UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

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Seasonal Variability in Microbial Contamination in Three Rivers of Mindo: A Comparative Analysis of the Saguambi, Mindo and Canchupí Rivers

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RESUMEN

Ecuador depende de fuentes de agua superficiales y subterráneas, siendo sus ríos el principal recurso hídrico. Lamentablemente, el aumento de la población y el turismo en ciertas zonas ha provocado la contaminación en estos cuerpos de agua. Una de estas zonas es la parroquia de Mindo, que no tiene acceso al agua potable y las aguas residuales son vertidas directamente en sus ríos. En respuesta a ello, este estudio analizó muestras de agua en dos puntos de recolección en los ríos Saguambi, Mindo y Canchupí durante tres épocas del año, evaluando si el agua es apta para el consumo y actividades recreativas. El estudio encontró altas concentraciones de *Escherichia coli* y coliformes totales en todos los ríos, superando los límites establecidos por varias directrices internacionales en la mayoría de los puntos de muestreo. Además, numerosos patógenos oportunistas y primarios fueron encontrados en los ríos, los cuales ponen en riesgo la salud animal y humana. El análisis detectó el ADN de varios patógenos, entre ellos *Mycobacterium tuberculosis, Mycobacterium leprae, Helicobacter pylori*, *Giardia* y *Cryptosporidium* spp., siendo *M. tubercolisis* el patógeno más prevalente. Especialmente durante la época seca, el río Canchupí después de la comunidad, presentó niveles de contaminación elevados, lo que sugiere una contribución significativa de patógenos procedentes de las zonas urbanas de Mindo. Adicionalmente, la secuenciación Sanger y la herramienta Blastn corroboraron los resultados de la PCR, a excepción de *Cryptosporidum* spp. Este estudio brinda información relevante sobre la variabilidad microbiológica estacional de tres ríos de Mindo, y subraya la necesidad de un tratamiento adecuado de aguas residuales y disponibilidad de agua potable.

Palabras clave: Río Saguambi, Río Mindo, Río Canchupí, *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Helicobacter pylori*, *Giardia* spp., *Cryptosporidium* spp., *Escherichia coli* y coliformes totales.

ABSTRACT

Ecuador relies on both surface and underground water reserves, with its rivers serving as the primary surface water resource. Unfortunately, the population growth and tourism in certain areas have led to contamination of these water bodies. One of those areas is the Mindo village, which does not have access to drinking water, and wastewater is directly discharged into the rivers. In response to this issue, studies were carried out analyzing water samples from three rivers passing through Mindo, these are the Saguambi, Mindo, and Canchupí Rivers. Water samples were taken before and after the river passes through the village, during three rivers during three seasons, assessing their suitability for human consumption and recreational activities. The study revealed a high concentration of *Escherichia coli* and total coliforms in all rivers, exceeding the permitted limits set by various international guidelines at most collection points. Furthermore, numerous opportunistic and primary pathogens were present in the rivers representing a serious risk to human and animal health. The analysis detected DNA of several pathogens, including *Mycobacterium tuberculosis, Mycobacterium leprae, Helicobacter pylori, Giardia* spp., *and Cryptosporidium* spp., with *M. tuberculosis* being the most prevalent. Particularly during the dry season, the Canchupí River after passing through the village exhibited the highest pollution levels, suggesting a significant contribution of pathogens from the urban areas. Additionally, Sanger sequencing, and Blastn corroborated the PCR results except for *Cryptosporidium* spp. This study provides relevant information on the seasonal microbiological variability among three rivers of Mindo, emphasizing the need for proper wastewater treatment and access to clean drinking water.

Keywords: Saguambi River, Mindo River, Canchupí River, *Mycobacterium tuberculosis*, *Mycobacterium leprae, Helicobacter pylori*, *Giardia* spp., *Cryptosporidium* spp., *Escherichia coli, and total coliforms*.

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1. INTRODUCTION

1.1.Ecuador´s water wealth and contamination risks

Ecuador stands out as one of the wealthiest countries in South America in terms of water resources (25.931m^3 per person per year). This figure exceeds the Latin American average by 8.3 times, underscoring Ecuador's remarkable abundance of water sources (Auquilla, 2020). Ecuador is supplied by surface and underground water, with rivers constituting its primary surface water resource. Unfortunately, these water bodies face severe contamination from biological and chemical pollutants. This contamination endangers the quality of water, exacerbating the situation as the population continues growing (Cabrera et al., 2012).

1.2.Water management across Ecuador.

At the global level, 80% of wastewater is discharged untreated into water bodies, including lakes, rivers, and coastal zones. In Ecuador, this creates a significant issue, as surface water is sometimes used as an alternative to drinking water (Vinueza et al., 2021). According to the Metropolitan Public Company of Drinking Water and Sanitation of Quito (EMAPS, 2023), Quito, the capital of Ecuador with a population of 2,644,145 (INEC, 2017), has a low percentage of treated wastewater (3.44%). Concerns persist in small rural areas like Mindo with 6552 inhabitants (GAD parroquial Mindo, 2019), where reports from Politécnica Salesiana University described the direct discharging of wastewater into the Canchupí River (Castillo y Ullauri, 2022). Regarding drinking water, INEC (Instituto Nacional de Estadística y Censos) reported that Pichincha province has the highest coverage rate of 98.2% coverage of drinking water, surpassing other provinces (2023). However, this coverage is not uniform throughout the province. For example, in Mindo, only 58.07% of the population has access to the water supply, this service does not have standards for drinking (GAD parroquial Mindo, 2019).

1.3.Geographical data of Mindo

Mindo is located 70km from Ecuador's capital, Quito, and lies in the northwest of Pichincha province within the Mindo-Nabillo protected forest. Positioned at the central coordinates of 9994159.16 (Southern latitude), 7 47635.98 (West latitude), and 1250m.a.l. (GAD parroquial de Mindo, 2012). The area experiences a warm-wet climate with temperatures ranging from 12℃ to 26.8℃. Like many other places in Latin America, Mindo has become increasingly significant in tourism, being this activity the main source of economic income. Therefore, Rivers play a fundamental role in this type of tourism, and it is essential to maintain their good condition (GAD parroquial Mindo, 2019).

1.4.Rivers present in Mindo: Saguambi, Mindo and Canchupí

Mindo is renowned for its diverse river network, with notable rivers including Saguambi, Mindo, and Canchupí, all interconnected. The Mindo River serves as a conduit for drainage from various slopes to flow into the Blanco and Guayllabamba Rivers, which together form the Esmeraldas River. On the other hand, the Saguambi and Canchupí Rivers originate within the micro-basin of the Blanco River and merge into the Mindo River (Perfectura de Pichincha, 2017; Gobierno Autónomo Descentralizado Municipal del Cantón San Miguel de los Bancos, 2015). The Mindo River is surrounded by forested areas, providing a habitat for a variety of flora and fauna such as orchids, birds, amphibians, reptiles, and exotic butterflies. This river has gained recognition as a popular tourist destination, offering water-related sport activities such as rafting, kayaking, regatta, and among others. The Canchupí River has been utilized for bathing and laundry, so its ecosystem and surroundings have been significantly altered (Oliva, 2011). In contrast, the Saguambi River is characterized by supplying 60% of fresh water to the population (Rojas, 2021) and has several lodges in the surrounding area (Cevallos, 2015).

1.5.Sources of pollution in the rivers of Mindo

The population and tourist surge into Mindo have been steadily increasing in recent years, resulting in adverse impacts on the rivers of Mindo. These developments have led to the degradation of the natural environment and habitat fragmentation (Oliva, 2011). Each river exhibits a different type of pollution. The Saguambi River faces issues due to solid accumulation as a result of soil removal, urbanization, and wastewater discharges. The Mindo River appears to be affected by chemical and organic residuals, likely stemming from livestock activities, tourism, soil erosion, and direct discharges from households. Lastly, the Canchupí River, which crosses all the urban areas of Mindo, has higher anthropogenic contamination levels. It includes fecal bacteria and organic material from direct household discharges, in addition to the fact that the wastewater treatment system is not functioning effectively (Rojas, 2021). An inadequate management of domestic water has resulted in the pollution of surface water bodies which is particularly noticeable when the rivers are closer to the urban area, providing water without any treatment to the population (Custode, 2016).

1.6.Microorganisms and their health implications

When evaluating river contamination, it is crucial to consider the analysis of microorganisms such as fecal indicators, pathogen bacteria, and parasites. *E. coli* and total coliforms are typically derived from human and animal intestines being used as; fecal indicators and enable the presence of further primary and opportunistic pathogens. Among the pathogen bacteria, various *E. coli* pathotypes are detected in numerous studies in fresh water resources (Vinueza et al., 2021), including *enteropathogenic E. coli* (EPEC), *enterohemorrhagic E. coli* (EHEC), *enteroinvasive E. coli* (EIEC), and *enteroagregative E .coli* (EAEC). All these pathotypes cause diarrhea with some variants and EHEC is the most hazardous public health menace (Ogura et al., 2009). EHEC could cause diarrhea with blood and hemolytic uremic syndrome (Eichhorn et al., 2015). Parasites, such as *Giardia* and

Cryptosporidium spp. are pathogenic microorganisms that inhabit several human and animal intestines, causing zoonotic diseases (Cuatindioy, 2019). Likewise, these parasites can also spread through food consumption, contaminated water, and direct contact with the fecal matter of an infected individual (Idowu and Rowland, 2006.). *Giardia* infection, known as giardiasis, presents with symptoms such as abdominal pain, diarrhea, nausea, vomiting, and fatigue (Saleh and Nigm, 2022) Furthermore, *Cryptosporidium* infection leads to cryptosporidiosis, which is also characterized by diarrhea, abdominal pain, anorexia, and vomiting (Khaliq and Alqaisi, 2023). *Helicobacter pylori* is a pathogen bacterium known to cause a range of gastrointestinal disorders, including peptide ulcers, and chronic inflammation, and it is related to the development of long-term stomach cancer (Roszczenko-Jasińska et al., 2020). Symptoms associated with *Helicobacter pylori* could include stomach pain, nausea, poor appetite, weight loss, burps, and abdominal swelling (Abbas et al., 2018). Other bacterial pathogens are *Mycobacterium tuberculosis* and *Mycobacterium leprae*, being opportunistic pathogens (Percival & Williams, 2014). These bacteria can be transmitted from person to person or through contact with contaminated objects (Mladenova and Durazzo, 2018). *Mycobacterium tuberculosis*, causes tuberculosis, primarily affecting the lungs with symptoms such as intense cough, fever, weight loss, night sweats, chest pain, weakness, and lack of appetite (Guetahum et al., 2015). *Mycobacterium leprae* is another significant pathogen responsible for leprosy, being a neurological and dermatological illness (Mungroo, et al., 2020). The symptoms are erythema, numbness, eyebrow hair loss, painless nor pruritic skin lesions, and tubercles (Chen et al., 2021). Due to the noticeable increase in the human population of Mindo, there have been significant changes in the ecosystem, with rivers particularly impacted. The pollution of these rivers has been on the rise, increasing the need for microbiological analysis of the three rivers in Mindo. This analysis aims to determine their suitability for consumption and recreational activities.

2. METHODS

2.1. Sample collection.

The water samples were collected from three rivers flowing through the town of Mindo, more exactly: Saguamby, Mindo, and Canchupí Rivers, which were chosen based on prior studies realized by Rojas (2021) and accessibility upstream and downstream of the town (see **Table 1**). The dates of the water collection were February and June for the transitional season, April and May for the Rainy season, and August and September for the dry season. The water sample collection protocol was according to Borja-Serrano et al. (2020). Each sample was collected in a glass jar, previously sterilized, and autoclaved at 121℃ for 15 min. The flacks were submerged to a depth of 0.3 m below the water surface to avoid solid remains and foam, collecting 800 ml of water at each sampling point and stored at 4℃ until transported to the Microbiology Institute at the San Francisco de Quito University (MI-USFQ).

2.2. Filtration

The water samples were filtered using a 0.45-μm nitrocellulose membrane (Millipore) with a vacuum pump (Chemical Duty Pump), ensuring aseptic conditions. This filtration method was adapted from Dobrowsy et al. (2014) with minor adjustments. Subsequently, the nitrocellulose membrane was transferred to a sterile falcon tube containing 20mL of distilled water. The falcon tube was vortexed for 15 min to separate soil particles from the microorganisms. Following this, the membrane was removed, and the tubes were centrifuged for 15 min at 5000rpm precipitating the sediments, resulting in a pellet that was suspended in 2ml of distilled water This sample was divided into three aliquots of 500µl each: one for DNA extraction using a DNeasy® PowerSoil® Pro kit proportioned by QIAGEN, the second for bacterial growth cultures, and the third for storage.

2.3. Growth cultures of *E. coli* and total coliforms.

The media culture employed was Chromocult agar for quantifying *Escherichia coli* and total coliforms through successive dilutions (see **Table 2**), as outlined by Borja-Serrano et al. (2020). The drop method was utilized, involving the culturing of 10µl (Arana et al., 2010). In this case, three drops of 10µl were cultured for each dilution at every sampling point and incubated at 35℃ for 24 h.

2.4. DNA extraction

DNA was extracted following the instructions provided by the DNeasy® PowerSoil® Pro kit provided by QIAGEN. This kit features a bead tube and optimized chemistry specifically designed for enhanced bacterial lysis, therefore ensuring efficient DNA extraction. Additionally, it contains an inhibitor removal technology to eliminate inhibitor compounds (such as humic acids) commonly encountered in environmental samples.

2.5. Molecular identification

Once the DNA was extracted from different samples, specific primers were employed to identify bacterial genera by Polymerase chain reaction (PCR) (see **Table 3**) for additional information). The PCR Mastermix consisted of a final volume of 15 µL and all the amount of the reagents depended on the type of pathogen genera or species. Briefly, the final PCR mixture included 3 µl of Green GoTaq Flexi Buffer (Promega, Madison, WI, USA), 0.90-1.8µl of 2.0mM $MgCl_2$ (Promega, Madison, WI, USA), 0.30-0.60 µl of 0.2 mM dNTPs mix (Promega, Madison, WI, USA), 0.45-0.75µl for each PCR primer, 0.08-0.10µl of 0.5U Go Taq Flexi DNA polymerase (Promega, Madison, WI, USA), 1-2µl of DNA and remaining volume was completed with DNA-free water. It is noteworthy, *Mycobacterium tuberculosis*, *Giardia* spp., and *Cryptosporidum* spp. detection required a pre-nested PCR and nested PCR. Meanwhile, 16S conserved rRNA gene, *E.coli* pathotypes, *Helicobacter pylori*, and *Mycobacterium leprae* detection was performed with merely single-

step PCR.

2.6. PCR Product analysis

The PCR products were visualized using electrophoresis with 1.5% agarose gel and TBE 1% except for *Giardia, Cryptosporidium,* and *M. tuberculosis*, for which a 2% agarose gel was used. The positive and negative controls were provided by (IM-USFQ). Subsequently, the positive PCR products of *Giardia, Cryptosporidium, Helicobacter pylori, M.leprae,* and *M. tuberculosis* were subjected to Sanger sequencing (Macrogen, South Korea; see **Table 4**).

2.7. Community surveys

Eighteen people who attended a seminar in Mindo were surveyed. They were asked seven close-ended questions and one open-ended question. The survey was related to topics such as contamination levels, activities to prevent contamination, and government actions in the Rivers of Mindo.

3. RESULTS

3.1. *Escherichia coli* and total coliform counts.

The study analyzed two collection points (before (1) and after (2) the community of Mindo) in the Saguambi, Mindo, and Canchupí Rivers during three seasons (See **Table 1** and **Figure 1**) demonstrating high concentrations of *E. coli* and total coliforms in all rivers. **Table 2** shows the concentration of both *E.coli* and total coliforms, while, **Figure 2** illustrates that most collection points overpassed the limit set by various international guidelines. The United States of America standard values of the Recreational Water Quality Criteria is the most strict regulation for *E.coli* and total coliforms (EPA,2012). Additionally, this study took into account Brazilian guidelines for bathing waters established by the Conama Resolution No.274 of November 29, 2000 (Ambiente, 2001) as well as European Union guidelines (Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006) for bathing water quality (European Union Law, 2006).

As shown in **Table 3**, the highest concentrations of *E.coli* and total coliforms were found in Canchupí River during the dry season with 1.50×10^7 and 1.79×10^7 CFU/100mL values respectively. In the Saguambi River, the highest concentration of Escherichia coli was detected after the community in the transitional season with 9.42×10^4 CFU/100mL, while the highest concentration of total coliforms was detected before the community in the transitional season, with a value of 2.58×10^5 CFU/100mL. In contrast, the Mido River showed the highest values of both *E.coli* and total coliforms after the community sample collection point in the transitional season with 7.15×10^5 and 5.85×10^5 CFU/100mL respectively. From this data, it is clear that the concentration of *E.coli* and coliforms increased after the community, except for Saguambi River which showed higher values before the community in the transitional season.

3.2. Molecular identification of pathogens on superficial water of Saguambi, Mindo, and Canchupí rivers

Molecular identification was performed by polymerase chain reaction (PCR) to identify the DNA of pathogens in all superficial water samples of the three rivers. The best positive results were analyzed by Sanger sequencing (Macrogen, South Korea). As illustrated in **Figure 3,** the study found some clinically relevant and opportunistic pathogens. *Mycobacterium tuberculosis was* found in the three rivers across all seasons, while *Mycobacterium leprae* was found in the Saguambi River during the dry season, the Mindo River in all seasons, and the Canchupí River during transitional and dry seasons. *Helicobacter pylori* was found in the Mindo River during the dry season and in the Canchupí River in all seasons. *Giardia* spp. was found in the Canchupí River in all the seasons, while *Cryptosporidium* spp. was only found in the Mindo River during the dry season. The study did not reveal the presence of any analyzed *Escherichia coli* pathotypes in the three rivers or any of the seasons. Furthermore, the Canchupí River during the dry season evidenced the highest level of pathogens after the community. Finally, Mindo and Saguamby Rivers demonstrated a higher number of pathogens before the community.

As shown in ***Table 4,*** Sanger sequencing validated the initial molecular identification by PCR assays and identified some of the species of the detected pathogens. More exactly, the selected *M. tuberculosis* samples showed an identity of 99.32% (T2 sample) and 98.33% (T3 sample) of identity. Only one of the four selected samples (L1 sample) for DNA sequencing evidenced an identity of 98.57% for *Mycobacterium leprae*. Meanwhile, *Helicobacter pylori* was identified in two selected samples showing identity values of 96.18% (P1 sample) and 87.95% (P4 sample). Finally, one out of the three *Giardia* spp. samples was successfully identified as *Giardia intestinalis* with an identity of 89.80%, but Cryptosporidium spp. samples could not be identified by DNA Sanger sequencing.

3.3. Community surveys

As illustrated in **Figure 4**, ten of the surveyed individuals have lived in Mindo for less than a year, and most of them are not employed in the activities that were mentioned in the survey. Also, 14 people believed that humans can damage nature for their gain and 15 people are familiar with or have visited the three rivers studied, more exactly: Sagumabi, Mindo, and Canchupí Rivers. The majority of people considered inadequate water treatment, population growth, tourism, and trash to be important factors to increase pollution in the rivers of Mindo. Only 3 people agree that competent authorities are taking appropriate action regarding water treatment. Finally, 15 people consider that adequate water treatment, environmental education, urban, and recreational planning are crucial in preventing the contamination of Mindo.

4. DISCUSSION

In Mindo, the current pollution of water bodies is threatening the health of the local population as well as the tourism activity which represents a huge income for the community. Mindo is located in a region of great biodiversity and has been declared an area of conservation for birds (Calvache et al., 2016). The rivers are one of the most important fresh water resources for the Andean cloud forest. Due to hydrological connectivity, the rivers are highly sensitive to broad anthropogenic impacts (Castello et al., 2012). The Saguambi, Mindo, and Canchupí Rivers are all affected by different sources of pollution, being the wastewater discharges the primary cause (Rojas, 2021), with high levels of *E.coli*, total coliforms, and numerous potential pathogens that constitute a significant health risk.

Most collecting points across all seasons (transitional, rainy, and dry) (see **Table 1**) exceeded the permitted limit of *E.coli* and total coliforms (see **Table 2** and **Figure 2**) (United States Protection Agency (Recreational Water Quality, 2012), European Union guidelines (European Law, 2006), and Brazilian guidelines (Ambiente, 2001). All rivers evidence a higher amount of *E.coli* and total coliforms after the local community except for total coliforms in the Saguambi River during the transitional season. The Mindo River was previously studied by Borja- Serrano et al.(2020), who also reported high levels of *E. coli* (1.72×10^1 CFU/100mL) and total coliforms (6.78×10^1CFU/100mL). According to our results, these levels have increased in 2023, showing *E.coli* levels of 5×10^3 –7.15×10^5 CFU/100mL and total coliforms of 8×10^4 – 5.85×10^5 CFU/100mL. Castillo and Ullauri reported an average of 4.8×10^4 CFU/100mL of total coliforms in Canchupí River but the study did not cover *E.coli* levels. In our results, we evidenced a higher amount of total coliforms in Canchupí River, ranging from 6×10^4 to 1.79×10^7 CFU/100mL. However, there is no current information to compare the freshwater quality of the Saguambi River, nor is there any data available for different seasons and sample collection points for the three rivers

as carried out in the present study.

Local and international studies found similar levels of *E.coli* and total coliforms which exceeded international limits. Studies carried out in other Ecuadorian rivers such as as Machángara, Guayllabamba, and Zamora, among others, showed; *E. coli* and total coliforms levels higher than 1.0×10^4 CFU/100mL of *E.coli* and total coliforms (Borja-Serrano et al., 2020; Vinueza et al., 2021). It is worth noting that the sample collection points of these studies were taken near urban areas. Meanwhile, a microbiological study conducted in the Caribbean Sea of Colombia also revealed that the levels of *E.coli* was approximately 7.2×10^3 CFU/100mL, and the amount of total coliforms was around 5.9×10^7 CFU/100mL in freshwater sources from Salgar River, and Pradomar beach, among others (Sánchez et al., 2019). In Honduras, the Choluteca River demonstrated a similar total coliform concentration of 6×10^4 CFU/100mL (Mendoza et al.,2023). Likewise, the Larut River in Malaysia showed E.coli and total coliform levels of 4.1×10^5 CFU/100mL and 4.7×10^5 CFU/100mL respectively. Purohit et al. (2020) reported lower average levels of *E.coli* (5.2×10^3 CFU/100mL) and total coliforms (9.4×10^4 CFU/100mL) in Kshipra River (India) when compared to our study. Therefore, both the literature and this study demonstrated that water is unsafe for human consumption and recreational activities. The fecal contamination was suspected to be caused by human activities (Bong et al., 2022), such as livestock where studies indicate that levels of coliforms could be related to the fluvial system, season, and land use (Díaz-Gavidia et al., 2022). According to Maes et al. (2022), in Sweden rivers like tourism is one of the activities that increase *E.coli* levels. In Mindo, this could be related to water sports like rafting, kayaking, and regatta, among others (Olivia, 2011). Urbanization also leads to an increase of *E. coli* levels in the rivers of Thailand and causing the rise of antibiotic resistance (Honda et al., 2016).

Furthermore, present study found numerous opportunistic and primary pathogens in

all the rivers representing a serious risk to human and animal health (see **Figure 3**). When pathogens are found before the community, they are usually provided from urban areas nearby such as houses and hospitals (Mtetwas et al., 2022). *Mycobacterium tuberculosis* DNA was found in all rivers and seasons. This pathogen can be spread within and between livestock and wildlife populations, and its presence in water resources represents a high health risk due to its persistence in the environment. However, only DNA presence was confirmed and further studies should realized to validate these preliminary results and to narrow its contamination origin, where *M. tuberculosis* contamination source could be due to wild animals (Cuatindioy, 2019). *M. tuberculosis* has also been found in water resources in southern Spain, where cachectic animals were found to be the original hosts (Barasona et al., 2016). Additionally, rivers near Hospital areas in Nigeria, (Nwaloize, 2018), and the Yellow River in China (Zhou et al. in 2017) observed the same situation. Furthermore, the literature suggests that *M. tuberculosis* can survive in rivers until 50 days at temperatures of 8 to 20℃ (Mtetwa et al., 2022). In addition, *Mycobacterium leprae* DNA was found in the Saguambi River during the dry season, the Mindo River in all seasons, and the Canchupí River during transitional and dry seasons. The source of infection is known to be untreated leprosy patients and animal reservoirs such as armadillos (Tió-Coma et al., 2019). So, further studies must also be carried out in future research. Moreover, recent studies in Brazil, India, Indonesia, Surinam, England, and Bangladesh have shown that *M.leprae* can survive outside the human body until 46 days in moist soil, potentially becoming a source of infection. However, the mode of transmission is not well understood yet (Turankar et al., 2022). In a previous study by Turankar et al. (2011), samples from sick people and animals of the surroundings, such as monkeys and armadillos. This emphasizes the importance of understanding the ecology to explain the mode of transmission. The DNA of *Helicobacter pylori* was found in the Mindo River during the dry season and in the Canchupí River in all

seasons. This primary pathogen is one of the most common causes of gastric infections worldwide being also correlated to the development of gastric cancer (Roszczenko-Jasińska et al., 2020). Studies have found evidence of the bacteria in various freshwater sources, such as the Bogotá River in Colombia (Vesga, 2023) and the Nairobi River basin in Kenya (Dinda and Kimang'a, 2016). There is also, evidence of the prevalence of *H.pylori* in Iran (Farhadkhani et al., 2019), and in Michigan (USA) even in processed wastewater (Bai et al., 2016). This high prevalence of *H.pylori* in water sources can provoke an increase of several gastrointestinal diseases in the population. Regarding parasite DNA, *Giardia intestinalis* was found in the Canchupí River after the community during the transitional season, while *Cryptosporidium* spp. DNA was found in the Mindo River during the dry season after the community. Both protozoa can cause numerous gastrointestinal symptoms in animals and humans, in particular in children (Fradette, 2022). Moreover, potencial source of *Giardia* and *Cryptosporidium* spp. contamination before the community could be due to livestock (López-Angulo, 2017). Borja-Serrano et al. (2020), also identified the presence of *Cryptosporidium* spp. DNA in the Mindo River, as well as the Pisque and Alambi Rivers in Pichincha. On the other hand, San Pedro, Machángara, and Monjas Rivers among others in Pichincha province showed the presence of *Giardia* spp. The occurrence of *Giardia* and *Cryptosporidium* spp. has been reported in various rivers worldwide, such as the Kuan River in Thailand (Chuah et al., 2016) and several rivers of the Qinghai Tibetan plateau area in China (Ma et al., 2019). *Giardia intestinalis* was found in the Vistula River in Poland (Lass et al., 2017) and the Negro River in Brazil (Coronato et al., 2016).

The conducted survey indicates that there is a huge level of consciousness about the factors that could affect the rivers of Mindo and their potential treatment despite the number of questionnaires being low. However, according to GAD parroquial Mindo (2019), water treatment needs urgent attention from the local government.

5. CONCLUSIONS

In summary, Canchupí River was the most polluted river in Mindo by the results obtained in the present longitudinal study. This observation is consistent with *E.coli* and total coliform levels. These high levels of fecal contamination can be attributed to various causes of pollution, such as livestock, agriculture, tourism, soil erosion, urbanization, and waste water discharges. On the other hand, Canchupí River possesses more contact with waste water when compared to the Mindo and Saguambi Rivers. The Canchupí River flows through the urban area of Mindo and the local community reported concerns about the direct discharge of households and the lack of proper wastewater treatment. Finally, during the dry season, the water volume decreaseds in the Canchupí River which cause pollutants to become more concentrated and visible in the water. Additionally, the Canchupí River showed a higher number of pathogens in the water samples after the community, which suggesting that most pathogens are provided from urban areas. On the contrary, the Saguambi and the Mindo Rivers demonstrated a higher number of pathogens before the community, supporting the presence of contamination sources outside the urban Mindo area.

For future projections, it is essential to study the origin of *M.tuberculosis* DNA due to its high prevalence among all the rivers and seasons. In addition, microbiological studies need to be supplemented with a physico-chemical analysis to enhance the results. Furthermore, with the increasing antibiotic resistance, an analysis of the spread of microbial resistance will be necessary.

6. TABLES

Table 1. General information about collection sampling from the Saguambi, Mindo, and Canchupí Rivers.

River	GPS Coordinates	Season	Date of Collection sampling
Saguambi	(1) 0°3'8" S 78°45'47" W (2) 0°03 28.6"S 78°46'31.6"W	Transitional	2/4/2023 6/25/2023
		Rainy	4/2/2023 5/27/2023
		Dry	8/26/2023 9/23/2023
Mindo	(1) 0°4'26" S 78°45'16" W (2) 0°3'29" S 78°46'49" W	Transitional	2/4/2023 6/25/2023
		Rainy	4/2/2023 5/27/2023
		Dry	8/26/2023 9/23/2023
Canchupí	(1) 0°2'3" S 78°45'21" W (2) 0°3'11" S 78°46'59" W	Transitional	2/4/2023 6/25/2023
		Rainy	4/2/2023 5/27/2023
		Dry	8/26/2023 9/23/2023

Legend: Sample collection point (1) before the community sample collection point; (2) after the community.

Table 2. The average amount of *Escherichia coli* and total coliforms of Saguambi, Mindo, and Canchupí rivers across three seasons.

River	Season	Collection	Collection	CFU/100ml [(a)]	Average amount	SD[(c)]	CFU/100ml [(a)]	Average amount CFU/100mL[(b)]	SD[(c)]
Saguambi	Transitional	Before the community	S1.T1	3.33E+03	1.67E+03	2.36E+03	2.43E+05	2.58E+05	2.12E+04
		Before the community	S1.T2	0.00E+00			2.73E+05		
		After the community	S2.T1	8.50E+04	9.42E+04	1.30E+04	2.10E+05	1.82E+05	4.01E+04
		After the community	S2.T2	1.03E+05			1.53E+05		
	Rainy	Before the community	S1.R1	0.00E+00	0.00E+00	0.00E+00	5.00E+04	4.25E+04	1.06E+04
		Before the community	S1.R2	0.00E+00			3.50E+04		
		After the community	S2.R1	5.00E+03	4.17E+03	1.18E+03	1.37E+05	1.40E+05	4.71E+03
		After the community	S2.R2	3.33E+03			1.43E+05		
	Dry	Before the community	S1.D1	0.00E+00	0.00E+00	0.00E+00	1.53E+05	1.52E+05	2.36E+03
		Before the community	S1.D2	0.00E+00			1.50E+05		
		After the community	S2.D1	1.00E+05	6.75E+04	4.60E+04	3.05E+05	2.10E+05	1.34E+05
		After the community	S2.D2	3.50E+04			1.15E+05		
Mindo	Transitional	Before the community	M1.T1	6.67E+03	6.67E+03	0.00E+00	6.00E+04	8.00E+04	2.83E+04
		Before the community	M1.T2	6.67E+03			1.00E+05		
		After the community	M2.T1	1.40E+06	7.15E+05	9.69E+05	1.00E+06	5.85E+05	5.87E+05
		After the community	M2.T2	3.00E+04			1.70E+05		
	Rainy	Before the community	M1.R1	3.33E+03	5.00E+03	2.36E+03	7.67E+04	1.06E+05	4.12E+04
		Before the community	M1.R2	6.67E+03			1.35E+05		
		After the community	M2.R1	0.00E+00	6.67E+03	9.43E+03	9.50E+04	1.48E+05	7.42E+04
		After the community	M2.R2	1.33E+04			2.00E+05		
	Dry	Before the community	M1.D1	1.67E+04	2.67E+04	1.41E+04	1.00E+05	1.65E+05	9.19E+04
		Before the community	M1.D2	3.67E+04			2.30E+05		
		After the community	M2.D1	1.33E+04	2.67E+04	1.89E+04	8.33E+04	1.69E+05	1.21E+05
		After the community	M2.D2	4.00E+04			2.55E+05		
Canchupí	Transitional	Before the community	C1.T1	0.00E+00	1.67E+03	2.36E+03	6.50E+04	7.00E+04	7.07E+03
		Before the community	C1.T2	3.33E+03			7.50E+04		
		After the community	C2.T1	1.60E+06	2.05E+06	6.36E+05	2.40E+06	7.20E+06	6.79E+06
		After the community	C2.T2	2.50E+06			1.20E+07		
	Rainy	Before the community	C1.R1	3.33E+03	1.67E+03	2.36E+03	2.50E+04	8.00E+04	7.78E+04
		Before the community	C1.R2	0.00E+00			1.35E+05		
		After the community	C2.R1	1.25E+05	1.81E+06	2.39E+06	1.40E+05	5.32E+06	7.33E+06
		After the community	C2.R2	3.50E+06			1.05E+07		
	Dry	Before the community	C1.D1	0.00E+00	0.00E+00	0.00E+00	6.00E+04	6.00E+04	0.00E+00
		Before the community	C1.D2	0.00E+00			6.00E+04		
		After the community	C2.D1	6.00E+06	1.50E+07	1.27E+07	2.15E+07	1.79E+07	5.07E+06
		After the community	C2.D2	2.40E+07			1.43E+07		

Legend: (a) Amount of *Escherichia coli* and coliforms as CFU/100mL (colony-forming unit per 100ml). (b)The average amount of *Escherichia coli* and coliforms measured as CFU/100 mL taking into account duplicate samples collected. (c) SD-Standard deviation values.

Table 3. Primers and PCR cycling parameters for potential pathogens.

Organism	Primer name	Primer sequence (5′–3′)	PCR mixture	PCR cycling parameters	Gene (size [bp])	References
Single PCR assays						
Universal	Forward: fDD2	CCGGATCCGTCGACAGAGTTTGATCITGGCTCAG	3 µl of Green GoTaq Flexi Buffer, 1.2µl of 2.0 mM MgCl_2, 0.30 µl of 0.2 mM dNTPs mix, 0.75 µl for each PCR primer, 0.10 µl of 0.5U Go Taq Flexi DNA polymerase, 2 µl of DNA.	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	1.5 µl of 2.0 mM MgCl_2, 0.38 µl of 0.2 mM dNTPs mix, 0.75 µl for each PCR primer, 0.10 µl of 0.5U Go Taq Flexi DNA polymerase, 2 µl of DNA	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				
EAEC	Forward:AggRKs1	GTATACACAAAAGAAGGAAGC	3 µl of Green GoTaq Flexi Buffer, 1.2 µl of 2.0 mM MgCl_2, 0.30 µl of 0.2 mM dNTPs mix, 0.75 µl for each PCR primer, 0.10 µl of 0.5U Go Taq Flexi DNA polymerase, 2 µl of DNA	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				
Mycobacterium leprae	Forward: S13	CTCCACCTGGACCGGCGAT	0.90 µl of 2.0 mM MgCl_2, 0.30 µl of 0.2 mM dNTPs mix , 0.60 µl for each PCR primer, 0.10 µl of 0.5U Go Taq Flexi DNA polymerase, 2 µl of DNA	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)
	Reverse: S62	GACTAGCCTGCCAAGTCG				
Nested PCR assays						
Mycobacterium tuberculosis	Forward: Mpb1	TCCGCTGCCAGTCGTCTTCC	0.90 µl of 2.0 mM MgCl_2, 0.30 µl of 0.2 mM dNTPs mix , 0.50 µl for each PCR primer, 0.10 µl of 0.5U Go Taq Flexi DNA polymerase, 2 µl of DNA	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	1.8 µl of 2.0 mM MgCl_2 , 0.60 µl of 0.2 mM dNTPs mix, 0.45 µl for each PCR primer, 0.10 µl of 0.5U Go Taq Flexi DNA polymerase , 1 µl of DNA	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	1.8 µl of 2.0 mM MgCl_2 , 0.60 µl of 0.2 mM dNTPs mix, 0.45 µl for each PCR primer, 0.10 µl of 0.8U Go Taq Flexi DNA polymerase , 1 µl of DNA	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	1.8 µl of 2.0 mM MgCl_2 , 0.60 µl of 0.2 mM dNTPs mix, 0.45 µl for each PCR primer, 0.10 µl of 0.5U Go Taq Flexi DNA polymerase , 1 µl of DNA	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	1.8 µl of 2.0 mM MgCl_2 , 0.60 µl of 0.2 mM dNTPs mix, 0.45 µl for each PCR primer, 0.10 µl of 0.8U Go Taq Flexi DNA polymerase , 1 µl of DNA	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: The methods are shared with the project carried out by Cabrera (2023) and Yepéz (2023).

Table 4. Sanger sequencing to identify pathogens.

Sample	Collection Time	Consensus sequence [a]	Result	Identity (%) [b]
T2	C1.S1	TGGGACCAATACCTGGGTTGGGCCGGCTGCTTCGGGCAGCAACTCCCCCGGGTTGAAGAAGAAAATCACCCCGTCGTTCGTGACTGCGAAGTTCTGATAATTCACCGGGTCCTAGCCGGCATTCGGCGCTATCGATACCTGTTGTCCG	*Mycobacterium tuberculosis*	99.32
T3	S1.T1	CGCCGAATGCCGGCTTTGGACCCGGTGAATTATCAGAACTCGCAGTCACGAACGACGGGGTGATTTTCTTCTTCAACCCGGGGGAGTTGCTGCCCGAAGCAGCCGGCCCAA*TCCCA***GGT	*Mycobacterium tuberculosis*	98.33
L1	M1.T2	GAGGCCTACACATTCTGGGTCACCCGGATGCTGGCTTATGTCATCGACAACATCTCAGCCACGGTCCTGCTCGGCATTGGCATGTTGATTCACACGCTCACGAAGCAAGAGGCGTGCGTCACTGATATCACGCAGTACAATGTTA*TCAG TACTGTGCTACTCAGCCTACCGGCATCGGCATGTTGGCGTTCTGGTTCGCATGGTTGATGGCGACGGCCTACCTGGTCTGGAACTACGGCTATCGCCAGGGCGCCACCGGCTCCAGCATTGGCAAGACGGTAATGAAGTTCAAGGTGATC AGCGAGGCTACTGGGCAGCCAATCGGTTTCGGTATGTCGGTGGAGCGCCA	*Mycobacterium leprae*	98.57
L3	M2.S2	C*TGTTGGTGTGCA*CGCCATCTACTCCGGGACGT*GCCGGGCGGTTCTAGAACTGTCAAGTCAT*GACTGTGCATGA*CCTA**CA**CCAGCCGGGGGATGCACGCGTTGATCGACCGGGCGTTTTATCTGCCGAAGTCCTGGACCGATG ACCCGGCACGCTG**CGCC**GAGGCCGGCGTGCCCGCCGGGATCGAGTTCGCGACCAAACCCGCCCTGCTCACAGCGATGATCCTCGGCGCGTTCGATGCCGCATTCCCGTTCGGGTGGGTGGCCGGCGACGAGGTGTACGGCGCGG ACCCCGATCTGCAAACAACCCTGCAGGACAGGCAAATCGGCTACGTGCTGGCGATCGTTTGCGACCGACG**GGATAACCACCGAGGCGGGTC*ACACCC GGTCAGCGATCTGGCCGCCG	-	-
L4	M1.L2	ACTAGCCCCGCAAGTCGGATAGTCCGCTGCCGGTAGTGATCCTGAGATATTTTTCGATTCGACATCCGCTGGCGATTGTGACCATAAAACGGCCCTCGCACTCACCATGCTCCTTGCA*GAGGTTCAGCCC*GCGACGGGCCTCTCGCTT TTGGTT*TATTCGCATGTCGATTCCC*TGGAAGCAACCGACCTTGAGGTCGAGAGCGATCATCTTCTGAGTATGGGTCCTTGGAGACCCTTTTCCCGCAGATTTCGCGCCCAAATCGCTTGACGGCTCAGAGGAGACGCAAATCGAAGTGC GCTCCCCCTGCCATCCGAAAGATTTCGATTCGCCGGCATGACTTG*ACCTGAAATTCCCAGCGCC*GGAAAAT*ATGGCCGGCAGCAAAC*GAAGAAGTCATACCCAACGCCGGTTTCAGAGCAAGTCGAGGAGCTGACATTCTCCCA GTCGTCCCGCGA	-	-
L5	C2.S1	ACTCCGGCTACGCTCATTTCATCTCGAAAACTGCCCCCTCGGTCCTGGTCTGCATTACCATCTCCGTCCGGACCCTCTCTTGATCAACCGGGCGTGTCATCTATCTCACTCCTGGAATATAATCGGAACGGTCCTCCACTCAGGCCGGCG TGCATCGGC**AGTCTTC**GAGGCTCTGGCT*ACACGATTCTGGTTAAGCCCTAATCCTAGACGGGCTCGAGTCATCGTCACGATCCGAGCCAA**TCGGCTGGCTCG*GCGTTACCAAGACGCATGTTGA***AGA*CCCGGTC*ACGAA GCTAGAGGCGTGCGGCAC*CGCATGTCAGGCAGTACAATGCTGACCATCAGCTGTGCGA*CGCGGAATATCC*TGCCAGG*GCATCTGCATCTG*CGCT*TTGTGGC*CGCCTGGACGT*ACGGAGAAAGACCTGGACCGGCAGAAAACT AGCAGAGATC**ATGGGGGACGAAACTCGACCAGTCTCCAGCCGG*ATCTGACAGGGTGCCACAACTTGCGGGGCTAGTCCAAAAAAAAAAAAAAAAAAAAAAAAAGTGTCCTTGGTATGCAATTGGTTCGGCGATGTGTGCGGAGCGGAC CCCGGGGGCACTAACAC	-	-
P1	C1.L2	TGACTGACTATCCCGGCCTGACTGGTATCCAGCGGCC**GACTGACTATCCCGTCGGTCCCCCCCTTCCTCCGCATTACGGAGGCAGGACATCGGGAGAGTTCTCAG*CATGACCTGTTAGCAACTAAGAAAGGGGGTTGCGCTCGGTTG CGGGGCTTAACCCAAACATCTCACGACATGAGCTGACGACAGCCGTGCAGCACCTGTTTTCAAGGTCTAGCAAGCTAGA*CACTCCACTATTTCTAGCGGATTCTCTCAATGTCAAGCCTAGGTAA**GGTTCTTCG*TGTATCTTCGAAT TAAACCAC***ATGCTCC**ACCGCTTGTGCGGTTCCCCGTCTATTCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGGCGGGATGCTTAATGCGTTAGCGGGTTTACTGGAGAGACTAAGCCCTCCAACAACTAGCATCCATCGTTT AGGGCG*****GGACTACCAGGGTATCTAATCCTGTTAGCTCCCCACGCTTTCGCGCAATCAGCGTCAGTAATGTTC*CAGCAGGTCGCCTTCGCAATGAGTATTCCTCTTGATCTCTACGGATTT**TACC****CCTAC****** AC*******	*Helicobacter pylori*	96.18
P4	C2.S2	AACTAGTTCGGTG*T*TAGTCGAGATCGCATCTTGATGACTT***GTCGTAGGTCCCACCTTACTC*CTCATTACGGAAACAGTATCCTTAGAG*TTCTCAGCATGACCTGTTAGCCACTAAGAAAGGGGGCTGCGTTCGTTGTATCA*GGCT TAACCCAACCTCTAACGATTCGA*GTGCTGACAACAGCCGTGTTGAAGCCCATGTTTGC*AAGGACTAACAAGCTAGACGCTCCACTATTTCTAGCGGATTTTCTCAATGGCTAACCTAGGCAAGGTTCTTCGTGTATCTTCGAATTAAAC CACATGCTCCACCG**CTTG*TGCGGACCC*CGTCTATTCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGTATGCTTAATGCGATAGATGCATTACTGGAGAGACT**AAGACCTCCAACAACTAGCATTGATCGTTTACGTCG TGTCCTACCAGCCCA**TCTGATCCTGTTTGCTCCCCACGCTTTCCCGCAATCAGCGACAGTAACGTCCCAGCCAGTGGCCTTCCCAATGAGTACTCTCTCTTGATCTCTACGGATTTGCCCCTACACCAGGAATTCCACCTACCTATTCC ACACTCTAGA*TAGTAGAT*CAA*ATGC	*Helicobacter pylori*	87.95
A1	C2T2	AGGGGAATTTTTTTTGATCATACTTTTTTTCTTGAACTAGTGTCTTAAGGCCCGCCTGGCCTTTCCGTCTGCGAAATCGGGCAAGCTCG*AAAACGGAATAATTCAG*ATGCTTGTGCAAGCTAGGTGAGTCGTGTGCATT*CTATTCTCCGA GATTCTCGACTCATGCTAGGTGTTTTGGCCA*CCTAATCCGGGCTTATGTCTTTTTGGATTGGTGAAA*TGAAC*GAATTGGGTGAAAA*TAG*CGGCGCTT*TAGGATTG*CGTCGCGGGAATTGTTGATTGGAAGGTTGACC*CGAGTGTTG AGATGCT*TCTGGACCTGGGCCTCACCCAACCTGCGGCCCCG*TAATAAAACGCCAACCCCCCACGAGAGCACCGGTACAAAAACGGGGGAGCAAACCAACGAGCAGAGAGCCCAAGTAGCCTAAGCGAGCTCTGGAGAAGGGCATG ATGGTCATCTTCTGCACTGGGGAGACACTGGACGAGCGCAAGGCCGACAAGACTATGGATGTGAACATTGCACAGCTCGAGGTCCTTAAAAAGAAACCCGATATCGATAAATGCTCTGGAAGAGTGTCCTCATCGCCCTACGA	-	-
A2	C2.S2	CGACTGAACTTGAATTGAGGGGTTTAGCCAGGAGGGGACGTCTCGAAGGTTTCTTAGAGACGATTCAGCTGGGCGCACACCTGAATTTTATTCAACATTCGCAGCTCTTCGGAAGCCGCCAAATGCTCTCAATTA*******TCCCTTCTAC CTTCCGGATCTTTATCTTACTGTGGC*AAGTTCCGGGGGTTTCTTGTCACCGT*G**CC*AG*TGG*T*TGCCCCCGA**GAGCAGCGGGAACCAACGTCGCGACGACGTGCTT****CTGCCGAATTCGCAGGAGGTCGTACAAGGTGGCCT GGATTGCTTCCAGAACCGTCTTCATCGCTACGGCGGATTGCTGGTGCCCACA**CGTGCCGCGTCGACAAGAAGTCGCCCCCCCAAACAAAATCCGCGACCATGTGCGA*TTC*GCCTTTACCATCTGCAGCAG*GTCTTGCCGAAGCCT* GCTGTCCTAGCGAAAGC*AGATCAAGCACCGGCGATAGGCGAAAGGGACTCCCGGGTTGTGCACAAATTTTTCCCCCCTTTAAATCACGATCCTGAAGGGAGGAGGGATCATCTAGAGCATCAACGGTTTCCCAAGACCAGGAAAGTAA AAAGAAATAACGAGTGAACTAGACATGAATCGTATCTGACAAAACTCCGAACCAAGGGGACTCCAATTAGCCTAAACTGAAAGTTAACC	-	-
A3	C2.L2	CGTAGATTCCCCACTCTGGGGATGAGATTGTTGAGTCCTGGTGTGGAGAGTGTGTGCGGGCATCATTCCGAACCCTTATCTTTTCTTGCCGACAATTTCAGAGCGCCCTAGAC*TGTACACT*TCCCAAACCATAGTG*TGTGGAATGATGC AAGTCTATCATTCCT*TCTAGAACATGGTCCGGAGTCGGGAATTGTCGTTGACCACTGAAGGTACC***CCCGTCATACCTACTCCGGCCGCCAACTGGATGGATTG**GCTAACCTGTACGTCATCCGAGTCCCTCATGGGAGGGCCCGT CTACAGAACCATCGCATAGACACCTATG*GAGCCT***TTCC**CAGAAGTCTGTGATGTGAAGACATACATGT*CT*G*GGCTGTTAAAAAGGGACGGAAAAAACGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAT GCTCGCCAGTCATCC CGCTCAACACCCTCCCCGACGGGCTTCCGCCATCCCCCCCCCAAGGGGGGGGAACCAGGGAG	-	-
C1	M1.S2	CGTAGATTCCCCACTCTGGGGATGAGATTGTTGAGTCCTGGTGTGGAGAGTGTGTGCGGGCATCATTCCGAACCCTTATCTTTTCTTGCCGACAATTTCAGAGCGCCCTAGAC*TGTACACT*TCCCAAACCATAGTG*TGTGGAATGATGC AAGTCTATCATTCCT*TCTAGAACATGGTCCGGAGTCGGGAATTGTCGTTGACCACTGAAGGTACC***CCCGTCATACCTACTCCGGCCGCCAACTGGATGGATTG**GCTAACCTGTACGTCATCCGAGTCCCTCATGGGAGGGCCCGT CTACAGAACCATCGCATAGACACCTATG*GAGCCT***TTCC**CAGAAGTCTGTGATGTGAAGACATACATGT*CT*G*GGCTGTTAAAAAGGGACGGAAAAAACGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAT GCTCGCCAGTCATCC CGCTCAACACCCTCCCCGACGGGCTTCCGCCATCCCCCCCCCAAGGGGGGGGAACCAGGGAG	-	-

Legend: (a) Consensus sequences are the correct order of a sequence. (b)Percentage of identity with other sequences in BLASTn (NIH, n.d).

7. FIGURES

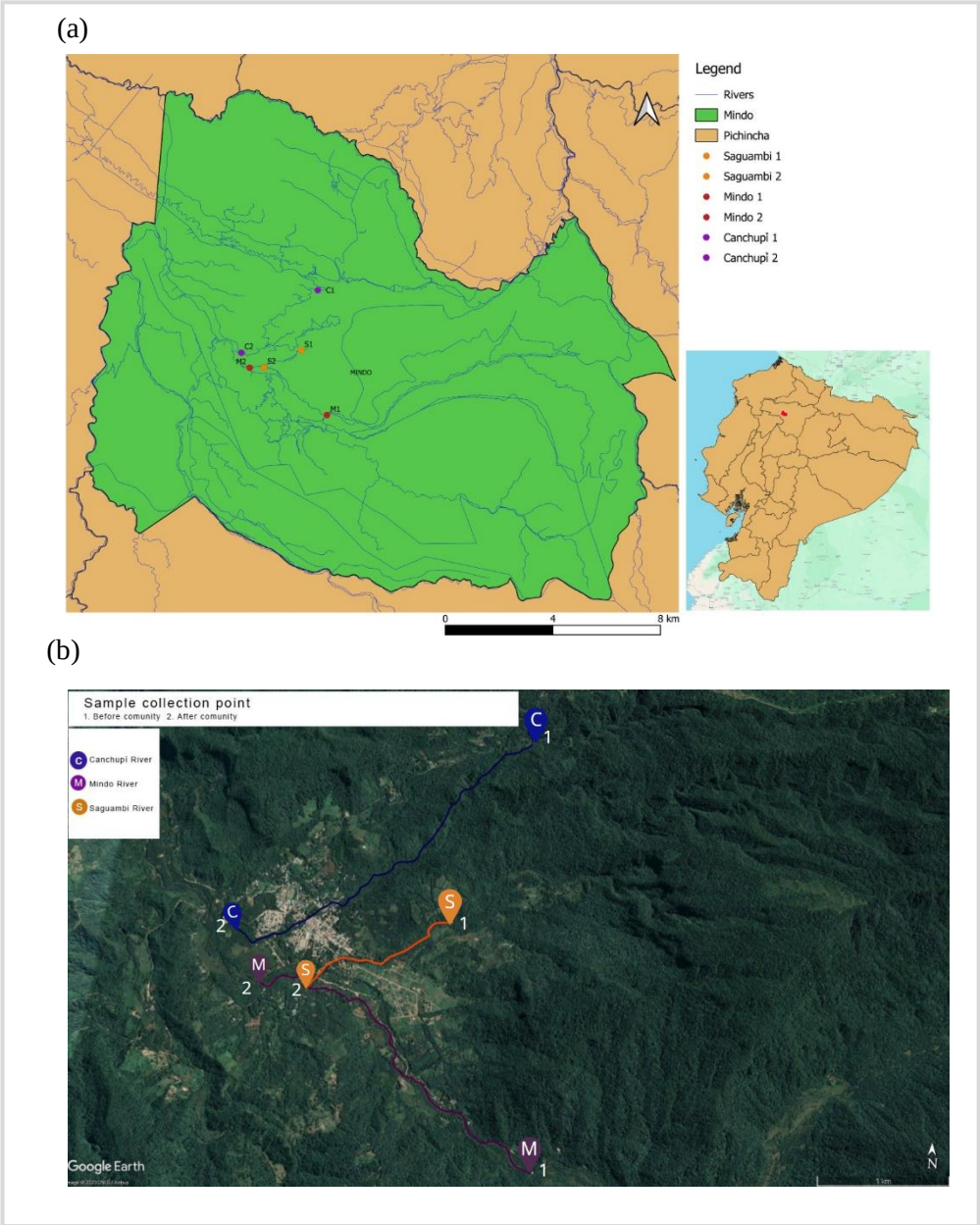

Figure 1. General map of Mindo with collection sampling points of Saguambi, Mindo, and Canchupí Rivers (a), illustrating the selected sampling points before the Mindo community are represented by number 1, and after the Mindo community are represented by number 2 (b)

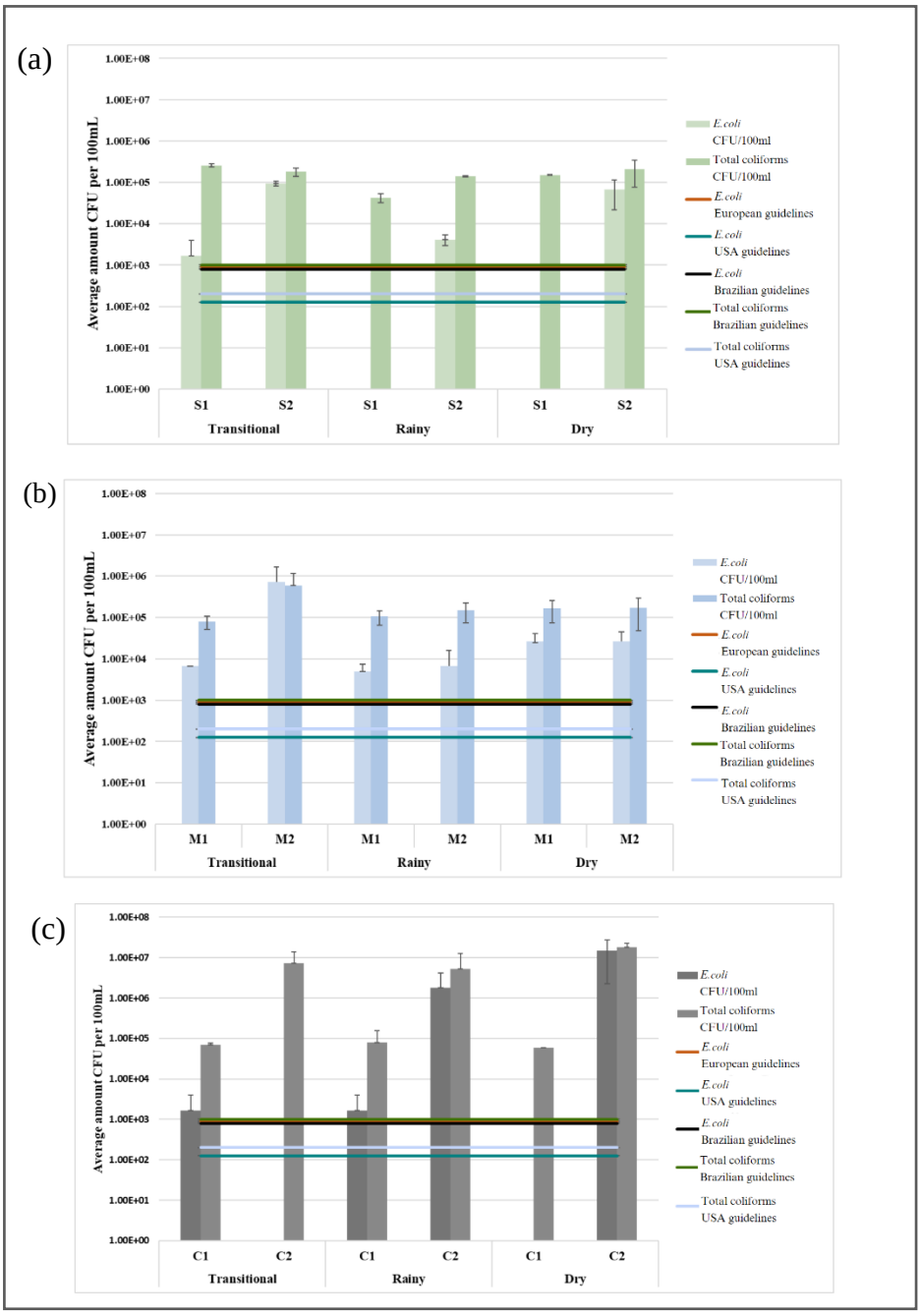

Figure2. The average amount of *Escherichia coli* and total coliforms in (a) Saguambi River, (b) Mindo River, and (c) Canchupí River. Limit values (—) *E. coli* at ≤ 900 CFU/100mL (Council of the Europen Union (2006). "Directive 200/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water and repealing Directive 76/160). (—) *E. coli* at ≤ 126 CFU/100mL; (—) total coliforms at ≤200 CFU/100mL (Recreational Water Quality Criteria U.S. EPA, 2012). (—)*E. coli* at ≤ 800 CFU/100ml; (—) total coliforms≤ 1000 CFU/100ml (The Brazilian guidelines for bathing waters were established by Resolution CONOMA n°274 of 29 November).

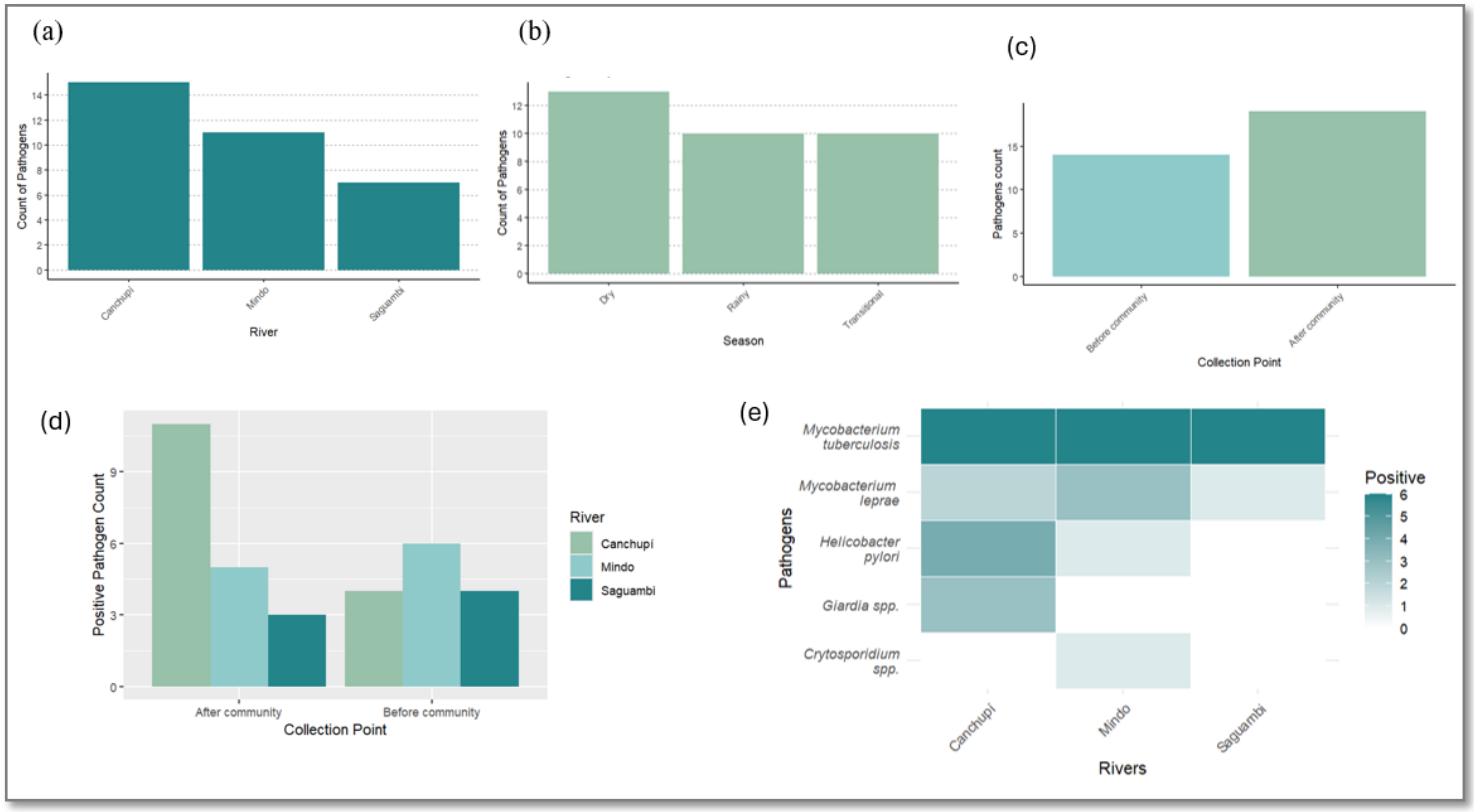

Figure 3. Detection of *Mycobacterium tuberculosis, Mycobacterium leprae, Helicobacter pylori, Giardia, and Cryptosporidium* spp. in the Saguambi, Mindo, and Canchupí Rivers. (a) Total count of pathogens in all three rivers. (b) Count of pathogens found in different seasons. (c) General count of pathogens before and after the Mindo community sample collection points. (d) Pathogen count before and after the Mindo community sample collection points for the Samguambi, Mindo, and Canchupí Rivers. (e) Heatmap show the count of pathogens in each river.

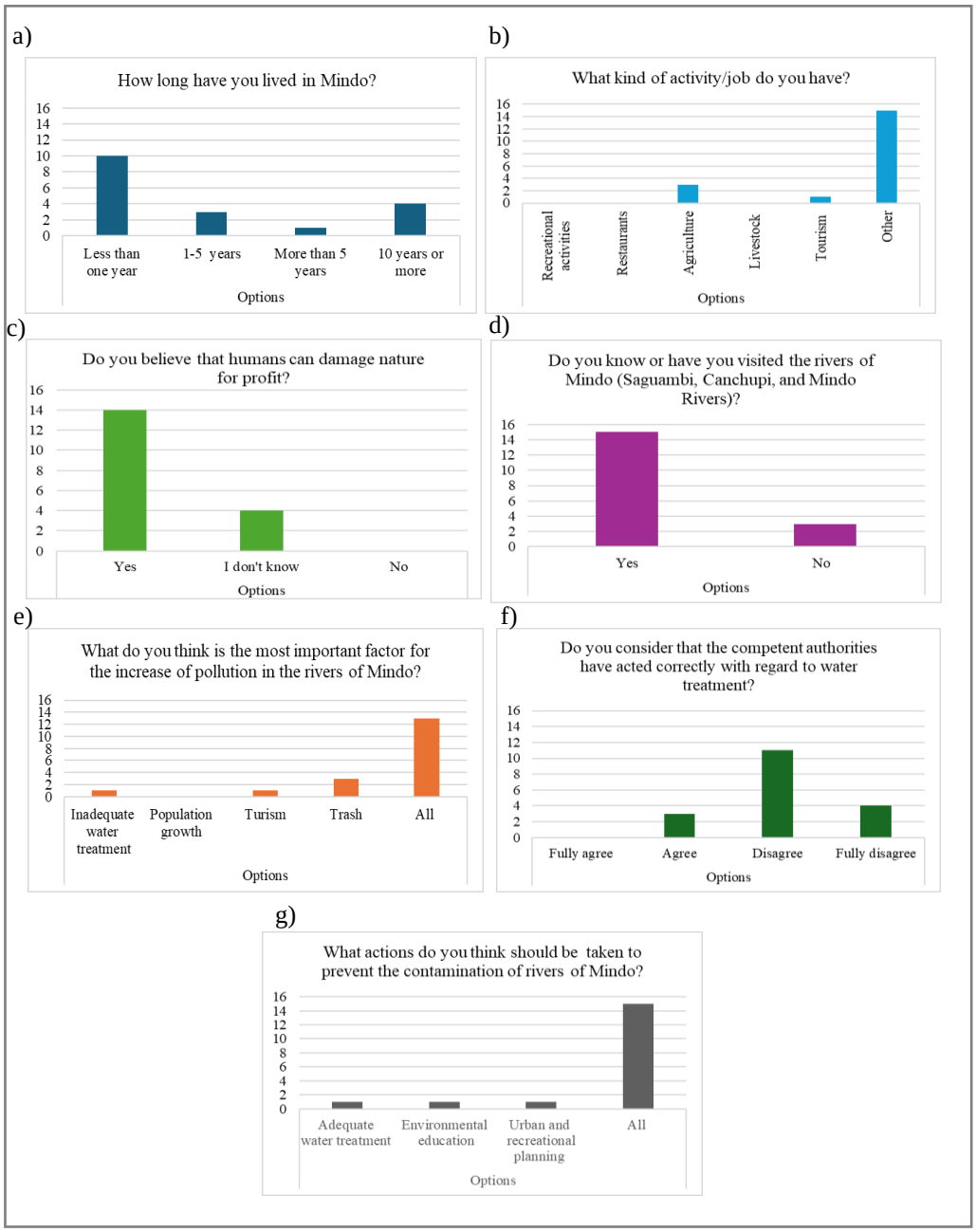

Figure 4. Illustration of the main results obtained through the questionnaire realized among the population of Mindo.

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