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Evaluation of the microbial and chemical load in rivers from the province of Pichincha in Ecuador

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Evaluation of microbial and chemical load in 18 rivers from the province of Pichincha in Ecuador

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RESUMEN

La contaminación de los recursos naturales de agua es uno de los mayores problemas en salud a nivel mundial. Esta contaminación puede ser causada por químicos, metales o agentes microbianos (*Salmonella*, *Pseudomonas*, *Legionella*, *Shigella* spp. y patotipos de *Escherichia coli*). El objetivo de este estudio fue el de analizar la calidad de dieciocho ríos ubicados en la provincia de Pichincha en Ecuador, por medio del análisis de parámetros fisicoquímicos y microbianos. El contaje de *E. coli* y coliformes totales se realizó por un procedimiento de contaje en medios de cultivo. La identificación de los géneros microbianos previamente mencionados se realizó por Reacción en Cadena de la Polimerasa (PCR), también se utilizó este método para *Candida albicans* y dos parásitos (*Cryptosporidium* y *Giardia* spp.) y para patotipos de *E. coli*, específicamente, *E. coli* enterohemorrágica (EHEC), *E. coli* enteroagregativa (EAEC), *E. coli* enteroinvasiva (EIEC) y *E. coli* enteropatogénica (EPEC). Adicionalmente, se detectaron parámetros *in situ* como pH, conductividad, turbidez, temperatura y oxígeno disuelto (OD). Por otro lado, parámetros como demanda química de oxigeno (DQO), solidos totales (ST), solidos suspendidos totales (SST), cloruros, amonio, nitrato, fosfato, sulfato, metales y elementos mayores fueron analizados en laboratorio. Los resultados obtenidos en este estudio demostraron que la mayoría de los ríos de Pichincha no tienen niveles aceptables de parámetros fisicoquímicos, microbianos y de metales, para su consumo, uso en agricultura, en industrias o en actividades recreativas. De los cuatro patotipos de *E. coli*, se detectaron tres (EIEC, EHEC y EAEC) en algunos ríos, exactamente: el río Monjas tuvo la presencia de EIEC y EHEC; el río Machángara demostró la presencia de EAEC y EIEC; y finalmente, el río Guayllabamba mostro la presencia de EIEC. En cuanto a la carga microbiana, los ríos más contaminados fueron Monjas, Machángara, Pisque y Pita. Así mismo, en cuanto al análisis de parámetros fisicoquímicos y metales, el río Monjas y Machángara mostraron los niveles más alto de ciertos parámetros (como, pH, OD, DQO y SST) y concentraciones de metales (como, manganeso y aluminio). Este estudio preliminar revela la diversa y severa contaminación del agua en estos ríos de acuerdo a la legislación ecuatoriana. Se recomienda realizar estudios a futuro para evaluar las posibles fuentes de contaminación y su impacto en la salud de la población.

Palabras clave: Ríos, *Escherichia coli*, Coliformes totales, Patotipos de *Escherichia coli*, *Candida albicans*, Parásitos, Parámetros fisicoquímicos, elementos mayores y metales, Pichincha, Ecuador.

ABSTRACT

Contamination of natural water sources is one of the main health problems worldwide. This contamination could be caused by chemicals, metals or microbial agents (*Salmonella, Pseudomonas, Legionella, Shigella* spp., and *Escherichia coli* pathotypes). The aim of this study was to analyze the quality of eighteen rivers located in the province of Pichincha in Ecuador, through physical-chemical and microbial parameters. *E. coli* and total coliforms assessment was performed by a counting procedure in growth media. The identification of microbial genera previously mentioned was performed with Polymerase Chain reaction (PCR), as well as *Candida albicans*, two parasites (*Cryptosporidium* and *Giardia* spp.) and *E. coli* pathotypes, more specifically, enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC) and enteropathogenic *E. coli* (EPEC). Additionally, physical-chemical parameters, such as pH, conductivity, turbidity, temperature, dissolved oxygen (OD) were detected *in situ* while chemical oxygen demand (COD), total solids (TS), total suspended solids (TSS), chlorine, ammonium, nitrate, phosphate, sulfate, metals and major elements analysis was performed in the laboratory. The results obtained in this study showed that most of the rivers in Pichincha do not possess acceptable levels of microbial, physical-chemical and metals parameters for drinking water, agricultural, industrial or even recreational activities. Furthermore, three of the four analyzed *E. coli* pathotypes (EIEC, EHEC and EAEC) were detected in certain rivers, more exactly: Monjas river showed the presence of EIEC and EHEC; Machángara showed the presence of EAEC and EIEC; and finally, Guayllabamba showed the presence of EIEC. In terms of microbial biodiversity, the most polluted rivers were Monjas, Machángara, Pisque and Pita. Similarly, in terms of physical-chemical and metal analysis, Monjas and Machángara rivers showed the highest levels of certain parameters (such as, pH, DO, COD and TSS) and metal concentrations (such as, manganese and aluminium). This preliminary study revealed diverse and severe water contaminations in these rivers by Ecuadorean legislation. Further studies should evaluate the possible sources of contamination and public health impact in the population.

***Keywords*:** Rivers, *Escherichia coli*, Total coliforms, *Escherichia coli* pathotypes, *Candida albicans*, Parasites, Physical-Chemical Parameters, Metal and Major Elements, Pichincha, Ecuador.

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1 INTRODUCTION

1.1 Global context

The discharge of wastes and chemical compounds into the rivers is one of the biggest sources of environmental contamination, mainly in developing countries due to the lack or few treatment of wastewaters (Kora et al., 2017; Noorhosseini et al., 2017; Olguín et al., 2010). The absence of water treatment generates an accumulation of environmental pollutants, which could lead to severe health public issues (Zhang, Wu, & Gu, 2015). Also, the pollution in rivers can affect different economical sections, such as agriculture, cattle raising, industrial production and recreational activities (Paul, 2017; Staley et al., 2014; WHO, 1996). The increase of microorganisms and anthropogenic contaminants enhances the risk of pathogens outbreaks, bacteria antibiotic resistance and public health costs (Ramírez et al., 2013; Valencia et al., 2014). Due to population and industrialization growth, urban rivers are the most affected ones and its composition can be easily modified by the release of untreated wastewaters (Hem, 1985; Noorhosseini et al., 2017; Valencia et al., 2014).

1.2 Pollution of freshwater resources

Globally more than 80% of residual waters are released to the environment without any adequate treatment (UNESCO, 2017). It has been reported that worldwide around 2 until 5 million of people die annually by water related diseases (Gleick, 2003). In 2000, Ecuador evidenced more than 2 thousands cases of diseases associated with water pollution, which most of those cases consisted on diarrhea or dysentery associated with certain pathogens, such as *Escherichia coli*, *Entamoeba histolytica* and others (Fretes et al., 2003; Vasco et al., 2014). In fact, Ecuador has been reported to use higher

amounts of agrochemicals when compared to other Latin America countries (Fretes et al., 2003). All these factors increases the rates of morbidity and mortality in Ecuador by water contamination (Fretes et al., 2003), as well as, the need to monitor the contamination of microbial load and chemical effluents in the rivers.

1.3 Pichincha province

Pichincha is one of the most important twenty-four provinces of Ecuador, which is located the capital town of Ecuador (Quito). Pichincha contains approximately a population of 2.576.287 and 86.9% of inhabitants actually reside in Quito (INEC, 2010; Sistema Nacional de Información, 2010). The domestic and industrial wastes produced in Quito ends in four main rivers (Gomez et al., 2014), more exactly, Machángara, Monjas, San Pedro and Guayllabamba rivers. It is known that almost 81% of contamination is due to domestic wastewaters discharge, while the remaining 19% of pollutes is due to industrial wastes (Gomez et al., 2014), such as chemicals and oils. Quito did not possess a treatment plant until 2018, however, it remains only partially operational on the south section of the town (EPMAPS, 2013). It is important to mention that Pichincha is located in the Andean region and it is surrounded by Coastal and Amazonian regions. This geographical location attributes Pichincha with a variety of climates and ecosystems, such as Andean deserts, valleys and semitropical zones (MECN, 2009).

1.4 Natural water resources in the province of Pichincha

Although the contamination of Pichincha rivers is clearly visible nowadays, few studies were published about their microbial and chemical quality (Perez Naranjo et al., 2015; Vizcaíno et al., 2016; Voloshenko et al., 2014). The last study done by the municipal

water service of Quito (EPMAPS) revealed that most of the rivers in the south part of the capital overpass the authorized microbial limits of potable water by 3000% (Campaña et al., 2017). In 2014, Voloshenko and colleagues found emerging organic pollutants along the San Pedro, Guayllabamba and Esmeraldas rivers, such as carbamazepine and acesulfame. Also, the same study revealed the increment of concentration of the pollutants in the surroundings of Quito (Voloshenko et al, 2014).

1.5 Analysis of water quality in natural water resources

Most of the studies on water quality uses biological indicators such as *Escherichia coli* and total coliforms counting (Liang et al., 2016). However, other potentially opportunistic or even pathogenic microorganisms can be identified in the recollected samples and used as biological indicators, such as: *Salmonella, Pseudomonas, Shigella,* and *Legionella* spp.; as well as parasites, *Giardia* and *Cryptosporidium* spp. (Dobrowsky et al., 2014; Gallas-Lindemann et al., 2016; Law et al., 2014). Furthermore, water quality can also be evaluated in terms of its physical and chemical properties (Perez Naranjo et al., 2015; Reyes et al., 2016), such as metals (which can be found by natural or anthropogenic causes). The natural causes for elevated metal concentration could be due to the erosion of rocks or precipitation of sediments, while the anthropogenic causes could be due to industrial, mining and agricultural activities or even by untreated sewage discharges (Paul, 2017; Perez Naranjo et al., 2015; Reyes et al., 2016).

The identification of potential pathogenic microorganisms and the microbial load evaluation are usually done by microbiological classic methods (Ahmed et al., 2012) and sometimes by biological molecular techniques (Law, Ab Mutalib, Chan, & Lee, 2014). *Escherichia coli* is known to be a commensal bacterium, nevertheless some

strains can be pathogenic for human or animals (Ramírez et al., 2013) and considered as potential public health risk (Kora et al., 2017). Therefore, several studies evaluated certain *E. coli* pathotypes (Ahmed et al., 2012; Ramírez Castillo et al., 2013), such as enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and enteropathogenic *E. coli* (EPEC). The *E. coli* pathotype identification is usually done by using molecular microbiology methods, such as Polymerase Chain Reaction (PCR) (Dobrowsky et al., 2014; Law et al., 2014; Stanwell-Smith et al., 2003). On the other hand, the counting of commensal *E. coli* and total coliforms is traditionally done by classical methodology through a specific culture medium (Ahmed et al., 2012; Law et al., 2014).

2 JUSTIFICATION

In the last years, some studies demonstrated the existence of high levels of contamination in the rivers from Pichincha province, specifically, near the highly populated zones. However, these studies were just limited to the physical-chemical analysis (Campaña et al., 2017; Vizcaíno et al., 2016). For this reason, it is also important to perform a further analysis on the microbial load and biodiversity of these rivers. Ecuadorean legislation (Texto Unificado de Legislación Secundaria del Medio Ambiente 2015) establishes that the detection of microbial contamination in the rivers must be performed through analysis of certain microbiological parameters, such as the counting of total and fecal coliforms (*Escherichia coli*). Despite of microbial load quantification, it is also important to detect the presence of potentially opportunistic or even pathogenic microorganisms for humans in the rivers. Therefore, this study expected to create awareness in the scientific community as well as to the competent authorities, demonstrating the negative microbial impacts in the rivers by the continuous discharge of untreated wastewaters. The majority of the rivers analyzed in this study are usually used in several human and economic activities, leading to the augmentation of public health risks and the prejudice of several industrial activities.

3 STUDY AREA

The recollection of water samples from the eighteen rivers was realized in a single sampling point of each analyzed river. Each sampling point was selected by population density of the territory of Pichincha Province (see Figure 1).

Figure 1. Map of the sample recollection points for the eighteen rivers from Pichinchaprovince analyzed in this study.

As previously referred, the sampling points of the eighteen rivers were selected by population density and also economical activities realized around them (see Table 1), such as, recreational, farming and industry activities.

Table 1. Name of the rivers and location on the map of Pichincha analyzed in this study, with its coordinates and water samples recollection date.

Location	***Rivers***	***Coordinates***	**Dates of *recollection***
1	Machángara	0°14'2"S 78°30'54"W	31/01/2016-18/06/16
2	Guayllabamba	0°04'01.8"S 78°22'27.3"W	18/05/2016-31/05/16
3	San Pedro	0°22'17.7"S 78°30'13.1"W	27/01/2017-18/11/17
4	Pita	0°18'16.3"S 78°27'03.6"W	27/01/2017-18/11/17
5	Monjas	0°01'48.5"S 78°26'57.4"W	27/01/2017-13/04/17
6	Blanco	0°00'23.7"N 78°54'12.6"W	10/02/2017-21/01/18
7	Mindo	0°03'33.4"S 78°46'16.2"W	10/02/2017-21/01/18
8	Cinto	0°06'46.2"S 78°47'13.1"W	10/02/2017-21/01/18
9	Pisque	0°01'27"S 78°20'0"W	03/03/2017-10/01/18
10	Chiche	0°11'36.3"S 78°22'25.1"W	03/03/2017-18/11/17
11	Pilatón	0°22'9"S 78°49'60"W	17/03/2017-13/01/18
12	Pachijal	0°09'41.9"N 78°56'14.9"W	24/03/2017-26/01/18
13	Alambi	0°07'59"N 78°40'16"W	24/03/2017-13/04/17
14	Caoní	0°04'31"N 79°02'60"W	24/03/2017-26/01/18
15	Mashpi	0°11'18.5"N 78°55'35.1"W	24/03/2017-06/01/18
16	Guachalá	0°0'19"N 78°10'28"W	07/04/2017-03/12/17
17	Granobles	0°3'22"N 78°9'50"W	07/04/2017-10/01/18
18	Pedregales	0°29'26"S 78°32'25"W	07/04/2017-07/01/18

4 OBJECTIVES

4.1 General objective

Evaluate the microbial and chemical load of the natural water resources around the province of Pichincha.

4.2 Specific objectives

- Quantify *Escherichia coli* and total coliforms load through classical methods of microbiology.
- Analyze the microbial biodiversity through Polymerase Chain Reaction (PCR) detection of the genera *Pseudomonas, Legionella, Shigella, Salmonella, Legionella* and the species *Candida albicans.*
- Identify the presence or absence of well-known parasites (*Cryptosporidium* and *Giardia* spp.) by PCR.
- Detect the presence of certain *E. coli* pathotypes by PCR, more specifically, enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and enteropathogenic *E. coli* (EPEC).
- Estimate the actual conditions of pollution in several rivers of Pichincha through the physical-chemical, metal and major elements analysis.

5 MATERIALS, REAGENTS AND EQUIPMENTS

5.1 Sample collection

- Glass containers
- Refrigerant Gel Pack
- Coolers
- Global Positioning Systems (GPS)
- Autoclave

5.2 Analysis *in situ* of physical-chemical parameters

- Multiparameter Thermo Scientific Model A329 (Thermo Fisher Scientific, Waltham, MA, USA)
- Turbidimeter Thermo Scientific Model AQUAFast AQ4500 (Thermo Fisher Scientific, Waltham, MA, USA)
- Teflon bottles
- Hypochlorhydric acid

5.3 Analysis of physical-chemical parameters in laboratory

- Spectronic 20D+ spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA)
- ThermoScientific iCAP 7400 ICP-OES
- Certificated reference material (CRM 1640a)
- Nitric acid
- Vacuum pump (Milipore)
- 0.45 µm cellulose filters (Milipore)

- Polyethylene bottles

5.4 Filtration of water samples

- 0.45 µm nitrocellulose membrane (Milipore)
- Vacuum pump (Milipore)
- 50mL sterile falcon tubes
- Distilled water
- Vortex
- Centrifuge for falcon tubes
- 2mL eppendorf tubes
- Micropipettes
- Tips for micropipettes
- Globes

5.5 Growth media for quantification and isolation of microorganisms

- *Legionella* CYE Agar Base (Difco)
- Chromocult Agar médium (Merck)
- MacConkey Agar (Difco)
- Biggy Agar (Difco)
- Sterile swabs
- Inoculating loop with handle
- Incubator
- Cryopreservation tubes
- Brain Hear Infusion BHI (Difco) + glycerol 15%
- Ultra-freezer -80°C

- Globes

5.6 DNA extraction

- PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc)
- Nanodrop (Thermo Scientific)

5.7 Molecular identification of the microbial biodiversity

- Green GoTaq Flexi Buffer (Promega, Madison, WI, USA)
- $MgCl_2$ (Promega, Madison, WI, USA)
- GoTaq Flexi DNA polymerase (Promega Madison, WI, USA)
- DNA free water
- PCR primers for *Pseudomonas* spp., *Legionella* **s**pp., *Shigella* spp., *Salmonella* spp. and *Candida albicans*
- Nester PCR primers for *Cryptosporidium* and *Giardia* spp.
- Positive controls
- Bio-Rad Thermocycler

5.8 Molecular identification of *E. coli* pathotypes

- Green GoTaq Flexi Buffer (Promega, Madison, WI, USA)
- $MgCl_2$ (Promega, Madison, WI, USA)
- GoTaq Flexi DNA polymerase (Promega Madison, WI, USA)
- DNA free water
- PCR primers for *E. coli* pathotypes (EHEC, EPEC, EAEC, EIEC)
- Positive controls
- Bio-Rad Thermocycler

5.9 PCR product analysis

- Electrophoresis equipment
- Agarose 1.5% for microorganisms diversity and *E. coli* pathotypes
- Agarose 2.0% for parasites
- TBE 1%
- Ethidium bromide 0.1%

6 METHODS

6.1 Sample collection

The water samples were collected from several rivers located in the province of Pichincha, Ecuador (see Figure 1). The samples were collected in glass containers previously sterilized by autoclaving them at 121°C for 15 minutes. For each river it was collected a total volume of 800 mL, and the samples were maintained at 4°C until its arrival to the Microbiology Institute at Universidad San Francisco de Quito (Dobrowsky et al., 2014).

Additionally, for the chemical analysis, water samples were taken in an acid-clean 1 L Teflon bottle previously washed with 10% HCl and then rinsed with distilled water. This samples were conserved at 4°C until its arrival to the Environmental Laboratory at Universidad San Francisco de Quito (LIA-USFQ). The phases were separated immediately by vacuum filtration with a 0.45 μm cellulose filter. For metal analysis, the filtrate was transferred to polyethylene bottles and then preserved with a high purity concentrated nitric acid (LobaChemie, Mumbai, India) to obtain a final concentration of 2% w/w.

6.2 Sample preparation

Surface water samples were filtered using a 0.45 μm nitrocellulose membrane (Milipore) with a vacuum pump under aseptic conditions (Chemical Duty Pump, Milipore Inc). The following procedure was adapted from the study realized by Dobrowsky and colleagues (2014) with minor modifications. The membrane was removed and placed in a sterile falcon tube with 20 mL of distilled sterile water. The tube was vortexed during a period of 15 minutes to suspend the soil particles and the

microorganisms. Then the membrane was removed and the tubes were centrifuged at 5000 rpm for 15 minutes to precipitate the sediments. Once the pellet was obtained, it was suspended in 2 mL of distilled sterile water. This sample was divided in two aliquots of 1 mL, being one for bacterial DNA extraction using Power Soil Extraction Kit (MO BIO Laboratories, Inc.) and another for bacterial growth cultures.

6.3 Cultivation, quantification and isolation of dominant microorganisms from river samples

Different media cultures were used to isolate or count the microorganisms found in the samples. More accurately, 20 µL of sample were incubated on MacConkey Agar (Difco) at 37°C for 18 to 24h for the recovery of the genera *Escherichia*, *Salmonella*, *Shigella* and *Pseudomonas*. Another 20 µL were incubated on *Legionella* CYE Agar Base (Difco) at 35°C for 48 h, for the isolation of *Legionella* spp., and on Biggy agar (Difco) to isolate Candida spp.. Finally, for the quantification of *Escherichia coli* and total coliforms, successive dilutions of the initial aliquot were cultured in Chromocult Agar medium (Merck; Biolab, Wadeville, Gauteng) at 35°C for 24 to 48h.

6.4 DNA extraction

DNA from the collected water samples was extracted using the instructions of the commercial PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc). A volume of 500 µL of the pellet obtained from the filtrations was placed in the PowerBead Tubes. These PowerBead Tubes contain a buffer that disperse the soil particles and dissolve humic acids, also it protects nucleic acids from the degradation. Then 60 µL of Solution C1 was placed and vortexed for 15 minutes. This solution contains SDS and other agents required for the complete cell lysis, also the vortex step ensures the complete

homogenization and cell lysis of the samples. Later the tubes were centrifuged at 10000*xg* for 30 seconds at room temperature. A total volume of 500 µL was taken of the supernatant and placed in a 2 mL collection tube, subsequently 250 µL of Solution C2 were added and the total volume of the tubes was incubated a 4°C for 5 minutes. Solution C2 contains a reagent that precipitates non-DNA organic and inorganic molecules. The tubes were centrifuged at 10000*xg* for 1 minute at room temperature. Later, 600 µL of supernatant from each tube were transferred to a new 2 mL collection tube with 200 µL of Solution C3. This solution allows to precipitate additional non-DNA organic and inorganic molecules. The tubes were centrifuged at 10000*xg* for 1 minute at room temperature. Afterwards, 750 µL of supernatant were mixed with 1200 µL of Solution C4, which contains a high concentration of salts. Half of the volume was place inside a spin filter and centrifuged at 10000*xg* for 1 minute at room temperature. Then, the liquid was discharged, and the previous step was repeated twice. Subsequently, 500 µL of Solution C5 were added inside the Spin Filter and centrifuged at 10000*xg* for 30 seconds at room temperature, the liquid from each tube was discharged. Solution C5 contains ethanol which helps to clean DNA bounded to the membrane filter. The tubes were again centrifuged at 10000*xg* for 1 minute at room temperature, removing all the residual solutions. Finally, the Spin Filter was placed on a new 2 mL collection tube and 100 µL of solution C6 were added to the center of the filter membrane. This solution contained a sterile elution buffer that releases DNA from the membrane. The tubes were centrifuged for 30 seconds at 10000*xg* and the spin filter was discarded. The DNA solution of each tube was stored at -20°C for the further PCR analysis.

6.5 Molecular identification of the microbial biodiversity

Bacterial genera and Candida albicans

Once the genomic DNA was extracted from the different samples, 16S conserved rRNA genes were amplified. The PCR mixtures consisted of a final volume of 20 μL and contained 4 μL of 1x Green GoTaq Flexi buffer (Promega, Madison, WI, USA), 1.60 μL of 2.0 mM MgCl2 (Promega, Madison, WI, USA), 0.40 μL of 0.2 mM dNTPs mix (Promega, Madison, WI, USA), 1.0 μL of each PCR primer (Table 2), 0.2 μL of 0.5U GoTaq Flexi DNA polymerase (Promega, Madison, WI, USA), 2 μL template DNA and the remaining volume of DNA-free water. For *Shigella, Salmonella, Legionella* and *Pseudomonas* spp., the same reaction mixture was used with the exception that 0.09 μL of 0.5U GoTaq Flexi DNA polymerase were added. Additionally, for the identification of *Candida albicans* the same reaction mixture was used, with the exception that 2 μL of 2.0 mM MgCl2, and 0.18 μL of 0.5U GoTaq Flexi DNA polymerase were added. The PCR methodology was performed in a thermocycler (Bio-Rad) with the procedure illustrated in Table 2.

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

Microorganism	*Primer name*	*Primer sequence (5'–3')*	*PCR cycling parameters*	*Gene (size [bp])*	*References*
Universal	Forward: fDD2	CCGGATCCGTCGACAGAGTTTGATCITGGCTCAG	3 min at 94°C; 30 cycles of 94°C for 30 s, 53°C for 30 s, 72°C for 1.5 min	16S rRNA (1,600)	Dobrowsky et al., 2014
	Reverse: rPP2	CCAAGCTTCTAGACGGITACCTTGTTACGACTT			
Shigella spp.	Forward: IpaH-F	CCTTGACCGCCTTTCCGATA	2 min at 95°C; 35 cycles of 94°C for 1 min, 62°C for 1 min, 72°C for 2.5 min, 72°C for 3 min	*Invasion plasmid antigen* H (606)	Dobrowsky et al., 2014
	Reverse: IpaH-R	CAGCCACCCTCTGAGGTACT			
Legionella spp.	Forward: JFP	AGGGTTGATAGGTTAAGAGC	5 min at 95°C; 40 cycles of 94°C for 1 min, 57°C for 1.5 min, 72°C for 1 min, 72°C for 5 min.	*Attachment invasion locus gene* (386)	Dobrowsky et al., 2014
	Reverse: JRP	CCAACAGCTAGTTGACATCG			
Salmonella spp.	Forward: IpaB-F	GGACTTTTTAAAAGCGGCGG	2 min at 95°C; 35 cycles of 94°C for 1 min, 62°C for 1 min, 72°C for 2.5 min, 72°C for 5 min.	*Invasion plasmid antigen* B (314)	Dobrowsky et al., 2014
	Reverse: IpaB-R	GCCTCTCCCAGAGCCGTCTGG			
Pseudomonas spp.	Forward: PA-GS-F	GACGGGTGAGTAATGCCTA	2 min at 95°C; 35 cycles of 94°C for 20 s, 54°C for 20 s, 72°C for 40 s, 72°C for 5 min	16S rRNA (618)	Dobrowsky et al., 2014
	Reverse: PA-GS-R	CACTGGTGTTCCTTCCTATA			
Candida albicans	Forward: CALB1	TTTATCAACTTGTCACACCAGA	5 min at 95°C; 35 cycles of 94°C for 30 s, 58°C for 30 s, 72°C for 30 s, 72°C for 10 min.	*ITS*-1, *ITS*-2 (278)	Luo & Mitchell, 2002
	Reverse: CALB2	ATCCCGCCTTACCACTACCG			

***Cryptosporidium* and *Giardia* spp.**

For the molecular identification of certain well-known parasites, more exactly, *Cryptosporidium* and *Giardia* spp., a nested PCR was performed using two sets of primers for each parasite. The PCR mixtures consisted of a final volume of 25 μL and contained 5 μL of 1x Green GoTaq Flexi buffer (Promega, Madison, WI, USA), 3 μL of 3.0 mM MgCl2 (Promega, Madison, WI, USA), 1.00 μL of 0.4 mM dNTPs mix (Promega, Madison, WI, USA), 0.75 μL of each PCR primer (Table 3), 0.07 μL of 0.35U GoTaq Fexi DNA polymerase (Promega, Madison, WI, USA), 1 μL template DNA and the remaining volume of DNA-free water. The nested PCR used the same reaction with the difference that the product of PCR from the pre-nested one, was used as template DNA. The PCR methodology was performed in a thermocycler (Bio-Rad) with the procedure illustrated in Table 3.

Table 3. Primers and PCR cycling parameters for the detection of *Cryptosporidium* and *Giardia* spp.

Microorganism	*Primer name*	*Primer sequence (5'–3')*	*PCR cycling parameters*	*Gene (size [bp])*	*References*
Cryptosporidium spp.	Forward: Cry 15	GTAGATAATGGAAGAGATTGTG	10 min at 95°C; 45 cycles of 94°C for 30 s, 52°C for 30 s, 72°C for 50 s.	*COWP* (550)	Salza, 2014; Yu et al., 2009
	Reverse: Cry 9	GGACTGAAATACAGGCATTATCTT			
	Forward: Cowpnest F	TGTGTTCAATCAGACACAGC	10 min at 95°C; 32 cycles of 94°C for 30 s, 60°C for 30 s, 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 45 s, 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 45 s, 55°C for 30 s, 72°C for 1 min.	*TPI* (530)	
	Reverse: AL3545	GTGGCCACCACTCCCGTGCC			

Escherichia coli pathotypes

For the molecular identification of *E. coli* pathotypes the PCR mixtures consisted of a final volume of 20 μL. The volume contained 4 μL of 1x Green GoTaq Flexi buffer (Promega, Madison, WI, USA), 1.60 μL of 2.0 mM MgCl2 (Promega, Madison, WI, USA), 0.40 μL of 0.2 mM dNTPs mix (Promega, Madison, WI, USA), 0.5 μL of each PCR primer (Table 2), 0.18 μL of 0.5U GoTaq Fexi DNA polymerase (Promega, Madison, WI, USA), 2 μL template DNA and the remaining volume of DNA-free water. The PCR methodology was performed in a thermocycler (Bio-Rad) with the procedure illustrated in Table 4.

Table 4. Primers and PCR cycling parameters for the detection of *E. coli* pathotypes accordingly to a previous realized by Ramirez Castillo and colleagues (2013).

***E. coli* pathotypes**	***Primer name***	***Primer sequence (5'–3')***	***PCR cycling parameters***	***Gene (size [bp])***
EAEC	Forward: AggRKs1	GTATACACAAAAGAAGGAAGC	Stage 1, initial denaturing at 95°C for 2 min; stage 2, denaturing at 95°C for 1 min, primer annealing at 54°C for 1 min, and elongation at 72°C for 1 min; for 30 cycles, and stage 3, final elongation step at 72°C for 10 min.	*aggR* (254)
	Reverse: AggRkas2	ACAGAATCGTCAGCATCAGC		
EHEC	Forward: VTcomU	GAGCGAAATAATTTATATGTG		*stx* (518)
	Reverse: VTcomd	TGATGATGGCAATTCAGTAT		
EPEC	Forward: SK1	CCCGAATTCGGCACAAGCATAAGC		*ege* (881)
	Reverse: SK2	CCCGGATCCGTCTCGCCAGTATTCG		
EIEC	Forward: IpaIII	GTTCCTTGACCGCCTTTCCGATACCGTC		*ipaH* (619)
	Reverse: IpaIV	GCCGGTCAGCCACCCTCTGAGAGTAC		

6.6 PCR product analysis

The PCR products were visualized using electrophoresis with 1.5 % agarose gel and staining with ethidium bromide 0.1%., excepting for *Cryptosporidium* and *Giardia* spp., which it was used a 2% agarose gel. The negative and positive controls used were provided by the Microbiology Institute at Universidad San Francisco de Quito.

6.7 Analytical methods

Parameters such as conductivity, pH, temperature, dissolved oxygen (DO), and turbidity were measure in situ and triplicate in the surface water of the rivers in all the recollection points (see Figure 1). Conductivity, pH, temperature and DO were measured using a multiparameter Thermo Scientific Model A329 (Thermo Fisher Scientific, Waltham, MA, USA). The turbidity was measured with a turbidimeter Thermo Scientific Model AQUAFast AQ4500 (Thermo Fisher Scientific, Waltham, MA, USA). The analysis of physical-chemical parameters was conducted by following the standardized protocols for analysis of residual wastes (APHA, 2014). The total chemical oxygen demand (CODT) was measured by colorimetric methods using a Spectronic 20D+ spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Total suspended solids (TSS) and the total solids (TS) were measured by gravimetric methods. The analysis of major and trace elements was performed by inductively coupled plasma optical emission spectrometry (ICP-OES) using a ThermoScientific iCAP 7400 ICP-OES at the Environmental Engineering Laboratory at Universidad San Francisco de Quito (LIA – USFQ). Calibrations curves were created from a multielement standard solution (Sigma Aldrich, US). The detection limits were obtained by measuring the blanks 8 times for each run and were calculated by

multiplying the standard deviation of the blanks per three. For the quantification limit the previous procedure was repeated but the difference was that the standard deviation was multiplied by ten. Quality control for major and trace elements analysis was conducted by employing certificated reference material (CRM 1640a), every ten samples (NIST, Gaithersburg, Maryland). The recovery percentages were calculated to determine the matrix effects and to measure the accurateness of the method. All the concentrations were corrected based on the percentage of recoveries obtained in each analysis.

7 RESULTS

7.1 *Escherichia coli* and total coliforms counts.

The counting of *Escherichia coli* and total coliforms for the eighteen rivers analyzed in this study is shown in Table 5. All the rivers analyzed presented concentrations of both *E. coli* and total coliforms that overpassed the permitted limit according to the United States Environmental Protection Agency (EPA, 2012), except for Caoní river on *E. coli* quantification. These quality limits focuses on both marine and freshwaters intended for full or partial contact with humans, such as the rivers from this study. In the World Health Organization and the Ecuadorian legislation (TULSMA, 2015), the standard values of *E. coli* and total coliforms is only specified for quality of drinking water, where they mention that there should not be any of both microbiological parameters present in 100 mL.

Table 5. Amount of *Escherichia coli* and total coliforms in the analyzed rivers.

RIVER	*Escherichia coli* (CFU/mL) 126 CFU per 100 mL[a]	Total coliforms (CFU/mL) 200 CFU per 100 mL[b]
Machángara	2.25×10^2	3.25×10^2
Guayllabamba	1.25×10^2	3.13×10^2
San Pedro	9.60×10^1	2.25×10^2
Pita	1.00×10^2	3.50×10^2
Monjas	9.18×10^2	5.15×10^3
Blanco	1.83×10^0	4.25×10^0
Mindo	1.72×10^1	6.78×10^1
Cinto	2.98×10^1	7.30×10^1
Pisque	1.71×10^1	4.00×10^1
Chiche	1.25×10^2	3.68×10^2
Pilatón	1.79×10^0	4.88×10^0
Pachijal	7.75×10^0	2.32×10^1
Alambi	7.08×10^0	2.58×10^1
Caoní	1.17×10^0	3.95×10^0
Mashpi	2.58×10^1	7.35×10^1
Guachalá	1.29×10^2	2.98×10^2
Granobles	1.67×10^1	2.46×10^1
Pedregales	1.17×10^1	2.29×10^1

[a] The permitted level for Surface Water Partial-Body Contact (for *Escherichia Coli*) United States Environmental Protection Agency (EPA, 2012).
[b] The permitted level for Surface Water Partial-Body Contact (for Total Coliforms) United States Environmental Protection Agency (EPA, 2012).

As shown in Figure 2, the highest concentration of *E. coli* and total coliforms were found in Monjas, Machángara and Chiche rivers, showing levels of *E. coli* and total coliforms between 1.25 x 10^2 – 9.18 x 10^2 and 3.68 x 10^2 – 5.15 x 10^3 CFU/mL, respectively.

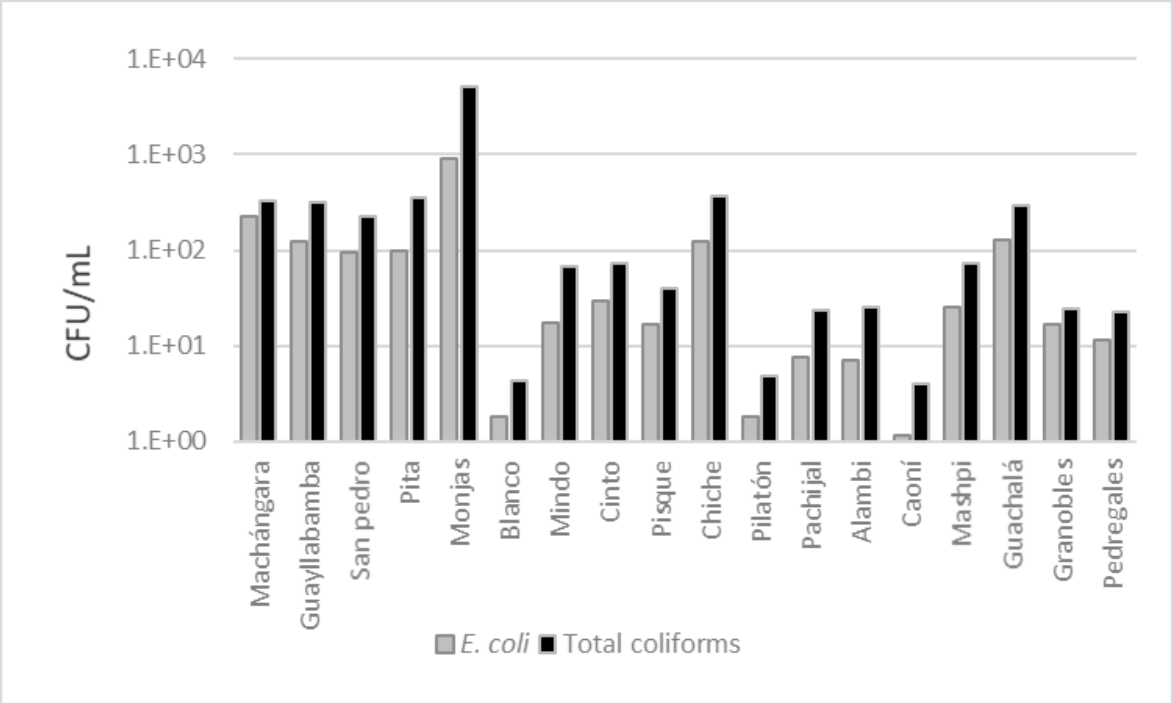

Figure 2. *E. coli* and total coliform counts of the eighteen rivers analyzed in the present study.

Although all analyzed rivers overpassed the legal permitted levels for surface water partial-body contact (see Table 5), except for *E. coli* counting on Caoní which had a value of 1.17 x 10^0 UFC/mL. Moreover, Caoní and Pilatón rivers evidenced the lowest values of *E. coli* and total coliforms of the study set, more exactly, 1.17 x 10^0 – 1.79 x 10^0 and 3.95 x 10^0 – 4.88 x 10^0 CFU/mL, respectively.

7.2 Detection of microbial genera, *Candida albicans* and *E. coli* pathotypes

Molecular analysis was conducted by PCR to confirm the presence or absence from the following microbial genera: *Legionella*, *Pseudomonas*, *Salmonella*, *Shigella*,

Cryptosporidium and *Giardia*. In relation to parasites genera, three rivers showed the presence of *Cryptosporidium* spp., more exactly, Mindo, Pisque and Alambi rivers (see Figure 3). While, eight rivers showed the presence of *Giardia* spp., more precisely: Machángara, San Pedro, Monjas, Blanco, Mindo, Pisque, Pilatón and Guachalá rivers.

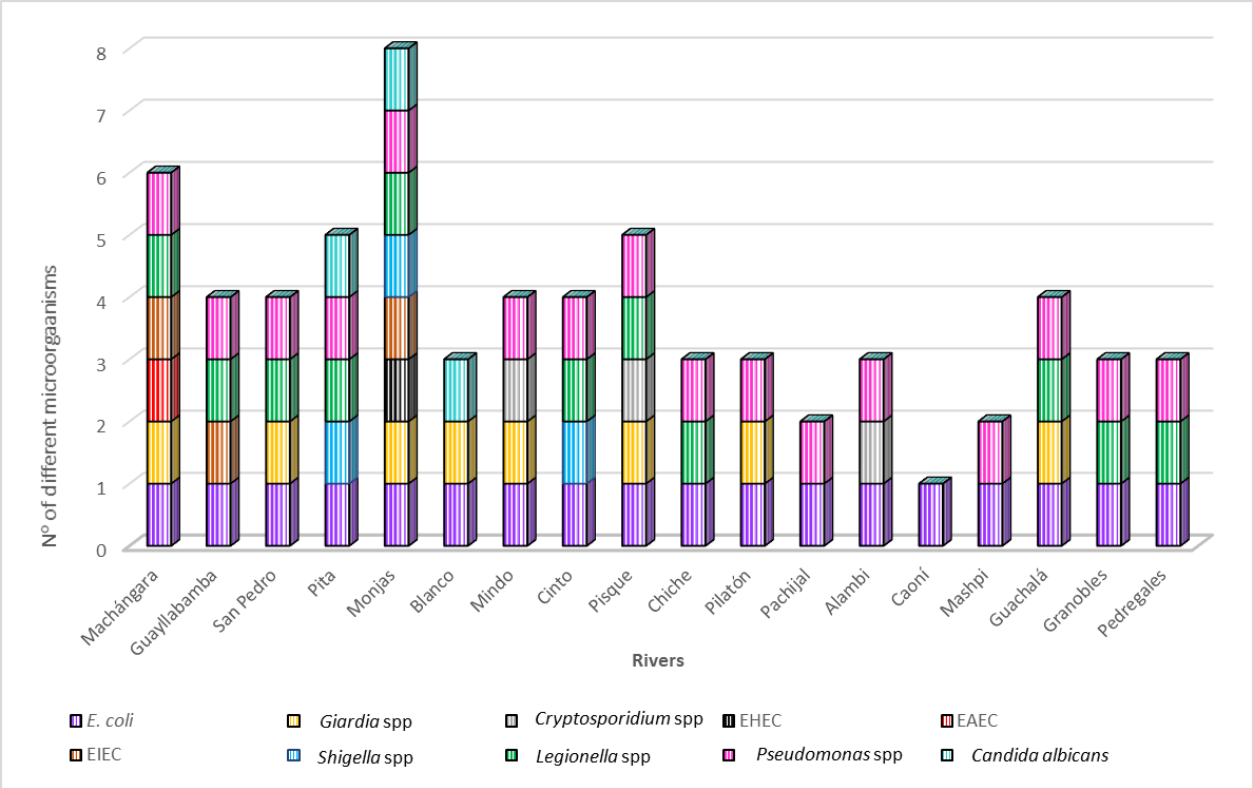

Figure 3. Molecular detection of microbial genera, *Candida albicans* and *E. coli* pathotypes of the eighteen rivers analyzed in the present study.

Also, the presence and absence of different bacterial genera such as *Pseudomonas*, *Salmonella*, *Legionella* and *Shigella* was also analyzed in the study set. None of the rivers showed presence of *Salmonella* spp. and all rivers showed the presence of *Pseudomonas* sp. excepting in Blanco and Caoní rivers. The second most prevalent bacteria genera detected in our study was *Legionella* spp. showing its presence on eleven from eighteen analyzed rivers. Specifically, Blanco, Mindo, Pilatón, Pachijal, Alambi, Caoní and Mashpi rivers were the exception in *Legionella* spp. detection in our

study set. At last, three rivers showed the presence of *Shigella* spp., more exactly, Pita, Monjas and Cinto rivers.

The presence of *Candida albicans* was also evaluated in this study, evidencing its existence on Pita, Monjas and Blanco rivers. Furthermore, the detection of four *E. coli* pathotypes was performed for all the analyzed rivers, more exactly: enteroaggregative *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enteroinvasiva *E. coli* (EIEC) and enteroaggregative *E. coli* (EAEC). Our analysis showed EIEC pathotype as the most prevalent pathogen in the study set, illustrating positive results in Machángara, Guayllabamba, and Monjas rivers. Meanwhile, EHEC and EAEC were only detected in one river, more precisely, Monjas and Machángara rivers, respectively. Finally, EPEC pathotype was not detected in any of the eighteen rivers evaluated during this study.

7.3 Analysis of physical-chemical parameters

Besides the microbiological analysis previously performed in the eighteen rivers, we also analyzed the physical-chemical parameters presented in Table 6. The reported values were obtained by triplicated measurements of each analyzed river. These parameters were selected as good indicators of water safety according to the parameters presented in the Ecuadorean Legislation (TULSMA), which indicated the maximum contaminant levels (MCL). In relation to pH, Pisque and Machángara rivers showed the highest pH values above the legal MCL, specifically 9.55 and 9.11, respectively. No pH value below legal MCL was detected in the study set. In fact, the minimum pH value was of 7.15 in both Chiche and Pachijal rivers. In contrast, conductivity, turbidity and ORP are *in situ* parameters without MCL in the Ecuadorian Legislation (TUSLMA). However, conductivity values ranged extremely since 19.87 μS/cm in

Caoní river until 616.00 μS/cm in Monjas river. While, turbidity measurements also varied tremendously between 1.23 NTU in Blanco river and 881.33 NTU in Machángara river. But ORP values ranged slightly between 297.13 mV in San Pedro river to 489.53 mV in Alambi river (see Table 6). Next, the remaining *in situ* parameters (DO and temperature) had MCL values and thus certain results were outside the standard levels. More exactly, Monjas river was the only river with DO value (5.36 mg/L) below MCL (TULSMA) and, however no maximum level is stipulated by TULSMA, the highest value of 10.32 mg/L was obtained in both Pachijal and Chiche rivers. Similarly, in the case of temperature, the values ranged from a minimum of 12.40°C in Mashpi river, to a maximum of 22.30°C in Caoní river. As previously referred, the following physical-chemical parameters was then conducted by standardized protocols at LIA-USFQ, more precisely, total chemical oxygen demand (COD_{Total}), total solids (TS), total suspended solids (TSS), chloride (Cl^-), ammonium (NH_4^+N), nitrate (NO_3^-N), phosphate ($PO_4^{3-}P$) and sulfate (SO_4^{2-}). As shown in Table 6, only two rivers showed values of COD_{Total} superior to 250 mg/L (MCL), more exactly, Machángara (692 mg/L) and Monjas (318 mg/L). Although all analyzed rivers showed TS values within the permitted limits, five rivers showed TSS values superior to 130 mg/L (MCL), more precisely, Machángara (520 mg/L), Alambi (367 mg/L), Chiche (300 mg/L), Pisque (237 mg/L) and Monjas (154 mg/L). Next, the remaining physical-chemical parameters, such as chloride, phosphate and sulfate, were within the allowed MCL values. Nevertheless, chloride values varied between 1.06 and 40.32 mg/L from Mashpi and Monjas rivers, respectively. Phosphate values ranged since 0.05 mg/L in Cinto river until 3.93 mg/L in Monjas river at last, sulfate measures were quantified between 2.00 and 11.66 mg/L from Pachijal and Caoní rivers, respectively. It is important to mention that neither ammonium nor nitrate had a legal MCL by

TULSMA. However, ammonium values were quantified between 0.13 and 27.48 mg/L in Pedregales and Monjas rivers, respectively. While nitrate showed a range between 0.57 and 11.66 mg/L in Cinto and Caoní rivers, respectively.

Table 6. Analysis of physical-chemical parameters of the eighteen rivers analyzed in this study.

River **MCL**	**pH** 6.5 – 9[a]	**Conductivity (µS/cm)** N/A	**DO (mg/L)** Not<6[a]	**Turbidity (NTU)** N/A	**ORP (mV)** N/A	**T (°C)** <32[a]	**COD_{Total} (mg/L)** 250[b]	**TS (mg/L)** 1600[b]	**TSS (mg/L)** 130[b]	**Cl^- (mg/L)** 120[b]	**NH_4^+N (mg/L)** N/A	**NO_3^-N (mg/L)** N/A	**$PO_4^{3-}P$ (mg/L)** 10[b]	**SO_4^{2-} (mg/L)** 1000[b]
Machángara	**9.11***	297.97	6.77	881.33	362.70	15.20	**692.00***	1359.00	**520.00***	37.27	20.36	6.40	0.17	29.00
Guayllabamba	7.90	365.00	7.42	56.50	402.23	18.20	33.00	397.00	90.00	26.51	2.54	5.13	1.17	11.50
San Pedro	8.00	529.77	8.23	22.17	297.13	13.43	20.00	470.00	52.00	23.78	7.16	6.95	1.19	65.85
Pita	8.41	221.80	8.10	10.73	346.70	13.80	8.00	280.00	45.00	4.45	0.23	1.93	0.50	71.62
Monjas	8.04	616.00	**5.36***	136.00	323.17	19.60	**318.00***	632.50	**153.50***	40.32	27.48	3.43	3.93	103.72
Blanco	7.32	53.53	8.76	1.23	310.00	20.97	20.00	470.00	6.67	1.11	4.19	0.63	0.05	3.50
Mindo	8.37	139.67	8.27	1.76	323.70	17.87	2.00	280.00	8.33	9.31	0.19	0.70	0.11	6.00
Cinto	7.20	232.93	8.06	5.34	306.00	20.37	2.00	632.00	6.67	21.39	0.39	0.57	0.05	29.00
Pisque	**9.55***	273.43	8.02	306.67	408.20	16.63	180.00	806.00	**236.67***	14.04	0.27	10.98	0.11	6.00
Chiche	7.15	44.80	10.32	5.89	412.23	21.40	206.00	597.00	**300.00***	28.17	1.01	6.31	0.18	3.50
Pilatón	8.15	101.67	8.77	56.10	372.23	17.23	2.16	182.00	54.00	3.93	0.22	0.95	0.12	11.00
Pachijal	7.15	44.80	10.32	5.89	412.23	21.40	2.00	61.00	3.33	1.24	0.22	0.86	0.11	2.00
Alambi	8.15	72.07	8.92	251.33	489.53	18.50	65.00	521.00	**366.67***	3.42	0.24	1.25	0.21	3.00
Caoní	7.33	19.87	9.35	25.93	397.07	22.30	7.00	45.00	20.00	2.31	0.21	11.66	0.09	3.50
Mashpi	8.15	33.72	9.87	11.07	435.40	N/A	9.00	36.00	8.33	1.06	0.22	1.19	0.06	4.00
Guachalá	8.11	147.00	7.78	7.60	381.40	12.40	2.00	407.50	21.67	2.53	0.29	2.60	0.27	14.00
Granobles	7.78	159.00	6.91	16.70	424.23	13.80	13.00	182.50	28.33	4.69	0.29	4.97	0.59	6.50
Pedregales	7.67	194.00	6.72	11.60	328.83	13.53	2.00	222.00	18.33	13.26	0.13	1.56	0.30	6.00

[a] Quality criteria acceptable for the preservation of flora and fauna in fresh waters, cold or warm, and marine waters and estuary. Annex I, Book VI of the TULSMA reformed on the Acuerdo Ministerial 97 on July 30, 2015 (see Table 3).

[b] Maximum allowable discharge limits to a fresh water body. Annex I, Book VI of the TULSMA reformed on the Acuerdo Ministerial 97 on July 30, 2015 (see Table 12).

*Values that exceed the quality criteria

7.4 Analysis of metal and major parameters

The analysis of metal and major elements of the eighteen rivers is shown in Table 7. The metals analyzed in this study were copper (Cu), iron (Fe), chromium (Cr), manganese (Mn), aluminium (Al), lead (Pb), lithium (Li) and zinc (Zn). While the major elements analyzed were calcium (Ca), sodium (Na) and magnesium (Mg). All these parameters were also compared to the MCL of TULSMA in Table 7. All metal values obtained for copper, chromium and lithium were below the MCL. The recollected iron values were detected below the MCL (5.0 mg/L) excepting for Pisque (6.76 mg/L), Chiche (12.69 mg/L) and Machángara (13.88 mg/L). In relation to manganese, only Monjas river reported a higher value than the MCL (0.20 mg/L), more exactly, 0.21 mg/L. The remaining rivers registered values below MCL, where the lowest values were found at Pachijal, Caoní and Mashpi rivers with 0.00 mg/L. In contrast, eight rivers showed aluminium concentrations above the MCL (5.0 mg/L), more precisely: Pilatón (13.12 mg/L), Cinto (17.30 mg/L), Pisque (17.53 mg/L), Mindo (17.66 mg/L), Machángara (18.05 mg/L), Chiche (18.08 mg/L), Granobles (18.12 mg/L) and Guachalá (18.25 mg/L). Meanwhile, lead concentrations were superior than the MCL (0.05 mg/L) in three rivers of our study set, such as Machángara (0.06 mg/L), Alambi (0.08 mg/L) and Pedregales (0.08 mg/L). Finally, all the rivers showed zinc values below the MCL (2.0 mg/L), excepting in Pedregales (3.72 mg/L). The Ecuadorean legislation does not possess MCL for the major elements, such as calcium, sodium and magnesium. However, the analysis and importance of these major elements is well-known in several studies worldwide. So, in this study, the calcium measurements varied since 3.70 to 170.26 mg/L in Caoní and Pedregales rivers, respectively. Meanwhile, sodium concentrations ranged between 4.59 and 73.15 mg/L in Caoní and San Pedro rivers, respectively. At last, the magnesium values were also quantified from 2.37 until 32.21 mg/L in Caoní and San Pedro rivers, respectively.

Table 7. Concentrations values of each metal and mayor elements from the eighteen rivers analyzed in this study

River MCL	Copper (mg/L) 2.0 mg/L[a]	Iron (mg/L) 5.0 mg/L[a]	Chromium (mg/L) 0.1 mg/L[a]	Manganese (mg/L) 0.2 mg/L[a]	Aluminium (mg/L) 5.0 mg/L[a]	Lead (mg/L) 0.05 mg/L[a]	Lithium (mg/L) 2.5 mg/L[a]	Zinc (mg/L) 2.0 mg/L[a]	Calcium (mg/L) N/A	Sodium (mg/L) N/A	Magnesium (mg/L) N/A
Machángara	0.03	**13.88**	0.05	0.17	**18.05**	**0.06**	0.01	0.43	21.2	31.76	6.05
Guayllabamba	0.01	1.31	0.00	0.07	0.49	N/A	0.02	0.10	17.86	30.71	13.33
San Pedro	0.00	0.71	0.00	0.05	0.03	N/A	0.04	0.05	29.32	73.15	32.21
Pita	N/A	0.79	N/A	0.04	0.16	N/A	0.02	0.00	16.07	17.73	10.73
Monjas	0.01	0.64	0.00	**0.21**	0.18	N/A	0.03	0.15	24.09	58.19	9.28
Blanco	0.02	0.84	0.03	0.01	5.07	N/A	0.00	0.18	7.92	8.46	2.72
Mindo	0.02	1.05	0.03	0.01	**17.66**	N/A	0.01	0.07	15.96	12.01	4.91
Cinto	0.01	1.47	0.03	0.06	**17.30**	N/A	0.01	0.07	17.74	16.76	9.00
Pisque	0.02	**6.76**	0.04	0.02	**17.53**	N/A	0.01	0.08	46.16	28.62	12.39
Chiche	0.01	**12.96**	0.04	0.03	**18.08**	N/A	0.01	0.09	12.71	20.15	7.08
Pilatón	0.01	1.74	0.04	0.02	**13.12**	N/A	0.01	0.10	11.67	8.78	4.42
Pachijal	0.00	0.11	0.00	0.00	N/A	N/A	0.00	0.07	5.82	4.82	3.28
Alambi	0.00	3.84	0.00	0.04	2.06	**0.08**	0.01	0.10	10.98	8.90	4.40
Caoní	0.00	0.25	0.00	0.00	0.08	N/A	0.00	0.06	3.70	4.59	2.37
Mashpi	N/A	0.27	0.00	0.00	N/A	N/A	0.00	0.08	5.82	4.99	3.22
Guachalá	0.01	2.21	0.04	0.02	**18.25**	0.01	0.01	0.09	15.23	14.57	6.66
Granobles	0.02	2.80	0.04	0.05	**18.12**	N/A	0.01	0.15	13.77	14.61	6.63
Pedregales	0.01	2.54	0.00	0.14	0.39	**0.08**	0.01	**3.72**	170.26	17.81	10.62

[a]Quality criteria for water for agricultural use, Annex I, Book VI of the TULSMA reformed on the Acuerdo Ministerial 97 on July 30, 2015 (see Table 6).
N/A: not available
*Values that exceed the quality criteria.

8 DISCUSSION

8.1 *Escherichia coli* and total coliforms counts

In the current study, most of the rivers showed *E. coli* and total coliforms levels above the permitted limits stablished by the United States Protection Agency (EPA, 2012), as well as the allowed limits according to TULSMA (2015), excepting for Caoní river on *E. coli* values. The obtained results are in agreement with other previous studies performed in Latin American countries, such as, Brazil (Carvalho & Stapelfeldt, 2004), Chile (Rivera, Encina, & Mejias, 2004) and Mexico (Sandoval Villasana et al., 2009). In fact, the minimum and maximum values for *E. coli* and total coliforms counting obtained in this study and other previous studies are showed on Table 8. Furthermore, the *E. coli* levels obtained in the rivers of this study (1.17-9.18x10^2 CFU/mL) are similar to the results reported in Brazil (4.20-2.40x10^2 CFU/mL), Chile (4.67x10^{-2}-7.90 CFU/mL) and are lower than the ones reported in Mexico (2.20x10^1-3.08x10^5 CFU/mL). Although Sandoval Villasan and colleagues (2009) did not analyze total coliforms in Mexico, Carvalho et al. (2004) and Rivera et al. (2004) showed lower results with total coliforms in Brazil and Chile, respectively, when compared to the present study. On the other hand, studies from United States (Staley et al., 2014) and Canada (Khan, Husain, & Lumb, 2003) reported lower levels of *E. coli* (in USA: 5.00x10^{-2}-3.00 CFU/mL) and total coliforms (in Canada: 3.70x10-7.40x10^2 CFU/mL), respectively, when compared the present study or even to other Latin American countries (see Table 8). In the same way, other studies from USA (Bower et al., 2005; Shehane et al., 2005) reported several amounts of water samples below the permitted value of *E. coli* by EPA (2012). For example, Bower and colleagues (2005) reported that 28 of 79 analyzed samples did not exceed the permitted limited; while Shehane and colleagues (2005) reported that none of their analyzed samples exceed the recommended levels. Studies performed in countries of Europe, such as Croatia, Italy and Poland, reported

similar levels of *E. coli* (Dragun, Kapetanovic, Raspor, & Teskeredžic, 2011; Ferronato et al., 2013; Lenart-Boroń, Wolanin, Jelonkiewicz, & Żelazny, 2017), more exactly, ranging from 0.03 to 4.10 x 10^2 CFU/mL. These studies showed similar contamination levels when compared to the results obtained in the present study (see Table 5). Likewise, other countries of Asia (India and Malaysia) and Africa (Nigeria, Ghana and Egypt) also showed similar levels of *E. coli* (see Table 8), when compared to the studies of Latin America countries, including this study. The reported levels of *E. coli* from Asia range from 4.33 until 7.94 x 10^2 CFU/mL (Al-Badaii et al., 2013; Gowrisankar et al., 2017), whereas the described levels of *E. coli* from Africa were between 3.36 and 6.40 x 10^2 CFU/mL (Karikari et al., 2006; Onyekuru et al., 2014; Rawway et al., 2016). A possible explanation for the slight difference of Latin American values with developed countries, such as USA, Canada and Italy, could be the lack of water treatment plants; while the unique climate and biodiversity of Ecuador could also explain superior microbial load in relation to other developing countries (Doherty et al., 2017), such as Ghana and Egypt. However, most countries of Asia and Africa also possess treatment plants, leading to less polluted water sources (Nikiema et al., 2013; Panswad et al., 1988). In Pichincha, most of the industrial and domestic effluents are directly discharged into the rivers, without any previous microbial or chemical treatment. Recently, a wastewater treatment plant opened in Pichincha, but it cannot supply the treatment required for all the rivers analyzed in this study (EPMAPS, 2013).

8.2 Prevalence of microbial genera, *Candida albicans* and *E. coli* pathotypes

As previously described in results, this study stated the presence of three of the total four *E. coli* pathotypes analyzed the rivers of Pichincha, more specifically EAEC, EPEC, EHEC and EIEC. The most prevalent pathogen was EIEC showing positive results in three of the eighteen rivers, more precisely, Machángara, Guayllabamba, and Monjas rivers. While, EHEC and

EAEC were only detected in one river, more exactly, Monjas and Machángara rivers, respectively. When compared to the present study, other countries, such as Australia, South Africa and Nigeria, reported the presence of the four *E. coli* pathotypes (Nontongana et al., 2014; Sidhu et al., 2013; Titilawo et al., 2015). In fact, Hamelin and colleagues (2007) reported the presence of EAEC and EPEC in Canada, while Ramírez Castillo and colleagues (2013) identified EAEC as the most prevalent *E. coli* pathotype in their recollection water set in opposite to our results. In the same way, a study performed in Japan reported the presence of EPEC and EAEC (Gomi et al., 2015), while a study performed in Germany reported the presence of EIEC and EPEC in low percentages (Stange et al., 2016). It is important to mention that the higher amount of *E. coli* pathotypes found in tropical or sub-tropical countries can be explained due to warmer water conditions that facilitates the survival rate of *E. coli* pathotypes (Barcina et al., 1986). So, climate variety could explain why developed countries and developing countries could be affected by different *E. coli* pathotypes. This situation could be dangerous to public health due to countries legislations that usually only control microbial and chemical levels, instead to also control the presence of pathogenic bacteria (Pandey et al., 2014). Also, most of the rivers received discharges from several and different sources, such as, agricultural farms, livestock or breeding farms and also wastewaters from industries. Therefore, it is difficult to determine the origin of the pathogens positive in the water analysis and control. It is important to mention that heavy rain or similar events of tropical countries may increase the number of pathogens by sediments from the rivers and recollected contaminants of nonpoint sources (such as fecal material from domestic and wild animals) in the main river, as previously described in other studies (Pandey et al., 2014; Sidhu et al., 2013). In Pichincha, several rivers are located near agricultural or livestock farms and also receive influxes from industries wastewaters and municipal sewage without any treatment (Gomez et al., 2014). Finally, these rivers are commonly used for recreational activities, agriculture,

livestock feeding or even domestic activities (such as bathing, washing clothes and even consuming as potable water) (Noorhosseini et al., 2017), leading to severe health public issues mainly diarrheal-associated diseases (Vasco et al., 2014).

Furthermore, the bacterial genera were also detected in this study, such as *Pseudomonas* and *Legionella*. These results were not surprising because both genera have been shown to be abundant or commensal on water resources (Dobrowsky et al., 2014; Stanwell-Smith et al., 2003). However, some species of both *Pseudomonas* and *Legionella* genera have been associated with diseases, more exactly, *P. aeruginosa* and *L. pneumophila* (Pandey et al., 2014; Stanwell-Smith et al., 2003), respectively. Nonetheless, other non-bacterial species have been reported in water sources (Bakhiet et al., 2016; Olorode et al., 2015; Pandey et al., 2014), such as, *Candida albicans* and parasites (*Giardia* and *Cryptosporidium* spp.). In this study, *Candida albicans* was detected in a low percentage (3 of the 18 analyzed rivers). Even though *Candida* sp. has been associated with freshwater, the obtained result was expected because this yeast is commonly found on mucocutaneous areas and alimentary tracts of mammals and birds (Cook & Schlitzer, 1981). In fact, Cook and Schlitzer (1981) revealed that the presence of *Candida albicans* in rivers comes from a recent source of contamination of human or animals' feces. Other species of *Candida*, such *as C. parapsilosis, C. krusei, C. glabrata* and *C. tropicalis*, have also been associated with fresh water (Medeiros et al., 2012) and to opportunistic infections (Luo & Mitchell, 2002). In addition, in the case of parasites, *Cryptosporidium* and *Giardia* spp. were detected in three and eight from eighteen rivers, respectively, evidencing a greater parasites contamination in rivers of Pichincha than from *Candida albicans*. In Germany, a study on Rhine river showed similar results, isolating a bigger percentage of *Giardia* than *Cryptosporidium* species (Gallas-Lindemann et al., 2016). Most studies lack parasite detection on their water analysis or show low levels of contamination (Dobrowsky et al., 2014; Staley et al., 2014). This lack of information or positive results could be happening

because of inhibitory compounds from the river that usually affect the nested PCR necessary to the parasites detection (Dobrowsky et al., 2014; Gallas-Lindemann et al., 2016). To avoid these type of troubleshooting, it is recommended to treat the samples with sodium or hypochlorite to reduce the possible effects of inhibition (Gallas-Lindemann et al., 2016). Another possible methodical troubleshooting, mainly in *Cryptosporidium* oocysts, could be the loss of parasite sample by adsorption of the recollection recipients or laboratory material and filtration steps (Huck et al., 2001). So further studies should be done to isolate pathogenic species from these rivers and fully characterize their virulence properties against public health.

Table 8. Summary of physical-chemical parameters and coliforms counting obtained in studies of rivers (including this study).

N°	Country	Study Group (n)	Counting		Physical-chemical parameters				Metal and mayors elements						References
			E. coli (CFU/mL)	Total coliforms (CFU/mL)	pH	DO (mg/L)	COD_T (mg/L)	TSS (mg/L)	Iron (mg/L)	Aluminium (mg/L)	Zinc (mg/L)	Calcium (mg/L)	Sodium (mg/L)	Magnesium (mg/L)	
1	Ecuador	18	1.17 – 9.18 x 10^2	3.95 - 5.15 x 10^3	7.15 – 9.55	5.36 – 10.32	2.0 – 692.0	3.33 – 520.00	0.210 – 13.880	0.030 - 18.050	0.000 – 3.720	3.70 - 170.26	4.59 – 58.19	2.37 – 32.21	This study
2	Brazil*	1	4.20 – 2.40 x 10^2	4.60 x 10 – 2.40 x 10^2	5.48 – 7.30	0.90 – 7.80	<10.0 – 9324.0	56.00 - 608.00	0.030 – 24.090	0.100 - 0.370	0.030 - 3.880	NA	NA	NA	Carvalho et al., 2004
3	Chile*	2	2.00 x 10^{-2} – 7.90	1.70 – 5.40 x 10	7.00 – 8.50	8.0 – 12.70	2.0 – 406.0	10.87 – 260.00	0.210 – 0.560	0.002 – 0.055	0.020 – 0.140	2.73 – 8.95	1.60 – 8.43	0.95 – 1.69	Rivera, Encina et al., 2004
4	Mexico*	1	2.20 x 10 – 3.08 x 10^5	NA	7.00 – 8.00	1.70 – 8.60	22.0 – 1841.0	8.00 – 343.00	0.510 – 0.530	< 5.000	< 0.100	NA	NA	NA	Sandoval Villasana et al., 2009
5	USA	1	5.00 x 10^{-2} – 3.00	NA	6.89 – 8.10	NA	NA	13.13 – 139.42	0.040 – 1.590	0.080 – 1.180	0.020 - 0.210	11.50 – 112.79	1.63 – 21.39	3.63 – 55.54	Staley et al., 2014
6	Canada	3	NA	3.70 x 10 – 7.40 x 10^2	3.20 – 9.00	9.20 – 14.70	NA	NA	0.009 – 4.200	0.000 – 21.000	0.000 – 1.000	NA	0.30 – 17.30	NA	Khan et al., 2003
7	Poland	5	1.58 – 1.18 x 10^2	3.80 – 2.98 x 10^2	7.40 – 7.70	NA	NA	223.00 – 518.00	0.080 – 4.400	NA	NA	NA	4.00 – 33.50	0.80 – 5.40	Lenart-Boroń et al., 2017
8	Italy	4	3.00 x 10^{-2} – 4.10 x 10^2	0 – 1.30 x 10^2	6.90 – 8.80	1.70 – 18.40	4.0 – 87.0	4.00 – 64632.00	0.013 – 0.530	0.003 – 0.809	0.001 – 4.410	NA	NA	NA	Ferronato et al., 2013
9	Croatia*	3	1.00 x 10^{-1} – 2.97 x 10^2	1.01 x 10 – 6.67 x 10^3	7.82 – 8.24	NA	NA	NA	0.016 – 0.520	0.013 – 0.072	NA	58.80 – 77.30	2.12 – 88.30	9.30 – 27.10	Dragun et al., 2011

Table 8. (*continued*).

N°	Country	Study Group (n)	Counting		Physical-chemical parameters				Metal and mayors elements						References
			E. coli (CFU/mL)	Total coliforms (CFU/mL)	pH	DO (mg/L)	COD_T (mg/L)	TSS (mg/L)	Iron (mg/L)	Aluminium (mg/L)	Zinc (mg/L)	Calcium (mg/L)	Sodium (mg/L)	Magnesium (mg/L)	
10	India	2	3.16×10^2 – 7.94×10^2	6.30×10^2 – 6.31×10^6	7.10 – 8.00	NA	NA	172.00 – 1820.00	0.380 - 2.050	0.008 – 2.710	0.490 – 1.020	18.00 – 137.00	18.00 – 406.00	8.00 – 55.00	Gowrisankar et al., 2017
11	Bangladesh	1	NA	NA	7.24 – 7.61	1.22 – 3.66	NA	239.00 – 1349.00	1.400 – 3.290	NA	0.080 – 0.190	NA	NA	NA	Islam et al., 2013
12	Malaysia	1	4.33 – 2.73×10^3	NA	5.23 – 8.41	4.13 – 7.44	8.6 – 63.0	17.66 – 80.00	NA	NA	NA	NA	NA	NA	Al-Badaii et al., 2013
13	Nigeria	1	2.50 x 10 – 6.40×10^2	NR - 1.60×10^2	6.84 – 7.20	NA	NA	8.63 – 11.36	2.970 – 4.800	NA	NA	8.44 – 11.48	2.77 – 4.10	6.06 – 8.66	Onyekuru et al., 2014
14	Ghana	1	3.36 – 7.39	1.13 x 10 – 1.88 x 10	7.20 – 7.48	6.60 – 7. 16	NA	142.00 – 225.00	0.610 – 1.190	NA	0.014 – 0.100	NA	NA	NA	Karikari et al., 2006
15	Egypt*	1	3.79 – 7.03	7.13 – 1.42 x 10	7.60 – 8.70	NA	4.9 – 21.2	NA	NA	NA	NA	68.00 - 93.00	NA	32.00 - 56.00	Rawway et al., 2016

NA: Not analyzed in the study.
NR: Not reported in the study.
* All these studies used NPM/mL for counting. According to Ecuadorean legislation the limit for *E. coli* and total coliforms for recreational water use is of 200 MPN/100mL and 1000 MPN/100mL respectively. Annex I, Book VI of the TULSMA reformed on the Acuerdo Ministerial 97 on July 30, 2015 (see Table 9).

8.3 Physical-chemical analysis

As previously referred in Table 6, most of the physical-chemical parameters analyzed in this study were below the maximum allowed level by the Ecuadorean legislation (TULSMA, 2015). Nevertheless, certain parameters were outside of the authorized range by legislation, more precisely, pH (9.11 to 9.55 > 9), DO (5.36 mg/L < acceptable minimum of 6 mg/L), total COD (318 mg/L to 692 mg/L > 250 mg/L) and TSS (153.50 mg/L to 520.00 mg/L > 120 mg/L). When compared to other studies, some countries of Latin America, such as Chile (Rivera et al., 2004) and Mexico (Sandoval Villasana et al., 2009), showed pH values inside the range of this study (see Table 8), more exactly, between pH 7.00 and 8.50. While, in Brazil, Carvalho and Stapelfeldt (2004) reported lower pH levels ranging from 5.48 to 7.30. However, studies from North America (USA and Canada) showed pH values in more superior range varying from 3.20 until 9.00 (Khan et al., 2003; Staley et al., 2014). Nevertheless, several studies from European countries, such as Croatia, Italy and Poland, registered pH levels inside the specified limits (see Table 8) (Dragun et al., 2011; Ferronato et al., 2013; Lenart-Boroń et al., 2017). However, in Italy, Ferronato and colleagues reported the broadest of pH range in these countries, more exactly, a pH range between 6.9 and 8.8. Similarly, studies realized in different countries of Asia (India, Bangladesh and Malaysia) and Africa (Nigeria, Ghana and Egypt) showed pH values inside the legal limits (see Table 8) excepting, in Malaysia, where Al-Badaji et al. (2013) reported pH range between 5.23 and 8.41. Therefore, the present study showed the highest pH value (9.55) reported in the water analysis from a river, when compared to the several studies worldwide. Usually, higher pH values are associated with carbonate rocks of the geographical region and also with wastewaters from residual municipal or industrial discharge effluents (Meybeck & Helmer, 1989; Sandoval Villasana et al., 2009). Next, the dissolved oxygen (DO) values obtained in this study were within the permitted range of the

legislation (more than 6 mg/L) excepting, in Monjas river, where the DO value was of 5.36 mg/L. When comparing these results with other countries, it is possible to observe that certain countries of Latin America, such as Brazil (Carvalho et al., 2004) and Mexico (Sandoval Villasana et al., 2009), showed lowest values of DO, more exactly, 0.90 and 1.70 mg/L, respectively. However, in Chile, Rivera and colleagues (2004) reported DO values between 8.0 and 12.7 mg/L (> 6 mg/L). Similarly, in a study from Canada, the DO value ranged from 9.2 to 14.7 mg/L (Khan et al., 2003). However, several countries worldwide, such as Italy (Ferronato et al., 2013), Blangladesh (Islam et al., 2013) and Malysia (Al-Badaii et al., 2013), registered extremely low DO values (see Table 8), more precisely, 1.7, 1.22 and 4.13 mg/L, respectively. These low DO values could be caused by the discharge of untreated wastewaters increasing the organic matter and thus decreasing the dissolved oxygen in these waters (Minnesota Pollution Control Agency, 2009). In Ecuador, when studying water quality parameters in Machángara River on a longitudinal analysis (DO, biodegradability index (BOD/COD) and total nitrogen), Vizcaíno and colleagues (2016) observed that highest temperatures had a negative effect on DO by decreasing its value. So, longitudinal studies should be realized to clarify variables associated with physical-chemical parameters inconsistency.

In this study, two rivers (Monjas and Machángara) showed high levels of total chemical oxygen demand (COD_{Total}), more precisely, 318.00 and 692.00 mg/L, respectively; while the remaining analyzed rivers were below the legal maximum level (250 mg/L) being 2.00 mg/L the lowest COD_{Total} level detected in two rivers (Pachijal and Pedregales). As shown in Table 8, Rivera and colleagues (2004) detected similar COD_{Total} levels within a range between 2.00 and 406.00 mg/L. However, in Mexico and Brazil, studies reported greater differences in COD_{Total} range reaching contamination levels of 1841 and 9324 mg/L (Carvalho et al., 2004; Sandoval Villasana et al., 2009), respectively. In relation to other countries worldwide, such as Italy

(Ferronato et al., 2013), Malaysia (Al-Badaii et al., 2013) and Egypt (Rawway et al., 2016), the obtained values for COD_{Total} were all inside the permitted limit (see Table 8). In addition, some authors postulated that these highest COD_{Total} values can be related to wastewaters and agricultural activities which increase the number of organic matter in the river (Zhang et al., 2015).

Finally, the values of total suspended solids (TSS) were also measured in our study showing five of eighteen rivers (27.78%) with TSS values above the maximum legal level (130 mg/L), more exactly, Monjas (153.50 mg/L), Pisque (236.67 mg/L), Chiche (300.00 mg/L), Alambi (366.67 mg/L) and Machángara (520.00 mg/L). Although some countries worldwide, such as Malaysia (Al-Badaii et al., 2013) and Nigeria (Onyekuru et al., 2014), reported TSS levels within the legal range, most of the countries worldwide registered TSS in high levels and overpassed the maximum permitted limit (see Table 8). In relation to the present study (3.33–520.00 mg/L), similar high levels of TSS were reported in Brazil (Carvalho et al., 2004) and Poland (Lenart-Boroń et al., 2017), more exactly, 56.00– 608.00 mg/L and 223.00– 518.00 mg/L, respectively. However, studies of other American countries, such as USA (Staley et al., 2014), Chile (Rivera, Encina et al., 2004) and Mexico (Sandoval Villasana et al., 2009), demonstrated lower overpassed levels of TSS, more precisely, 13.13–139.42 mg/L, 10.87–260.00 mg/L and 8.00–343.00 mg/L, respectively. In addition, Karikari et al. (2006) also showed lower overpassed levels of TSS in Ghana (Africa), specifically, 13.13–139.42 mg/L. On the other hand, other countries worldwide, such as Bangladesh (Islam et al., 2013), India (Gowrisankar et al., 2017) and Italy (Ferronato et al., 2013), evidenced greater levels of contamination by TSS, more exactly, 239.0–1349.0 mg/L, 172.00–1820.0 mg/L and 4.0–64632.0 mg/L, respectively. It is important to mention that high values of TSS could be associated with several climates and geographical conditions (Al-Badaii et al., 2013), such as

recent rainfalls, organic or inorganic particles suspended in the river and even higher rates of soil erosion produced by human activities.

8.4 Analysis of metal and major elements parameters

The concentration of metal and major elements was also measured in the samples of the eighteen rivers, as previously shown in Table 7. From our metal analysis, certain metals were overpassed the legal values by Ecuadorian legislation (TUSLMA, 2015) in some of the analyzed rivers, more precisely, iron (6.76 mg/L – 13.88 mg/L > 5 mg/L), magnesium (0.21mg/L > 0.2 mg/L), aluminium (13.13 – 18.25 mg/L > 5.0 mg/L), lead (0.06-0.08 mg/L > 0.05 mg/L) and zinc (3.72 mg/L > 2.0 mg/L). In relation to iron overpassed levels, only Carvalho and colleagues (2004) obtained similar iron values in Brazil, when compared to the present study. The remaining countries worldwide reported iron values within the permitted limits (see Table 8). A possible explanation for the high values of iron in the present study could be the discharge of untreated effluents from industries located nearby Machángara, Pisque and Chiche rivers (Onyekuru et al., 2014). On the other hand, only Monjas river presented higher concentration of manganese (0.21 mg/L; see Table 7) than the established in the Ecuadorian legislation. When comparing this result with other countries, Gowrisankar and colleagues (2017) reported high range of manganese levels (0.75 – 5.78 mg/L) in India. This study suggests that high concentrations of metals such as aluminium, iron and manganese could be obtained from domestic sewage contaminants for instance metals scraps, batteries, paints or oils from service stations (Gowrisankar et al., 2017). Moreover, this study also showed higher concentrations of aluminium (0.03–18.05 mg/L) in comparison with other American countries, such as Mexico (Sandoval Villasana et al., 2009), Brazil (Carvalho et al., 2004) Chile(Rivera, Encina et al., 2004) and USA (Staley et al., 2014), European countries (Italy, Croatia) and India (Ferronato et al., 2013; Dragun et al., 2011; Gowrisankar et al., 2017); which none of them

overpassed the maximum legal value (see Table 8). However, in Canada, Khan and colleagues (2003) showed similar contamination levels of aluminium (0.00 mg/L – 21.00 mg/L). Moreover, three of eighteen rivers showed high levels of lead (0.06-0.08 mg/L), more exactly, Machángara, Alambi and Pedregales rivers. In 1985, a study demonstrated that rivers located near volcanic zones usually contain higher concentrations of metals, such as aluminium, lead and iron (Hem, 1985). Due to most of the rivers located in Pichincha have its origin on the highlands and are located nearby volcanoes (MECN, 2009), this could be a possible explanation for the overpassed values of both metal. Nonetheless, the high concentrations of aluminium could also be associated with discharges of industrial wastewaters, as already postulated in a previous study (Guibaud & Gauthier, 2003). Furthermore, only one of eighteen rivers showed an overpassed zinc measure (3.72 mg/L), more precisely, Pedregales river. When compared to this river, other two studies also obtained similar values of zinc in Brazil (3.88 mg/L; Carvalho et al., 2004) and Italy (4.41 mg/L; Ferronato et al., 2013). While several studies in other Latin American countries (Mexico and Chile), North American countries (USA and Canada), Asian countries (India and Bangladesh) and an African country (Ghana) reported zinc values inside the legal range (see Table 8). However, these overpassed zinc concentrations could be associated with mining industries (Reyes et al., 2016), where Ecuador, Brazil and Italy already had been also associated in previous studies (Barbafieri et al., 2011; Belli et al., 1989; Gurmendi et al., 2003). As shown in Table 8, three major elements were also analyzed in this study revealing high range of concentrations of calcium (3.70 – 170.26 mg/L), sodium (4.59 – 58.19 mg/L) and magnesium (2.37 – 32.21 mg/L). Although no maximum legal value of these major elements is described in Ecuadorian legislation, the presented study showed the highest values of calcium when compared to other studies worldwide in Table 8. In relation to sodium, it is possible to observe that only Croatia (Dragun et al., 2011) and India (Gowrisankar et al., 2017) evidenced level ranges superior to the present study, more exactly, 2.12 – 88.30

and 18.00 – 406.00 mg/L, respectively. However, some studies of India (Gowrisankar et al., 2017), USA (Staley et al., 2014) and Egypt (Rawway et al., 2016) reported concentrations of magnesium almost twice from the highest value from the present study (32.21 mg/L), more precisely, 55.00, 55.54 and 56.00 mg/L, respectively. Similarly, these high concentrations of major elements had been described in rivers located near volcanos, accordingly to Meybeck and Helmer (1989). Also, it is important to note that Pedregales river showed the higher concentration of calcium and it is also located in an industrial area of dairy products, which are often enriched with calcium (Cisneros & Machuca, 2014). Consequently, the wastewaters of this industrial activity could pollute the river with high levels of calcium through its untreated effluent discharges into Pedregales river.

9 CONCLUSIONS

In summary, the present study revealed a diverse and severe contamination in most of the eighteen rivers located in Pichincha. The level of contamination was characterized by different types of parameters, more exactly, microbial load and genera, physical-chemical parameters and metal levels. These eighteen rivers are usually used for potable water, recreational, agricultural and industrial activities. The initial analysis of the microbial parameters in eighteen rivers from Pichincha showed high levels of fecal contamination (*E. coli* and total coliforms), except for Caoní river only in *E. coli* levels, also it revealed the presence of several microbial species (*Pseudomonas*, *Legionella* and *Shigella* spp., *Candida albicans*, *Cryptosporidium* and *Giardia* spp.) and *E. coli* pathotypes (EAEC, EHEC and EIEC). Monjas and Machángara rivers showed the highest number of *E. coli* pathotypes. In both cases, two pathotypes were identified by PCR, more precisely, in Monjas was discovered EHEC and EIEC while in Machángara was detected EAEC and EIEC. The physical-chemical results showed high levels of COD_{Total}, TSS and certain high metal concentrations, more exactly, lead, aluminium and iron. In addition, both microbial and physical-chemical analysis revealed that the most contaminated rivers were Monjas, Machángara and Chiche rivers while Caoní and Pilatón rivers demonstrated a lower level of fecal contamination as well as most of the physical-chemical parameters were in low levels.

10 RECOMMENDATIONS

Further studies should be done through longitudinal analysis of these rivers (Monjas, Machángara and Chiche rivers), clarifying the effect that climate conditions could have in the levels of each microbial and physical-chemical parameter. Finally, an additional study should analyze the pollution impact in the public health of the population around these rivers.

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