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Aeromonas hydrophila in surface water and their removal using a POU technology for drinking in rural communities

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ABSTRACT

Aeromonas hydrophila is a common emerging pathogenic bacterium in natural waters that affects the quality of drinking water. This becomes a challenge for the development and innovation of alternative technologies that provide safe water to families in rural communities. The present study evaluated the ability to inactivate natural *A. hydrophila* from surface waters using a drinking water treatment system at the point of use (POU). It is based on 2 thermofused polypropylene filters followed by a UV disinfection lamp, and it was installed in houses in two rural communities in Colombia. Experimental results showed that the POU systems removed *A. hydrophila* with a log reduction value (LRV) of 3.60 ± 0.02 in the synthetic waters, and removal values greater than 2 LRV in tests performed with natural surface waters with detected naturally occurring *Aeromonas* and quantified in these communities with LRV of 3.1 ± 0.6 and 2.2 ± 0.6, respectively, with a detection limit of 1 CFU/100mL. The concentrations of *E. coli* and total coliforms (TC) were also monitored in 54 homes all equipped with the above-described POU systems for 12 months. Given the efficacy found in this study and the availability, accessibility, and affordability of the elements used to design and manufacture the POU systems, it is feasible to propose their use to provide safe water to vulnerable families living in rural communities that lack water treatment systems.

1. Introduction

Access to safe water is fundamental for a sustainable and dignified life. Access to it in good conditions of quality, quantity, accessibility, and affordability is very limited by economic and geographic reasons (Pessoa et al., 2019). Globally, 2.1 billion people didn't have access to safely managed drinking water in 2020. Instead, they relied on untreated and non-protected water sources, which are frequently contaminated. This issue is exacerbated in rural areas, where still 8 in 10 people lack access to improved water services worldwide (WHO\UNICEF, 2021). In Colombia, 80.5% of the urban population and 40.2% of the rural have access to improved drinking water by 2020 (Anon, 2020). The existing rural drinking water treatment plants have been reported to have a high risk (45%) of quality failures; while, in most cases, treatment plants don't even exist (Zambrano, 2020). The Colombian Ministry of Health reports that about 1300 children die each year from diarrheal diseases caused by drinking poor-quality water (Bedoya et al., 2021). The drinking water supply has played a fundamental role in reducing the incidence of many waterborne infectious diseases. However, water contamination influences the availability of the resource for human consumption. For this reason, it is necessary ensuring the adequate treatment of water for human consumption by installing and operating proper drinking water treatment systems in rural populations (Herschy, 2012).

The genus *Aeromonas* comprises a group of bacteria ubiquitous in freshwater rivers and lakes (Chaix et al., 2017; Khor et al., 2015). It is widespread in natural habitats such as soil, fresh and brackish waters, in warmer climates (Seidler et al., 1980). The presence of *Aeromonas* has also been reported in sewage and wastewater in general (Kühn et al., 1997). The importance of detecting *Aeromonas* has increased due to public health concerns and their prevalence and distribution in natural water (Razzolini et al., 2008) and food (Kirov, et al., 1990), as well as their potential pathogenicity (Cisneros-Sureda et al., 2022). *Aeromonas* has been isolated from different food samples (Kirov et al., 1990) and has been frequently found in chlorinated and non-chlorinated water (Maes et al., 2019; Fish and Boxall, 2018; Janda and Abbott, 2010;

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Razzolini et al., 2008; Uyttendaele et al., 2004). *Aeromonas* are considered emerging pathogens responsible for health issues for example gastroenteritis, septicemia, or wound infections. Initially thought to be an opportunistic pathogen of low virulence, it is now recognized as a primary pathogen in food and water (Cisneros-Sureda et al., 2022).

Enteropathogenic *Aeromonas* can produce potential infections and increase disease factors. They have been found in domestic water supplies even after chlorination (Chopra et al., 2009; Chauret C. et al., 2001; Massa et al., 1999). *Aeromonas* are environmental bacteria reported as frequent inhabitants of biofilms in the distribution pipes of chlorinated and non-chlorinated water (Bomo et al., 2004). Some studies reported that treating these biofilms using chlorine is difficult due to the protection provided by the persistent polysaccharides that coat this bacterium (Torres-Armendariz et al., 2015). Williams and Braun-Howland (2003) found that the recommended dose of HClO dose of 1 ppm is not enough to inactivate this bacterium in biofilms, which could help explain the difficulties encountered in removing this group of bacteria in the water networks.

A. hydrophila caused several gastroenteritis outbreaks via contaminated drinking water contamination within the range between 1 and 10^4 CFU/100mL (Lontsi et al., 2013; Silvestry-Rodriguez et al., 2008; Fernandez et al., 2000). Their high prevalence in drinking water systems can be attributed to benthic growth in sediments and/or the growth of biofilms on pipe walls, followed by transmission to water (Liu et al., 2014). This proliferation is favored by organic matter, temperature, high contact time, and the absence of residual chlorine in water treatment systems (Liu et al., 2014).

A. hydrophila has been included on the Candidate Contaminant List (CCL) reported by the United States Environmental Protection Agency (US EPA) (Embrey et al., 2002) as an 'agent of concern' in drinking water. Canada and USA request the *Aeromonas* monitoring in bottled water (EPA, 2006), while the Netherlands uses *Aeromonas* as a quality criterion for potable water, which is limited to values below 20 CFU/100mL (median value over 1 year) (Figueras and Beaz-Hidalgo, 2014; Standards, 2000; Van der Kooij and Hijnen, 1988).

Although the global incidence of human infections caused by *Aeromonas* is unknown, incidence rates of 10.5 cases per million inhabitants were reported in England in 2004 and 1.5 cases per million inhabitants in France in 2006 (Lamy et al., 2009). The presence of *Aeromonas* in water for human consumption has been demonstrated as a public health issue. Estupiñan et al. (2017) reported the presence of *Aeromonas* in 63% of the public pools analyzed in Bogotá (Colombia). Didugu et al. (2015) determined the presence of *Aeromonas* by PCR in 125 water samples (well, tap, and bottled) in the Municipal Corporation of Greater Hyderabad Telangana (India). They reported the presence of *Aeromonas* in 52% of the samples from well water, 20% in tap water and 16% in bottled water, with an overall prevalence of 28.8% (Didugu et al., 2015). The presence of *A. hydrophila* in rural waters and the detection of *A. hydrophila* strains resistant to ampicillin, tetracycline, chloramphenicol, and sulfonamides has been also reported (Khan et al., 2012). Recently, metataxonomic studies carried out by Bedoya et al. (2021) in Curití, La Linda, and El Carmelo (Colombia), analyzed the microbiota of surface waters used for consumption and reported the presence of bacteria such as *Legionella, Mycobacterium, Yersinia, Burkholderia, Rickettsia, Aeromonas, Streptococcus, Staphylococcus, Corynebacterium, Treponema, Leptospira, Escherichia / Shigella*. These are same communities where this research was performed. They evidenced the risk for the communities supplied by these water sources and the need to include *Aeromonas* monitoring and inactivation in any program to protect and provide safe drinking water in these communities (Bedoya et al., 2021).

Some factors limit the use of technologies that provide safe water to rural communities. In many cases, the availability, accessibility, and affordability of technologies that currently provide safe water efficiently are unknown, which limits their compliance with what is required established in the Sustainable Development Goal number 6 of the United Nations. Many of these technologies have not been deployed in a rural or a low-income context, due to socio-economic, geographic, and political issues. Other limitations are related to engineering and market aspects. Most of the potabilization products and technologies have not been specifically designed to operate and be sustainable in low-income communities. In these scenarios, poor electricity supply, lack of simple components for the water distribution systems on-site, or lack of skills and capacities for routine and period maintenance and microbial monitoring in the field make impossible the use of these technologies (Baldasso et al., 2021). The most used POU technologies for low-income communities include simple and low-cost solutions like chlorination, filtration, solar disinfection, and UV lamps, usually applied to reduced volumes of water (2 to 20 L) with limited efficiency, depending on the method (Clasen et al., 2007).

In this work, we have used a combination of them, i.e., a sedimentation tank pre-treatment, followed by multiple filtration (5 and 1 μm) and an UVC lamp. This POU system was built in 54 rural households providing the potable water required for each family (150 L d^{-1} $house^{-1}$). This system has been proven to improve the quality of surface water to potable (Pichel et al. 2021); its economic, technical, and environmental sustainability is still under investigation. This contribution shows the evaluation of 6 POU water treatment systems, installed in households of Curití and El Carmelo communities, for the removal of *Aeromonas* from surface waters. This was initially evaluated under ideal conditions, without other microorganisms neither organic nor inorganic matter, to determine the susceptibility of *Aeromonas* to UVC; prior to challenging the POU for testing in the field. Then, the POU was assessed for 3 months under real conditions in the field using similar POU systems for disinfection of natural surface water spiked with *Aeromonas*. The objective was to determine the efficacy of this POU to remove *Aeromonas* beyond the standard quality indicators, *E. coli* and TC, and provide drinking water quality to the users minimizing their exposure to this emerging pathogen.

2. Materials and methods

2.1. Household point-of-use (POU) system

The POU system (Fig. 1) used for this work was installed in the lab, for testing under controlled conditions, and in the households for field testing. It consists of a sedimentation tank of 20 L (laboratory system) or 150L (field system), followed by a filtration unit consisting of 2 pleated 2 × 10-inch thermofused polypropylene filters (Purikor, Mexico) of 5 and 1 μm pore size respectively, and followed by a UV disinfection commercial unit (Evans®) for drinking water equipped with a 16 W LP-UVC lamp, 5.2 mW/cm^2 (λ=254 nm). The UVC fluence of the flow system, or UVC irradiance per surface area (mW/cm^2), was determined by KI-iodate actinometry (Rahn, 1997), which determines the irradiance in the photoreactor at 254 nm. The actinometry method is reported in the supplementary data. The treated water was safely stored in a closed tank of 20 L (lab tests) or 150 L (field tests). The POU system worked as one flow system operated with a flow rate of 3.8 L/min using a diaphragm pump (Seaflo SFDP1-012-035-21, China).

2.1.1. POU maintenance activities

The maintenance of the POU systems included cleaning the tanks and filters periodically. For the raw water tanks, it was recommended to wash the bottom and walls of the tanks every 15–30 days depending on the quality of the raw water, which varied seasonally. For the treated water tanks, the walls, bottom, and lid were cleaned with a damp cloth rinsed in a diluted chlorine solution, after that the tanks were rinsed with plenty of treated water to remove the excess chlorine. For the dirty filters, they were washed with treated water until the excess dirt was removed. Washed filters were reused until clogged, in that case they were disposed and replaced by new ones.

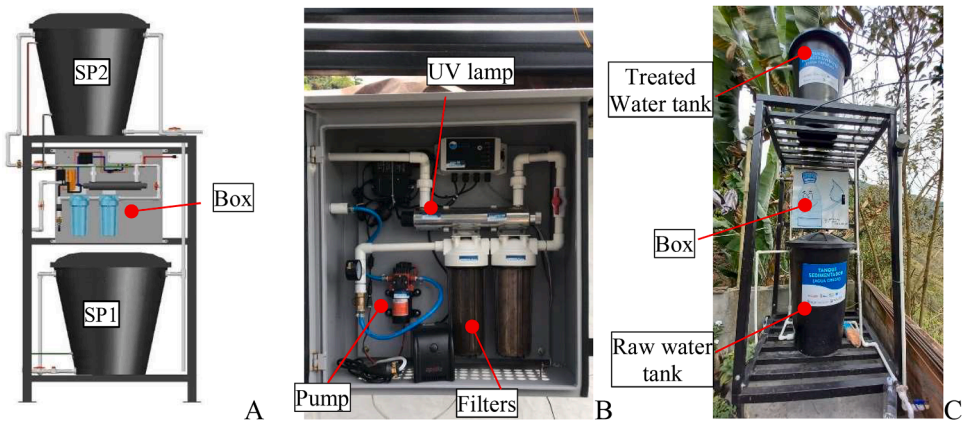

Fig. 1. SAFEWATER POU system (A) general diagram, indicating the sampling points SP1 in the raw water tank (RWT), before sedimentation, and SP2 in the treated water tank (TWT), and box (B) box containing the pump, filters, UV-lamp and electrical connections, (C) SAFEWATER POU system on a large scale installed in a house in Curití, Antioquia.

2.2. Lab testing of POU system with synthetic water

The lab prototype of the POU system was evaluated with synthetic water prepared with saline solution NaCl 0.9% spiked with 10^3 CFU/100mL of *A. hydrophila*, similar to those reported in natural waters (Lontsi et al., 2013; Silvestry-Rodriguez et al., 2008; Fernandez et al., 2000).

Nine water treatment trials were conducted in triplicate, with a volume of 20 liters of synthetic water. To determine the efficiency of the treatment system, water samples were taken from the raw water tank (RWT, SP1 point) before any treatment, and the treated water tank (TWT, SP2 point) to quantify the concentration of *Aeromonas hydrophila* (Fig. 1). To evaluate the effect of storage temperature on the regrowth of *A. hydrophila* in the treated water (SP2), *A. hydrophila* counts were performed at 24 and 48 hours with samples stored in the dark at room temperature (24 ± 2 °C) and under refrigerated conditions (8 °C).

2.3. Field testing of the POU system

Six POU systems were installed in two rural communities: Curití (Liborina; 6°40′59″N, 75°48′0″W) and El Carmelo (El Peñol; 6°13′08″N, 75 ° 14′31″ W) (Antioquia, Colombia). Three of them were installed in El Carmelo (CaVi01, 6°11′38.63"N, 75°14′41.97"W; CaVi02, 6°11′36.22"N, 75° 14′55.71"W; CaVi03, 6°11′35.44"N, 75°14′43.42"W) that are supplied with surface water from the El Pozo micro-basin and three systems in the rural community of Curití (CuVi01, 6°40′19.67"N, 75°48′1.44"W; CuVi02, 6°40′15.98"N, 75°48′2.80"W; CuVi03 6°40′20.85"N, 75°47′58.61"W) which are supplied with surface water from the El Obo, Fundungo and El Guineal streams that are stored in a tank that distributes the water to the homes. To monitor the effectiveness of the POU systems, monthly monitoring was carried out in quadruplicate for a period of 3 months between September and November 2021. Water samples were taken from the RWT (SP1) and the TWT (SP2) (Fig. 1). Water turbidity, electrical conductivity, pH, temperature, and transmittance at 254 nm (UVT_{254}) were measured for each sample. Temperature, conductivity, and pH were measured using a HACH® HQd Field case multiparameter, turbidity and using a turbidity meter (HACH® 2100Qis), and % transmittance at 254 nm (UVT_{254}) using UV/VIS Spectrophotometer (Genesys 150 thermoscientific®) (Table 1). The concentration of *A. hydrophila,* TC, and *E. coli* present in natural waters was monitored following the methods described (Section 2.5).

2.4. Disinfection performance POU system

The disinfection efficiency of the target microorganisms was calculated by determining the LRV by Eq. 1

$$LRV = logN_0 \ - log\ N_t = log_{10}\left(\frac{N_0}{N_t}\right) \quad (1)$$

Where LRV represents the logarithmic reduction value obtained between points SP1 and SP2 after a specific UVC irradiation time, N_0 is the concentration of microorganisms (UFC/mL) at the initial time (in SP1) and N_t is the concentration of microorganisms (UFC/mL) at time t (in SP2).

Table 1
Raw water characteristics in the communities.

Field location	Parameter	Mean ± standard deviation			Median [range]		
El Carmelo		CaVi01	CaVi02	CaVi03	CaVi01	CaVi02	CaVi03
	Turbidity (NTU)	5.9 ± 0.4	4.9 ± 0.3	3.3 ± 0.1	7.4 [26.4]	4.4 [2.1]	2.7 [3.2]
	Conductivity (μS/cm)	29.7 ± 2.0	53.7 ± 1.0	19.7 ±0.6	28.8 [4.0]	54.2 [1.9]	19.4 [1.3]
	pH	7.2 ± 0.2	7.5 ± 0.2	7.6 ± 0.3	7.1 [0.5]	7.4 [0.4]	7.5 [0.7]
	Temperature (°C)	22.8 ± 4.2	26.1 ± 0.2	24.5 ±3.6	22 [8.5]	26 [0.5]	26 [7.3]
	UVT_{254} (%T)	92.2 ± 0.7	99.1 ± 0.1	89.9 ±0.3	95.4 [14.2]	99.1 [8.8]	88.4 [14.9]
Curití		CuVi01	CuVi02	CuVi03	CuVi01	CuVi02	CuVi03
	Turbidity (NTU)	4.40 ± 0.13	9.6 ± 0.2	6.3 ± 0.6	4.4 [1.3]	9.6[0.0]	5.7 [1.9]
	Conductivity (μS/cm)	109.1 ± 2.8	105.3 ±2.0	108 ± 3	107.0 [16.9]	103 [8.4]	104 [11.7]
	pH	8.5 ± 0.4	8.5 ± 0.3	8.3 ± 0.2	8.6 [0.8]	8.7 [0.5]	8.3 [0.4]
	Temperature (°C)	24.8 ± 2.8	24.5 ± 2.0	26 ± 3	23.0 [0.8]	23.4 [0.5]	23.8 [4.8]
	UVT_{254} (%T)	91.5 ± 0.2	91.1 ± 0.6	91.6 ±0.2	91.5 [1.4]	91.8 [2.2]	91.5 [0.8]

The statistical analyses of all the data were performed with the IBS SPSS Statistics version 25 software. The Shapiro-Will test was implemented to demonstrate the normality of the data. To identify the difference between the medians of the raw water tank and treated water, the Mann- Whitney U test was performed ($p<0.05$). Kruskal-Wallis test was used to compare the medians of the three groups analyzed (Curití, Carmelo, and synthetic water). Spearman's Rho correlation tests were performed to determine whether the physicochemical parameters had any correlation with the disinfection performance of the POU systems.

2.5. Microorganisms culture and quantification

Escherichia coli, total coliform bacteria (TC), and *A. hydrophila* were used as microbiological target indicators to test the efficiency of the POU systems. *A. hydrophilic* as it is an emerging pathogenic bacterium of public health concern and *E. coli* as the most common fecal indicator tested. *A. hydrophila* (ATCC 35654) and *E. coli* (ATCC 25922) strains were used for laboratory testing with synthetic waters. They were maintained on nutrient agar (Merck), grown at 37 °C with periodic subculturing every 30 days. For field testing, TC was also monitored.

The membrane filtration technique was used for the detection and enumeration of TC and *E. coli.* Samples were filtered in triplicate through sterile cellulose nitrate membrane filters (0.45 μm) and transferred to Petri dishes with Chromocult Agar (Chromocult® Coliform Agar). Petri dishes were incubated at 36 ± 2 °C for 21 ± 3 h. Fuchsia, dark blue to violet-colored colonies were enumerated as TC and dark blue to violet as *E. coli* with a detection limit of 1 CFU/100mL.

The membrane filtration technique and the RYAN selective medium (Oxoid) supplemented with ampicillin (5 mg/L) were used for the detection and enumeration of *A. hydrophila* (US Environmental Protection Agency, 2001; Ryan, 1985; Rogol et al., 1979). Samples of 100 ml of water were taken from SP1 (RWT) and SP2 (TWT) (Fig. 1A). The samples were filtered in triplicate through sterile cellulose nitrate membrane filters (0.45 μm) and transferred to Petri dishes with RYAN selective medium supplemented. Opaque green colonies with dark centers obtained on the filter surface were enumerated as *A. hydrophila* with a detection limit (DL) of 1 CFU/100mL. Confirmatory Oxidase, Indole, and Gram biochemical tests were performed on 5 colonies identified as *A. hydrophila* and one of these colonies was given the Vitek test (Elbehiry et al., 2019) to verify the presence of *A. hydrophila.*

2.5.1. Incubation temperature variation tests for A. hydrophila enumeration

To select the incubation temperature to differentiate *E. coli* colonies from *A. hydrophila* colonies present in natural waters in RYAN differential medium, *A. hydrophila* (ATCC 35654) and *E. coli* (ATCC 25922) strains were grown independently with synthetic water and in mixture and incubated at 30 and 37 ± 0.5 °C for 24 ± 2 h.

3. Results and discussion

3.1. Standardization of Aeromonas hydrophila quantification

The initial quantification analyses of the natural waters of the communities using the RYAN selective medium with incubations at 37 °C for 24 ± 2 h resulted in the growth of colonies of various colors ranging from yellow to green (Fig. 2). The biochemical tests (Table 2) carried out on the 5 colonies showed the presence of *Aeromonas* (isolate 4) and *E. coli* (isolate 2), generating difficulties in quantification. The Vitek test carried out on isolate 4 confirmed the presence of *A. hydrophila* in the natural waters of the communities.

Incubation temperature variation tests performed with pure strains of *A. hydrophila* and *E. coli* grown in RYAN medium at 30 or 37 °C showed differences in their behavior. *Aeromonas* grown at 30 °C presented large colonies easy to quantify and no *E.coli* growth was observed. These results can be attributed to the fact that *Aeromonas* are freshwater bacteria with better growth ability at lower temperatures than *E. coli* (Awan et al., 2018; Mizan et al. 2018). Given the results obtained, the *Aeromonas* incubations were performed at 30 °C for 24 ± 2 h.

3.2. POU system performance assessment in synthetic water (lab test)

All the tests carried out with synthetic waters were spiked with *A. hydrophila* at an initial concentration between 3467 and 4400 CFU/100 mL and then with the POU systems at flow rates of 3.8 L/min, the

Table 2
Biochemical tests were performed on colonies isolated from the natural waters of the rural community of Curití, analyzed by the RYAN method with incubation at 37°C.

Isolated #	Indol	Oxidase	Gram	MO
1	Negative	Negative	Negative	
2	Positive	Negative	Negative	Consistent with *E. coli*
3	Negative	Negative	Negative	
4	Positive	Positive	Negative	Consistent with *Aeromonas*
5	Negative	Positive	Positive	

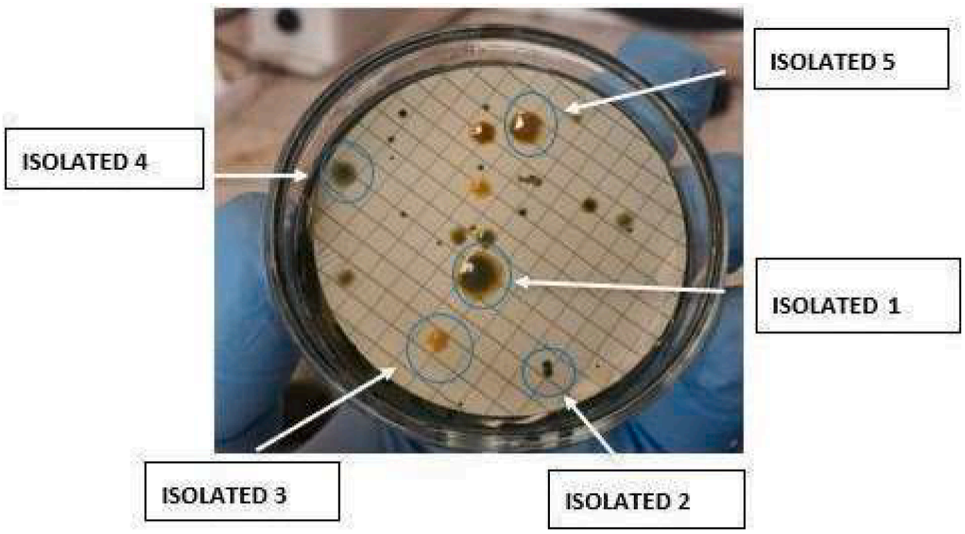

Fig. 2. Growth of bacteria from natural waters of the rural community of Curití in the RYAN differential medium after incubation at 37 °C for 24 h.

detection limit was reached and an LRV greater than 3.5 was obtained. (Table 3).

The regrowth tests carried out with synthetic waters treated with the POU system did not show regrowth of *A. hydrophila* at 24 or 48 hours when the samples were stored under refrigerated (8 ± 2 °C) and dark conditions. Regrowths occurred at 24 hours in water samples that were stored in the dark at room temperature (22 ± 2 °C), although preliminary studies of *E. coli* regrowth in synthetic waters treated with the same POU system and stored under dark conditions at room temperature did not show any regrowth of *E. coli* (Pichel et al., 2021). This difference may be associated with the natural behavior of these microorganisms. *E. coli* is an enteric bacterium that is cultivated at 37 °C under laboratory conditions, while *Aeromonas* are natural inhabitants of water and grow well at room temperature (Chaix et al., 2017; Khor et al., 2015).

There is plenty of evidence that demonstrates that *Aeromonas spp*. is one of the opportunistic premise plumbing pathogens (OPPP). They are native to the plumbing environment and present an emerging infectious disease problem (Hayward et al., 2022). *Aeromonas spp*. has been reported as a prevalent of OPPP detected in residential drinking water infrastructures by 20 studies up to date. They demonstrated their prevalence in drinking water from 0.7 to 32.4% with concentrations from 5 to 333.3 CFU/mL; meanwhile their prevalence in biofilms was from 3.9 to 77.5% (Hayward et al., 2022). On the other hand, the detection of *Aeromonas* spp. (like many other opportunistic pathogens) in environmental samples becomes a very challenging task since there are limitations in culture-based detection methods, today considered the "gold standard". Often these methods underestimate microbial concentrations due to difficulties in detecting viable non-culturable bacteria. This is compounded by the lack of standardization of culture methods for the quantification of many of these opportunistic bacteria. These difficulties open the possibility for significant variation in sampling techniques and the development of enumeration protocols in drinking water. *Aeromonas* prevalence in collected rainwater in Australian cities has been also reported (Chubaka et al., 2018). *Aeromonas* appeared in 10–33% of the sources tested, although none of them was associated with epidemiological evidence between the years 1981 and 2009 (Chubaka et al., 2018). *Aeromonas spp*. have been isolated from wastewater, natural water sources, aquacultures, as well as urban drinking water (Piotrowska and Popowska, 2014), becoming an emerging concern for human health due to the highly frequent presence of antimicrobial-resistant genes (ARG) in these opportunistic pathogens and their ability to share and transfer ARG between different genera of bacteria (Piotrowska and Popowska, 2014).

3.3. POU system performance assessment in natural water (field tests)

When analyzing the variation of the physicochemical parameters generated by the treatment with the POU systems (Table 4), no changes in temperature, pH y conductivity were found. On the contrary, significant differences were found between the medians in Turbidity (NTU) ($p<0.000$) because of treatment in both rural communities, with a reduction of 85% (7.1 NTU to 1.1 NTU) in Curití and 71% (6.5 NTU to 1.9 NTU) in El Carmelo. In the case of UVT_{254}, significant differences between medians were only found in the Curití community ($p<0.000$), in Carmelo, there were no significant differences between means for UVT_{254} ($p<0.054$).

When analyzing the quality of the water supply of the systems in the two communities, it was observed that the microbial counts were lower in Curití (*E. coli*: 29 ± 36 CFU/100mL, TC: 101 ± 512 CFU/100mL, *A. hydrophila*: 2870 ± 702 CFU/100mL) than in El Carmelo (*E. coli*: 100 ± 121 CFU/100mL, TC: 1260±3086 CFU/100mL, *A. hydrophila*: 3333 ± 362 CFU/100mL) (Table 5), these fecal indicator differences (CT and *E. coli*) between communities can be attributed to the population density in the communities and the type of use and management that these communities give to the micro-watersheds that supply water to homes. When analyzing the efficiency of the POU system installed in the rural communities of Curití and El Carmelo, differences were found in the response to the inactivation of TC, *E. coli,* and *A. hydrophila* bacteria present in natural waters (Table 5). For *E. coli*, the initial concentration of bacteria in rural communities was low, in El Carmelo it was 100 ± 121 CFU/100mL with an LRV of 2.0 ± 0.3, and in Curití, the initial concentration was 29 ± 36 with an LRV of 1.3 ± 0.5, for both communities the inactivation reached the detection limit.

For TC reduction, in El Carmelo the inactivation was LRV = 3.2 ± 0.6, however complete inactivation was not achieved, and the concentration after treatment was 45 ± 92 CFU/100mL. In Curití, the efficiency of the system to remove TC was LRV = 1.0 ± 0.7 ($p < 0.026$) with a final TC concentration after treatment of 7 ± 345 CFU/100mL.

When comparing the efficiency of the POU for the removal of the different microorganisms, it was clear that it was lower for the TC, possibly because it is a heterogeneous group of wild bacteria. The efficiency in the elimination of *Aeromonas* in El Carmelo was LRV = 3.1 ± 0.6 ($p<0.000$) with an after-treatment concentration of 2 ± 11 CFU/100mL, while in Curití, the LRV was 2.2 ± 0.6 ($p<0.000$) and the post-treatment concentration was 28 ± 29 CFU/100mL. In both rural communities, it was not possible to reduce the counts of *Aeromonas* below the DL.

In general, the differences in the LRV for each bacteria group between the 2 communities are due to the values in the initial concentrations. The higher the initial concentration of bacteria, the more removal was observed (Table 5). Residual concentrations of bacteria in the treated water tanks were also observed. This was attributed to the poor maintenance and cleaning of the POU system. As stated by Baldasso et al. (2021) one of the main problems with UVC disinfection in rural developing regions is the need to monitor the operation of the system to guarantee the effectiveness of disinfection without frequent microbiological tests. For this reason, the training and awareness of the community in this aspect were considered essential to guarantee the quality of the treated water. Interestingly, Bernedo et al. (2020), indicated that wild *Aeromonas* are more resistant to UVC, which would limit the performance of the POU systems, with a final stage of UVC disinfection, in natural waters compared to synthetic waters (Bernedo et al., 2020).

The POU systems were efficient for the elimination of *Aeromonas* present in natural waters, reducing the average numbers in 4 of the POU systems installed to values below 20 CFU/100mL (Table 5) complying with the standards of the maximum level allowed for this bacterium in the water leaving the treatment plant in The Netherlands and, additionally, all treated water complied with the drinking water quality allowed in the distribution systems (200 CFU/100mL) (Standards, 2000; Figueras and Beaz-Hidalgo, 2014; Van der Kooij and Hijnen, 1988). The limitations of these results are also clear; the elimination of *Aeromonas* in natural water did not reach the DL and their LRVs were lower than those found for synthetic waters.

Table 3
A. hydrophila removal from synthetic water using the POU.

	Synthetic Water					
	A. hydrophila [CFU/100mL]			Regrowth		
Test #	RWT	TWT	LRV	8± 2°C		22± 2°C
				24h	48h	24h
1	4143±0.2	BDL	3.62±0.02	-	-	+
2	3467±0.3	BDL	3.54±0.04	-	-	+
3	3795±0.1	BDL	3.58±0.01	-	-	+
4	4083±0.2	BDL	3.61±0.02	-	-	+
5	4400±0.2	BDL	3.64±0.02	-	-	+
6	4133±0.2	BDL	3.62±0.03	-	-	+
7	3867±0.2	BDL	3.59±0.02	-	-	+
8	3900±0.3	BDL	3.59±0.03	-	-	+
9	3667±0.3	BDL	3.56±0.04	-	-	+

BDL: below the detection limit (1 CFU/100 mL), using membrane filtration.

Table 4
Physicochemical parameters before and after the treatment in Curití and El Carmelo.

Rural Community	Temperature (°C)		pH		UVC254 (%)		Conductivity (μS/cm)		Turbidity (NTU)	
	RWT	TWT	RWT	TWT	RWT	TWT	RWT	TWT	RWT	TWT
CuVi01	27.5±0.7	27.6±0.6	8.4±0.3	8.5±0.2	92.2±1.5	95.2±1.0	108.2±6.2	110.1±6.3	4.6±0.6	1.0±0.4
CuVi02	27.1.0±0.8	27.3±0.6	8.5±0.3	8.5±04	90.9±1.1	95.7±0.6	107.4±5.2	111.2±5.5	8.3±2.4	1.1±0.5
CuVi03	27.5±1.0	27.4±1.3	8.3±0.2	8.4±0.2	91.7±0.4	95.2±2.0	106.9±4.2	107.6±4.7	8.3±1.4	1.3±0.0
Mean Curití	27.4±0.7	27.4±0.85	8.4±0.3	8.5±0.3	91.8±1.0	95.4±1.3	106.9±5.9	110.6±6.2	7.1±1.5	1.1±0.3
CaVi01	22.8±3.3	23.3±3.8	7.2±0.2	7.6±0.3	89.6±5.1	89.9±4.1	28.7±0.1	36.2±1.4	11.4±10.2	1.5±0.3
CaVi02	25.1±0.2	26.1±0.3	7.5±0.2	7.4±0.2	91.7±4.7	92.1±4.2	43.9±0.7	46.2±0.1	4.9±1.0	2.4±0.2
CaVi03	23.5±2.73	26.2±1.3	7.5±0.3	7.7±0.4	89.8±6.7	90.1±3.8	19.7±0.6	20.2±0.2	3.3±1.4	2.0±0.6
Mean El Carmelo	23.6±2.1	25.2±1.8	7.4±0.2	7.6±0.3	89.5±5.5	90.2±3.2	30.8±0.5	34.2±0.5	6.5±4.2	1.9±0.4

Table 5
Summary of the results (mean and standard deviation) of the physicochemical, and microbiological analyses and p-value for the median difference of water samples before (RWT) and after treatment (TWT) with the POU systems.

Parameter		EL CARMELO				CURITÍ					LAB			
		Natural surface water				Natural surface water					Synthetic water			
	POU	RWT (M ±SD)	TWT (M ±SD)	LRV	p-value	POU	RWT (M ±SD)	TWT (M ±SD)	LRV	p-value	RWT (M ±SD)	TWT (M ±SD)	LRV	p-value
Turbidity (NTU)	Cavi 01	11.4 ±10.2	1.5±0.3			Cuvi 01	4.6±0.6	1.0±0.4						
	Cavi 02	4.9±1.0	2.4±0.2			Cuvi 02	8.3±2.4	1.1±0.5						
	Cavi 03	3.3±1.4	2.0±0.6			Cuvi 03	8.3±1.4	1.3±0.0						
Mean		6.5± 4.2	1.9± 0.5		0.000		7.1±1.5	1.1±0.3		0.000	–	–		–
UVT_{254} (%)	Cavi 01	89.6±5.1	89.9 ±4.1			Cuvi 01	92.2 ±1.5	95.2 ±1.0						
	Cavi 02	91.7±4.7	92.1 ±4.2			Cuvi 02	90.9 ±1.1	95.7 ±0.6						
	Cavi 03	89.8±6.7	90.1 ±3.8			Cuvi 03	91.7 ±0.4	95.2 ±2.0						
Mean		89.5±5.5	90.2 ±3.2		0.054		91.8 ±1.0	95.4 ±1.3		0.000	–	–		–
A. hydrophila (CFU/100mL)	Cavi 01	3802 ±1756	2±2			Cuvi 01	3315 ±740	53±34						
	Cavi 02	2958 ±957	11±19			Cuvi 02	2688 ±714	16±23						
	Cavi 03	2808 ±1433	5 ±5			Cuvi 03	2561 ±561	35±24						
Mean		3333 ± 362	2 ± 11	3.1 ±0.6	0.000		2870 ± 702	28 ± 29	2.2 ±0.6	0.000	3900 ± 265	BDL	3.60 ±0.02	0.000
E. coli (CFU/ 100mL)	Cavi 01	85±41	BDL			Cuvi 01	66± 40	1±1						
	Cavi 02	200±200	BDL			Cuvi 02	44± 31	BDL						
	Cavi 03	93±24	1±2			Cuvi 03	10± 8	BDL						
Mean		100± 121	BDL	2.0± 0.3	0.000		29 ±36	BDL	1.3± 0.5	0.000	–	–		–
TC (CFU/100mL)	Cavi 01	1180 ±881	11±21			Cuvi 01	756 ±744	476± 490						
	Cavi 02	6850 ±2300	BDL			Cuvi 02	195± 158	11± 17						
	Cavi 03	1138 ±227	125 ±133			Cuvi 03	34± 9	3± 5						

The microbiological analyses carried out in this study with natural waters showed the prevalence of *Aeromonas* during the 3 months of testing, with average values of 3333 ± 362 CFU/100mL in El Carmelo and 2870 ± 702 CFU/100mL in Curití (Table 5), which confirms previous findings in these communities (Bedoya et al., 2021). These values are considered high in the Netherlands, Australia, Portugal, and the United States, in which the presence of *Aeromonas* has been reported in sources of water for human consumption and networks of non-chlorinated purification systems (Van Bel et al., 2021; Fernandez--Bravo and Figueras, 2020; Solaiman et al., 2020; Zdanowicz et al., 2020). The prevalence of *Aeromonas* in natural water and its persistence in treated water suggests the need to include these microorganisms as an agent of concern in drinking water and the importance of placing it on the Candidate Contaminant List (CCL) as stated by the EPA (Embrey et al., 2002) and the importance of including this microorganism in tests for the development of new technologies to provide safe water to rural communities.

The median comparison analysis for non-parametric data (Fig. 3) confirmed significant differences between the LRVs of synthetic and natural waters from the rural communities. Since the natural waters didn't present a normal distribution of means between the groups analyzed, it is suggested that there may be physicochemical or biological

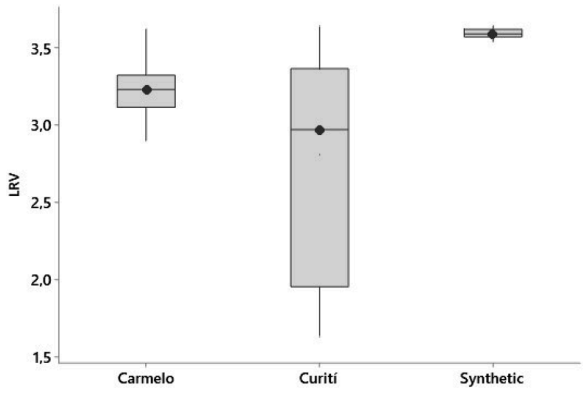

Fig. 3. Box-plot of Log reduction ratio (LVR) in *A. hydrophila* in El Carmelo, Curití, and synthetic water treated by the POU systems.

parameters could influence the performance of the POU system. The Rho Spearman test indicated there was no statistically significant correlation between the physicochemical parameters analyzed and the LRV of *Aeromonas* obtained, suggesting that there are other causes (bilateral correlation $\geq$ 0.05), i.e. biological. One of these causes could be the presence in natural waters of wild *Aeromonas* more resistant to UVC treatment, as discussed earlier.

Aeromonas regrowth tests were not carried out with natural waters since they did not reach the detection limit in treated waters in both Curití and El Carmelo. However, the samples of the synthetic waters treated that were stored in the dark at room temperature (22 $\pm$ 2 °C) for 24 h showed regrowth of *Aeromonas*, while at 8 °C, there was no evidence of regrowth at 24 h, nor at 48 h, indicating that the treated water should be stored under refrigerated conditions, or consumed within 48 h of treatment to prevent *Aeromonas* regrowth and minimize health risks. Some authors suggested that long-term water storage could lead to benthic growth of *Aeromonas* in sediments and/or in biofilms on the walls of storage tanks and/or pipes, releasing *Aeromonas* into non-chlorinated water (Van Der Wielen et al., 2016; Liu et al., 2014).

The different bacterial counts observed (Table 5) demonstrated the variability of the water quality of both communities. The periodic maintenance and cleaning that each family carried out in the POU system was also a very important factor that affected the efficiency of disinfection. The recommendations provided were supported by a visual inspection of the raw water tank to decide whether to flush the system. The cleaning frequency varied according to the rainy season, which increased the turbidity and color of the water, increasing the frequency of washing. However, follow-up visits showed visual differences in the maintenance of the POU systems. The differences were reflected in the presence of bacteria in the treated water samples in both El Carmelo (TC: 5/12 tanks; *E. coli*: 1/12 tanks) and Curití (TC: 8/12 tanks; *E. coli*: 3/12 tanks) reducing the efficiency of the POU systems by up to 37% as observed in the TC counts in TWT in the CuVi01 system in Curití (Table 5). *Aeromonas* sp. has a high sensitivity to UVC radiation and is characterized by rapid growth but low resilience under stress (Vadstein et al., 2018). The susceptibility of *Aeromonas* sp. was reported in studies of pathogens transmitted by milk have been related to the germicidal action of UVC radiation (Crook et al., 2015). In aquaculture systems, an LRV of 5 was reported for *A. hydrophila* under UVC doses between 8.5 and 9.83 mJ cm^{-2}, depending on the wavelength used, either 262 or 268 nm (Moreno-Andrés et al., 2020; Liltved et al., 1995). This rapid inactivation is attributed to the high uptake of nucleic acids in this range that causes the inactivation of pathogens (Hull and Linden, 2018). Compared to other waterborne opportunistic pathogens, *Aeromonas* sp. has a high sensitivity to UVC radiation requiring lower doses (in mJ cm^{-2}) for a similar reduction. For example, 4 *E. coli* LRVs can be achieved with 18-19 mJ cm^{-2}, which can vary slightly depending on the wavelength used between 254 and 285 nm (Hijnen et al., 2006); *Legionella pneumophila* requires a dose of 30 mJ cm^{-2} to reduce 4 LRV using an LP mercury lamp, ie 254 nm (Hijnen et al., 2006); *Pseudomonas aeruginosa* required 7.8 mJ cm^{-2} for a 4 LRV using UVC-LED treatment at 265 nm (Song et al., 2016). For *Aeromonas*, our results showed LRV of 2.2 and 3.1, these values varied with the water source.

The literature reports a germicidal fluence determined with a collimated beam configuration, which is far from flow systems, like in this work. This study presents a one-pass through UVC lamp reactor operated at a flowrate of 3.8 L min^{-1}. According to Baldasso et al. (2021) flow UVC disinfection systems have added difficulty to calculate and determine the delivered fluence due to obvious reasons of hydrodynamic and mass transfer within the reactor, and light scattering inside the systems. Experimental actinometric measurements are the best solutions in these cases to avoid laborious modeling tasks (Baldasso et al., 2021). In this work, the actinometric tests and the measurement of transmittance at 254 nm permitted to estimate the fluence delivered by the UVC lamp at 3.8 L min^{-1} as 51.2 and 53.5 mJ cm^{-2} for water with 91.5 and 95.7% for UVT_{254}, respectively (Supplementary materials). It is well recognized, that estimated fluences in flow systems are higher than in static collimated beam systems (Baldasso et al., 2021; Pichel et al., 2021).

4. Conclusions

The high concentrations and persistence of *Aeromonas* in natural waters found in this study demonstrate the health risk in rural communities and the need to evaluate environmental pathogens present in water. It is highly important to incorporating this group of bacteria as an indicator of environmental contamination in drinking water regulations. It is also necessary to develop new alternatives for the quantification of *Aeromonas* in water, for the estimation of the biological risks of this bacterium in drinking water, and for quantification by the Ryan method; the culture temperature should be modified to 30°C to avoid the growth of other bacteria. The POU systems have been demonstrated to be efficient in the inactivation of *Aeromonas* and *E. coli*, which makes it possible to deliver safe water to families in rural communities that lack treatment systems. Social appropriation in the maintenance and cleaning of the POU systems is essential to guarantee their proper functioning and avoid the growth of microorganisms in the treated water tank. Finally, this field study has also permitted us to highlight the importance of evaluating environmental pathogens beyond the microorganisms recommended by the WHO for POU technologies.

CRediT authorship contribution statement

Liliana Botero: Conceptualization, Methodology, Visualization, Writing – review & editing. **Laila Galeano:** Methodology. **Luis Javier Montoya:** Methodology. **Alexander Machado:** Methodology, Investigation, Data curation, Formal analysis, Writing – original draft. **John Anthony Byrne:** Writing – review & editing, Funding acquisition. **Pilar Fernandez-Ibañez:** Writing – review & editing, Funding acquisition. **Margarita Hincapié:** Conceptualization, Methodology, Investigation, Data curation, Validation, Formal analysis, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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