

## Indicators of Contamination by Fecal Bacteria and Escherichia Coli in Sewage Water for Human Consumption

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### Abstract

*To date there are few reports available on enteropathogenic micro-organisms that confirm the impact of the water body of the Santiago Apostol swamp complex on public health, although some data reveal daily discharges of millions of liters of wastewater, most without the slightest treatment. The objective was to determine the microbiological quality of the water of the swamp complex from drains and canals that flow into both sides of the swamp by detecting indicator organisms of faecal contamination, which allow the presence of intestinal pathogens to be inferred. Indicators of total coliform bacteria and Escherichia coli were quantified using the membrane filtration technique. The results showed that 100% of the samples showed the presence of faecal bacteria indicators. Based on the fact that these indicators should be zero in drinking water and using references for water bodies, the microbiological quality could be deduced, the samples being unsafe neither for human consumption activities nor for agricultural use. At present, the undisputed presence of enteropathogens in the water of the Santiago Apóstol swamp complex constitutes a risk to public health, mainly for the farmers who handle it and in irrigation due to the contamination of agricultural products for human consumption, as well as for people who come into contact with the swamp water.*

**Keywords:** *Bacteria, enteropathogens, contamination, public health.*

### INTRODUCTION

According to the National Plan for Municipal Wastewater Management in Colombia, there is a deficit of approximately 80% in the treatment and disposal of wastewater generated by agriculture, industry and domestic wastewater, which has generated significant accumulated pollution in various ecosystems, affecting their integrity and causing problems of health and physical, chemical and microbiological water quality in several regions.

According to Seoanez, 1999, the excessive increase in population and the development of civilization has led to the unregulated dumping of waste into water bodies, contaminating them with intestinal pathogenic microorganisms from human and animal faeces (Solarte et al., 2006). Contamination of water bodies with waterborne pathogens must be considered by communities in each region, implementing water conservation strategies. Human and animal wastes often contain intestinal pathogenic bacteria, which enter water

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from a variety of sources such as sewage treatment plants, septic systems, livestock operations, wildlife, runoff from rural and urban lands, and agricultural activities. The greatest impact of water pollution on human health is through ingestion, acting as a major vehicle for the transmission of intestinal diseases.

According to the report "Analysis of the Health Situation in Sucre 2011" by the Secretary of Health of the Government of Sucre, 31% of the municipalities in the department of Sucre do not have a wastewater treatment system or oxidation ponds, which means that the liquid waste generated in these communities is discharged directly into the soil and/or surface water sources, causing environmental pollution and affecting public health due to the proliferation of diseases and insects generated by this inadequate practice.

Indicators of faecal contamination are used to determine water quality. The most commonly used are total coliforms and *Escherichia coli* as bacterial indicators and somatic phages as viral indicators.

The present study was developed with the objective of determining the quality of the water of the Santiago Apóstol Cenotage Complex in the department of Sucre, Colombia, through the use of the indicators total coliforms and *Escherichia coli*, whose presence and number makes it possible to determine whether the water of the Cenotage complex constitutes a risk as a disseminator of intestinal pathogens and a public health and environmental problem for the community in general.

## MATERIALS AND METHODS

Sampling. The sampling site included stream discharge sites in the water body of the Santiago Apóstol swamp complex in the department of Sucre. Table 1 shows the sampled discharge sites along the swamp complex:

Table 1. Sites and location of sampling sites for microbiological water quality analysis in the Santiago Apóstol Cenagoso Complex, Department of Sucre, Colombia.

SITE	LATITUDE	LONGITUDE
1	8° 59'30,5"	74° 55'07,4"
2	8° 59'35,5"	74° 54'46"
3	8° 59'51,8"	74° 54'31,1"
4	9° 00'03,5"	74° 54'43,1"
5	9° 00'09,0"	74° 54'51,5"
6	8° 59'56,5"	74° 54'43,2"

Isolation of enteropathogenic bacteria. The proposed technique for the isolation of enteropathogenic bacteria was carried out using the protocol proposed by Olivás et al. (2011), which consisted of the following: The filtration equipment consisted of a reusable sterile filter holder, in which a sterile 47 mm diameter, 0.45 µm pore membrane bacteriological filter with a sterile grid was assembled. Then 100 mL of water sample was filtered, the membrane was separated from the filter holder and placed on an MI medium plate (DIFCO), with the grid facing upwards, marking the box. Serial dilutions of 1:100, 1:1000, 1:10 000, 1:100 000 and 1:1 000 000 were performed following the same filtration and seeding methodology for each dilution. All plates were incubated at 37 °C for 24 hours. At this time, all Petri dishes were removed from the incubator and read. All blue colonies, corresponding to *E. coli*, were counted and the results recorded. White colonies, corresponding to total coliforms, were also counted on each filter. Only counts of less than 200 colonies were recorded as total coliforms (TC) and *E. coli* (EC). Calculations to obtain the total number of organisms from the dilutions were performed

using the number of E. coli and total coliform colonies on the plates multiplied by the respective dilution.

Analysis of results. The presence of total bacteria and Escherichia coli was determined at each sampling site in the Santiago Apóstol swamp complex. The results obtained were evaluated taking into account the maximum values according to ARTICLE 11°. - MICROBIOLOGICAL CHARACTERISTICS of RESOLUTION NUMBER 2115 OF 22 JUN 2007 of the MINISTRY OF SOCIAL PROTECTION MINISTRY OF ENVIRONMENT, HOUSING AND TERRITORIAL DEVELOPMENT.

## RESULTDS AND DISCUSSION

Figure 1, shows the sites and geo-referencing sampled in the Santiago Apóstol Complex in the department of Sucre.

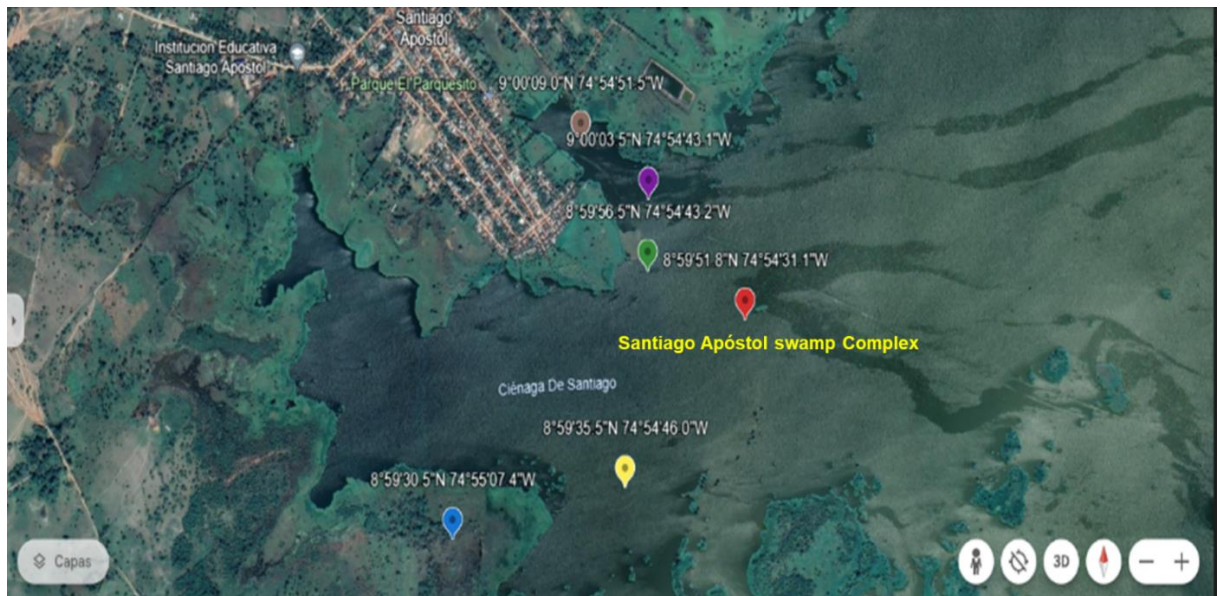


Figure 1. Sites and location of sampling for microbiological quality determination of the Apóstol Drainage Complex in the department.

According to Sánchez et al., (2022) and supported by Aguilera, (2011), in the Colombian Caribbean region there are important water resources, including large swamp complexes<sup>1</sup>. According to the report of the Disaster Risk Management Plan, San Benito Abad - 2012, these marshes are of great importance for the subsistence, economy and use for agricultural and livestock exploitation of the communities in the region, so they are subject to great anthropic pressures. One of these complexes is the Santiago Apóstol, which is located in the municipality of San Benito Abad, in the south of the department of Sucre in the sub-region of San Jorge 3, where fish diversity plays an important ecological role, and constitutes a fundamental fishing resource for the populations associated with the swamp complex. According to the findings described by Sánchez et al., (2022), the data obtained after evaluating physical, chemical and microbiological parameters for the Olaya Swamp Complex, infer that the AGC and the CCSA are under greater anthropic pressures that affect water quality, and consequently the ecosystem.

Figure 2 shows the results of the total coliform and E. coli counts, showing that most of the sampling sites have a very poor quality of contamination, not suitable for human consumption or irrigation activities for agricultural production; the presence of E. coli found was  $1.2 \times 10^3 \pm 5.6 \times 10^3$  CFU/ 100mL and faecal coliforms of  $1.1 \times 10^3 \pm 5.3 \times 10^3$  CFU/ 100mL in all the sites of the swamp complex analyzed.

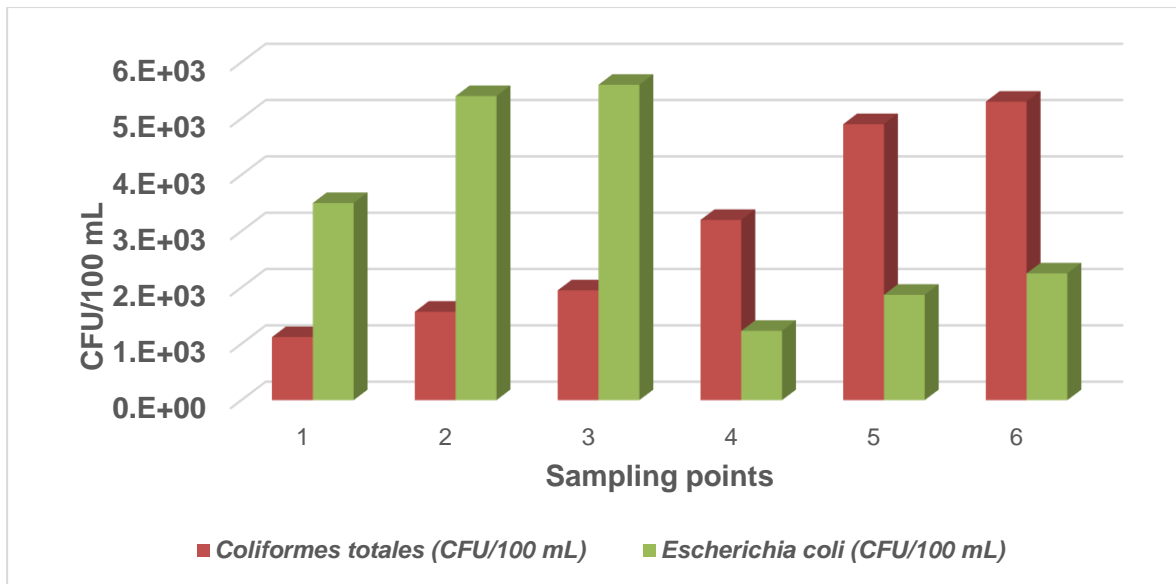


Figure 1. Total coliforms and *Escherichia coli* in waters of the Santiago Apóstol swamp complex, department of Sucre, Colombia.

The concentrations of total coliforms, expressed as geometric mean, found in the sampling sites, do not comply with Resolution 2115/2007, in relation to water used for drinking water treatment by conventional systems.

For the total coliform count, all colonies were counted, the salmon-red colonies plus the dark blue colonies. The result is obtained by multiplying the total number of colonies in the most representative box by the inverse of the dilution used. For the *E. coli* count, only dark blue-violet colonies were taken into account without taking into account total coliforms (salmon red). Doubtful *E. coli* colonies are confirmed by performing the indole test, adding 2 drops of Kovacs reagent, which produces a red halo.

Total coliforms included all colonies both salmon red (total coliforms) plus violet or blue (*E. coli*) and multiplied by the inverse of the dilution tested. For *E. coli*, only violet or blue colonies are counted.

Total coliforms and *E. coli* are used as indicators of faecal contamination in water, soil and sludge. They are normal inhabitants of the human gastrointestinal tract and when eliminated by faecal matter are found in water bodies in high concentrations, remaining longer than pathogenic bacteria. These characteristics make these microorganisms the most widely used as indicators of faecal contamination (Ramos et al., 2008). Total coliforms are Gram-negative bacteria in bacillary form that ferment lactose at 37°C producing acid and gas (CO<sub>2</sub>); they are classified as aerobic or facultative anaerobic, are oxidase negative, do not form spores and have β-galactosidase enzymatic activity. This group is composed of the genera *Escherichia*, *Enterobacter*, *Klebsiella*, *Serratia*, *Edwardsiella* and *Citrobacter*, which live as independent saprophytes or as intestinal bacteria and belong to the family Enterobacteriaceae (Pulido, 2005).

*Escherichia coli* belongs to the faecal coliform group, it is a facultative aerobic, Gram-negative, non-sporulating bacillus characterised by specific enzymes such as β-galactosidase and β-glucuronidase. It is the specific microbiological indicator of faecal contamination in drinking water (Odonkor et al. 2013).

According to a report by the United States Center for Disease Control and Prevention (CDC), the pathogenic microorganisms that can be found in contaminated water and food irrigated with this water and that also have a significant impact on human health either by consumption, aspiration or contact with the skin, are mainly bacteria that cause diarrheal

diseases such as Escherichia coli, Shigella, Campylobacter, Vibrio cholerae, among others. They can cause harmful effects on health. (Beer, et al. 2015).

## CONCLUSION

100% of the samples analyzed from the Santiago Apóstol swamp complex showed total coliforms and faecal coliform phylum Escherichia coli. The concentrations of total coliforms and E. coli found in the Santiago Apóstol swamp complex in the department of Sucre exceed the concentrations permitted by Resolution 2015 of 2007.

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