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Evaluation of the microbial and chemical load in rivers from several provinces of Ecuador

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RESUMEN

Uno de los más grandes problemas de salud a nivel mundial es la contaminación de fuentes naturales de agua con compuestos tóxicos y bacterias patógenas humanas, específicamente, algunos patotipos de *Escherichia coli, Campylobacter, Legionella, Pseudomonas, Shigella* y *Salmonella* spp. El objetivo de este estudio es analizar la calidad de los recursos hídricos naturales en áreas urbanas en ecuador en base a parámetros microbianos y físico-químicos, para comparar las regiones costera, andina y amazónica y así evaluar las posibles correlaciones entre estos parámetros. La cuantificación de *Escherichia coli* y coliformes se realizó a través de medios de cultivo y reacción en cadena de la polimerasa (del inglés *Polymerase Chain Reaction*, PCR) para cada género antes mencionado y patotipos de *E. coli*, específicamente: *E. coli* enteroagregativa (EAEC), *E. coli* enterohemorrágica (EHEC), *E. coli* enteropatogénica (EPEC) y *E. coli* enteroinvasiva (EIEC) en muestras triplicadas de diferentes ríos. Mientras tanto, los parámetros ambientales en aguas superficiales como pH, conductividad y oxígeno disuelto se determinaron *in situ* en cada punto de muestreo, mientras que la demanda química de oxígeno (DQO), sólidos totales (TS), sólidos suspendidos totales (TSS), amonio, nitrato, sulfato, análisis de fosfato y metales fueron medidos en el laboratorio de ingeniería ambiental. Este análisis inicial mostró que la mayoría de ríos evaluados no muestran niveles microbianos, fisicoquímicos y metálicos aceptables para el consumo de agua o incluso agua apropiada para actividades recreativas y agrícolas. Además, todos los ríos mostraron niveles de *E. coli* y coliformes totales por encima de la legislación, lo que evidencia la presencia de patotipos en seis de los doce ríos analizados en Ecuador. Además, tres de los cuatro patotipos de *E. coli* analizados (EAEC, EPEC y EIEC) fueron detectados, el río Machángara mostró la presencia de dos patotipos diferentes (EAEC y EIEC). Cuando se comparó la carga bacteriana del conjunto de estudio, los ríos Zamora, Esmeraldas y Machángara fueron los más contaminados. Además, en el análisis fisicoquímico y de metales, el río Guayas presentó el mayor número y niveles de parámetros de todos los ríos seleccionados, demostrando altos niveles en cinco de los catorce parámetros físico-químicos analizados (conductividad, COD_{total}, TS, TSS, Cl^-) y dos metales en concentraciones más altas (Aluminio y Hierro). Este estudio ofrece un análisis preliminar sobre la calidad del agua de los ríos en ecuador y alerta sobre la necesidad de medidas inminentes para reducir la contaminación fecal y metálica de los recursos hídricos nacional. Además, este estudio indicó la necesidad de una observación cercana de la salud pública de la población en el entorno del río y su aplicación en diferentes actividades. Son necesarios más estudios para evaluar un escenario futuro de reversión de estas altas tasas de contaminación microbiana y química con las medidas legales actuales del gobierno ecuatoriano.

Palabras clave: Recursos Hídricos, *Escherichia coli*, Coliformes Totales, Patotipos de *Escherichia coli*, Reacción en Cadena de la Polimerasa (PCR), Parámetros Físico-Químicos, Elementos Mayores, Metales Traza.

ABSTRACT

One of the major worldwide health problems is the contamination of natural water sources with toxic compounds and human pathogenic bacteria, specifically, some pathotypes of *Escherichia coli, Campylobacter, Legionella, Pseudomonas, Shigella* and *Salmonella* spp. This study aims to analyze the quality of natural water resources in urban areas in Ecuador based on microbial and physical-chemical parameters, in order to compare the Costal, Andean and Amazon regions and evaluate possible correlations between these parameters. *Escherichia coli* and coliforms quantification was conducted through growth media and Polymerase Chain Reaction (PCR) for each aforementioned genera and *E. coli* pathotypes, more exactly, enteroaggregative *E. coli* (EAEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), and enteroinvasive *E. coli* (EIEC) in triplicate samples from different rivers. Meanwhile, environmental parameters in surface waters such as pH, conductivity and dissolved oxygen were determined in situ in each sampling point, while chemical oxygen demand (COD), total solids (TS), total suspended solids (TSS), ammonium, nitrate, sulfate, phosphate and metal analysis were measured at environmental engineering laboratory. Our initial analysis showed that several rivers of Ecuador do not evidence acceptable microbial, physico-chemical and metal levels of drinking water or even water appropriate for recreational and agriculture activities. In addition, all rivers showed levels of *E. coli* and total coliforms above the legislation, evidencing the presence of pathotypes in six of the twelve analyzed rivers in Ecuador. Also, three of the four analyzed *E. coli* pathotypes (EAEC, EPEC, EIEC) were detected in national rivers, in which Machángara river showed two different pathotypes (EAEC and EIEC). When compared the bacterial load from study set, Zamora, Esmeraldas and Machángara rivers were the most polluted in this study. Furthermore, in the physico-chemical and metal analysis, Guayas river showed the most elevated number and levels of parameters from all selected rivers, demonstrating high levels of five from fourteen physico-chemical parameters analyzed (Conductivity, COD_{TOTAL}, TS, TSS, Cl^-) and two metals in higher concentrations (Aluminum and Iron). This preliminary analysis, offers a despicable idea on the water quality of the rivers in Ecuador and alerting for imminent measures to reduce fecal and metal contamination of our hydric resources. Also, our study indicated the need to a close observation of the population public health in the river surroundings and its application in different activities. Further studies are essential to evaluate a future scenario of reversing these high rates of microbial and chemical contaminations with the present legal measures of Ecuadorian Government.

Keywords: Water Resources, *Escherichia coli*, Total Coliforms, *Escherichia coli* Pathotype*s,* Polymerase Chain Reaction (PCR), Physico-Chemical Parameters, Major Elements, Trace Metals.

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1 INTRODUCTION

1.1 Global Context

The generation and discharge of effluents are of major concern worldwide, especially in developing countries where the majority of untreated domestic wastewaters are directly discharged into receiving bodies of water, resulting in severe impacts to the receiving ecosystems and posing a risk to public health (Dobrowsky, van Deventer, et al., 2014; Kora, Rastogi, Kumar, & Jagatap, 2017; Levy, Nelson, Hubbard, & Eisenberg, 2012; Tchounwou, Kishinhi, Tchounwou, & Farah, 2013).

Increased pollution in rivers leads to high health costs and low yields of agricultural and industrial production (Ferronato et al., 2013; Karikari & Ansa-Asare, 2006; Staley et al., 2014). High costs are usually due to increased bacterial and chemical contamination, leading to chronic diseases and persistence of microorganisms with microbial resistance (Ramírez Castillo et al., 2013). This contamination is more evident in greater population density areas, where both domestic and industrial wastes are discharged directly into water bodies without previous treatment (Almeida et al., 2014; Kora et al., 2017) . All these circumstances leads to more serious Public Health consequences(Palamuleni & Akoth, 2015).

1.2 Pollution of the Natural Water Resources

The continuous discharge of untreated effluents favors the microbial proliferation (either commensal, opportunistic or even pathogen microorganisms) and chemical contamination (Dobrowsky, De Kwaadsteniet, Cloete, & Khan, 2014)

Consequently the water of this type of natural resources is usually used for in drinking or agriculture and livestock farming, leading therefore to serious potential public health risk (Aracic et al., 2015; Gorchev & Ozolins, 2011; Mason, Canter, Gillies, Paisie, & Roberts, 2016). According to the United Nations Water Statistics, in developing countries, 90% of the domestic streams are discharged directly into rivers, lakes and coastal zones without treatment; and Ecuador is not an exception (United Nations Statistic Division, 2011).

1.3 National Context

Quito is the capital city of Ecuador with a population of 2.239.191 people based on the last census conducted in 2010 (INEC, 2013). Surprisingly, Quito does not have a wastewater treatment plant (WWTP) and, currently, 97% of domestic effluents are being discharged directly into Machángara River and Monjas River without prior treatment (EPMAPS, 2017). There are few studies presented in the literature regarding the quality of the rivers in Ecuador. Voloshenko-Rossin et al. (2015) investigated about some characteristics associated to water quality as well as some physical-chemical parameters in the San Pedro, Guayllabamba and Esmeraldas rivers. They determined that four wastewater streams from Quito were found to pollute the San Pedro River (Voloshenko-Rossin et al., 2015)

1.4 General Water Quality Analysis in Natural Resources

Several studies have analyzed the water resources through the general indicators of water quality as *Escherichia coli* and total coliforms counting (Liang et al., 2016). In addition, others potentially pathogenic microorganisms to human health

and even industrial production may also be evaluated such as *Pseudomonas, Shigella, Salmonella*, *Legionella* and *Campylobacter* spp. (Dobrowsky, De Kwaadsteniet, et al., 2014; Gliska-Lewczuk et al., 2016). Also, the water characteristic could be evaluated in terms of the physical-chemical contaminants present in surface water such as metals (Pérez Naranjo et al., 2015; Reyes, Vergara, Torres, Díaz, & González, 2016; Smith, Cooper, Kosiara, & Lamberti, 2016). The presence of metals in the environment could be attributed to natural sources such as leaching from rocks, erosion and volcanic activities and to anthropogenic sources such as discharges of domestic and industrial effluents, agricultural runoff, atmospheric deposition, among others (Pérez Naranjo et al., 2015; Reyes et al., 2016).

Although *Escherichia coli* is a commensal bacteria in water samples, the microbial load analysis should include the determination of certain *E. coli* pathotypes, more exactly, enteroaggregative *E. coli* (EAEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), and enteroinvasive *E. coli* (EIEC) (Dobrowsky, De Kwaadsteniet, et al., 2014). Few studies have been reported in Ecuador on contamination of water sources with potentially pathogenic microorganisms for human health (Gerhard, Choi, Houck, & Stewart, 2017; Levy et al., 2012). Currently, the microbial load evaluation in water samples used classic and molecular methodologies. *E. coli* and total coliforms counting are usually applied as classic techniques (Ahmed, Goonetilleke, & Gardner, 2010). Meanwhile, molecular techniques, such as polymerase chain reaction (PCR), allow a rapid detection of microorganisms in water samples (Law JW, Mutalib, Chan, Lee, 2015).

1.5 Natural water resources in Ecuador

Nowadays, little is known about the water quality in middle and low-income country, such as Ecuador. Due to the fauna and flora biodiversity of Ecuador, it is imperative to evaluate the quality in natural water resources (Gerhard et al., 2017; Levy et al., 2012) Additionally, it is expected to estimate the current situation of contamination of water resources in Ecuador with pathogens that could affect the prevalence of bacterial diseases that affect human health (Chandran & Mazumder, 2013; Karikari & Ansa-Asare, 2006; Kolawole, Ajayi, Olayemi, & Okoh, 2011).

2 JUSTIFICATION

Nowadays, a low percentage of people are really aware of the importance of caring for natural water sources and the increasing thee need of bigger water supply for the general population and their application in several economic sectors. In fact, the development of industry, livestock activities and the population growth are factors that increase the pollution rates in the main water resources of Ecuador. All direct discharge of effluents from anthropogenic activities to surface water sources without prior adequate treatment is now a problem of global interest, especially since this can be a potential source of public health risks for the population. In Ecuador, few studies have been reported on contamination of water sources with potentially pathogenic microorganisms for human health and chemical contaminants. For this reason, it is important to carry out a preliminary study to evaluate the current status of the country's water sources by microbiological, physico-chemical and metal standards. This evaluation could allow to understand what possible contamination are occurring in each Ecuadorian region (Costal, Andean and Amazon region) and finally establish some correlations between microbial load and chemical or metal parameters.

3 STUDY AREA

For collection, twelve sampling points corresponding to twelve of the main rivers of Ecuador distributed throughout the national territory were selected (Figure 1).

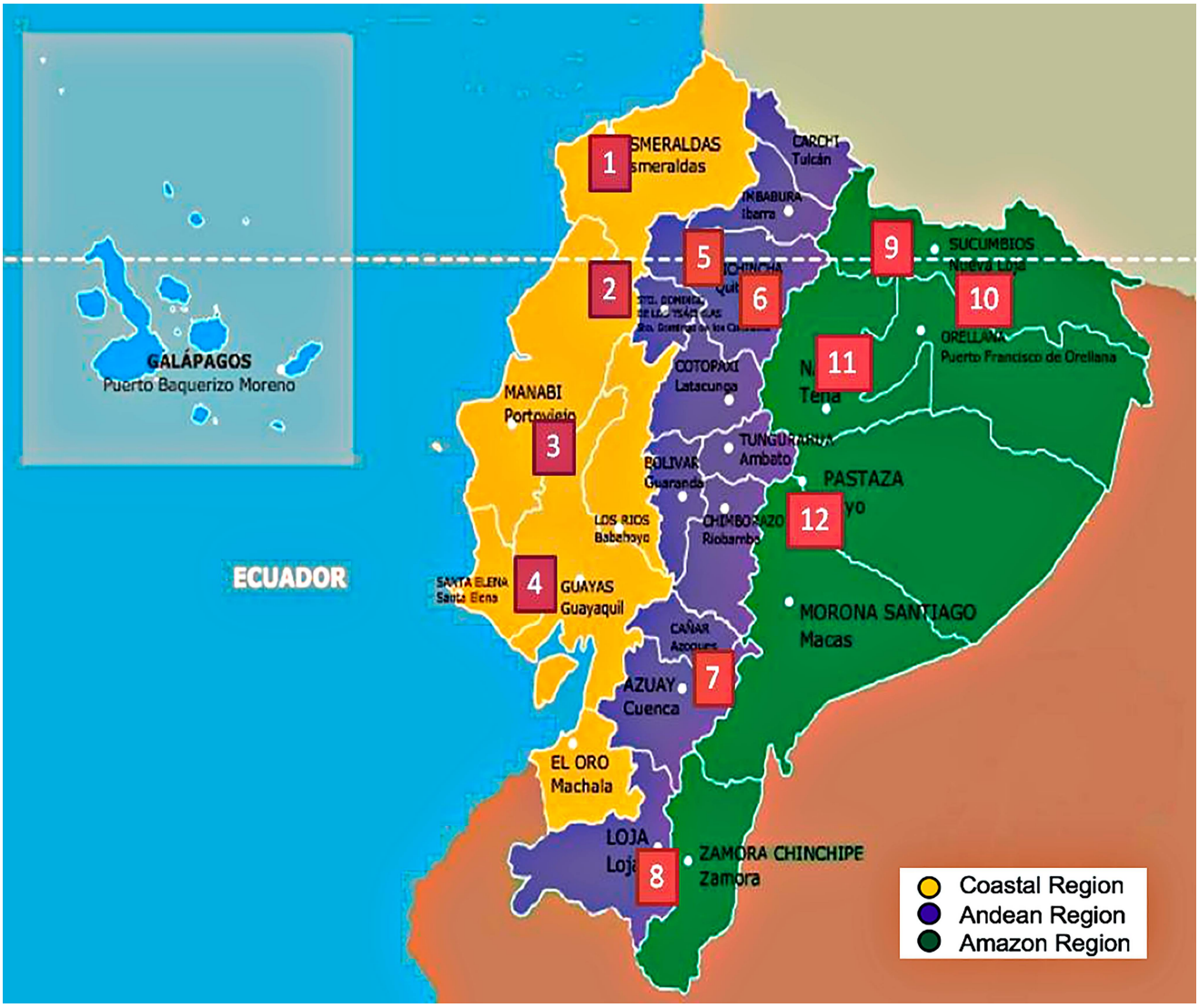

Figure 1. Map of the Republic of Ecuador.

The criteria for sampling were based on the duplicate and/or triplicate collection of water samples, specifically at urban points of high population density in the middle of large cities. Continental Ecuador is divided into three regions: Coast, Sierra and Amazonia, four of the main rivers were selected from each region for further analysis as detailed below (Table 1).

Table 1. Name of rivers and location on the map of Ecuador.

Location	River	Region
1	Machángara	Andean
2	Guayllabamba	Andean
3	Tomebamba	Andean
4	Zamora	Andean
5	Esmeraldas	Coastal
6	Toachi	Coastal
7	Chone	Coastal
8	Guayas	Coastal
9	Aguarico	Amazonian
10	Coca	Amazonian
11	Napo	Amazonian
12	Pastaza	Amazonian

4 OBJETIVES

4.1 General objective

Analyze the microbiological and chemical quality of the Natural Water Resources of Ecuador.

4.2 Specific objectives

- Quantify the microbial load of *Escherichia coli* and total coliforms through classical methods of microbiology.
- Analyze the microbiological quality in water resources of Ecuador through Polymerase Chain Reaction (PCR) detection of the genera *Pseudomonas, Shigella, Salmonella, Legionella, Campylobacter*.
- Detect the presence or absence of *E. coli* pathotypes, more exactly, enteroaggregative *E. coli* (EAEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), and enteroinvasive *E. coli* (EIEC).
- Estimate the current state of pollution of the main rivers of Ecuador through the analysis of physical-chemical parameters, major elements and trace metals in order to establish correlations between them.

5 MATERIALS, REAGENTS AND EQUIPMENT

5.1 Sample Collection

- Glass bottles
- Coolers
- Refrigerant Gel Pack
- Thermometer
- Global Positioning System (GPS)
- Autoclave

5.2 Analysis of Physical and Chemical Parameters *in situ*

- Teflon bottles
- Hypochlorhydric acid
- High density polyethylene bottles (Nalgene)
- Vacuum filtration (Milipore)
- 0.45 µm cellulose filter (Milipore)

5.3 Analysis of Physical-Chemical Parameters in Laboratory

- Nitric acid
- Multiparameter (Thermo Scientific Model A329)
- AGUAFast (Thermo Scientific (Model AQ4500)
- iCAP inductively coupled plasma (Thermo Scientific Model 7400)

5.4 Filtration of River Water

- Vacuum pump (Chemical Duty Pump, Milipore Inc.)
- Nitrocellulose membrane 0.45µm (Milipore)
- Vortex
- Centrifuge
- Falcon tubes 50 mL
- Distilled water
- Phosphate Buffered Saline (PBS)
- Micropipettes
- Tips for micropipettes
- Eppendorf tubes 1500 µl

5.5 Growth Media for Quantification and Isolation of Bacteria

- MacConkey Agar (Difco)
- Salmonella-Shigella agar (Difco)
- Legionella CYE Agar Base (Difco)
- Campylobacter agar (Difco)
- Chromocult Agar medium (Merck)
- Incubator
- Sterile swabs
- Handles
- Brain Heart Infusion BHI (Difco) + glicerol 15%
- Cryopreservation tubes
- Ultra-freezer -80ºC

5.6 DNA Extraction

- Power Soil extraction Kit (MO BIO Laboratories, Inc.)
- Nanodrop (Thermo Scientific)

5.7 Molecular Identification of Bacterial Genera

- Green GoTaq Flexi buffer (Promega)
- $MgCl_2$ (Promega)
- dNTP Mix (Promega)
- GoTaq Flexi DNA polymerase (Promega)
- PCR primers For *Pseudomonas* spp., *Legionella* spp., *Campylobacter* spp. *Shigella* spp. and *Salmonella* spp.
- Positive controls
- Thermocycler (Bio- Rad)

5.8 Molecular Identification of *E. coli* Pathotypes

- Green GoTaq Flexi buffer (Promega)
- $MgCl_2$ (Promega)
- dNTP Mix (Promega)
- GoTaq Flexi DNA polymerase (Promega)
- PCR primers For *E. coli* pathotypes (EAEC, EHEC, EPEC, EIEC)
- Positive controls (well-known bacterial strains from Microbiology Institute collection)
- Thermocycler (Bio- Rad)

5.9 PCR Product Analysis

- Electrophoresis equipment
- Agarose 2%
- TBE
- Ethidium bromide 0.1%

5.10 Statistical Analysis

- Software SPSS version 23.0 package

6 METHODS

6.1 Sample Collection

Water samples were recolected from rivers located along several provinces of Ecuador (see Figure 1), twelve rivers were selected due to their geographic regions of the country: Coast, Sierra and Amazon. Samples were taken in previously sterilized glass containers by autoclaving at 121 °C for a period of 15 minutes. A total volume of 800 mL was collected from each river.

6.2 Analysis of Physical-Chemical Parameters *in situ*

Surface water samples were collected in acid clean 1 L teflon bottles washed with 10% hydroclorhidric acid, and later washed with distilled water. The dissolved and suspended phases were separated after collection with the use of a vacuum pump and a nitrocellulose membrane of 0.45 μm. For metal analysis, the filtrate was transferred to acid cleaned high density polyethylene Nalgene bottles and preserved with high purity concentrated nitric acid (LobaChemie, Mumbai, India) to obtain a final concentration of 2% w/w.

Physical-chemical parameters such as: conductivity, pH, temperature, dissolved oxygen and turbidity were measured in situ in surface water samples in all sampling sites. Conductivity, pH, dissolved oxygen and temperature were measured using a multiparameter Thermo Scientific Model A329 (Thermo Fisher Scientific, Waltham, MA, USA). Turbidity was measured using a Thermo Scientific Model AQUAFast AQ4500 (Thermo Fisher Scientific, Waltham, MA, USA).

6.3 Analysis of Physical-Chemical Parameters in Laboratory

For the physical-chemical analysis of the collected samples, protocols already established for wastewater analysis were followed (APHA, 1998). For the analysis of chemical oxygen demand (COD), a colorimetric method was used using the Spectronic 20D + spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Total solids (TS) and total suspended solids (TSS) were measured by gravimetric methods.

Metal analysis on filtered and acidified water samples was conducted with a ThermoScientific iCAP 7400 inductively coupled plasma – optimal emission spectrometry ICP-OES at the Environmental Engineering laboratory (LIA – USFQ) at Universidad San Francisco de Quito. The instrument operated according to the parameters shown in Table 2. Standard solutions were prepared in dilute nitric acid from commercial standards (Sigma Aldrich, Trace-CERT multielement standard solution 6, Missouri, USA). The detection and quantification limits were calculated by analyzing blank samples with at least 8 replicates, and multiplying the standard deviation by 3 to obtain the detection limit and by 10 to obtain the quantification limit, respectively.

6.4 Filtration of River Water

Under aseptic conditions, the samples were filtered through a nitrocellulose membrane 0.45µm (Milipore) into a vacuum pump (Chemical Duty Pump, Milipore Inc.). Then, the remaining protocol was adapted from the previous study realized by Dobrowsky and colleagues (2014) with slight modifications. Briefly, the membrane was removed and placed in a sterile falcon tube with 20 ml of distilled water. The tube was vortexed over a period of 15 minutes to suspend the soil particles and

microorganisms in the water. The membrane was removed and then tubes were centrifuged at 5.000 rpm during 15 minutes to precipitate the sediments. The obtained pellet was suspended in 500 µl of distilled water and previously autoclaved. Subsequently this sample was then divided for both bacterial DNA extraction with the use of Power Soil extraction Kit (MO BIO Laboratories, Inc.) as well as for bacterial growth cultures.

6.5 Cultivation, Quantification and Isolation of Dominant Bacteria

Bacterial growth were realized by different media cultures to isolate or counting the most diverse microorganisms in the samples. More precisely, a portion were incubated on MacConkey Agar (Difco) at 37 °C for 18 to 24 h for the recovery of the genus *Escherichia*; Salmonella-Shigella agar (Difco) for the cultivation of *Salmonella* and *Shigella* genera at same conditions; a culture in Legionella CYE Agar Base (Difco) at 35 °C for 48 h for obtaining *Legionella* spp.; and Campylobacter agar (Difco) for the isolation of *Campylobacter* spp at 37 °C for 18 to 24 h. Finally, for the quantification *Escherichia coli* and Total Coliforms the Chromocult Agar medium (Merck; Biolab, Wadeville, Gauteng) was used for results validation. All bacterial growth were observed at 24-48 hours of both *Escherichia coli* and total coliforms.

6.6 DNA Extraction

The DNA from the collected water samples was extracted following the instructions of the commercial kit PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc). Briefly, 250 µL of the pellet obtained from the sample water filtration was placed in the PowerBead tubes. The PoweBead tubes contained a buffer that dispersed the soil particles and facilitated to dissolve humic acids and protect nucleic acids from degradation. Later, solution C1 was placed, that contained SDS

and other solutions that help to obtain complete cell lysis. Then, a step of 20 minutes vortexing was performed for homogenization and cell lysis in the samples. Subsequently, the tubes were centrifuged at 10.000 x g for 30 seconds. A total volume of 500 µl of the supernatant was taken and placed in 2ml Collection Tube, afterwards 250 µl of solution C2 was added and the total volume in the tubes was incubated at 4 °C for 5 minutes. Solution C2 it contained a reagent which serves for the precipitation of organic and inorganic substances and other pollutants including huminous acids. The tubes were centrifuged at 10.000 x g for 30 seconds. The supernatant volume of 600 from each tube was transferred to a new 2ml Collection tube with 200 µl of solution C3. Solution C3 allowed to precipitate additional non-DNA organic and inorganic material. The tubes were centrifuged at 10.000 x g for 30 seconds and 750 µl of the supernatant was mixed with 1.2 ml of Solution C4. Half volume was placed inside Spin Filter and centrifuged at 10.000 x g for one minute. Afterwards, the liquid was discarded and the previous step was repeated twice with the remaining volume. In the next step, 500 µl of the C5 solution was added inside the Spin Filter and centrifuged at 10.000 x g for 30 seconds and discarded the liquid in each tube. The tubes were again centrifuged at 10.000 x g for one minute at room temperature, removing the residual Solution C5. Carefully the Spin Filter was placed on a New 2ml Collection Tube. Finally, 100 µl of solution C6 sterile elution buffer were added to the center of the filter membrane. Then the tubes were centrifuged for 30 seconds at 10.000 rpm. The DNA solution of each tube was stored at -20 °C for further PCR analysis.

6.7 Molecular Identification of Bacterial Genera

Once the genomic DNA had been obtained from the different samples, 16S conserved rRNA sequences were amplified. The PCR mixtures consisted of a final volume of 20 µl and contained 4 µl of 5X Green GoTaq Flexi buffer (1X final concentration; Promega), 1.6 µl of $MgCl_2$ (2.0 mM final concentration Promega), 0.2 µl of dNTP Mix (0.1 mM final concentration, Promega), 1.0 µl of each PCR primer (0.5 µM final concentration) (Table 2) and 0.3 µl (1.5 U final concentration) GoTaq Flexi DNA polymerase (Promega), 2.0 µl template DNA and the remaining volume of DNA-free water. For *Shigella* spp. and *Salmonella* spp, the same PCR mix was used, with the exception that 0.2 µl of each PCR primer (0.1 µM) were added. For *Pseudomonas* spp., *Legionella* spp., and *Campylobacter* spp. again, the same reaction mixture was used, with the exception that 0,8 µl, 1 µl and 0.6 µl of the respective forward and reverse PCR primers (0.3 µM) were added. The PCR methodology was performed in a thermocycler (Bio- Rad) with the standard procedure illustrated in Table 2.

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

Organism	Primer name	Primer sequence (5′–3′)	PCR cycling parameters	Gene (size [bp])	Reference(s
Universal	Forward:fDD2	CCGGATCCGTCGACAGAGTTTGATCITGGCTCAG	3 min at 94°C; 35 cycles of 94°C for 30 s, 54°C for 30 s, 72°C for 1.5 min	16S rRNA (1,600)	(Rawlings, Trib & Hansford, 1
	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	Invasion plasmid antigen H (606)	(Kong, Lee, L Law, & Wu, 2
	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	Attachment invasion locus gene (386)	(Jonas, Rosenb Weyrich, & Bh 1995)
	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	Invasion plasmid antigen B (314)	(Kong et al., 2
	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	16S rRNA (618)	(Spilker, Coer Vandamme, LiPuma, 200
	Reverse: PA-GS-R	CACTGGTGTTCCTTCCTATA			
Campylobacter spp.	Forward: IC-F	CTAGAGTACAAACTAATAAGTCTC	3 min at 95°C; 30 cycles of 94°C for 45 s, 52°C for 45 s, 72°C for 45 s	Flanking regions of ITS gene (700)	(Khan & Edge,
	Reverse: IC-R	ATTCTAAAACGCATCACTTCCTTG			

(Dobrowsky, De Kwaadsteniet, et al., 2014)

6.8 Molecular Identification of *E. coli* Pathotypes

For the molecular identification of *E. coli* pathotypes the PCR mixtures consisted of a final volume of 20 µl and contained 4 µl of 5X Green GoTaq Flexi buffer (1X final concentration; Promega), 2 µl of $MgCl_2$ (2.5 mM final concentration Promega, Madison, WI USA), 0.4 µl of dNTP Mix (0.2 mM final concentration, Promega). For EAEC 0.6 µl, for EHEC 1 ul, for EPEC 0.5 µl and for EIEC 0.8 of each PCR primer (0.5 µM final concentration) (Table 3) and 0.5 µl (2.5 U final concentration) GoTaq Flexi DNA polymerase (Promega, Madison, WI US), 2 µl template DNA and the remaining volume of DNA-free water. The positive control strains utilized in the present study were obtained from the Microbiology Institute at Universidad San Francisco de Quito, Quito-Ecuador.

Table 3. Primers and PCR cycling parameters for the detection and identification of *E. coli* pathotypes.

Organism	Primer name	Primer sequence (5′–3′)	PCR cycling parameters	Gene (size [bp])	Reference(s)
EAEC	Forward: AggRKs1	GTATACACAAAAGAAGGAAGC	2 min at 95°C; 35 cycles of 95°C for 1 min, 54°C for 1 min, 72°C for 1 min	aggR (254)	(Toma et al., 2003)
	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			

(Dobrowsky, van Deventer, et al., 2014)

6.9 PCR Product Analysis

The result of PCR was observed with the use of an electrophoresis equipment in gels of 2% agarose, 0.1% ethidium bromide, with the respective use of negative and positive controls provided by the Institute of Microbiology of the University San Francisco de Quito.

6.10 Statistical Analysis

For the statistical analysis of the data obtained, the statistical software package SPSS version 23.0 was used. Linear regressions were performed between the concentration of *E. coli* and coliforms, the physico-chemical parameters and the detection of metals. (IBM Corp, 2013).

7 RESULTS

7.1 *Escherichia coli* and Total Coliform Counts

The counts of *Escherichia coli* and total coliforms were obtained through the Chromocult agar dilution method with the water samples from the 12 analyzed rivers. As shown in Table 4, all the analyzed rivers show concentrations of *E. coli* and total coliforms above the reference or standard values of the Arizona Department of Environmental Quality (126 UFC/ml for *E. coli*). These standard values of Arizona and Michigan Department Quality Divisions focus on surface waters with full contact or partial contact with humans, such as the analyzed rivers in this study. Unfortunately, the world health organization and Ecuadorian legislation only have a standard value on quality of drinking water for human consumption where it is stated that there should be no presence of *E. coli* or total coliforms. However, this comparison cannot be extrapolated to sources of river water, for this reason was used the current norm for Arizona and Michigan in the United States. It is important to mention that the amount of *E. coli* and total coliforms illustrated in Table 4 represented the average value of the total bacteria counting. In fact, all bacteria counting evaluated *E. coli* and coliforms through triplicate results from duplicate or even triplicate recollected water samples. As expected, all rivers evidenced higher concentrations of total coliforms than *E. coli*.

Table 4. Amount of *Escherichia coli* and total coliforms in the analyzed rivers.

River	*Escherichia coli* (UFC/ml)	Total coliforms (UFC/ml)
	126 UFC/ml[a]	
Esmeraldas	80000	160000
Toachi	55000	110000
Chone	75000	120000
Guayas	40000	143333
Machángara	90000	130000
Guayllabamba	50000	125000
Tomebamba	60000	113333
Zamora	100000	255000
Aguarico	25000	125000
Coca	20000	85000
Napo	45000	130000
Pastaza	25666	110000

[a] The permitted level for Surface Water Partial-Body Contact (for *Escherichia coli*) Arizona Department of Environmental Quality (U S Environmental Protection, 2008).

Figure 2 shows that the most contaminated rivers at the microbiological level were: Zamora, Machángara and Esmeraldas presenting values of *E. coli* from $1.00x10^5$ UFC/ml, $9.00x10^4$ UFC/ml y $8.00\ x10^4$ UFC/ml respectively. The rivers that report the lowest rates of microbial contamination were the rivers: Coca, Aguarico and Pastaza with concentrations of *E. coli* from $2.00\ x10^4$ UFC/ml, $2.50\ x10^4$ UFC/ml y $2.56\ x10^4$ UFC/ml respectively.

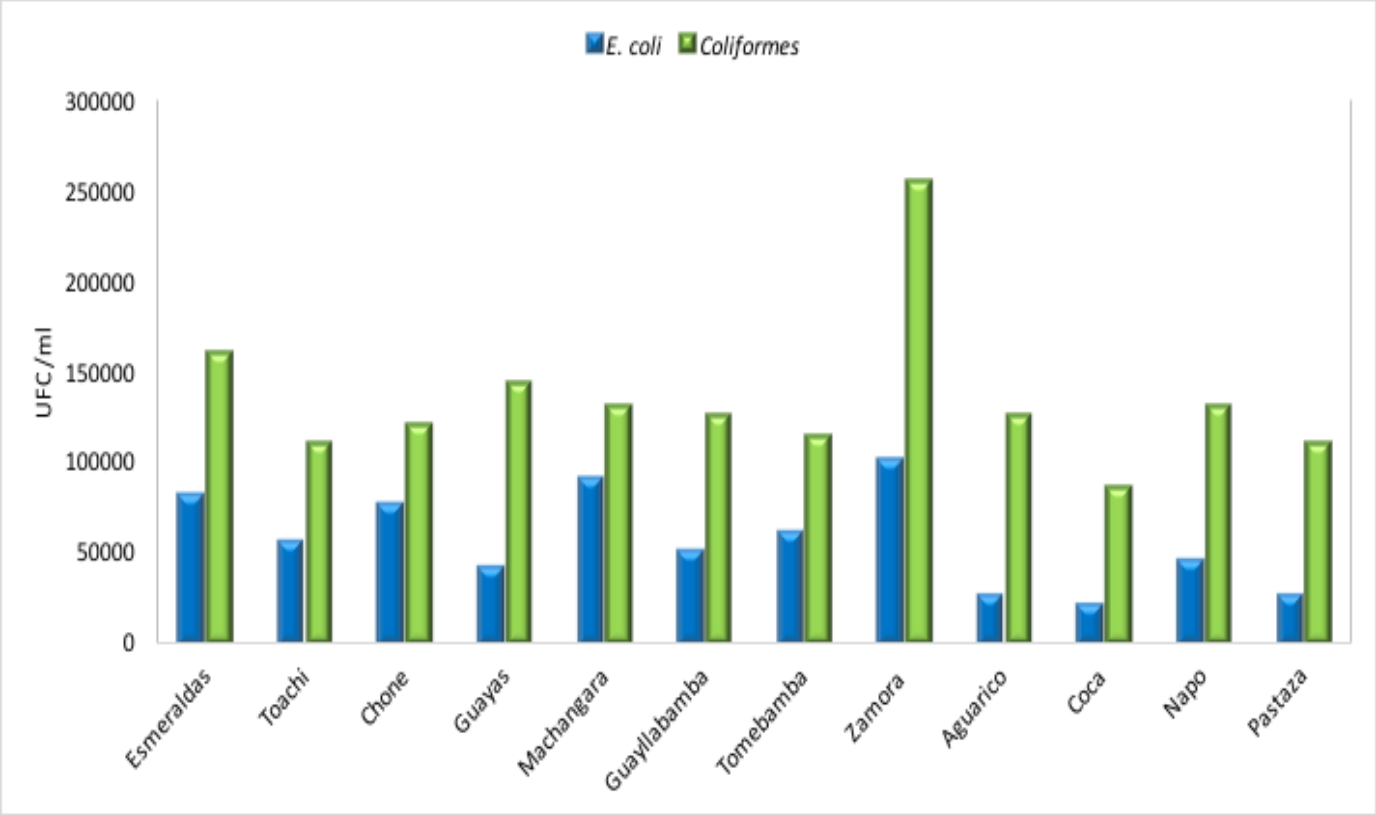

Figure 2. *E. coli* and coliform counts in the 12 rivers analyzed.

7.2 Cultivation, Quantification and Isolation of Dominant Bacteria

Bacterial growth was performed using various culture media. Figure 3 shows the result of the use of MacConkey agar, which is a selective and differential medium that allows the growth of enteric Gram-negative bacilli allowing differentiation based on lactose fermentation. Figure 4 shows the result of the culture using the medium Salmonella-Shigella agar that like the MacConkey medium allows a selective and differential culture due to the presence of bile salts that do not allow the development of Gram-positive bacteria. The *Salmonella* and *Shigella* genera do not ferment lactose, therefore they have clear colonies and Salmonella is able to produce sulfuric acid in black color. Figure 5. shows the Chromocult medium used for the quantification of *E. coli* and coliforms, the BCYE agar medium used for the isolation of *Legionella* sp., which corresponds to lead-colored colonies and finally the

Campylobacter agar medium to isolate brown small colonies corresponding to *Campylobacter* sp.

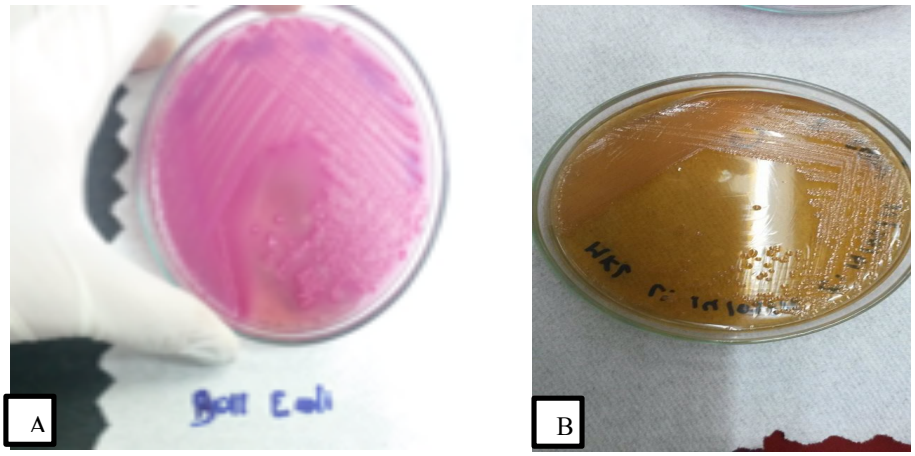

Figure 3. Culture medium MacConkey Lactosa (A) Lactose positive bacteria suspected of *E. coli* (B) Lactose negative bacteria suspected of *Pseudomonas* sp.

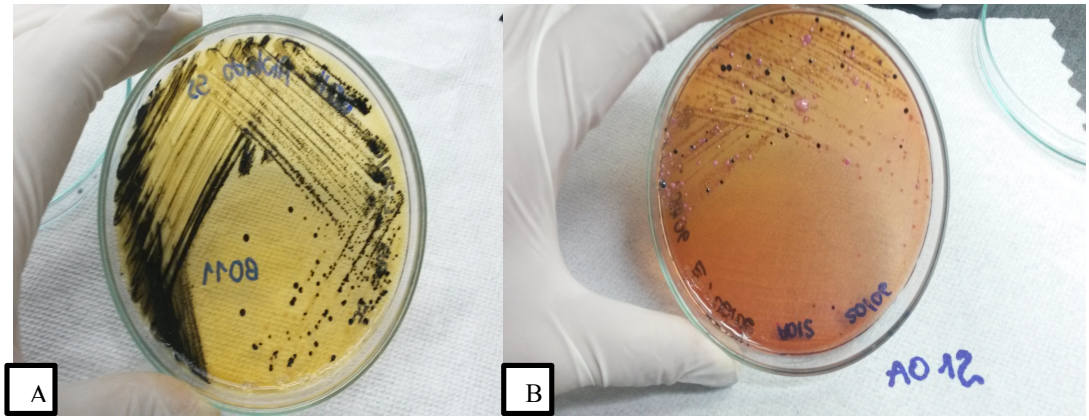

Figure 4. Culture medium Salmonella-Shigella agar (A) Suspicious bacteria of *Samonella* sp. (B) Suspicious bacteria of *Shigella* sp.

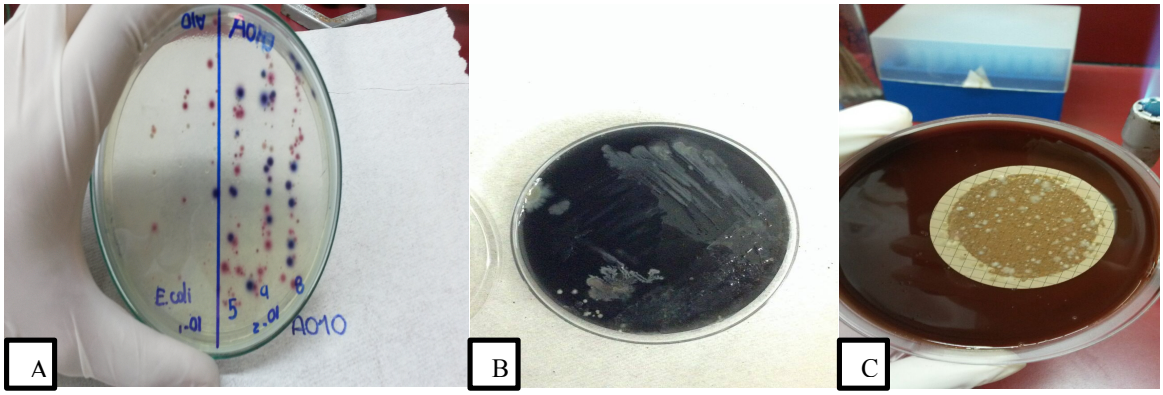

Figure 5. (A) Culture medium Chromocult agar for *E. coli* (violet colonies) y coliformes (pink colonies) (B) BCYE agar for *Legionella* sp.(C) BD Campylobacter Agar for *Campylobacter* sp.

Once the bacterial cultures were obtained, the bacteria suspected of the different genera were cryopreserved with the use of the Brain Heart Infusion (BHI) medium with 15% glycerol in order to cryopreserve the samples for subsequent molecular identification at level of species. The number of isolated strains is detailed in Figure 6.

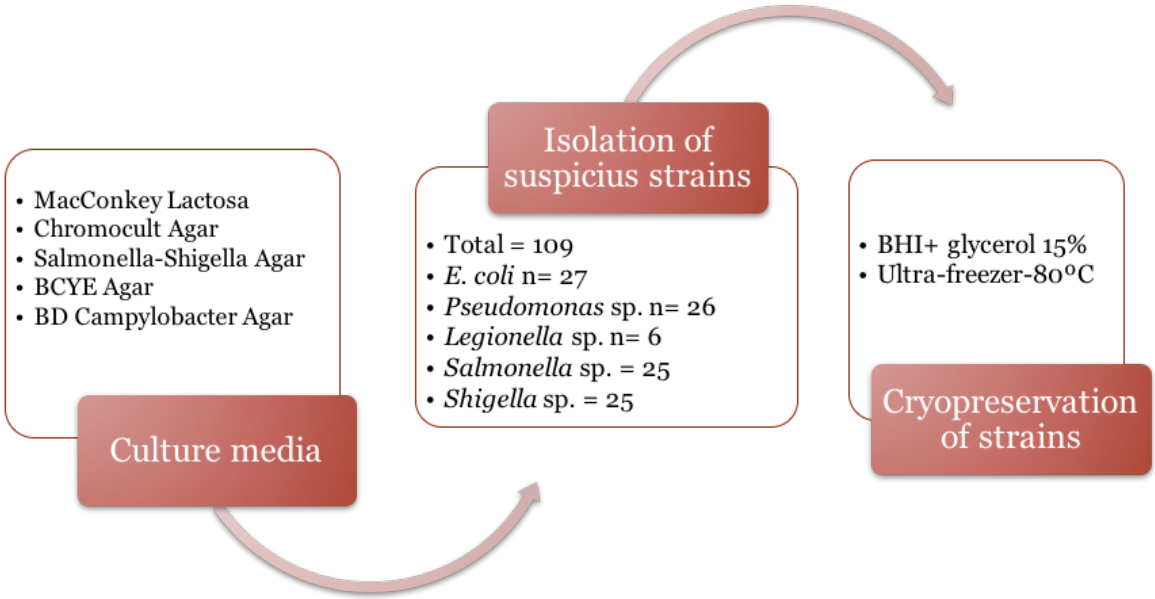

Figure 6. Isolation of bacterial strains.

7.3 Prevalence of Bacterial Genera and *Escherichia coli* Pathotypes

Following the *E. coli* and Coliforms counting, we proceed to the evaluation of the presence or absence from the following bacteria genera: *Legionella, Pseudomonas, Salmonella, Shigella, Campylobacte*r. None of the rivers showed presence of *Salmonella, Shigella* or even *Campylobacter* sp., nevertheless all rivers revealed the presence of *Pseudomonas* and *Legionella* sp. However, the presence of *E. coli* pathotypes were analyzed in all studied rivers, more exactly: enteroaggregative *E. coli* (EAEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC) and enteroinvasive *E. coli* (EIEC). Our results evidenced EIEC pathotype in the Esmeraldas, Chone, Machángara, Guayllabamba and Napo rivers. Meanwhile, EPEC pathotype was detected in the Zamora River and EAEC pathotype in the Machángara River. However, EHEC pathotype was not detected in any of the analyzed rivers.

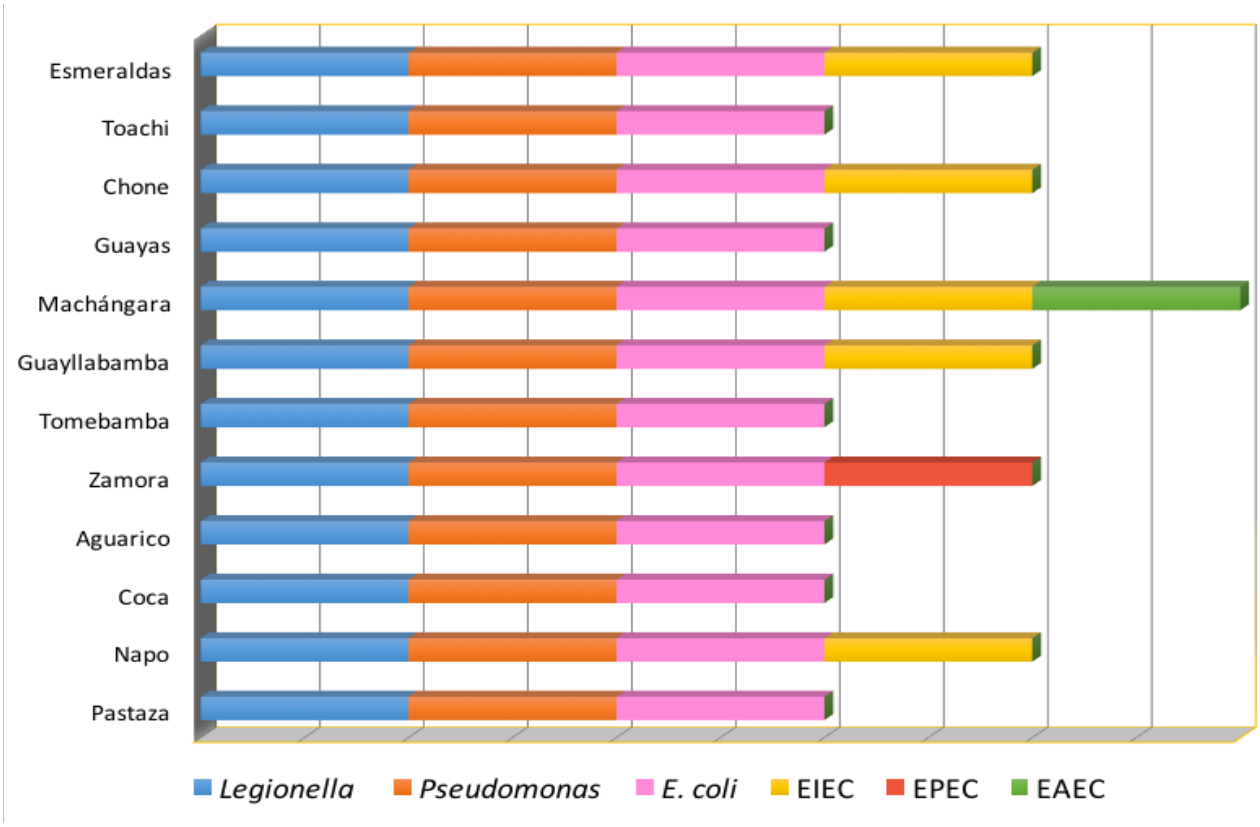

Figure 7. Molecular detection of several bacterial genera including some pathotypes of *E. coli.*

7.4 Analysis of Physico-Chemical Parameters

Additionally, to the microbiological analysis previously done from rivers into urban areas, we also analyze the physical-chemical parameters detailed in Table 5. These parameters were selected as good indicators of contamination indexes or safety of a water sample and according to the legal Ecuadorian limits (Unified Text of Secondary Legislation, TULSMA) as shown in Table 5. According to the Ecuadorian regulation, all water samples have pH, conductivity, DO, turbidity. ORP, ammonium, nitrate, sulfate and phosphate values within the normal range parameters, although each river shows certain variance due to their geographical region. Meanwhile, the Toachi River shows a higher temperature of the limit by the established index, but it must be taken into account that this river is located in the coast region of Ecuador where high ambient temperatures occur. Moreover, Esmeraldas and Guayas rivers from the coastal region of Ecuador show high concentrations of total solids (TS) and chlorides. While Guayas, Zamora, Coca, Pastaza, Machángara and Guayllabamba rivers have total suspended TSS values above those stipulated in the Ecuadorian norm, despite their different geographical or environmental region. Finally, it is important to note that only Guayas and Zamora rivers evidence high rates of total COD. In this way, were able to establish comparisons and prevalence of a certain parameter in comparison to the *Escherichia coli* index and total coliforms reported in each river analyzed (see in discussion section).

Table 5. Analysis of physico-chemical parameters obtained in Environmental Engineering laboratory (LIA – USFQ).

River	pH	Conductivity (uS/cm)	DO (mg/L)	Turbidity (NTU)	ORP (mV)	T (ºC)	COD_{TOTAL} (mg/L)	TS (mg/L)	TSS (mg/L)	Cl^- (mg/L)	NH_4^+-N (mg/L)	NO_3^--N (mg/L)	PO_4^{3-}-P (mg/L)	S((m
MCL	6.5 - 9[a]	N/A	Not < 6[a]	N/A	N/A	> 20 o >32[a]	250[b]	1600[b]	100[b]	120[b]	N/A	N/A	10[b]	10
Esmeraldas	7.92	938.53	6.53	34.6	314.967	27.3	48.37	**1657.50***	27.5	**204.91***	0.98	0.72	0.15	24
Toachi	8.13	206.47	7.34	13.47	328.53	22.5	33.61	127.5	80	0.07	0.17	0.4	0.07	12
Chone	8.14	623.5	8.3	5.3	313.53	**32.7***	76.56	5		24.23	1.16	0.49	0.49	25
Guayas	7.31	**4137.33***	6.08	925	310.93	26.8	**292.67***	**3667.50***	**939***	**769.58***	8.38	1.13	0.46	43
Machángara	7.4	501.1	6.69	60,5	349.9	14.5	133.58	370	**132.5***	104.12	5.15	1.42	3.91	8
Guayllabamba	7.75	474.63	6.84	31.57	371.17	15.4	114.34	160	**137.50***	36.43	1.38	1.18	2.98	9
Tomebamba	7.54	104.83	6.85	2.48	304.5	15.2	94.74	95	92.5	3.2	0.09	0.42	0.14	5
Zamora	7	101.8	6.24	5.71	288.53	16	**349.73***	867.5	**697.50***	5.75	0.47	0.42	0.34	3
Aguarico	7.15	57.01	7.9	82.33	282.6	19.3	24.83	242.5	92.5	8.73	0.15	0.49	0.98	6
Coca	7.22	77.33	7.27	105	412.77	18.9	69.63	225	**182.50***	2.17	0.08	0.32	0.18	8
Napo	6.89	365.83	7.64	124.67	62.44	22	19.72	592.5	65	1.39	0.11	0.3	0.91	5
Pastaza	6.99	48.37	6.08	2.5	343.27	23.4	26.85	80	**237.50***	3.72	0.18	0.48	0.04	3

[a] Quality criteria acceptable for the preservation of flora and fauna in fresh water, cold or warm and marine waters and estuary. TULSMA, Book VI, Annex I (see Table 3).

[b] Maximum allowable discharge limits to a fresh water body. TULSMA, Book VI, Annex I (see Table 12)

7.5 Analysis of Metal Parameters

The analysis of major elements and trace metals is showed in Table 6. In this study, the following metals were analyzed: Copper (Cu), Iron (Fe), Chromium (Cr), Manganesium (Mn), Aluminum (Al), Lead (Pb), Lithium (Li) and Zinc (Zn). The maximum limits were taken from the Ecuadorian legislation Unified Text of Secondary Legislation, known as TULSMA (Ministerio del Ambiente del Ecuador, 2015). This preliminary analysis showed that Aluminum were the most elevated elemental metal in the analysis (see Table 6). In fact, the Aluminum levels were between 4 and 6 times higher than maximum legal concentrations (5.0 mg/L), showing its highest level in the Guayas River (30.8 mg/L). This river was also the only analyzed river that simultaneously showed higher concentrations of Iron (6.84 mg/L). All metal contaminants belonged to elemental metals and Aluminum elemental as primal source of contamination independent of the studied region (Costal, Andean and Amazonia). These high levels of contamination in Aluminum level by Guayas river (Costa region) was then followed by the rivers Chone (Costa region), Tomebamba (Andean region) and Esmeraldas (Costa region). The remaining metals were below the Ecuadorian legislation and none of the trace metals (Cu, Cr, Mn and Pb) were near to a high concentration in this set of study. Finally, three rivers (Toachi, Pastaza and Aguarico) were not possible to analyze the samples due to contamination and transportation complications involved in the recollection of samples during our study. Therefore, only eight from twelve rivers were possible to obtain full metal analysis.

Table 6. Concentration values of each metals analyzed in Environmental Engineering laboratory (LIA – USFQ).

River	Copper (mg/L)	Iron (mg/L)	Chromium (mg/L)	Manganesium (mg/L)	Aluminium (mg/L)	Lead (mg/L)	Litium (mg/L)	Zinc (mg/L)
	2.0 mg/L	**5,0 mg/L**	**0.1 mg/L**	**0.2 mg/L**	**5.0 mg/L**	**0.05 mg/L**	**2.5 mg/L**	**2.0 mg/L**
Esmeraldas	0	0.03881	0.00	0.00	**22.26***	0	0.01	0.03
Toachi	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Chone	0	0.11758	0.00	0.02	**22.45***	0.01012	0.01	0.09
Guayas	0.15467	**6.84***	0.00	0.07	**30.80***	0.01073	0.02	0.09
Machángara	0	0.01145	0.01	0.16	**22.17***	0.01082	0.01	0.04
Guayllabamba	0.01017	1.31183	0.00	0.08	0.491	0	0.02	0.10
Tomebamba	0	0.09811	0.00	0.01	**22.44***	0	0.00	0.13
Zamora	0	0.2843	0.00	0.09	**22.25***	0	0.00	0.05
Aguarico	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Coca	0	0.16869	0.01	0.01	**22.11***	0	0.01	0.07
Napo	0	0.01844	0.00	0.00	**22.16***	0	0.00	0.05
Pastaza	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

[a]Quality criteria for water for agricultural use. TULSMA, Book VI, Annex I
N/A: not available

8 DISCUSSION

8.1 Escherichia coli and Total Coliform Counts

The water contamination is nowadays a concern in the global environment studies (Ahiarakwem, 2011; Karikari and Ansa-Asare, 2006; Yasin et al., 2015a). As previous referred in Results, all analyzed rivers showed *E. coli* above standard concentrations, in concordance with others studies in Latin America countries, such as Colombia (Ávila & Estupiñán, 2012), Mexico (Ramírez Castillo et al., 2013) and Perú (Rodriguez et al., 2017). Furthermore, some studies in USA reported lower levels of *E. coli* and total coliforms contamination than those reported in Latin America (Mason et al., 2016; Palamuleni & Akoth, 2015; Staley et al., 2014; Tchounwou et al., 2013). In particular, the recent study of (Bower, Scopel, Jensen, Depas, & Mclellan, 2005) showed total coliform levels 235 CFU/100ml of *E. coli* inferior than the standard legal limits 126 CFU/ml (U S Environmental Protection, 2008). Meanwhile, in some studies reported in Asia and Europe, *E. coli* is usually detected in different levels, being 3.1x 10^3 -6.4x 10^3 CFU/mL in Asia (India, Nepal and Iran) (Ewaid & Abed, 2017; Kolawole et al., 2011; Levy et al., 2012) and 4.2x 10^2 -5.4x 10^2 and CFU/mL in Europe (Spain and France) (Almeida et al., 2014; Di Blasi et al., 2013; C. Kittinger et al., 2013). Therefore, the contamination levels are less than the results obtained in our study 1.0x 10^4 - 1.0x 10^5 CFU/mL (see Table 5). One possible explanation for theses contamination levels could be the lack of water treatment plants in several developing countries in Latin America (Doherty et al., 2017), or even the geographical location in the tropical zone that increment the bacteria proliferation (United Nations Statistic Division, 2011). In Ecuador, the discharge of effluents is directly deposited in superficial water without any previous treatment and thus high levels of contamination are currently observed in published studies (Pérez Naranjo et al., 2015; Voloshenko-Rossin et al., 2015).

8.2 Prevalence of Bacterial Genera and *Escherichia coli* Pathotypes

Next, we reported the presence of three from a total of four *Escherichia coli* pathotypes analyzed in our study, more exactly, enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC) and enterohemorrhagic *E. coli* (EHEC). EHEC was not detected in any of the water samples for our rivers set. The EIEC pathotype was the most prevalence among our molecular analysis, being found in five rivers in different sample recollections. On the other hand, the EPEC and EAEC pathotypes were only detected in one river, more specifically, Zamora and Machángara rivers, respectively. In addition, several studies commonly reported EIEC, EPEC and EAEC as microbial water contamination (AbdelRahim, Hassanein, & Abd El Azeiz, 2015; Dobrowsky, van Deventer, et al., 2014; Levy et al., 2012). These *E. coli* strains are more commonly found in developing countries (Bouzari et al., 2012; Liang et al., 2016; Sidhu, Ahmed, Hodgers, & Toze, 2013), although some developed countries could also be found these pathotypes (Ahmed et al., 2010; Carvalho et al., 2015). These findings represent a potential public health problem taking into account the type of hydric distribution of untreated water (Bouzari et al., 2012; Dobrowsky, van Deventer, et al., 2014; Thani et al., 2016).

Furthermore, untreated water is usually direct or even indirectly correlated with several health public problems in communities (Levy et al., 2012; Vyas, Hassan, Vindhani, Parmar, & Bhalani, 2015). Due to communities that live in the surrounding area, they eventually used the untreated water for food, agricultural and recreational activities (Chandran & Mazumder, 2013; Gerhard et al., 2017), leading therefore to systematic and chronic health issues. *Legionella* and *Pseudomonas* genera were detected in all analyzed rivers, as expected, due to the normal environmental microbiota already published in several studies worldwide (Dobrowsky, van Deventer, et al., 2014; Clemens Kittinger et al., 2016; Musefiu, Olasunkanmi, & Tope, 2014). However, some studies found opportunist pathogen

strains from these genera (Ahmed et al., 2010; Dobrowsky, De Kwaadsteniet, et al., 2014; Clemens Kittinger et al., 2016), such as, *L. pneumophila* and *P. aeruginosa.* Future research should analyzed the isolated *Legionella* and *Pseudomonas* sp. obtained in the water samples of our study.

8.3 Analysis of Physico-Chemical Parameters

Regarding the physical-chemical parameters analyzed (see Table 6), mostly values were below the maximum legal values (pH, conductivity, DO, turbity, ORP temperature, NH_4^+-N, NO_3^-N, PO_4^{3-} P, SO_4^{2-}) excepting for TSS, COD, TS and Cl^- measures. The most recurrent irregular parameter was TSS in six rivers meanwhile COD, TS and Cl- were only elevated in two rivers each one. In Ecuador, few studies were realized with these types of parameters. Recently, Volshenko-Rossin and colleagues (2015) studied some physical-chemical parameters in the Napo, Pichincha and Esmeraldas rivers, obtaining similar values of pH, conductivity, DO and turbity when compared to the same rivers or even to the remain rivers analyzed in our study (see Table 6). Furthermore, other studies in Latin American countries also analyzed these basic parameters, such as in Brazil (Bortoletto, Silva, & Tavares, 2015; Carvalho et al., 2015); where similar levels of temperature, pH and turbidity were detected.

The dissolved O_2 range was found to be suitable for the natural waters depending on turbulence, temperature, salinity, and altitude (U S Environmental Protection, 2008). Furthermore, it is postulated that the range of DO between 4 to 6 mg/L ensures better aquatic life in the water body (Ferronato et al., 2013). Meanwhile, studies in Nigeria (Africa) reported different levels of physical-chemical parameters usually detected in our study. For example, these studies showed values of pH (7.62 to 9.82), Conductivity (303–8972us/cm), Turbidity (0.76–52.7Ntu), Dissolved Oxygen (0.0–7.6mg/L), Total Suspended Solid (79–

2560mg/L) (Kora et al., 2017; Palamuleni & Akoth, 2015; Purposes, 2008). Although, these parameters results are within the legal limit (Ministerio del Ambiente del Ecuador, 2015), when compared to our study, the average values of pH, DO and TSS were notably inferior from Nigeria, while Conductivity and Turbidity showed superior values.

Moreover, Yasin et al. (2015) found high levels of TSS in USA, which eventually induced harmful effect to the public health, such as problems on nervous system, provoking irritability and dizziness. So, it is important to note that similar TSS values were detected in our study in five rivers with TSS higher concentrations and no further evaluation to the public health was realized in those areas, in our best knowledge. High TSS levels were previously correlated with the presence of synthetic organic chemicals even in small concentrations (Chang, 2005). Therefore, future studies should analyze this parameter as well as the concentration of dissolved total solids.

8.4 Analysis of Metal Parameters

Water contamination by metals is been showed to affect drastically food security and public health (Bhardwaj, Gupta, & Garg, 2017; Ferronato et al., 2013; Yasin et al., 2015b). In our study, only Aluminum (Al) and Iron (Fe) were detected in high levels than legally postulated by TULSMA (Ministerio del Ambiente del Ecuador, 2015). Several studies reported the same metal analysis realized in our study (Bhardwaj et al., 2017; Karikari & Ansa-Asare, 2006; Pérez Naranjo et al., 2015; Smith et al., 2016; Yasin et al., 2015b), where large concentrations of Fe, Mn, Al, Pb, Zn are reported. Due to discharges of contaminated water from different anthropogenic activities (industrial, oil, agricultural, among others), the following public health issues were found in their communities: neurological problems, skin irritation, hormonal imbalances, atopic dermatitis, thyroid problems, among others (Pérez Naranjo et al., 2015; Reyes et al., 2016). High levels of heavy metals (such as, Pb, Cr, Cu and

Zn) generates a serious public health issues because they are not biodegradable and so accumulate in suspended particles (Pérez Naranjo et al., 2015).

In Latin America, several rivers with high concentrations of metals have been found in the last decades (Carvalho et al., 2015; Huaranga Moreno, Méndez García, Quilcat León, & Huaranga Arévalo, 2012; Reyes et al., 2016; Tchounwou et al., 2013). In Colombia, Cd and Pb values were the highest metal values found in crops of vegetables and legumes of the Orinoco and Magdalena rivers area (Reyes et al., 2016). Alerting to the scientific community to the close link between metal contamination in waters and food safety for future public consumption. Likewise, several studies have been developed in the United States allowing the comparison of their results with those found in Latin America (Howard, Dubay, & Daniels, 2013; Howard, Ryzewski, Dubay, & Killion, 2015; Smith et al., 2016). In USA, the low levels of metals is due to national regulations that control the heavy metal levels of effluents belonging to large industries (Smith et al., 2016; Vyas et al., 2015). Meanwhile, in our study, only Iron were at higher concentration that legislation in Guayas river (6.84 mg/L), being almost 10 times higher than previous as reported by the World Health Organization where it has been reported that the average iron concentration in rivers is 0.7 mg/L. Despite the fact that iron is considered an essential element in human nutrition, cases have been reported about intoxication due to the consumption of high concentrations of this metal (40mg / kg body weight of the person.) Health risks from intake of high concentrations Iron in humans include hemorrhagic necrosis, involvement of the stomach mucosa and submucosa (Huaranga Moreno et al., 2012).

Furthermore, in this study, the presence of high concentrations of Aluminum was reported in all the rivers except for Guayllabamba river. It is important to note that Aluminium is considered the most abundant metal in the earth's crust. This may be due to the geographical situation of the country, the diversity of soils that can be found in the three

continental regions of Ecuador and especially to the fact that Ecuador is considered a country with a large number of volcanoes that contribute to the Aluminum accumulation (Pourgheysari, Hajizadeh, Tarrahi, & Ebrahimi, 2015). In addition, the presence of Aluminum in water could be associated with the mining fields processing as well as metallic industrial production (Guilbaud and Gauthler, 2003). Exposure of this metal in low concentrations does not cause any harm, however, high concentrations can trigger complications in the kidney due to metal accumulation and also cases of infertility have been reported in animal models (Pourgheysari et al., 2015).

In Ecuador, the metal control and other physico-chemical parameters in the discharge of effluents is practically inexistent although, in recent years, a greater number of regulations have been promoted to control the metal levels on effluent discharges into hydric natural resources (Pérez Naranjo et al., 2015). In relationship to some European countries, it was found a progressive decrease in heavy metal concentrations in the last years (Almeida et al., 2014; Bhardwaj et al., 2017; Huaranga Moreno et al., 2012), showing similar results to others studies in the United States (Howard et al., 2013; Smith et al., 2016; Tchounwou et al., 2013). Finally, it is important to take into account the high average concentrations of Aluminum in several rivers in Ecuador that could generate health public problems in a near future.

9 CONCLUSIONS

- The main rivers with highest index of *E. coli* and coliforms were: Zamora, Machángara and Esmeraldas
- Three of the four *E. coli* pathotypes analyzed were detected, more exactly, EIEC, EPEC and EAEC.
- The most prevalent *E. coli* pathotype was EIEC, being found in six of the twelve rivers analyzed in our study.
- Only in MachÁngara river was simultaneously detected two *E. coli* pathotypes, more exactly, EIEC and EAEC.
- The presence of *Legionella* sp and *Pseudomonas* sp is reported in all rivers.
- Guayas River was the most physico-chemical contaminated river in our study (conductivity, turbidity, COD_{total}, TS, TSS, Cl^- and NH_4^+ N).
- MachÁngara River had high levels of conductivity, turbidity, TS, TSS, Cl^- and NH_4^+ N; while in the Zamora river had high levels of COD_{total}, TS and TSS.
- High concentrations of Aluminum were found in all the rivers analyzed, excepting in Guayllabamba river.
- Guayas river was the only to show high levels of Iron and Aluminum.

10 RECOMMENDATIONS

- To analyze with a greater number of samples from each individual province throughout the entire territory of Ecuador.
- To realize longitudinal studies in the most polluted rivers from this preliminary study
- To identify all isolate strains obtained from this study.
- To evaluate the impact of pollution on public health from the communities in further studies.

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