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Microbiological quality assessment of treated municipal wastewater effluents for crop irrigation purposes

Evaluación de la calidad microbiológica de efluentes de aguas residuales municipales tratadas con fines de riego de cultivos

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ABSTRACT

Massive crop production requires enormous volumes of water for irrigation. Water scarcity has led growers to seek suitable alternative water sources. Treated wastewater for agricultural purposes has been documented; however, high microbial loads pose a high risk to consumers of contaminated produce. This study aimed to evaluate the microbiological quality of treated wastewater from three artificial canals belonging to two treated municipal wastewater plants in Sinaloa, Mexico. Fecal coliforms, *Escherichia coli*, and *Salmonella* concentrations were quantified in 60 treated wastewater samples using standard methods. Fecal coliforms and *E. coli* ranged from $4.0x10^2$ to $3.9x10^7$ CFU/100 mL and $1.0x10^2$ to $1.0x10^7$ CFU/100 mL, respectively. *Salmonella* was isolated from 45 samples, with the highest value of 16.14 MPN/L. Most of the examined samples exceeded the national and international permissible limits for fecal coliforms in water used for agricultural purposes. Consequently, treated municipal wastewater from central Sinaloa, Mexico, is unsuitable for agricultural irrigation of horticultural products.

KEY WORDS: Crops irrigation, *E. coli*, Fecal coliforms, *Salmonella*, Treated municipal wastewater.

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RESUMEN

La producción masiva de cultivos demanda enormes volúmenes de agua para el riego. La escasez de agua ha llevado a los productores a buscar fuentes de agua alternativas adecuadas. Se ha documentado el uso de aguas residuales tratadas con fines agrícolas; sin embargo, las altas cargas microbianas representan un alto riesgo para los consumidores de productos agrícolas contaminados. Este estudio evaluó la calidad microbiológica de las aguas residuales tratadas de tres canales artificiales pertenecientes a dos plantas de tratamiento de aguas residuales municipales en Sinaloa, México. Se cuantificó la concentración de coliformes fecales, *Escherichia coli* y *Salmonella* mediante métodos estándar en 60 muestras de aguas residuales tratadas. Los coliformes fecales y *E. coli* se cuantificaron de $4{,}0x10^2$ a $3{,}9x10^7$ CFU/100 mL y de $1{,}0x10^2$ a $1{,}0x10^7$ CFU/100 mL, respectivamente. *Salmonella* se aisló de 45 muestras, con el valor más alto de 16,14 MPN/L. La mayoría de las muestras examinadas excedieron los límites permiscibles nacionales e internacionales de coliformes fecales en agua para fines agrícolas. Por lo tanto, las aguas residuales municipales tratadas del centro de Sinaloa, México, no son aptas para el riego agrícola de productos hortofrutícolas.

PALABRAS CLAVE: Riego de cultivos, *E. coli*, Coliformes fecales, *Salmonella*, Aguas residuales municipales tratadas.

Introduction

Natural resources and climatic conditions make Mexico a country with vast and firm land for high-quality vegetables and grain production. Notably, there are regions of intensive output, such as the northwestern state of Sinaloa, Mexico, with the highest production and export of horticultural products, totaling up to 11,39 million tons in a cultivated area of 1,029 million hectares during 2022 (CODESIN, 2023). Nevertheless, water scarcity has compelled farmers to explore new strategies for crop irrigation, and one potential alternative is the use of treated wastewater. The wastewater in question is a combination of domestic and industrial effluents from commercial centers, public institutions, urban drainage, and agricultural and livestock sources. This wastewater undergoes sanitation treatment, transforming it into treated wastewater (TWW), which is subsequently discharged into surface water bodies via canals before reaching coastal water bodies.

In recent years, both treated and untreated municipal wastewater have garnered increased attention as reliable water resources for crop irrigation in drought-prone areas (Belay *et al.,* 2020). This practice is particularly prominent in low-income countries where farmers seek alternatives, and treated wastewater reuse emerges as a viable irrigation strategy to achieve

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sustainable development (WHO, 2006; Drechsel & Evans, 2010; Akpor & Muchie, 2011). Some countries, such as Jordan and Saudi Arabia, have developed national policies for reusing their treated wastewater effluents, making considerable progress in their implementation. It is evident that these countries have reliable tertiary wastewater treatment systems, with primary applications in agricultural (landscape and crop irrigation), commercial, and industrial services (Ouda, 2016).

China has implemented treated municipal wastewater (TMWW) in agriculture for decades, and millions of hectares are irrigated with those effluents annually. The widespread acceptance of TWW in agriculture is grounded in agronomic and economic needs; however, low-quality TWW can pose a severe public health risk, mainly for irrigating crops vulnerable to acquiring pathogens (Xie, 2009). Pathogenic microorganisms such as *Escherichia coli* pathotypes, *Salmonella* spp., *Campylobacter* spp., Norovirus, Adenovirus, Hepatitis A virus, *Cryptosporidium* spp., *Cyclospora cayetanensis*, and *Giardia lamblia* have been frequently found in TMWW (Yin & Patel, 2018). Furthermore, some disadvantages and health problems associated with TWW reuse have been documented, including skin diseases (Trang *et al.,* 2007) and diarrheal diseases (Ferrer *et al.,* 2012). Additionally, indirect studies employing quantitative microbiological risk analysis have demonstrated the potential health risks associated with TWW use in crop irrigation (Dickin *et al.,* 2016).

Studies conducted in the Mezquital Valley, Hidalgo, Mexico, have reported severe consequences for human health in the region due to untreated wastewater (Cifuentes *et al.,* 1994; Jiménez *et al.,* 2005). Among the most common health issues faced by the local population are parasitic infections, such as ascariasis (Jiménez *et al.,* 2005).

Therefore, growers and the government must implement measures to minimize adverse health and environmental impacts, including reliable water treatment technologies and public policies to reasonably use TMWW (Akpor & Muchie, 2011; Ouda, 2016). Indicator organisms such as coliform bacteria, *Escherichia coli,* and fecal streptococci serve as indirect indicators of fecal contamination and are associated with the potential presence of specific pathogens in wastewater (Paillard *et al.,* 2005). In Mexico, the regulatory normative establishes fecal coliforms, *Escherichia coli*, and helminth eggs as microbiological criteria for the suitability of sewage for its discharge in national water bodies, as well as its use for agricultural purposes such as irrigation of urban parks and various crops, including fodder, grains, and vegetables (NOM-001-ECOL-1996; NOM-001-SEMARNAT-2021; NOM-003-ECOL-1997). However, based on the NOM-001-ECOL-1996, the suitability of wastewater for agricultural use should have fecal coliform counts of less than $2x10^3$ MPN/100 mL or CFU/100 mL, without specific limits for pathogenic bacteria.

Two treated municipal wastewater plants, located in the Culiacan and Navolato municipalities of Sinaloa, Mexico, specifically in the cities of Culiacancito and Villa Adolfo López Mateos, respectively, are responsible for treating the majority of wastewater generated by Culiacan town and several surrounding cities and villages. The North plant in Culiacancito employs an advanced biological primary system and can deliver up to 1.533 L/s, while the Villa Adolfo López Mateos plant utilizes biological oxidizing-stabilization lagoons, producing 12 L/s. Together, both plants collectively produce approximately 133.488 m^3/24 h. The drainage system consists of outdoor

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artificial canals of both plants, which fuse and discharge their water toward the coastal region (Figure 1). Along these canals are adjacent farmlands cultivating vegetables and grains that have the potential to be irrigated with this water. However, sources of point and non-point contamination contribute to an increased microbial load in the canals. The use of improperly treated municipal wastewater for agricultural irrigation poses health risks to consumers. This research aims to assess the suitability of TMWW for agricultural irrigation by evaluating the concentrations of fecal coliforms, *Escherichia coli*, and *Salmonella* spp., thus determining the microbiological quality of water from the two treated municipal wastewater plants in Sinaloa, Mexico.

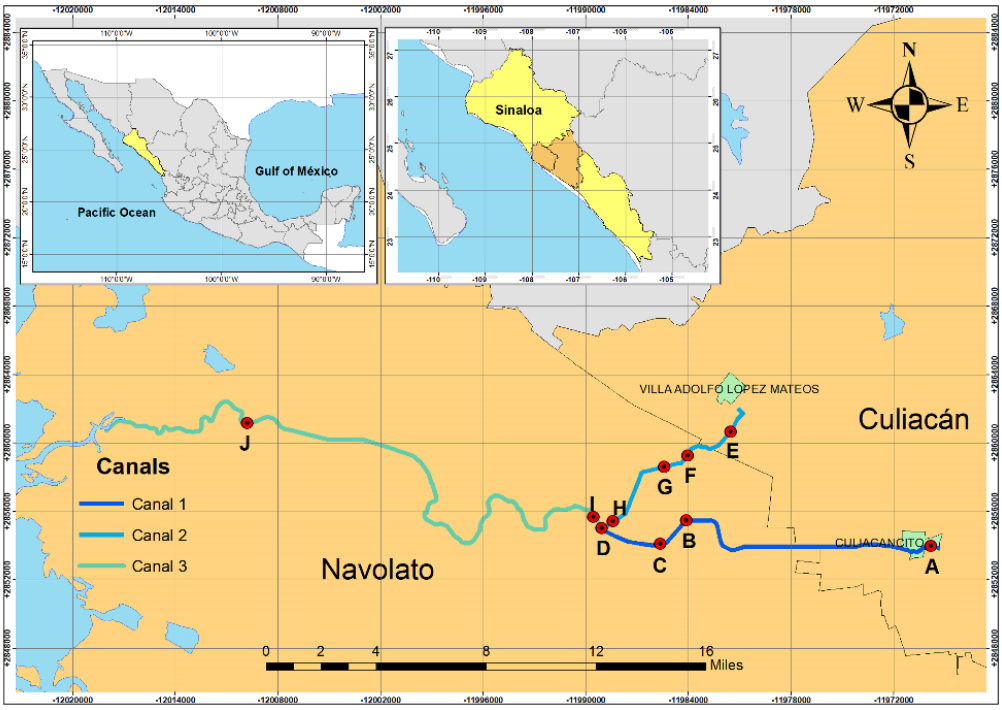

Figure 1. Geographical representation of the sampled points in the studied area.

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Material and Methods

Study setting

The study area encompasses the municipalities of Culiacan and Navolato in the state of Sinaloa, Mexico (see supplementary material Table 1). The North TMWW plant, situated in Culiacancito town (coordinates: 24° 49' 27" N; 107° 33' 08" W), receives nearly 85 % of the wastewater from Culiacan city. This plant employs an aerated lagoon system with a biological process and forced sedimentation, handling an influent of up to 1700 L/s and generating a discharge of 1533 L/s, which flows into the "Cedritos" artificial canal (referred to as canal 1). The "El Tamarindo" TMWW plant, located in the community of Villa Adolfo Lopez Mateos, Navolato (24°, 53', 26" N; 107°, 38', 16" W), is an oxidation pond plant (stabilization) and facultative anaerobic biological process, with an influent of 13 L/s and a discharge of 12 L/s, treating a daily volume of 1123 m3 (personal communication obtained from the Junta Municipal de Agua Potable y Alcantarillado de Culiacán office). The TMWW is discharged into the "El Papachal" canal (canal 2). Both canals follow their course and converge at the town of "El Paraiso" (after merging, they are collectively referred to as canal 3), which continues until it reaches its outlet in Santa Maria Bay, Navolato (Figure 1).

Sample collection

Treated municipal wastewater sampling and microbiological analysis were carried out between November 2018 and April 2019, corresponding to the period of most intensive agricultural activity and high demand for irrigation water for crops, including grains and vegetables. A total of 60 samples were collected, with one sample taken each month from 10 geographically referenced sampling points along the three canals leading to the coastal region of Navolato (supplementary material Table 1). One liter of treated wastewater in every sampling point was collected using polypropylene sterile bottles according to the sampling method described in PROY-NMX-AA-003/3-SCFI-2008. The samples were transported at 4 °C to the Laboratorio Nacional para la Investigación en Inocuidad Alimentaria (LANIIA) from the Centro de Investigación en Alimentación y Desarrollo, A.C. (CIAD), Culiacan station. The analysis was conducted within 24 hours of sample collection.

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Fecal coliforms and *E. coli* quantification

Fecal coliforms (FC) and *E. coli* analysis were performed using the membrane filtration method, following the Mexican standard NMX-AA-102-SCFI-2006, with some modifications. Briefly, the samples underwent serial dilutions (1:10) in buffered-phosphate solution (pH 7.2) and were then filtered through sterile 0.45 µm nitrocellulose membranes (GN-6 Metricel, Pall Corp., NY, USA). Subsequently, membranes were incubated in ECC chromogenic agar (CHROMagar™ ECC, Paris, France) for 24 h at 44 °C. Simultaneous quantification of *E. coli* and fecal coliforms was determined based on colony color differentials, as indicated by the manufacturer. FC was confirmed by gas production as indicated by the rising of Durham on Lactose-Peptone Broth (PLB Medium, Difco, Madrid, Spain) after 24-48 h incubation at 44.5 °C. *E. coli* was confirmed by gas production through inoculating presumptive colonies on EC broth (EC, Difco, Madrid, Spain) and incubation at 37 °C for 24 h. At the same time, presumptive colonies were inoculated in tryptone broth, followed by the addition of Kovacs reagent to test for indole production.

Salmonella quantification

The Most Probable Number (MPN) method, following protocol No. 1200 established by the United States Environmental Protection Agency (US-EPA, 2012), was employed for enumerating *Salmonella*. Different concentrations of Tryptic Soy Broth (TSB) series were prepared. To each of these, 20, 10, and 1 mL of TMWW samples were added, followed by incubation at 36 °C ± 1.5 °C for 24 hours. Subsequently, from the tubes exhibiting bacterial growth, 30 µL drops were spread onto Modified Semi-solid Rappaport-Vassiliadis Agar (MSRV, BD-DIFCO, Maryland, USA) and incubated at 42 °C for 24 hours. Colonies with *Salmonella* characteristics were isolated and streaked onto Xylose-Lysine Deoxycholate Agar (XLD, Bioxon, Mexico), and incubated for 24 hours at 37 °C. Colonies displaying *Salmonella* characteristics on XLD agar were cultivated on Tryptic Soy Agar (TSA), and biochemical tests were carried out using a set of Agar-Iron-Lysine (LIA-Difco Medium, Madrid, Spain), Triple Sugar Iron Agar (TSI-Medium, Difco, Madrid, Spain), and Urea Broth (Urea Broth, Difco Madrid, Spain). Biochemical tests were incubated at 37 °C for 24h, and presumptive *Salmonella* spp. were identified.

PCR *Salmonella* confirmation and serotyping

The end-point polymerase chain reaction (PCR) was carried out according to the previously designed and validated protocol in our laboratory (data not shown) using the *pfk* gene oligonucleotides sense-5'-ACACCTCCTCTTCTCACCAGCGTATC-3, and the antisense-5'-CGGCTTTGATTTCCGCCACCAGA-3' (Thermo Fisher Scientific, Waltham, MA, USA). Briefly, DNA extraction was conducted from presumptive *Salmonella* pure colonies (c.a. $1X10^6$ CFU) inoculated in 0.6 mL sterile tubes containing 100 µL of nanopure water (PISA, Mexico). The sample was vortexed for one minute and then lysed by thermal shock in a thermal cycler at 94 °C for 15 min, followed by ice incubation for 10 min. The cell lysates were centrifuged at 6.700 *x g* for 5 min, and the supernatant was recovered. The DNA concentration was adjusted to a final concentration of 50 ng/µL using a NanoDrop 2000c Spectrophotometer (Thermo Fisher

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Scientific, Waltham, MA, USA).

Ten microliters PCR reactions mix were made (Taq® DNA Pol 0.5 U, free $MgCl_2$ amplification Buffer 1.0 X, $MgCl_2$ 1.5 mM, dNTP's mix 400 mM [Promega®, USA], each sense and antisense primers 200 mM, 1 µL of the DNA sample (c.a. 50 ng), and nuclease-free water to reach a final volume of 10 µL). The PCR reactions were carried out in a Mastercycler® gradient thermal cycler (Eppendorf, Hamburg, Germany), including an initial step for 5 min at 95 °C for DNA denaturation; subsequently were included 30 cycles of 50 s at 95 °C, 50 s at 60 °C, and 1 min at 72 °C. A final 5 min cycle at 72 °C for final extension was carried out, and the reaction stabilization was at 4 °C. PCR products were immediately analyzed through electrophoresis in 0.8% agarose gels (Promega®, USA) in 1X TAE buffer (Eppendorf®, USA) for 40 minutes at 70 V and stained with GelRed®. Samples were considered positive when an amplified product of 170-180 bp was observed (suppl. material figure 1). From each sample, three to six PCR-*Salmonella* confirmed strains were selected and serotyped according to the Kauffmann–White scheme (Grimont & Weill, 2007) in the Public Health Laboratory, Faculty of Medicine, National Autonomous University of Mexico.

Results

Indicator bacteria counts

Fecal coliforms (FC) were found in 100 % of the 60 analyzed samples (Table 1). Canal 1 had the higher FC concentrations, fluctuating between $8.0x10^5$ CFU/100 mL (sampling point C in February) to $3.9x10^7$ CFU/100 mL (sampling point A in November). Canal 2 exhibited FC counts ranging from $4.0x10^2$ CFU/100 mL (sampling point E in March) to $1.0x10^6$ CFU/100 mL (sampling point F in November). Finally, canal 3 displayed FC levels fluctuating from $4.0x10^2$ CFU/100 mL (sampling point J in January) to $6.0x10^6$ CFU/100 mL (sampling point I in March); with the latter count recorded immediately after the merging of canals 1 and 2. The highest FC counts were quantified in November, corresponding to sampling point A in canal 1, the nearest point to the North-TMWW plant.

Escherichia coli was also quantified in 100% of the 60 analyzed samples (Table 1). The higher *E. coli* counts were recorded in canal 1, with counts between $4.0x10^5$ CFU/100 mL (sampling points B in March and point D in February) and $1.0x10^7$ CFU/100 mL (sampling point A in November). In canal 2, *E. coli* concentrations fluctuated from $3.4x10^2$ CFU/100 mL (sampling point G in April) to $1.3x10^5$ CFU/100 mL (sampling point G in January). In canal 3, the values fluctuated from $1.0x10^2$ CFU/100 mL (sampling point J in February) to $2.7x10^6$ CFU/100 mL (sampling point I in November).

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Table 1. Fecal coliforms and *Escherichia coli* concentrations in analyzed TMWW by location and period time evaluated.

Canals	Sampled point	Months/Fecal coliforms[§] and *Escherichia coli*[£] concentrations (CFU/100 mL)					
		Nov	Dec	Jan	Feb	Mar	Apr
Canal 1	A	$3.9x10^{7§}$ $1.0x10^{7£}$	$5.3x10^{6}$ $4.4x10^{6}$	$3.1x10^{6}$ $7.0x10^{5}$	$7.5x10^{6}$ $1.0x10^{6}$	$8.25x10^{6}$ $7.0x10^{5}$	$7.4x10^{6}$ $8.2x10^{5}$
	B	$6.5x10^{6}$ $5.2x10^{6}$	$2.3x10^{6}$ $6.0x10^{5}$	$4.7x10^{6}$ $1.4x10^{6}$	$8.0x10^{6}$ $6.1x10^{5}$	$2.25x10^{6}$ $4.0x10^{5}$	$3.2x10^{6}$ $5.0x10^{5}$
	C	$9.2x10^{6}$ $5.7x10^{6}$	$5.9x10^{6}$ $5.0x10^{5}$	$4.4x10^{6}$ $1.1x10^{6}$	$8.0x10^{5}$ $5.4x10^{5}$	$3.0x10^{6}$ $9.0x10^{5}$	$3.4x10^{6}$ $9.2x10^{5}$
	D	$1.0x10^{7}$ $6.7x10^{6}$	$7.2x10^{6}$ $2.3x10^{6}$	$7.8x10^{6}$ $8.0x10^{5}$	$6.3x10^{6}$ $4.0x10^{5}$	$4.0x10^{6}$ $5.0x10^{5}$	$4.5x10^{6}$ $6.3x10^{5}$
Canal 2	E	$1.3x10^{5}$ $8.0x10^{2}$	$6.7x10^{4}$ $2.1x10^{3}$	$5.8x10^{4}$ $4.0x10^{3}$	$2.5x10^{3}$ $2.0x10^{3}$	$4.0x10^{2}$ $4.0x10^{2}$	$5.3x10^{2}$ $5.2x10^{2}$
	F	$1.0x10^{6}$ $8.0x10^{2}$	$9.8x10^{3}$ $9.0x10^{3}$	$6.0x10^{2}$ $5.0x10^{2}$	$7.7x10^{3}$ $2.9x10^{3}$	$8.0x10^{2}$ $7.0x10^{2}$	$7.3x10^{2}$ $4.5x10^{2}$
	G	$1.2x10^{5}$ $5.1x10^{3}$	$5.4x10^{3}$ $4.0x10^{3}$	$7.7x10^{5}$ $1.3x10^{5}$	$9.5x10^{3}$ $1.0x10^{3}$	$5.5x10^{2}$ $4.0x10^{2}$	$4.8x10^{2}$ $3.4x10^{2}$
	H	$6.7x10^{4}$ $3.3x10^{3}$	$6.7x10^{4}$ $5.3x10^{4}$	$2.0x10^{3}$ $1.8x10^{3}$	$2.7x10^{3}$ $2.7x10^{3}$	$3.5x10^{3}$ $1.4x10^{3}$	$2.0x10^{3}$ $4.0x10^{2}$
Canal 3	I	$4.1x10^{6}$ $2.7x10^{6}$	$6.0x10^{5}$ $2.5x10^{6}$	$5.1x10^{6}$ $1.7x10^{6}$	$2.6x10^{6}$ $2.2x10^{5}$	$6.0x10^{6}$ $7.0x10^{5}$	$5.7x10^{6}$ $5.4x10^{5}$
	J	$4.6x10^{3}$ $1.2x10^{3}$	$7.6x10^{3}$ $3.0x10^{2}$	$4.0x10^{2}$ $2.0x10^{2}$	$5.0x10^{2}$ $1.0x10^{2}$	$8.5x10^{2}$ $8.5x10^{2}$	$7.0x10^{2}$ $3.0x10^{2}$

Salmonella quantification

Salmonella was detected in canal 1 during all sampling months, with the highest concentration of 16.14 MPN/L recorded at sampling point B in November. In contrast, the lowest *Salmonella* counts were recorded in canals 2 and 3 during the December sampling period (<0.6473 MPN/L). Interestingly, regardless of the sampling period, the *Salmonella* concentrations of canal 1 were consistently exceed those in canal 2 (Table 2). In November, canal 3 also showed the highest *Salmonella* values when it reached 14.3 MPN/L (sampling point I, immediately after joining canals 1 and 2). The data suggests that the higher *Salmonella* counts in canal 3 were primarily contributed by canal 1. Regarding sampling months, in November, the highest *Salmonella* concentrations were found, like FC and *E. coli* concentrations.

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Table 2. *Salmonella* concentration in MPN/L and serotypes by location and period evaluated.

Canals and sampling points		*Salmonella* MPN/L[¥] and serotypes[€] by months of sampling					
		Nov	Dec	Jan	Feb	Mar	Apr
Canal 1	A	12.45[¥] Enteritidis[€] Senftenberg	0.65 Agona	9.84 Goerlitz	3.97 Give	5.5 Give	2.79 Give
	B	16.14 Goerlitz	1.3 Goerlitz	7.14 Goerlitz	2.02 NS	3.6 Muenchen	4.57 NS
	C	8.22 Agona	0.65 Agona	5.01 NS	4.57 Enteritidis	<0.6473 Give	2.26 Agona
	D	12.53 Give	<0.6473 ND	11.81 Give	5.9 NS	1.8 Agona	3.39 ND
Canal 2	E	0.484 Muenchen	<0.6473 ND	0.234 NS	0.0775 NS	<0.6473 Saphra	<0.6473 ND
	F	3.01 NS	<0.6473 ND	0.456 NS	0.312 NS	10.3 Oranienbur Saphra	0.0325 Javiana
	G	2.02 NS	<0.6473 ND	1.39 Sandiego	0.036 NS	0.4 NS	0.0325 NS
	H	0.64 NS	<0.6473 ND	2.02 Senftenberg	2.09 NS	0.18 NS	0.144 NS
Canal 3	I	14.3 NS	<0.6473 ND	3.01 Muenchen	3.39 Oranienburg	3.9 Gatineau	4.8 Give
	J	8.1 Sandiego	<0.6473 ND	3.28 Sandiego	0.0775 Senftenberg	<0.6473 ND	<0.6473 ND

ND: Non-detected serotypes; NS: Non-selected for serotyping

From forty-five PCR-confirmed *Salmonella* strains, serotyping was reached in 33, corresponding to 11 *Salmonella* serotypes, with different frequencies and various sampling points throughout the sampling period (Table 2). Detected serotypes were Give (7 strains), Agona (5 strains), Goerlitz (4 strains), Muenchen, Senftenberg, and Sandiego (3 strains each), Enteritidis, Saphra, and Oranienburg (2 strains, each), Gatineau and Javiana (one strain, each).

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Discussion

In our study, fecal coliforms and *E. coli* exceeded the maximum limits allowed in most sampling points and periods, where a maximum of $2x10^3$ CFU/100 mL of fecal coliforms for agricultural irrigation is permissible according to Mexican and WHO regulations (NOM-003-ECOL-1997; NOM-001-ECOL-1996; NOM-001-SEMARNAT-2021; WHO, 2006). Similarly, the European Economic Community established that fecal coliforms must be less than $2x10^3$ CFU/100 mL for irrigation purposes in raw-eaten crops, sports fields, and public parks.

Our results demonstrate that canal 1, belonging to the North plant, consistently maintained the highest fecal coliform, *E. coli*, and *Salmonella* loads during the study at all sampling points. This indicates that canal 1 is the primary source of microbial pollution discharged into the system. While canals 2 and 3 exhibited lower microbial loads, none of them complied with national regulations. Therefore, based on our findings and normative recommendations, this water does not meet the criteria for the irrigation of vegetables. Although wastewater and drinking water undergo treatments to eliminate pathogenic microorganisms and prevent water-transmitted diseases, studies suggest that conventional wastewater treatment does not guarantee their complete elimination (Howard *et al.,* 2004). Advanced biological primary systems are expected to be better than biological stabilization lagoons (Rodríguez-Miranda *et al.,* 2015); however, in our investigation, we observed some point and non-point contamination sources through both canals but higher in canal 1 (for example, streams, agricultural field waste, and slaughterhouses discharges). Although we could not confirm the effects of point and non-point contaminations, it could influence higher microbiological contamination in canal 1.

Other phenomena could influence the microbial loads in water bodies at specific times. For instance, in December, the FC and *E. coli* counts decreased between one and two exponential units. In this regard, it is important to mention that torrential rain fell in the center of Sinaloa on December 4th, 2018 (three days before sampling), which could have caused the bacterial loads to be washed into the canals, leading to lower microbial counts in that particular month.

In our study, *Salmonella* was present in almost 50 % of the TMWW samples (as confirmed by *Salmonella* counts and serotypes detection, Table 2). Notably, there was a significantly higher concentration in canal 1, whereas the values for canal 2 were considerably lower. Among the selected *Salmonella* strains from every positive sample, it was possible to identify at least one serotype, and in some cases, multiple serotypes were identified within a single sample.

In terms of *Salmonella* presence, there are no specific Mexican regulations for maximum permissible limit; however, Wilkes *et al*. (2009) showed *E. coli* and fecal coliform to be the most appropriate indicators of the presence of *Salmonella*, compared to *Clostridium perfringens*, *Enterococcus*, and total coliform. TThey identified an *E. coli* threshold of 89 CFU/100 mL, which correlated with identifying around 10% of *Salmonella*-positive samples. Additionally, Paillard *et al*. (2005) mentioned that indicator organisms such as coliform, *Escherichia coli*, and fecal streptococci correlate with the possible presence of a particular pathogen in wastewater.

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Several of the *Salmonella* serotypes identified in our investigation were previously reported in rivers and river-derivative irrigation canals (Burgueño-Roman *et al*., 2019) and tomato fruits (Estrada-Acosta *et al*., 2014) in Culiacan, Sinaloa. These findings support the idea that irrigation water contaminated with *Salmonella* could lead to fruit contamination in the field. This phenomenon has potential repercussions: the occupational risk of agricultural laborers due to direct contact with TMWW with the presence of pathogenic bacteria, the possible food contamination, and the consequent threat to consumers. This information is also valuable since it provides new knowledge about the incidence and incidence of *Salmonella* serotypes in TMWW from Culiacan, Sinaloa, Mexico.

In a recent study by Yanagimoto *et al*. (2020) in Japan, 71 out of 72 analyzed samples (99 %) were positive for *Salmonella*. They obtained 689 *Salmonella* isolates belonging to 38 serotypes, including ten non-typeable strains. The most common serotype was Schwarzengrund (11 %), followed by Anatum (9 %) and Newport (5 %).

Several *Salmonella* serotypes found in our study (Enteritidis, Senftenberg, Agona, Give, Muenchen, Oranienburg, Javiana, and Sandiego) have previously been reported by the United States Centers for Disease Control and Prevention (US-CDC) as responsible for salmonellosis outbreaks. The potential *Salmonella* contamination sources were irrigation water and contaminated vegetables from various locations in Mexico (CDC, 2018). Mohle-Boetani et al. (1999) reported *Salmonella* Saphra as the cause of a salmonellosis outbreak in California in 1997, associated with consuming Cantaloupes grown and packed in Altamirano, Guerrero, Mexico. Although the exact origin of the contamination could not be identified, this study underscores the risk of infection from consuming foods contaminated with *Salmonella* serotypes similar to those found in our investigation. Other studies have reported *Salmonella* outbreaks by consuming fresh produce irrigated with poor-quality water (Jung *et al*., 2014; Adegoke *et al*., 2018).

While the health risks of reusing wastewater in agricultural activities have been studied, most studies have estimated infection risk based on total bacterial loads in sewage. Pathogenic bacteria in the field have received relatively little attention (Farhadkhani *et al*., 2018). Therefore, it is crucial to monitor pathogenic bacteria concentrations at various irrigation stages and in cultivated crops to assess potential human health risks associated with wastewater use.

Conclusion

Fecal coliforms and *E. coli* concentrations in most of the TMWW analyzed exceeded $1x10^3$ CFU/100 mL, which is the highest permissible limit for water used in agriculture according to Mexican regulations. Additionally, the presence of *Salmonella* concentrations and serotypes at various monitoring points indicates potential health risks for individuals who may consume fresh vegetables irrigated with this water. Although national regulations do not specify minimum limits for *Salmonella*, most international laws require non-detectable *Salmonella* in irrigation water. Therefore, this investigation demonstrated that TMWW from central Sinaloa, Mexico, is unsuitable for agricultural irrigation.

Authors contribution

Work conceptualization: author 2, author 9; Methodological performance: author 1, author 3, author 4, author 9; Software management: author 5, author 6; Experimental validation: author 1, author 4, author 9; Results analysis: author 1, author 4, author 5, author 9; Data management: author 1, author 9; Manuscript writing and preparation: author 1, author 5, author 6, author 9; Reading, revision, and editing: author 2, author 3, author 4, author 5, author 6, author 7, author 8; Project manager: author 2, author 9; Funding acquisition: author 2. All the authors read and accepted the public version of this manuscript: GRA, CC, NOA, MFJA, GGJP, GLI, SVNP, CCN, and LCO.

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Declaration of interests

The authors declare that there are no conflicts of interest.

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