. It is also crucial to highlight that the obtained PCR results will undergo further validation through Sanger sequencing. Subsequent studies should aim to identify the sources of these pathogens. Furthermore, ongoing research seeks to explore the relationship between the microbial load and physico-chemical parameters at Machángara River during seasons. This approach is essential for efficiently explaining the persistent presence of specific pathogenic microorganisms over time, facilitating the proposal of effective alternatives to mitigate their impact on the environment and public health in the country.

6. TABLES

Table 1. Collection points and dates from the Machángara River for microbial analysis in this study.

Sample Code	GPS Coordinates	Location (City)	Region	Season	Collection sampling	Name and additional data of INAMHI Station*
M0	0°20'8"S 78°34'58"W	Guamaní (Quito)	Andean	Rainy	11/11/2022 25/11/2022	M0325 Garcia Moreno 0°14′5"S/78°37′38"W 1950 meters high
				Transitional	11/03/2023 19/03/2023	
				Dry	15/06/2023 29/06/2023	
M1	0°13'18,1"S 78°29'12,4"W	Puengasí (Quito)	Andean	Rainy	11/11/2022 25/11/2022	
				Transitional	11/03/2023 19/03/2023	
				Dry	15/06/2023 29/06/2023	
M2	0°11'07.0"S 78°24'53.8"W	Nayón (Quito)	Andean	Rainy	14/11/2022 28/11/2022	
				Transitional	10/03/2023 17/03/2023	
				Dry	16/06/2023 30/06/2023	

Legend: [a] Data from the study conducted by Vinueza et al. (2021), ‘‘Determining the microbial and chemical contamination in Ecuador’s main rivers’’ Retrieved from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8531378/.

Table 2. Primers and PCR cycling parameters for the detection of various potential pathogens.

Organism	Primer name	Primer sequence (5′–3′)	PCR cycling parameters	Gene (size [bp])	References
Single PCR assays					
Universal	Forward: fDD2	CCGGATCCGTCGACAGAGTTTGATCITGGCTCAG	3 min at 94°C; 35 cycles of 94°C for 30 s, 54°C for 30 s, 72°C for 1.5 min	*16S* rRNA (1,600)	(Dobrowsky et al., 2014)
	Reverse: rPP2	CCAAGCTTCTAGACGGITACCTTGTTACGACTT			
Helicobacter pylori	Forward:	GCGGGATAGTCAGTCAGGTG	2 min at 94°C; 40 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 1 min	*16S* rRNA (706)	(Valenzuela & Machado, 2016)
	Reverse:	AAGATTGGCTCCACTTCGCA			
Campylobacter spp.	Forward: IpxA*C. coli*	AGA CAA ATA AGA GAG AAT CAG	2 min at 94°C; 35 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 45 s	*lpxA* (391)	(Klena et al., 2004)
	Forward: IpxAC. *jejuni*	ACA ACT TGG TGA CGA TGT TGT A		*lpxA* (331)	
	Forward: pxAC. *upsaliensis*	AAG TCG TAT ATT TTC YTA CGC TTG TGT G		*lpxA* (206)	
	Reverse: lpxARKK2m	CAA TCA TGD GCD ATA TGA SAA TAH GCC AT			
EAEC	Forward: AggRKs1	GTATACACAAAAGAAGGAAGC	2 min at 95°C; 35 cycles of 95°C for 1 min, 54°C for 1 min, 72°C for 1 min	*aggR* (254)	(Ramírez Castillo, Avelar González, Garneau, Díaz, et al., 2013)
	Reverse: AggRkas2	ACAGAATCGTCAGCATCAGC			
EHEC	Forward: VTcomU	GAGCGAAATAATTTATATGTG		*stx* (518)	
	Reverse: Vtcomd	TGATGATGGCAATTCAGTAT			
EPEC	Forward: SK1	CCCGAATTCGGCACAAGCATAAGC		*eae* (881)	
	Reverse: SK2	CCCGGATCCGTCTCGCCAGTATTCG			
EIEC	Forward: IpaIII	GTTCCTTGACCGCCTTTCCGATACCGTC		*ipaH* (619)	
	Reverse: IpaIV	GCCGGTCAGCCACCCTCTGAGAGTAC			
	Forward: S13	CTCCACCTGGACCGGCGAT			

Mycobacterium leprae	Reverse: S62	GACTAGCCTGCCAAGTCG	5 min at 95°C; 30 cycles of 94°C for 2 min, 58°C for 1 min, 72°C for 2min	*pra* (531)	(Arunagiri, Sangeetha, Sugashini, Balaraman, & Showkath Ali, 2017)
Nested PCR assays					
Mycobacterium tuberculosis	Forward: Mpb1	TCCGCTGCCAGTCGTCTTCC	5 min at 95°C; 30 cycles of 95°C for 30 s, 54°C for 30 s, 72°C for 30 s	*MPB64* (240)	(Madhavan, Therese, Gunisha, Jayanthi, & Biswas, 2000)
	Reverse: Mpb2	GTCCTCGCGAGTCTAGGCCA			
	Forward: Mpb3	ATTGTGCAAGGTGAACTGAG	5 min at 95°C; 35cycles of 95°C for 30 s, 58°C for 30 s, 72°C for 30 s	*MPB64* (200)	
	Reverse: Mpb4	AGCATCGAGTCGATCGCGGA			
Cryptosporidium spp.	Forward: Cry 15	GTAGATAATGGAAGAGATTGTG	10 min at 95°C; 45 cycles of 94°C for 30 seconds, 52°C for 30 seconds, 72°C for 50 seconds	*COWP* (550)	(Salza, 2014; Yu, Lee, & Park, 2009)
	Reverse: Cry 9	GGACTGAAATACAGGCATTATCTT			
	Forward: Cowpnest F	TGTGTTCAATCAGACACAGC	10 min at 95°C; 32 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 50 s.	*COWP* (311)	
	Reverse: Cowpnest R	TCTGTATATCCTGGTGGG			
Giardia spp.	Forward: AL3543	AAATTATGCCTGCTCGTCG	5 min at 94°C; 35 cycles of 94°C for 45s, 50°C for 45 s, 72°C for 1 min	*TPI* (605)	(Salza, 2014)
	Reverse: AL3546	CAAACCTTTTCCGCAAACC			
	Forward: AL3544	CCCTTCATCGGTGGTAACTT	5 min at 94°C; 35 cycles of 94°C for 45s, 53°C for 30 s, 72°C for 1 min	*TPI* (530)	
	Reverse: AL3545	GTGGCCACCACTCCCGTGCC			

Legend: As previously mentioned, the methods are shared with the project carried out by María Paula Yepez.

Table 3. The average amount of *Escherichia coli* and total coliforms in the Machángara River across the three seasons and water classification applied to bathing-water standards by USA, European, and Brazilian guidelines.

Season	Sample Code	GPS Coordinates	*Escherichia coli* (CFU per 100 mL ± SD)	Total coliforms (CFU per 100 mL ± SD)	USA guidelines (*E. coli*: ≤100-126 CFU per 100 mL[a]; No values are given for total coliforms)	European guidelines (*E. coli*: ≤500 CFU per 100 mL[b]; No values are given for total coliforms)	Brazilian guidelines (*E. coli*: ≤800 CFU per 100 Ml; Faecal (thermotolerant) coliforms: ≤1000 CFU per 100 mL[c]; No values are given for total coliforms
Rainy	M0	0°20'8"S 78°34'58"W	0.00E+00 (0)	1.67E+04 (2.36E+04)	Acceptable	Acceptable	Acceptable
	M1	0°13'18,1''S 78°29'12,4"W	8.17E+07 (5.89E+07)	2.05E+08 (1.25E+08)	Not acceptable	Not acceptable	Not acceptable
	M2	0°11'07.0"S 78°24'53.8"W	9.00E+07 (2.36E+07)	2.42E+08 (5.89E+07)	Not acceptable	Not acceptable	Not acceptable
Transitional	M0	0°20'8"S 78°34'58"W	2.00E+04 (2.83E+04)	3.00E+04 (3.77E+04)	Not acceptable	Not acceptable	Not acceptable
	M1	0°13'18,1''S 78°29'12,4"W	4.00+08 (9.43E+07)	7.5E+08 (7.07E+07)	Not acceptable	Not acceptable	Not acceptable
	M2	0°11'07.0"S 78°24'53.8"W	1.32E+08 (7.07E+06)	2.33E+08 (9.43E+06)	Not acceptable	Not acceptable	Not acceptable
Dry	M0	0°20'8"S 78°34'58"W	1.68E+03 (2.33E+01)	5.00E+03 (2.36E+03)	Not acceptable	Not acceptable	Not acceptable
	M1	0°13'18,1''S 78°29'12,4"W	5.83E+08 (7.07E+05)	1.13E+09 (9.43E+07)	Not acceptable	Not acceptable	Not acceptable
	M2	0°11'07.0"S 78°24'53.8"W	1.57E+08 (2.36E+05)	2.95E+08 (4.95E+07)	Not acceptable	Not acceptable	Not acceptable

Legend: SD – Standard deviation values; [a] Recreational Water Quality Criteria U.S. EPA, 2012. [b] Council of the European Union (2006). "Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC"). [c] Brazilian guidelines for bathing waters established by Resolution CONAMA n° 274 of 29 November 2000.

Table 4. Detection of different microorganisms by conventional PCR in the three sampling points of the Machángara River in the three seasons.

Microorganism	***Gene***	**Season**								
		Rainy			**Transitional**			**Dry**		
		M0	M1	M2	M0	M1	M2	M0	M1	M2
Universal	*16srRNA*	X	X	X	X	X	X	X	X	X
Cryptosporidium spp.	*COWP*	-	-	-	-	-	-	-	X	X
Giardia spp.	*TPI*	-	X	X	-	X	X	-	X	X
EAEC	*aggR*	-	X	X	-	X	X	-	X	X
EHEC	*stx*	-	X	X	-	X	X	-	X	X
EPEC	*ege*	-	-	-	-	-	-	-	X	X
EIEC	*ipaH*	-	X	X	-	X	X	-	X	X
Helicobacter pylori	*16srRNA*	-	-	-	-	X	-	-	-	-
Campylobacter jejuni	*IpxAC*		X	X	-	-	-	-	X	X
Campylobacter coli	*IpxAC*	-	-	-	-	-	-	-	-	-
Campylobacter upsaliensis	*IpxAC*	-	-	-	-	X	X	-	-	-
M. tuberculosis	*Mpb64*	X	X	X	X	X	X	X	X	X
M. leprae	*pra*	-	-	-	-	-	-	-	-	-

Legend: sampling collection points M0 Guamaní, control point; M1 Puengasí and M2 Nayón. The presence of microorganisms is represented with (X) and the absence with (-).

7. FIGURES

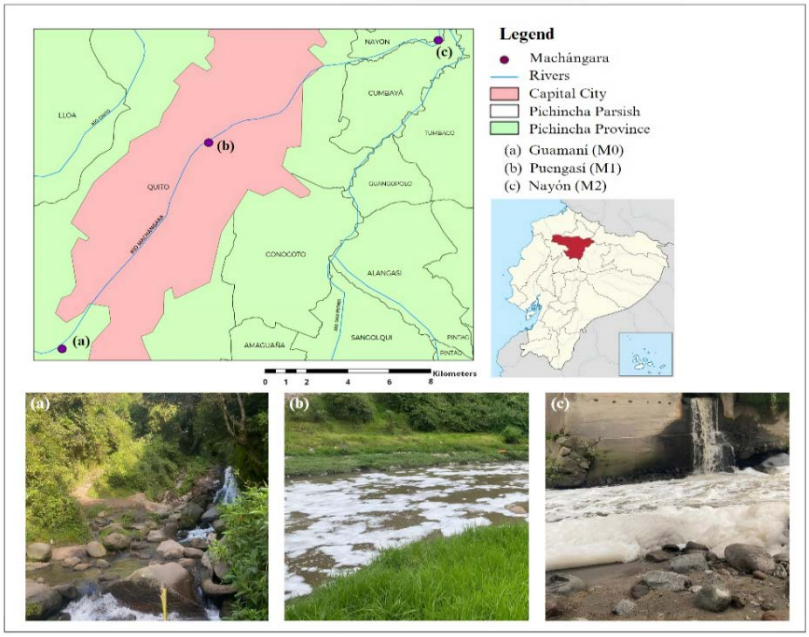

Figure 1. General map of the Machángara River with sample collection points (a) Guamaní, (b) Puengasí, and (c) Nayón. The map was made with ArcGIS Desktop software 10.8.

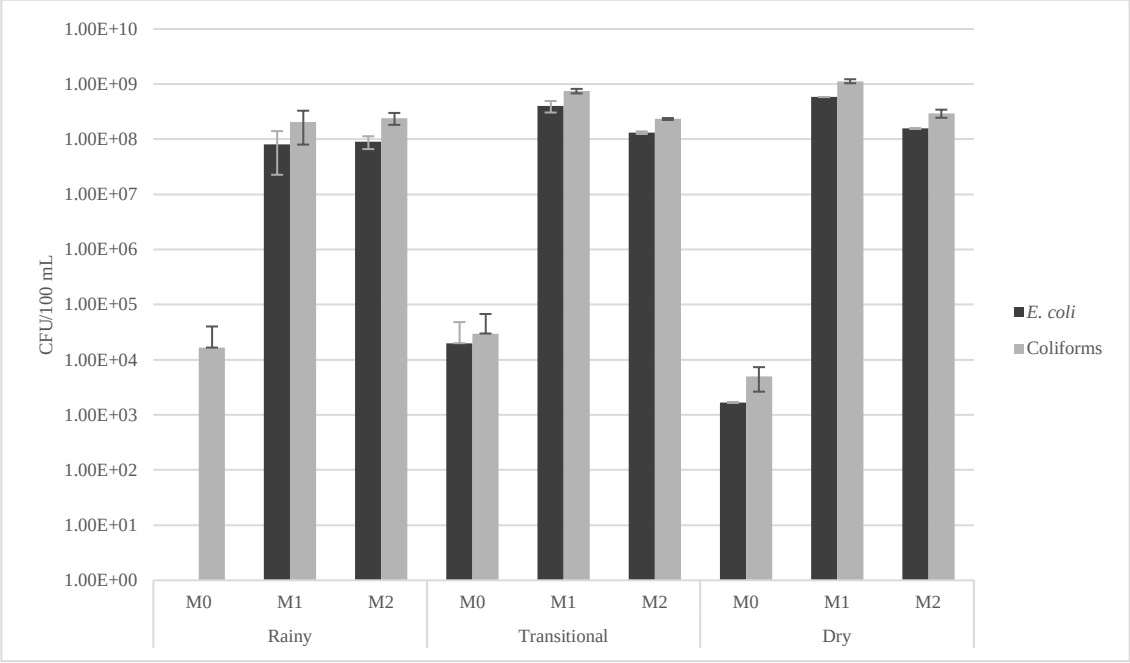

Figure 2. Average count of *E. coli* and total coliforms on Machángara River in different sampling points during rainy, transitional, and dry seasons. Sampling collection points were the following: M0 – Guamaní point; M1 – Puengasí point; and, M2 – Nayón point.

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