DETERMINATION OF BACTERIOLOGICAL CONTAMINATION IN SURFACE WATER BODIES: THE HIGHLANDS SOUTH OF JALISCO, MEXICO

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Abstract. The present work consisted of evaluating the quality of surface waters in The Highlands-South región in the state of Jalisco, Mexico where, through geographic information and field trips, the main hydrological features of this area were identified, establishing 35 specific sampling points distributed in the 12 municipalities that make up this region. For this, water samples were taken in the dry seasons of the years of 2020, 2021 and 2022, mainly from rivers and dams in the study área. Quantitatively determining microbiological parameters where greater attention was paid to total coliform organisms and fecal coliform organisms, since the presence of these microorganisms in the water are indicators of organic contamination of fecal origin, in the same way these can be important pathogens for the human being. Observing the results obtained, it is possible to appreciate that the sampling points in the municipalities of Tepatitlan (T3, T4, T6), Acatic (AC5), Arandas (AR2, AR3) and San Julian (SJ4) are where the amount of fecal coliform organisms they are considerable. This is directly related to the economic activities that take place in the affected municipalities as well as to the domestic wastewater discharges without adequate treatment coming from the population centers.
Keywords: *bacteriological contamination, surface water, water quality, Western Mexico*

Introduction

Water is one of the vital resources on planet earth, where human beings depend on it not only for domestic use, but also for socioeconomic development, energy, food production and for agricultural and industrial activities. It is a key element for the functioning and maintenance of natural ecosystems and their biodiversity, to the extent that its scarcity causes these habitats to degrade, limiting its contribution to balanced environmental maintenance. In the last decades with the high population growth, the activities to produce more and better goods and services, causes a strong increase in the demand for water resources, likewise another great challenge is the various degrees of contamination of this vital liquid since Lacking the minimum characteristics necessary for its use, its scarcity intensifies. The waters of surface and underground bodies are contaminated mainly by discharges without prior treatment, of municipal and industrial waters, as well as by runoff that comes from areas that practice agricultural and livestock activities (SEMARNAT, 2012). Undoubtedly, water contamination has repercussions on human activities; however, the repercussion that generates the most concern is regarding the health issue. Because polluted water and lack of basic sanitation hinder the eradication of extreme poverty and disease in the poorest countries.

In 2017, 2,000 million people in the world did not have basic sanitation facilities such as toilets or latrines; furthermore, 673 million people still practiced open defecation. According to the WHO/UNICEF Joint Monitoring Program for Water Supply and Sanitation, at least 2 billion people worldwide drink water that may be exposed to contamination from faeces. An even greater number consume water that is distributed through systems vulnerable to other types of contamination (UN, 2021). In general, water constitutes an essential part of every ecosystem, both in qualitative and quantitative terms; a decrease in the quantity and/or quality of available water generates serious negative effects on ecosystems (WWAP, 2013). Thus, water and all ecosystems are indisputably linked, because the presence of one cannot be explained without the presence of the other. Today it is customary to consider that ecosystems provide a service, but they are also a system, because through its cycle water plays several roles in the climate as well as in the chemistry and biology of our planet, it is difficult to conceptualize it only in terms of support, regulation or provision of services. Precipitation, in the form of rain or snow, is the source of water that maintains ecosystems; these in turn control the renewal of water, since they regulate precipitation and contribute to its distribution in the processes of evaporation, recharge and runoff. Likewise, and through these mechanisms, water maintains terrestrial and aquatic ecosystems (Cisneros et al., 2010).

The evidence indicates that our planet has an availability of water of 1,386 million cubic kilometers (MCK), distributed as follows: 97.5% salt water and only 2.5% fresh water (35 MCK) (*Figure 1*). Of that amount, only 0.007% of the total is available for human consumption, due to the fact that 69.7% of fresh water is frozen at the poles or in glaciers; 30% is buried below the surface in aquifers; and 0.3% in rivers and lakes. In addition, UNESCO points out that 80% of wastewater returns to the ecosystem without any type of treatment (Díaz Moreno, 2014). All bodies of water are interconnected, from the atmosphere to the oceans through the hydrological cycle. The main bodies of water that make up the Earth are: (1) Rivers; these bodies of water, commonly called streams, are characterized by the fact that they flow unidirectionally with relatively high average velocities that vary between 0.1 and 1 m/s. The flow in the rivers is highly variable and depends on the climatic conditions and the characteristics of the drainage area. In general, rivers are bodies of water which can be considered permanently mixed, and in most of them, the quality of the water is important in the direction of flow; (2) Lakes; in these aquatic systems, the average speed is relatively low: it varies between 0.01 and 0.001 m/s (surface values). This fact causes the water to remain in the system from a few days to several years. Regarding the quality of the water, it behaves or is governed according to the trophic state and the stratification periods; and (3) Groundwater; in aquifers the flow regime is relatively stable in terms of speed and direction. Average velocities can vary between 3 and 10 m/s and are governed by the porosity and permeability of the stratum. There is another type of transient water bodies that are characterized by their hydrodynamic variability among them, the most important are:

Reservoirs; they can be considered intermediate bodies of water between lakes and rivers and are characterized in that their hydrodynamics and water quality depend on the operating rules;

Swamps: they are ecosystems considered intermediate bodies of water between a lake and a phreatic aquifer; and

Estuaries; they are intermediate aquatic systems between river and sea.

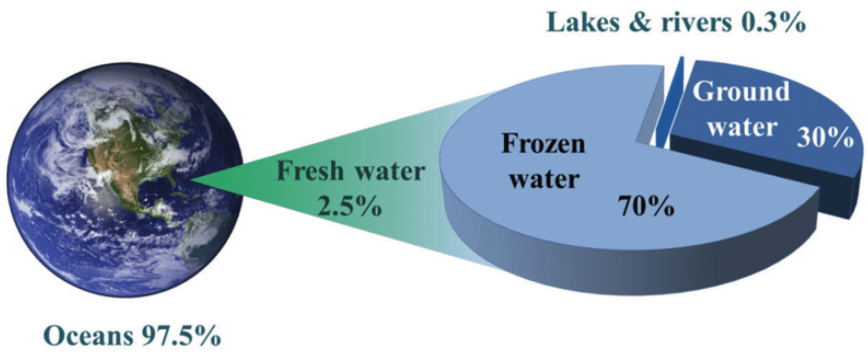

Figure 1. *Distribution of water on the planet.*

Surface waters (freshwater lakes, rivers, lagoons, swamps), which are what man uses to carry out his basic functions (supply of drinking water, navigation, recreation, among others), are unfortunately the most polluted due to because they directly receive wastewater discharges without any treatment. Many surface currents in the world are in advanced stages of contamination and have no use, except to receive waste (Ramírez, 2021).

Availability of water

Water stress, essentially calculated as water use versus available supply, affects many parts of the world, with more than two billion people living in countries experiencing water stress. An estimated 4 billion people live in areas that experience severe physical water scarcity for at least one month of the year. Some 1.6 billion people face "economic" water scarcity, which means that even if water is physically available, they lack the infrastructure to access it. Several of the world's major aquifers are under increasing stress and 30% of the world's largest groundwater systems are being depleted. Water withdrawals for irrigation are the leading cause of groundwater depletion worldwide (WWAP, 2013). In Mexico, 77% of the population lives in regions with little water, where 8 out of 10 Mexicans suffer or will suffer from a lack of water. According to the World Health Organization (WHO), a person requires 100 liters of water per day for their consumption and hygiene needs; however, the average water consumption per person is 380 liters per day, being up to almost 4 times more than recommended. In the Mexican state of Jalisco, the degree of water pressure is different depending on the region, because the areas with the largest population are the most vulnerable to scarcity, for example: the metropolitan area of Guadalajara and Los Altos (Jalisco State Government, 2021).

Main sources of water contamination in Jalisco

Much of the water bodies are polluted, to a greater or lesser extent. According to the information generated by the National Monitoring Network (NMN) operated by CONAGUA, the main currents of Jalisco and Lake Chapala show significant pollution problems, especially industrial areas. In Jalisco, water pollution originates mainly from untreated residual discharges of industrial, domestic, commercial, agricultural and agricultural return origin. In addition, there are other external sources of contamination, such as open-air garbage dumps, defective sanitary landfills, occasional and inappropriate discharges of chemical and petrochemical materials and substances, agricultural by-products, and construction debris (Hernandez-Alvarez et al., 2021). Part of the contamination of wastewater is due to the fact that they contain dissolved chemical elements and substances, as well as suspended solids, in variable concentration, which are discharged without treatment, which causes the contamination of surface water bodies. The main pollutants in municipal wastewater are nitrogen, phosphorus, organic compounds, fecal coliform bacteria, and organic matter, among others. It is estimated that worldwide between 80 and 95% of wastewater is discharged directly into rivers, lakes and oceans without receiving prior treatment (CONAGUA, 2017).

CONAGUA (2017), at the national level, between 2000 and 2005 the volume of discharges generated from municipal wastewater increased by just over 6% (from 250 to 265.6 m^3/s); trend that was reversed from 2006. For 2016, the volume of wastewater from municipal discharges was approximately 7.2 thousand cubic hectometers per year, equivalent to 228.9 cubic meters per second, of which were collected in the sewage systems. Sewerage 6.69 thousand cubic hectometers per year (212 m^3/s; 92.6% of the generated) and 3.9 thousand cubic hectometers were treated in the same period (123.6 m^3/s; 58.3% of the collected). In 2016, the entities that generated the highest municipal wastewater flows were the State of Mexico (31.7 m^3/s), Mexico City (21.4 m^3/s), Jalisco (14.3 m^3/s), Veracruz (13.4 m^3/s), Nuevo León (12.6 m^3/s) and Sonora (10.2 m^3/s), which together contributed around 45% of the national volume generated for that year. In general, there is a relationship between the generation of wastewater and the Gross Domestic Product (GDP): the entities that contribute the highest percentage to the national GDP are also the ones that generate the largest discharges of wastewater (Government of Mexico, 2018) (*Figure 2*).

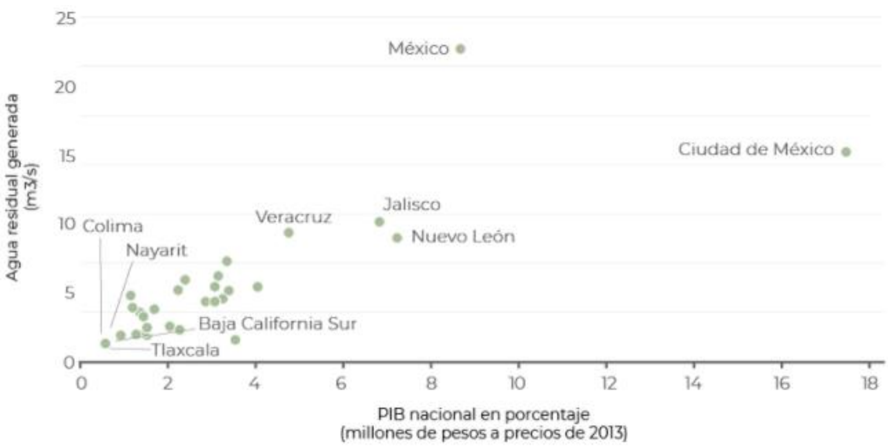

Figure 2. *State GDP and municipal wastewater generation in 2016.*

Highlands-South region of Jalisco (HSRJ)

HSRJ has an area of 6,667 km², which corresponds to 5% of the area of the state of Jalisco, made up of twelve municipalities: Acatic, Arandas, Cañadas de Obregon, Jalostotitlan, Jesus Maria, Mexticacan, San Julian, San Ignacio Cerro Gordo, San Miguel el Alto, Tepatitlan de Morelos, Valle de Guadalupe and Yahualica de Gonzalez Gallo, where Tepatitlan is located as the headquarters municipality of this region (IIEG, 2019) (*Figure 3*). HSRJ presents the following primary geopolitical limits: to the north, the Jalisco municipalities of Teocaltiche, San Juan de los Lagos and Union de San Antonio; to the east, the Jalisco municipality of San Diego de Alejandria and the State of Guanajuato; to the south, the Jalisco municipalities of Tototlan, Atotonilco el Alto, Ayotlan, and Degollado; and to the west, the state of Zacatecas and the Jalisco municipalities of Cuquio and Zapotlanejo (Regional Development Plan, 2020). In terms of hydrology, the region is located in the 12 Hydrological Region (12-HR) “Lerma-Santiago” hydrological region, and five basins benefit the region: Río Verde Grande, Río Lerma-Salamanca, Río Lerma-Chapala, Río Santiago Guadalajara; and a small portion of the municipality of Yahualica in the Río Juchipila basin. The area occupied by the main channel of the Verde River, comprises topo valley forms with terraces and corresponds to the most fertile part of the region (Regional Development Plan, 2020). The economic activities in HSRJ have been and are an important source of income necessary for the development and growth of the sectors, in addition to being the livelihood of numerous families in the region. Activities ranging from food production, extraction of raw materials to the provision of services, among others. The same that at some point come to represent a polluting factor towards the environment.

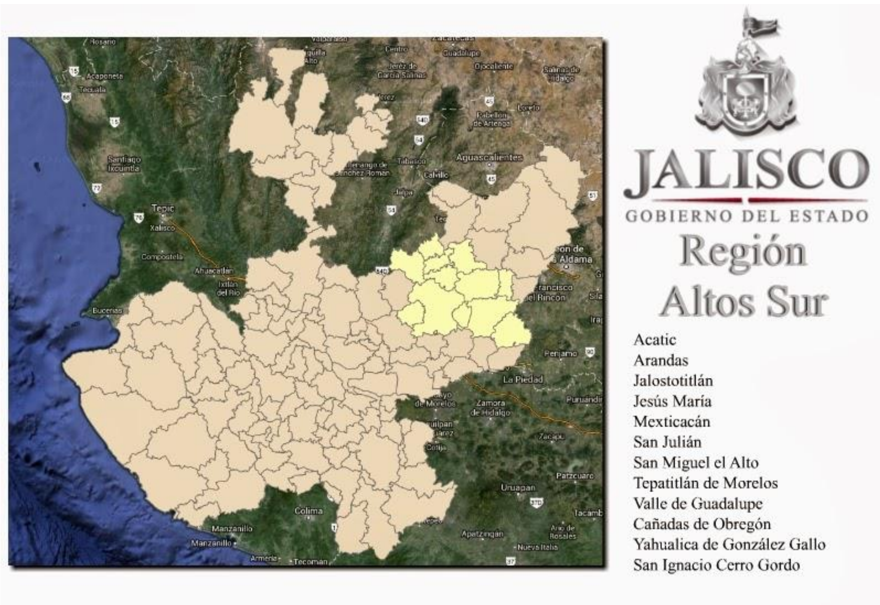

Figure 3. *Highlands-South region at Jalisco state, Mexico.*

Agricuture and livestock

“The state of Jalisco contributes to agricultural and fishing production a volume of more than 40 million 481 thousand tons, which makes it the leading state in production” (Government of Mexico, 2020). Jalisco in the livestock sector is a leader in milk, eggs (with more than 50% of national production) and pork. In addition to occupying leading positions in the production of beef, chicken, honey and in the inventory of female sheep. Among the agricultural products in which Jalisco has national leadership are: tequila agave, white corn (in the seasonal cycle), fodder corn, chia, lime, blueberry, raspberry, tamarind, sugar cane (in which it has second place), export watermelon etc. In addition, if Jalisco were a country, it would be among the five world producers of avocado, being the second national producer (Jalisco State Government, 2020). HSRJ in the period between 2013-2018, the value of agricultural production showed various fluctuations, having registered its highest levels in 2017 and 2018.

The value of agricultural production in the region in 2018 represented 14.2% of the total value of state agricultural production and had its largest share in 2014 with 16.6% (IIEG, 2019). In the same year, the main agricultural product was agave with 5,589 million 727 thousand pesos, followed by grain corn, with a production value of 2,269 million 907 thousand pesos per year, followed by green forage corn which represents 10.7%, green tomato 1.9%, avocado 1.5% and pastures and meadows with 1.3% (IIEG, 2019). On the other hand, livestock production in HSRJ has maintained a growing trend during the 2013-2018 periods, with 2018 being the year in which the highest growth in the value of livestock production in the region has been recorded, representing 34.4% of state production. Among the livestock products that stood out for their participation of HSRJ in 2018, the first place is the egg for plate, which contributes 47.4% of the total value of production in the region, followed by carcass meat from pork with 19.4%, bovine milk that participates with 17.2%, bovine carcass meat with 11.3% and poultry carcass meat with 4.6% of the regional total (IIEG, 2019).

Agricutural pollution

According to the Food and Agriculture Organization of the United Nations (FAO), agriculture demands 70% of the world's water and is responsible for the discharge of chemicals, organic matter, waste, sediments and salts. Global agriculture today is related to the use of pesticides and chemical elements such as potassium or magnesium that can reach the various bodies of water, in addition to maintaining agricultural yields, pesticides, fertilizers, fungicides, herbicides and fertilizers (agrochemicals), which filter into aquifers, others intentionally discharged into water flows together with organic remains and sediments, position agriculture as the main responsible for pollution by nitrates and phosphorus. In the last 20 years, a new class of pollutants has emerged in the form of drugs such as antibiotics and hormones used in livestock farming, which also often reach water sources and ecosystems, posing a health risk, according to In this study, intensive livestock farming contributes above all to pollution due to the type of waste, as well as animal excrement and other substances that end up degrading in the environment (FAO, 2018). Other important economic activities for HSRJ should also be highlighted: Among them, the production of traditional foods for local consumption, the varied manufacturing and artisan activity, the construction industry, the forest exploitation of oak, oak, willow, eucalyptus and mesquite, mining, fishing and the tequila factories that are located in the region. These activities generate a great economic contribution to families in HSRJ, so it is important to highlight the impact they have.

According to the information from the national statistical directory of economic units (INEGI), the southern highlands region has 21,245 economic units as of April 2019, where trade prevails with 45.45%, there is also 38.88 % in services, 12.37% in manufacturing industries, 2.13% in legislative activities, 0.58% in transportation, 0.26% in construction, 0.18% in electricity generation from water harvesting, and the lowest of all, agriculture with only 0.12% of the companies located in the region (IIEG, 2019). The general objective of the present study was by means of complementary bacteriological parameters to identify the main points or municipalities in HSRJ that has a greater contamination and that therefore requires better treatment in its waters to obtain a better quality, surface water, clarifying that the quality of the water will depend on its use. The specific objectives are: To identify, through previous research, the causes of surface water contamination in HSRJ. Identify enterobacteria present in the sampling points of the region to be analyzed at accordance with NOM-003-SEMARNAT-1997 and NOM-127-SSA1-1994, determine the use that can be given to the analyzed water (SEGOB, 2005).

In this study, bacteriological parameters are determined in order to evaluate and contextualize the impact that has been had on the surface water bodies of HSRJ, from various sources of contamination such as wastewater discharges derived from the activity domestic, agricultural and industrial, which is in direct correlation with the constant population growth of the region. The possible increase in water contamination, among others, affects biodiversity and ecosystems that are present in surface water bodies, affecting the environmental, economic and social environment.

Materials and Methods

Through geographic information and field trips, the main hydrological features of HSRJ were identified, establishing 35 regular sampling points in the 12 municipalities of the study area. The samplings were carried out in several campaigns and field trips in the time between the months of November and December of the year 2020, and in the months of January, February and March of the 2021, that is to say in the dry periods to avoid the dissolution of the samples in the main rivers and dams of all the municipalities that make up the study region, quantitatively determining parameters in the field using portable equipment, as well as collecting samples for later analysis in the laboratory, which will allow us to obtain greater certainty in microbiological measurements as well as complementary physicochemical parameters to establish the degree of quality of surface water.

Sampling techniques

For the sampling, the 500 ml sterile glass bottles with screw caps were filled in accordance with NOM-230-SSA1-2002, which indicates submerging the bottle in water with the neck down to a depth of 15 to 30 cm, uncover and then turn the bottle slightly allowing filling (*Figure 4*). Once the sample has been taken, the stopper or lid must be placed and the bottle removed from the water. It should be noted that when they are microbiological samples, the glass bottles should not be subjected to rinsing; the sample should be taken directly, leaving a space for aeration and mixing of 1/3 of the sampling bottle. The containers with the samples were labeled with date and origin, keeping them in refrigerated conditions until they arrived at the laboratory, where they were analyzed in less than 24 hours.

Figure 4. *Sampling for bacteriological analysis.*

Microbiological analysis

In this process, a diagnostic technique that uses biological, biochemical, molecular or chemical methods to quickly and easily identify the presence of microorganisms in a material. It is often applied to microorganisms responsible for disease and food spoilage. To obtain satisfactory results in the isolation and identification of bacteria, we must ensure microbiological analysis, where correct sampling and adequate transport of these must be carried out (*Figure 5*). The handling of the sample must always be carried out in a rigorously aseptic manner, using sterile material at all times. The control points that must be taken into account include: (1) the time elapsed since the collection; (2) the ideal container for each simple; (3) the amount; (4) preservation and conservation of the simple; and (5) possible contamination of this with material close to the collection site.

Figure 5. *Carrying out bacteriological analysis of the samples.*

At the same time, the correct functioning of the material and equipment is ensured, such as the temperature of stoves, refrigerators and freezers, incubation hoods, autoclaves, apparatus by which all material is sterilized by steam at high temperature, and sterilization stoves, pipettes, among other. Once the process is controlled, the appropriate technique is determined according to the microorganism to be analyzed. Some indicator microorganisms that are analyzed in water samples are Escherichia coli, total coliforms, fecal coliforms, mesophilic aerobes, sulfite-reducing microorganisms, among others. The indicator microorganisms that will be analyzed in this case are total coliform organisms and fecal coliform organisms.

Total and fecal coliforms

Coliforms are a group of bacteria belonging to the Enterobacteriaceae family. It owes its name to the best known member of this group, the bacterium Escherichia coli. However, coliform bacteria group four bacterial genera: Escherichia, Enterobacter, Klebsiella and Citrobacter. These bacteria are important because they are an indicator of the levels of contamination in water bodies. The presence of these bacteria indicates that the water is contaminated with fecal matter. Likewise, some are important pathogens for man.

Total coliforms organisms

The group of total coliform bacteria is made up of bacteria that are rod-shaped and gram-negative, in addition to being facultative anaerobes, which means that they can grow both in the presence and absence of oxygen. Total coliforms include all coliform bacteria, and there may be pathogenic genera, as well as others that are harmless or harmless to humans.

Thermotolerant or fecal coliforms

This is a subgroup within the total coliform bacteria. They are known as thermotolerant because they have the peculiarity of being able to ferment lactose at extremely high temperatures, between 44 and 45°C. These bacteria are also known as fecal coliforms because they generally originate in the intestine of some animals. Because of this, they are contained in fecal matter. The genera that make up the group of thermotolerant bacteria are Escherichia, Enterobacter and Klebsiella, the most representative of all bacteria being Escheric.

Materials

Microbiological analysis is a diagnostic technique that uses biological, biochemical, molecular or chemical methods to quickly and easily identify the presence of microorganisms in a material. It is often applied to microorganisms responsible for disease and food spoilage. To obtain satisfactory results in the isolation and identification of bacteria, we must ensure microbiological analysis, where correct sampling and adequate transport of these must be carried out. Some of the main materials used were: (1) bacteriological pipettes to distribute 10 and 1 ml (or if necessary 11 and 2 ml), with a cotton plug. Pipettes can be graduated in volumes equal to one tenth of their total volume; (2) 250 ml glass bottles with screw cap; (3) sterilizable utensils for obtaining samples: knives, tweezers, scissors, spoons, spatulas, etc.; (4) 20 x 200 mm and 16 x 160 mm culture tubes with metal or screw caps; (5) fermentation hoods (Durham tubes); (6) graduated bacteriological pipettes of 10 and 1 ml; (7) racks; and (8) platinum or nichromel loop approximately 3 mm in diameter.

Results and Discussion

The regular 35 sampling points established in the main bodies of surface water such as rivers and dams of the 12 municipalities that make up HSRJ (Mexico), are described in *Table 1*. Several campaigns were mainly carried out to collect samples, during the periods between November to December 2020, during January, February and March 2021 and in March 2022, that is, during the dry seasons to avoid dissolution of the samples. The microbiological parameters evaluated were: (1) TCO (Total Coliform Organisms) and (2) FCO (Fecal Coliform Organisms). While in *Table 2* shows the quantitative results of the microbiological content of the surface water bodies analyzed in the study area.

Table 1*. Seasonal variation in quantitative microscopy of A. Marmelos L. leaf.*

Municipality	Sample identification key	Sampling point location			Description of the water body
		N	W	Altitude (M)	
Tepatitlan	T1	20° 51’19.63”	102°42’51.35”	1904	Jihuite dam
	T2	20° 51’21.51”	102°48’11.33”	1888	Carretas dam
	T3	20° 48’ 15.35”	102°45’47.40”	1767	Tepatitlan river center of Tepatitlan
	T4	20° 47’20.67”	102°48’54.23”	1742	Tepatitlan river at the exit of the municipality
	T5	20° 49’21.0”	102°35’0”	2049	Capilla de Guadalupe dam
	T6	20°46’48.10”	102°48’16.31”	1744	Stream tributary river Tepatitlán
Acatic	AC1	20° 43’12.82”	102°49’19.35”	1738	La red Dam
	AC2	20° 41’25.15”	102°57’42.59”	1620	Calderon dam
	AC3	20° 45’41.79”	102°52’46.43”	1697	Lagunillas dam
	AC4	20° 45’40.25”	102°54’3.16”	1688	Tepatitlan River in

	AC5	20° 47’08.03”	102°56’56.58”	1670	the Acatic city Tepatitlan river after the city of Acatic
	AC6	20° 45’48.12”	102°54’21.45”	1689	Tepatitlan river tributary stream
Arandas	AR1	20° 44’5.39”	102°25’34.39”	2014	El tule dam
	AR2	20° 44’ 5.39”	102°20’23.93”	2059	Arandas river in the center of the city of Arandas
	AR3	20° 41’13.42”	102°19’55.75”	2026	Arandas river at the exit of the city of Arandas
	AR4	20°52’58.31”	102°21’03.16”	2057	Sta. Maria dam
Yahuaica	Y1	21° 10’59.03”	102°54’8.54”	1817	El Estribón dam
	Y2	21° 00’25.62”	102°49’5.39”	1473	Verde river
San Miguel el Alto	SMA1	20° 59’33.01”	102°24’23.81”	1835	San Miguel dam
	SMA2	21° 01’40.58”	102°23’59.43”	1843	San Miguel river
Cañadas de Obregon	CO1	21° 11’35.32”	102°42’04.12”	1612	Verde river
San Ignacio Cerro Gordo	SI1	20° 46’05.10”	102°32’07.73”	2069	El mezquite dam
Jalostotitlan	J1	21° 09’ 20.75”	102°27’26.84”	1749	Jalostotitlán dam
	J2	21° 09’47.23”	102°28’02.85”	1738	Jalostotitlán river
San Julian	SJ1	20° 58’13.18”	102°10’54.57”	2093	San Isidro dam
	SJ2	21° 00’46.57”	102°11’18.89”	2052	San Julian river at the entrance to the city of San Julian
	SJ3	21° 00’ 33”	102°09’02”	2099	San Julian dam
	SJ4	21°00’36.89”	102°10’12.43”	2057	San Julian river at the exit of the city of San Julian
Mexticacan	M1	21° 16’34.83”	102°46’41.91”	1756	La Paloma dam
	M2	21° 15’57.88”	102°46’26.30”	1756	Mexticacan river In the center of the city of Mexticacan
	M3	21°16’27.23”	102°46’34.21”	1759	Mexticacan river at the exit of the city of Mexticacan
Jesus Maria	JM1	20° 43’17.75”	102°09’00.33”	2207	Ojo Zarco dam
	JM2	20° 39’01.39”	102°08’49.26”	2171	La Luz dam
Valle de Guadalupe	VG1	21° 01’48.16”	102°42’01.04”	1812	El Salto dam
	VG2	21° 00’39.02”	102°37’09.21”	1820	Valle de Guadalupe river in the center of the city of Valle de Guadalupe

Table 2*. Results of microbiological analysis of surface water in HSRJ (2020-2021).*

Sample identification key	Indicator organisms (MPN/100ml)	
	TCO	FCO
T1	170	12
T2	<1.8	<1.8
T3	90,000	32,000
T4	9’200,000	3’500,000
T5	2,100	20
T6	135,000	27,500
AC1	540	13
AC2	330	40
AC3	22,000	400
AC4	46,000	4,000
AC5	1’700,000	700,000
AC6	4,900	3,300
AR1	28	2
AR2	22’000,000	2’700,000
AR3	4’300,000	520,000

AR4	160,000	22,000
Y1	33	23
Y2	130	17
SMA1	130	79
SMA2	280	31
CO1	94	46
SI1	350	22
J1	920	33
J2	250	250
SJ1	2,800	2
SJ2	1,700	700
SJ3	170	110
SJ4	24,000	24,000
M1	1,600	280
M2	79	23
M3	22	17
JM1	170	2
JM2	70	23
VG1	12	6.1
VG2	350	49

The municipalities that showed the highest concentrations of TCO and FCO were Tepatitlan (T4, T6 and T3), Acatic (AC5 and AC4), Arandas (AR2, AR3 and AR4) and San Julian (SJ4). The enterobacterial varieties detected in the monitored water bodies were mainly: Kluyvera ascorbata, Escherichia coli, Klebsiella pneumoniae, Enterobacter aerogenes, Serratia adorifera, and some varieties of Klebsiella (*Table 3*). Additionally, a sample was taken at the water inlet of the Tepatitlan treatment plant with coordinates 20°47'16.34” N 102°47'14.12” W and an altitude of 1749 m, in order to compare the amount of TCO and FCO that exist between the sampled points and this point in particular, which will be given an identification key "T8", which is where the wastewater discharge from the locality is located before being treated in the wastewater treatment plant. Tepatitlán, these results are presented in *Table 4*.

***Table 3**. Enterobacteriaceae identified in surface waters in HSRJ (2020-2021).*

Sample identification key	Enterobacteriaceae identified
T1	Citrobacter freundii, Enterobacter aerógenes
T2	Kluyvera ascorbata
T8	Serratia liquefaciens, Klebsiella pneumoniae, Klebsiella ozoenae
T4	Escherichia coli, Kluyvera ascorbata, Klebsiella pneumoniae, Klebsiella planticola, Klebsiella oxytoca
T6	Escherichia coli, Enterobacter aerogenes, Kluyvera ascorbata
AC2	Klebsiella pneumoniae, Klebsiella ornithinolytica, Klebsiella terrigena
AC1	Salmonella subgrupo 3b, Kluyvera ascorbata
AC5	Serratia adorifera, Kluyvera ascorbata
AC6	Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter cloacae, Kluyvera ascorbata
T5	Serratia adorifera, Serratia liquefaciens, Kluyvera ascorbata
AR1	Escherichia coli
AR2	Klebsiella pneumoniae, Enterobacter aerógenes
JM1	Kluyvera ascorbata
JM2	Kluyvera ascorbata
AR3	Enterobacter aerógenes, Klebsiella pneumoniae

AR4	Klebsiella pneumoniae, Kluyvera ascorbata
SM1	Kluyvera ascorbata
SM2	Kluyvera ascorbata
SJ2	Klebsiella planticola. Citrobacter amalonaticus, Enterobacter amnigenus
SJ3	Klebsiella pneumoniae, Kluyvera ascorbata
SJ1	Klebsiella pneumoniae, Kluyvera ascorbata
SJ4	Escherichia coli, Kluyvera ascorbata
VG2	Escherichia coli, Kluyvera ascorbata
VGI	Serratia adorifera, Kluyvera ascorbata
Y2	Serratia adorifera, Kluyvera ascorbata
Y1	Kluyvera ascorbata
M3	Kluyvera ascorbata
M2	Kluyvera ascorbata
M1	Escherichia coli, Kluyvera ascorbata
CO1	Escherichia coli, Kluyvera ascorbata

Table 4*. Reults of the microbiological parameters (TCO/FCO) at sampling point T8, at the entrance of the Tepatitlan wastewater treatment plant, 2020..*

Sample identification key	Indicator organisms (MPN/100ml)	
	TCO	FCO
T8	13’000,000	2100,000

Comparing the results, it can be seen that at points T4, AR3 and AC5 they exceed one million TCO, being the points closest to T8 with results of 9'200,000 (T4), 4'300,000 (AR3) and 1'700,000 (AC5), while AR2 far exceeds T8 with almost 70%. The surface water bodies that presented concentrations of FCO that exceed the maximum limits allowed in the current official regulations (NOM-001-ECOL-1996) were those of the municipalities of: Tepatitlán (T3, T4 and T6), Acatic (AC4, AC5 and AC6), Arandas (AR2, AR3 and AR4) and San Julián (SJ4). Taking into consideration the maximum permissible limit for FCO of 240 NMP/100 ml established in the Official Mexican Standard NOM-003-ECOL-1997 "reused water in services to the public with direct contact", the municipalities that comply were: Yahualica, San Miguel el Alto, Cañadas de Obregón, Jesus Maria and Valle de Guadalupe. In this standard is established the maximum permissible limits of contaminants for treated wastewater that is reused in public services, it is established, depending on the type of water reuse, maximum concentrations of FCO of 240 (services to the public with direct contact) and 1000 MPN/100 ml (services to the public with indirect or occasional contact); defining each type of reuse:

1. Services to the public with direct contact; it is the one that is intended for activities where the user public is exposed directly or in physical contact. The following reuses are considered: filling of recreational artificial lakes and canals with boat rides, rowing, canoeing and skiing; ornamental fountains, vehicle washing, irrigation of parks and gardens.

2. Services to the public with indirect or occasional contact; It is the one that is intended for activities where the general public is exposed indirectly or in incidental physical contact and that its access is restricted, either by physical barriers or security personnel. The following reuses are considered: irrigation of gardens and medians on highways, medians on avenues, ornamental fountains, golf courses, water supply

hydrants for fire-fighting systems, non-recreational artificial lakes, hydraulic security barriers and pantheons.

Considering this standard, the surface water bodies that exceed the limit of 240 MPN/100 ml for public reuse with direct contact were: Acatic (AC3), Jalostotitlan (J2), San Julian (SJ2), and Mexticacan (M1). The surface water bodies that presented quantities of FCO within the regulations were those of the municipalities of: Jesus Maria, San Ignacio Cerro Gordo, Mexticacan, Yahualica, Cañadas de Obregon, Valle de Guadalupe and San Miguel el Alto. These FCO values are comparable with the following sampling points: T4 (Rio Tepatitlan at the exit of the municipality), AR2 (Rio Arandas in the center of Arandas) and to a lesser extent AC5 (Rio Tepatitlan at the exit of Acatic), these high concentrations can be a potential risk to the health of the inhabitants. At the end of March 2022, the samples mentioned in the previous paragraph were sampled and analyzed, observing the following microbiological results (*Table 5*). Regarding FCO, the points that exceed the reference point T8 are T4 with 66.66% and AR2 with 28.57%, which constitutes a latent risk for the population of said localities.

Table 5*. Results of the three points with the highest presence of total and fecal coliform organisms in HSRJ (2022).*

Sample identification key	TCO (MPN/100ml)	FCO (MPN/100ml)
T4	11’300,00	4’250,000
AC5	18’400,000	2’100,000
AR2	22’000,000	1’900,000

The current official regulations for wastewater treated for reuse (NOM-003-SEMARNAT-1997) establish 240 to 1000 MPN of FCO/100 ml, for services to the public with direct contact and service to the public with indirect or occasional contact, respectively. When analyzing the data of this study, it was possible to notice that 61% (22 sampling points) do not exceed the established limits, so it has a quality comparable to treated wastewater for reuse in public service. The remaining 39% exceeded the permissible level for services to the public with direct contact. In the case of services to the public with indirect or occasional contact of the sampled points, 27.78% exceeds the number of fecal coliforms. There are studies that similarly present certain contamination by FCO in other places, for example, the results reported by Barrera-Escorcia et al. (2013), when analyzing the water bodies located in Patzcuaro, Xochimilco, Metztitlan and Zirahuen; it is found that more than 71% of the samples exceeded the permissible level for services to the public with direct contact and more than 43% with indirect or occasional contact.

However, the highest data found by them was 3,800 MPN of FCO/100 ml, while in table 9 it can be seen that in the Tepatitlan river at the exit of the municipality, 3’500,000 MPN of FCO/100 ml could be quantified, a figure very superior. Other studies of the microbiological quality of surface water bodies have also shown high counts of coliform bacteria. According to CONAGUA, the water quality of Bahía de Banderas/Puerto Vallarta between the years 2012-2015 presented a large amount of FCO, presenting up to 24,196 MPN/100 ml as a maximum value in Guayabitos Beach (La escollera), Rio Ameca (San Juan de Abajo), and Jarretaderas. On the other hand, in the Carretas dam in the same municipality of Tepatitlan, no total coliforms or fecal coliforms were detected, a quality that meets the criteria of the Modification to the Official Mexican Standard NOM-127-SSA1-1994 (Environmental health. Water for use and human consumption Permissible limits of quality and treatments to which the water must be submitted for its purification).

Enterobacteria are the family of the coliform group, an indicator widely used to detect microbial contamination of water. Coliforms are useful because many inhabit the digestive tracts of humans and other animals. Thus, its presence in the water indicates probable faecal contamination. Coliforms are defined as gram-negative, facultatively aerobic, non-sporulating rods that ferment lactose with gas production in less than 48 h at 35°C. However, this definition includes several bacteria that are not necessarily intestinal; for this reason, fecal coliforms are important in water safety assessments. Escherichia coli, a coliform whose only habitat is the intestine and which survives only a relatively short time outside it, is the key faecal coliform. The presence of E. coli cells in a water sample is taken as evidence of faecal contamination and means that the water is unfit for human consumption (Madigan et al., 2016). 63 species have been cited, the most common being: Escherichia, Shigella, Salmonella, Enterobacter, Klebsiella, Serratia, and Proteus (Carroll et al., 2016). Among the Enterobacteriaceae identified in this study are Escherichia coli, Salmonella spp, various species of Klebsiella, various species of Serratia, Kluyvera ascorbata, Enterobacter aerogenes and cloacae, Citrobacter freundii and amalonaticus.

There are several reports of the presence of Enterobacteria in surface water sources. In La Paz, Bolivia, the Choqueyapu River receives wastewater and urban, hospital, and industrial waste as it passes through the city. Escherichia coli, Salmonella enterica, Klebsiella pneumoniae and Shigella spp have been identified; specifically, enterotoxigenic E. coli (ETEC) strains were detected. Balcaza et al. (2020), studied the strains recovered from rivers and lagoons in Chaco Argentina, having determined that 14% of the isolated enteric bacteria were resistant to any of the antibiotics evaluated; According to these authors, although the percentage of resistant strains was low, it is worrying that water acts as a reservoir and means of transmission to man. The indiscriminate use of antibiotics to prevent and treat infections has led to the presence of resistance determinants. A study carried out by Mendoza Leon (2020), ensures that there is a broad relationship between antibiotic residues in different ecosystems and the increase in antibiotic-resistant bacteria. It is worth mentioning that in evaluations carried out previously of physicochemical parameters (2014 and 2016) such as: Dissolved Oxygen, Specific Conductivity, Salinity, Oxide-Reduction Potential, Ammonia Nitrogen and Chemical Oxygen Demand in the study area, the sampling points that present the greatest contamination coincide with those that in the present study resulted in the highest bacteriological loads and that correspond mainly to the municipalities of Tepatitlán (T3, T4, T5 and T6), Acatic (AC5) and Arandas (AR2 and AR3) (Villanueva, 2020).

Conclusion

In the present microbiological evaluation it is possible to appreciate the various degrees of organic contamination that exists in the bodies of water in the study area, with the following municipalities being the most contaminated: Tepatitlan with points T4 (Tepatitlan river at the exit of the municipality), T6 (tributary stream to the river) and T3 (Tepatitlan river in the center of the municipality); Acatic with point AC5 (Tepatitlan river at the exit of Acatic); and Arandas with points AR2 (Arandas river in the center of Arandas) and AR3 (Arandas river at the exit of Arandas). These municipalities being large producers in the region, in addition to the fact that Tepatitlan and Arandas have the largest population in the southern highlands (Jalisco). The high contents of coliform bacteria, as well as other organic and inorganic contaminants put the southern highlands on alert, evidencing the need to take care of the quality of the waters that are thrown into the bodies of water from industry, agricultural activities and by the population in general, which grows considerably over time.

Due to the fact that, by not being properly treated, the wastewater reaches the water bodies with a large amount of contaminants such as nitrogen and phosphorus, organic compounds and enteric bacteria, many of which are disease producers. With these already contaminated water resources, certain activities are carried out such as crop irrigation, animal husbandry and human recreational activities, thus closing the life cycle for these microorganisms, managing to reach a new host. Currently, of the 30 plants in the region, only 15 are operating. Being a region with vast economic and industrial activities, it is necessary to cover the highest percentage of plants in operation, in addition to the development of comprehensive water resources plan in order to reduce the impact on water resources and ecosystems dependent on it. It is of the utmost importance to continue conducting microbiological and physicochemical studies of the surface waters of the municipalities of HSRJ, to analyze the trend in the behavior of water, in which it is possible to measure the effects generated by population growth and economic, as well as actions and public policies in the comprehensive management of water resources, it being recommended that it be carried out at least every two years. As another recommendation, before the water is discharged into the surface water bodies, a previous treatment must be carried out because, as was observed in the present investigation, by not treating the water, a risk can be generated in the quality of the water of that zone. Likewise, involving institutions and research centers to be part of projects on the water issue will help to propose new alternative strategies for its treatment and reuse in a more ecological way.

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Conflict of interest

We declare that there is no conflict of interest between the authors of this study and any institution or previous publication.

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