Research Article/Civil and Sanitary Engineering http://doi.org/10.15446/ing.investig.100573

Helicobacter pylori Removal through Gravel Filtration in a Water Treatment System of the Municipality of Popayán, Cauca

Remoción de *Helicobacter pylori* a través de sistemas de tratamiento por filtración en gravas en el municipio de Popayán, Cauca

Cristina Ledezma[1], Javier Fernández[2], Patricia Acosta[3], and Javier Leyton[4]

ABSTRACT

Multi-stage filtration technology (MSFT) is an alternative that reduces the risk of fecal contamination, allowing for reliable water purification in rural water supplies. MSFT is composed of two gravel filtration (GF) stages: one including dynamic gravel filters (DyGF) and up-flow gravel filters (UGF), and a final stage with slow sand filters (SSFs). However, with the purpose of reducing construction costs, this technology is partially implemented, leaving SSFs for a later construction stage and limiting its treatment potential. To evaluate the removal capabilities regarding fecal contamination (especially that by the pathogen *H. pylori*) of a two-stage GF system, the quality of raw and treated water and the hydraulic parameters of Los Llanos treatment system (municipality of Popayán, Cauca) were monitored for 15 weeks. This system is operated and maintained by the community. The results showed the removal efficiency regarding turbidity to be 16% (p=0,045) and 34% (p=0,030) for the DyGF and the UGF, respectively. The color removed by the DyGF reached 19% (p=0,033), and the UGF reported a value of 30% (p=0,041). The reduction of total coliforms was limited by the system's operation and maintenance, exhibiting a tendency towards increased concentrations at the outlet. The presence or absence of *H. pylori* was determined via the PCR molecular technique. A greater presence was evidenced in treated water than in raw one, which may be associated with a limited operation and a low maintenance frequency of the system. The implementation of MSFT, without the complement of SSFs, is not reliable in ensuring quality of water, particularly from a perspective of microbiological control and *H. pylori* control.

Keywords: *Helicobacter pylori*, gravel filtration, multi-stage filtration technology, rural water supply

RESUMEN

La tecnología de filtración en múltiples etapas (FIME) es una alternativa que reduce el riesgo por contaminación fecal, permitiendo la potabilización del agua de manera confiable en acueductos rurales. La tecnología FiME está compuesta por dos etapas de filtros en grava (FG): una que incluye filtros dinámicos (FGDi) y filtros gruesos ascendentes (FGAC), y una etapa final con filtros lentos en arena (FLA). Sin embargo, con el fin de reducir los costos de construcción, esta tecnología se implementa de manera parcial, dejando los FLA para una etapa posterior de construcción y limitando su potencial de tratamiento. Con el fin de evaluar la capacidad de remoción de la contaminación fecal (especialmente del patógeno *H. pylori*) en un sistema de FG de dos etapas, se monitoreó durante 15 semanas la calidad del agua cruda y tratada y los parámetros hidráulicos del sistema de tratamiento Los Llanos en el municipio de Popayán, Cauca, el cual es operado y mantenido por la comunidad. Los resultados mostraron que la eficiencia de remoción respecto a la turbiedad es de 16 % (p=0,045) y 34 % (p=0,030) para el FGDi y el FGAC respectivamente. El color removido por el FGDi alcanzó el 19 % (p=0,033), y el FGAC reportó un valor de 30 % (p=0,041). La reducción de los coliformes totales se vio limitada por la operación y mantenimiento del sistema, presentando una tendencia a incrementar su concentración en la salida. La presencia o ausencia del *H. pylori* se determinó con la técnica molecular PCR. Se evidenció una mayor presencia en agua tratada que en agua cruda, lo cual puede asociarse con una limitada operación y una baja frecuencia de mantenimiento del sistema. La implementación de la tecnología FiME, sin el complemento de FLA, es poco confiable para garantizar la calidad del agua, particularmente desde el punto de vista microbiológico y el control del *H. pylori*.

Palabras clave: *Helicobacter pylori*, filtración en gravas, filtración en múltiples etapas, acueducto rural

Received: January 19th, 2022
Accepted: July 24th, 2023

[1] Chemist. MSc in Continental Hydrobiological Resources, Universidad del Cauca. Affiliation: Associate professor, Department of Environmental and Sanitary Engineering, Environmental Engineering research group, Universidad del Cauca, Colombia. Email cledezma@unicauca.edu.co

[2] Sanitary engineer. PhD in Engineering, Universidad del Valle. Affiliation: Full professor, Department of Environmental and Sanitary Engineering, Environmental Engineering research group, Universidad del Cauca, Colombia.

[3] Biologist. PhD in Environmental Sciences, Universidad del Cauca. Affiliation: Full professor, Department of Physiological Sciences, Applied Human Genetics research group, Universidad del Cauca, Colombia.

[4] Environmental engineer. Specialist in Water Resources, Universidad del Cauca. Affiliation: Research assistant, Environmental Engineering and Applied Genetics research groups, Universidad del Cauca, Colombia.

Introduction

In a worldwide systematic review by Hooi *et al*. (2017), it was revealed that 60% of the population is infected with *Helicobacter Pylori* (*H. pylori*), with variations in distribution. The regions with developing countries that show the highest prevalence of this infection are Africa (79,1%), Latin America and the Caribbean (63,4%), and Asia (54,7%), contrasting with a lower prevalence in developed countries in North America (37,1%) and Oceania (24,4%).

H. pylori infection is acquired by the population before the age of ten, and its prevalence increases with age (Ramos and Sánchez, 2009). In Colombian children, it is close to 75%, and, in the population older than 20, it reaches 86% (Moncayo *et al*., 2006). The incidence of this pathogen is related to gastric cancer figures. In Colombia, it represented 13,7% of all cancer deaths in the country, being the first cause in men and the third in women. The highest risk of mortality was reported in the departments of Quindío, Huila, and Cauca for men and in Cauca, Norte de Santander, and Huila for women (Pardo *et al*., 2017; Adrada *et al*., 2008).

Three routes of diffusion have been considered for this bacterium, oral-oral, gastro-oral, and fecal-oral (Aziz *et al*., 2015; Bartram and Cairncross, 2010; Goh *et al*., 2011). However, for the latter, water is considered as an intermediate vehicle for transmission, acting as an environmental reservoir (Hulten *et al*., 1996; Engstrand, 2001). Its presence was reported in surface sources used in water supply systems in Venezuela (Adrada *et al*., 2008; Fernández-Delgado *et al*., 2008) and, more worryingly, in water from storage tanks and in water distribution systems for consumption in Peru (Hulten *et al*., 1996), Portugal (Gião *et al*., 2008), United States (Baker and Hegarty, 2001), Mexico (Premoli *et al*., 2004), Costa Rica (Campos *et al*., 2011; Montero-Campos, 2019), Iran, and Pakistan (Samra *et al*., 2011; Al-Sulami *et al*., 2010), thus confirming the presence of the pathogen in drinking water as an important source of transmission. Additionally, some studies provide evidence that surface waters may contain considerable levels of viable *H. pylori* cells, (Acosta *et al*., 2018; Agustí *et al*., 2010; Santiago, 2016).

In Colombia, *H. pylori* has been found in the water sources used in the public supply systems of cities such as Bogotá (Vesga, 2018), as well as in rural areas of the department of Cauca (Ordóñez, 2015). In addition, the purification treatments used have great operating limitations in small municipalities, particularly in rural areas, where the necessary infrastructure for water treatment is non-existent or has only been partially implemented. This entails negative impacts on the quality of the water, which was reflected on the Water Quality Risk Index (IRCA) of 2019. This index showed low risk values for urban areas (5,73%) and medium risk values for the rural sector (16,23%). These figures do not include self-sufficiency, since most rural water supplies have not been legalized, and 42,4% of these systems are classified as having high risk (Ministerio de Vivienda, Ciudad y Territorio, 2020), evidencing a great gap between urban and rural areas in terms of the quality of water for human consumption.

The use of MSFT depends on the water sources' degree of contamination. This method can comprise two or three stages of filtration, *i.e.*, dynamic gravel filters (DyGF), up-flow gravel filters (UGF), and slow sand filters (SSF) (Galvis, 1999). The first two stages make up the pretreatment phase, which allows reducing the concentration of suspended solids as well as microbiological contamination. Upon reaching the SSF, a more effective removal of the remaining microbiological contaminants is achieved via biological predation mechanisms. Finally, low-turbidity waters are produced, which are virtually free of enterobacteria, enterovirus, and protozoan cysts (S. Haig, 2014; Haig *et al*., 2011; Sánchez *et al*., 2007; Raman *et al*., 1992). MSFT is considered to be a simple, reliable, and efficient technology that is suitable for rural communities due to its ease of operation and maintenance.

Considering a gradual and progressive approach, the implementation of this technology could be carried out in two phases. In the first phase, the pretreatment system would be built (DyGF and UGF), while, the SSFs would be constructed in the second one (Galvis *et al*., 1992; Galvis, 1999; Galvis *et al*., 1999; Sánchez *et al*., 2007).

Numerous studies carried out in Colombia and different parts of the world have shown that the combination of pretreatment systems involving gravel and slow sand filters yields water with low sanitary risk for users (Mushila *et al*., 2016; Vega, 2013; Nkwonta and Ochieng, 2009; Sánchez *et al*., 2007; Fernández *et al*., 2006; Ochieng *et al*., 2004; Galvis, 1999; Wegelin, 1996; Galvis *et al*., 1992). In all the aforementioned studies, pre-treatment systems have been able to achieve water qualities that would allow applying gradualness and progressiveness in rural treatment systems of water for human consumption, which could benefit the development of projects when there are no resources for a full implementation of MSFT.

MSFT is considered to be highly efficient in the elimination of pathogens, but there are no studies on the removal of *H. pylori* using this technology, much less in gravel prefiltration systems (DyGF and UGF), which, despite their high microbiological removal capabilities, may not be enough to achieve optimal levels of potability.

This research presents the results obtained by monitoring the removal of *H. pylori* in water pretreated with gravel filtration in Los Llanos water supply system in the municipality of Popayán, Cauca.

Methodology

This full-scale study was carried out at the drinking water treatment plant in the village of Los Llanos, which is part of Las Guacas water supply system and is located in the northeastern part of the municipality of Popayán.

The water treatment plant is composed of a DyGF, followed by a layered UGF (Figure 1). It does not have SSFs or employ a chlorination stage as a disinfection method. The raw water supply comes from the Velasquillo and San Isidro streams.

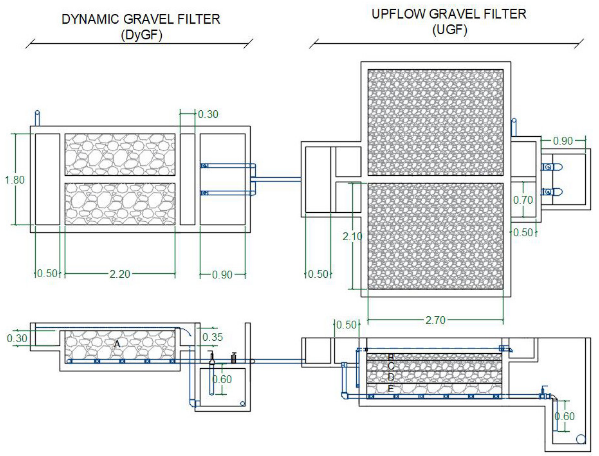

Figure 1. Scheme of treatment plant and gravel size
Source: Authors

Water quality monitoring

The physical-chemical and microbiological quality of the water was monitored by analyzing the parameters shown in Table 1.

Considering the configuration of the plant, the sampling points were as follows: i) raw water, ii) mixture of the effluent water from the two dynamic filters, and iii) outlet of the layered UGF. Physical and hydraulic parameters were measured twice a week, and microbiological aspects were evaluated once a week, for a total of 15 weeks.

Table 1. Measurement protocol for water quality parameters

Parameter	Unit of measurement	Equipment	Method
Turbidity	Nephelometric turbidity units (NTU)	Turbidity meter HACH 2100P (HACH, USA)	Nephelometric (SM 2130B)
Color	Platinum-cobalt units (PCU)	Spectrophotometer HACH DR2010 (HACH, USA)	Spectrophotometric (SM 2120C)
pH	Dimensionless	pH-meter (HACH, USA)	Potentiometric (SM 4500H+B),
Flow	L/s.	Volumetric material and stopwatch	Volumetric method (Ministry of the Environment, 2007)
Head loss	cm	Piezometers	Difference of levels
Total coliforms and *E. coli*	CFU/100ml	Membrane filtration equipment, laminar flow chamber, incubator	Membrane filtration, code: TP0314 IDEAM

Source: Authors

By means of a macro-drip system, 500 mL of water were collected for 30 min in amber glass containers and sterilized via a Gemmyco S232 autoclave at 121 ˚ C for 15 min. They were transported, in portable refrigerators at 4 ˚C, to the Applied Human Genetics laboratory of Universidad del Cauca, where they were processed for later *Helicobacter pylori* analysis. They were also transported to the SENA-Cauca's Environmental Analysis Laboratory, where the presence of total coliforms and *E. coli* was determined. Physical parameters were measured in the field. As operating control parameters of the treatment plant, the flow treated by the filtration units and the head losses were measured in the UGF.

Microbiological contamination indicators

The total coliforms and *E. coli* were measured by filtering 100 mL of water through sterile cellulose membranes with 0,45 μm pores and 47 mm in diameter (S/PAD, MS, MCE squared membrane, Sterile White). Chromocult Coliform Agar was used as the culture medium. As positive controls for *E. coli* and total coliforms, the *Escherichia coli* ATCC 25922 strain was used, in addition to *Pseudomona aeruginosa* ATCC 10145 strain as a negative control.

Helicobacter pylori detection

The water samples were concentrated by centrifugation in two stages. A first step at 4 000 rpm and 8 ^{0}C for 30 min (Heraeus Megafuge 1.0R centrifuge) was carried out to reach a concentration volume of 4,5 mL. Previously resuspended, this volume was transferred to 1,5 mL microcentrifuge tubes, which were in turn centrifuged at 12 000 rpm for 5 min, eliminating the supernatant until final volumes of 200 uL were obtained. This was done using an Eppendorf 5415C centrifuge.

Subsequently, DNA extraction was carried out. The concentrates were processed using the E.Z.N.A. Water DNA kit (Omega Bio-Tek, Doraville, USA) while following the manufacturer's recommendations. The isolated DNA was eluted in 200 μL of buffer solution and stored at -20 ˚C until amplification.

DNA amplification was carried out by means of polymerase chain reaction (PCR), using specific primers previously reported for *H. pylori:* VacA si/s2, VacA m1/m2, and CagA (Atherton *et al.*, 1995; Erzin *et al.*, 2006; Yamaoka *et al.*, 1999). Additionally, PCR specificity was verified using DNA from three strains of *H. pylori* (TX30a, 11638, and 11637). The amplification mix contained 0,36 μL of each primer (Mm), 5 μL of Qiagen Multiplex PCR, prepared according to the manufacturer's recommendations, and 7 μL of genomic DNA. The PCR tubes were briefly centrifuged at low speed in order to bring the entire sample to the bottom of the tube. The samples were taken to the thermal cycler (MyCycler Thermal Cycler, BIORAD) under the conditions shown in Table 2.

Table 2. PCR conditions for amplification

Step	Process	T (°C)	Time	Cycles
1	Initial denaturation	95	10 min	1
	Denaturation	94	30 s	
2	Coupling	57	90 s	15
	Extension	70	45 s	
	Denaturation	94	30 s	
3	Coupling	54	1 min	25
	Extension	72	1 min	
4	Final extension	72	5 min	1

Source: Authors

Finally, the amplified DNA was visualized in 1,5% agarose gels in 1X TAE buffer (Tris-Acetic-EDTA), using EZ-VISION THREE as a dye. Gel electrophoresis was performed at 80 V for 60 min. The presence of the amplified fragments was verified by visual inspection on the Gel DocTM XR + Imaging System (BIO-RAD, USA), taking the amplification bands of strain 11637 as reference.

The data were processed in the STATGRAPHICS Centurion XVI.I statistical software. The maximum efficiency of each filtration unit was determined through hypothesis tests, with a confidence level of 95% regarding the turbidity and color parameters. The reduction of microbiological characteristics (total coliforms and *E. coli*) was performed in logarithmic units.

Results and discussion

Raw water quality

The turbidity of the raw water ranged between 1,6 and 12,7 NTU, the average apparent color was 39,6 UPC, the total coliforms and the the average *E. coli* were 1 213 and 103 CFU/100 mL, respectively, the average temperature was 18,8 °C, and the pH ranged from 6,6 to 7,5. These quality conditions were recommended by Galvis *et al*. (1999) for the use of MSFT systems with DyGF, UGF, and SSF plus disinfection as a safety barrier. However, the treatment system only has the first two stages, which is usable within a scheme of progressivity for improving water quality if disinfection is performed collectively or individually in the households.

Treatment system operation

In general, some operating conditions limited the proper functioning of the different treatment structures. The DyGF did not exhibit a water level over the filter bed, which does not allow blocking the turbidity peaks that occur in raw water during the rainy season due to the dragging of solids in the basin. The flow treated by the plant during the study period was 3,05 L/s, which corresponded to a filtration rate of 2,94 m/h in the DyGF. Meanwhile, in the studied UGF, the treated flows per unit ranged between 0,84 and 2,04 L/s, with an average of 1,52 L/s, which represents a filtration rate range of 0,53-1,14 m/h, with an average value of 0,85 m/h. These rates are above those recommended (0,30 and 0,60 m/h) by Galvis *et al*. (1999), which may entail reduced removal efficiency and increased maintenance requirements due to a greater hydraulic head loss in the UGF.

It can be highlighted that there were periods of low maintenance frequencies in the treatment units, with an impact on hydraulic head losses. During the first five weeks, the UGF reached hydraulic head loss values of up to 4,7 cm, with large amounts of mud on the filter surface generated by a very low frequency of maintenance, which included only two bottom drainages without surface washing. Figure 2a shows substantial sludge buildup on the surface filter medium, while Figure 2b illustrates a clean filter medium.

Figure 2. Gravel filter with presence of mud on the surface. a) week 3; b) week 10.
Source: Authors

During the following five weeks, the maintenance frequency was increased to once a week, with deep washing and superficial washing every two weeks, showing reductions in hydraulic head loss (from 4,7 to 2,1). For the last four weeks of monitoring, the frequency of maintenance was reduced again (no bottom drainage or surface washing was evidenced), yielding a much greater increase in hydraulic head loss (between 2,6 and 5,8 cm).

These data, particularly those between the first five weeks and the last four, indicate that there was an accumulation of sludge within the filter medium of the UGF.

Treated water quality

The results obtained regarding turbidity, color, total coliforms, and *E. coli* are summarized in Table 3. None of these parameters reached the average quality value suggested by the World Health Organization (WHO, 2017) for drinking water, which shows low or no removal efficiency. These statistical results were obtained with a confidence level of 95%, through which the removal efficiency was estimated (Table 4).

As per the statistical analysis, with regard to turbidity and color, the DyGF exhibited removal values lower than 16 and 19%, respectively (Table 4). These results are consistent with the efficiency of other MSFTs treating similar raw water qualities. Galvis *et al*. (1999) reported a removal efficiency ranging from 24 to 38% for turbidity and between 17 and 21% for color. However, these values were obtained under appropriate DyGF operating and maintenance conditions.

Table 3. Quality of raw water and effluent of DyGF and UGF

Parameter	Statistical	Raw water	DyGF	UGF
Turbidity	Average	3,8	3,3	2,3
	SD	2,1	1,4	1,0
	Maximum	12,7	7,3	5,9
	Minimum	1,6	1,9	1,4
Apparent Color	Average	39,6	32,8	26,3
	SD	21,7	17,0	16,5
	Maximum	92,3	67,0	67,7
	Minimum	8,3	7,7	3,7
Total Coliforms	Average	1 213	1 343	1 184
	SD	732	958	736
	Maximum	3 300	4 100	3 069
	Minimum	483	154	392
E. coli	Average	103	83	57
	SD	71	63	39
	Maximum	263	239	148
	Minimum	5	20	8
H. pylori	Total samples	30	30	30
	Number of positives	3	6	6

Source: Authors

In the case of the UGF, the results indicate removal values of less than 34,0% for turbidity and 30% for color (Table 4), while the same study by Galvis *et al.* (1999) reported 31-36% for turbidity and 21-26% for color, with a filtration rate of 0,6 m/h, lower than that of our study. Posso (2012) reported 32 and 36% turbidity removals in the Golondrinas and Arroyohondo plants, respectively, with filtration rates between 0,45 and 0,57 m/h. This indicates that the UGF units under study exhibit a similar removal efficiency, despite the higher filtration rates used.

The results obtained regarding the removal of total coliforms and *E. coli* (Tables 3 and 4) show great variation and low performance in the filtration units. In 71% of the cases, there was a tendency towards an increased concentration of total coliforms at the outlet of the DyGF. In the remaining 29%, a reduction of 0,17 log was observed. The UGF exhibited a similar but less accentuated trend, with a reduction of 0,22 log for 64% of the cases (Table 4).

The performance of the DyGF in the removal of *E. coli* also exhibited limitations, as reductions were only achieved in 57% of the cases, reaching a value lower than 0,22 log (Table 4). Meanwhile, the UGF, managed to obtain reductions of less than 0,3 log for 79% of the cases. These results evidence the low performance of the system when compared to the reduction of microorganisms through gravel filters (Mushila *et al.*, 2016; Vega, 2013; Nkwonta and Ochieng, 2009; Sánchez *et al.*, 2007; Fernández *et al.*, 2006; Galvis *et al.*, 1999; Posso, 2012), where removals and/or reductions between 30 and 40% and between 0,6 and 2,5 log have been reported, The behavior of the studied units was favored by the environmental conditions caused by inadequate operation and maintenance,

Table 4. Efficiency of the filters in the removal and reduction of the parameters

Efficiency	Parameter	DyGF	UGF
% Removal, (p-value)	Turbidity	16 (0,045)	34 (0,030)
	Apparent Color	19 (0,033)	30 (0,041)
Reduction in Log, (p-value)	* Total Coliforms	0,17 (0,041)	0,22 (0,032)
	**E. coli*	0,21 (0,047)	0,30 (0,037)

* Includes only the values that caused reductions.
Source: Authors

The removal of particles in a gravel filter occurs by different mechanisms, with sedimentation being one of the most important. The efficiency of the filter depends on variables such as the filtration speed, the length and size of the filter medium, porosity, and the particle size to removed (Boller, 1993; Sánchez, 2017). The adherence of the particles to the surface of the filter medium is given by the interaction between van der Waals attraction forces and hydrodynamics. The accumulation of particles inside the pores reduces their size, which entails increased interstitial flow velocity, hydraulic head losses, and shear forces that allow for the detachment of the retained particles (Boller, 1993). Sánchez (2017) found that particles less than 5 µm in diameter can only be removed with efficiencies between 45 and 70% and a filtration rate of less than 0,5 m/h.

In this sense, the low or null efficiency found especially in the removal of coliforms and *E. coli* could be explained by the detachment of accumulated excess particles inside the gravel filters, which is caused by the low frequency of maintenance and high filter operation speeds (0,53-1,14 m/h), likely entailing shear forces large enough to generate drag in the retained particles and cause increases in both turbidity and color, as well as in the concentration of microorganisms, as described by Boller (1993).

Helicobacter pylori removal

The presence of *H. pylori* was detected in 15 of the 90 samples taken during monitoring. Three samples correspond to raw water, six to the outlet of the DyGF, and another six to the outlet of the UGF (Table 5). Considering that the bacteria can survive in water by employing several strategies, including the formation of biofilm (Campos *et al.*, 2011; Moreno *et al.*, 2007; Santiago, 2016; Chowdhury, 2012; Watson *et* al., 2004), conversion to coccoid form in a non-cultivable viable state (CVNC) (Azevedo *et al*., 2007; Oliver, 2005), or adherence to abiotic surfaces (Fernández *et al*., 2008), their greater presence at the outlet of the filters could be associated with the possible detachment of retained solids due to the high cutting forces generated by the accumulation of particles in the gravel. However, this finding should be studied in more detail.

As shown in Table 5, during weeks 1 to 5 and 12 to 15, in which maintenance was not adequately carried out, nor with the frequency required for this type of filtration units, the number of samples with presence of *H. pylori* was greater at the outlet of the gravel filters (DyGF and UGF). On the other hand, between weeks 6 and 11, during which maintenance activities were performed more frequently, no positive values were detected.

Table 5. Presence (+)/absence (-) of *H. pylori* per week

Sampling point	Week						Total samples
	*1 to 5 **		*6 to 11***		*12 to 15****		
	(+)	(-)	(+)	(-)	(+)	(-)	
Raw water	0	10	0	12	3	5	30
DyGF output	4	6	0	12	2	6	30
UGF output	2	8	0	12	4	4	30

Maintenance activities:
** Two bottom drainages without surface washing*
*** Weekly bottom drainages with surface washing*
**** Without bottom drainages or surface washing*
Source: Authors

Statistical analysis revealed a relationship between the lack of filter maintenance and the presence of *Helicobacter pylori*, with p-values of 0,036 and 0,018 for the DyGF and the UGF respectively. Meanwhile, no significant differences were found in terms of color, turbidity, *E. coli*, and total coliforms removal, with p-values greater than 0,27.

Conclusions

The results obtained for the studied system's operating conditions do not show significant microorganism removal (coliforms and *Helicobacter pylori*). On the contrary, there is a greater number of positive values at the outlet than at the inlet. It should be noted that the operation and maintenance of the treatment system was mostly inappropriate, which could explain the results. In general, when they are well operated and maintained, these systems are expected to improve quality of water. Thus, it is evident that the selection and implementation of treatment technologies must be thoroughly considered according to local conditions. In our case, the partial introduction of MSFT in Los Llanos water supply system, despite the fact that its operation and maintenance scheme is much simpler than that of other technologies, does not seem to be sufficient to guarantee a good quality of water for human consumption if interventions that facilitate the appropriation of technology by the community are not considered. Similarly, without the complement of SSFs, MSFT seems to be unreliable in ensuring water quality, particularly from a microbiological point of view.

These results also show that applying the concept of *graduality* to rural water treatment systems, which considers the construction of staged systems, must ensure quality of water for all users. This should also be complemented with disinfection techniques.

Although the findings regarding microbiological removal are linked to a low frequency of maintenance, a more detailed study is required to better explain the presence of *H. pylori* at the outlet of the filters.

Acknowledgments

The authors express their gratitude to the project titled *Validation of slow sand filters for the removal of the emerging pathogen Helicobacter pylori in rural water supply systems*, which is attached to the call for the funding of projects aimed at strengthening research groups classified in category C, which are recognized and registered by the Vice-Principalship for Research of Universidad del Cauca. The authors would also like to thank the community of Los Llanos, which is part of Las Guacas water supply system, for their valuable support in the execution of our research activities; as well as the Environmental Engineering and Human Genetics laboratory of Universidad del Cauca and the SENA-Cauca Environmental Analysis Laboratory.

CRediT author statement

All authors contributed to the conceptualization, methodology, validation, formal analysis, investigation, data curation, and writing of this work. They have approved the definitive version of this manuscript for publication.

References

Acosta, C. P., Codony, F., Fittipaldi, M., Sierra-Torres, C. H., and Morató, J, (2018). Monitoring levels of viable Helicobacter pylori in surface water by qPCR in Northeast Spain. *Journal of Water and Health*, *16*(5), 839-845. https://doi.org/10.2166/wh.2018.195

Adrada, J. C., Calambás, F. H., Díaz, J. E., Delgado, D. O., and Sierra, C. H. (2008). Características sociodemográficas y clínicas en una población con cáncer gástrico en el departmento de Cauca, Colombia. *Revista Colombiana de Gastroenterologia*, *23*, 309-314. http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0120-99572008000400004

Agustí, G., Codony, F., Fittipaldi, M., Adrados, B., and Morató, J. (2010). Viability determination of *Helicobacter pylori* using propidium monoazide quantitative PCR. *Helicobacter*, *15*(5), 473-476. https://doi.org/10.1111/j.1523-5378.2010.00794.x

Al-Sulami, A. A., Al-Taee, A. M. R., and Juma'a, M. G. (2010). Isolation and identification of helicobacter pylori from drinking water in Basra governorate, Iraq. *Eastern Mediterranean Health Journal*, *16*(9), 920-925. https://doi.org/10.26719/2010.16.9.920

Atherton, J. C., Cao, P., Peek, R. M., Tummuru, M. K. R., Blaser, M. J., and Cover, T. L. (1995). Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*, Association of specific vacA types with cytotoxin production and peptic ulceration. *Journal of Biological Chemistry, 270*(30), 17771-17777. https://doi.org/10.1074/jbc.270.30.17771

Aziz, R. K., Khalifa, M. M., and Sharaf, R. R. (2015). Contaminated water as a source of *Helicobacter pylori* infection: A review. *Journal of Advanced Research*, *6*(4), 539-547. https://doi.org/10.1016/j.jare.2013.07.007

Baker, K. H., and Hegarty, J. P. (2001). Presence of *Helicobacter pylori* in drinking water is associated with clinical infection. *Scandinavian Journal of Infectious Diseases*, *33*(10), 744-746. https://doi.org/10.1080/003655401317074536

Bartram, J., and Cairncross, S. (2010). Hygiene, sanitation, and water: Forgotten foundations of health *PLoS Medicine*, *7*(11), e1000367. https://doi.org/10.1371/journal.pmed.1000367

Boller, M. (1993). Filter mechanisms in roughing filters. *Aqua*, *42*(3), 174-185.

Campos, M., Soto, H., Meléndez, M., Sandoval, C., Santamaría, G., Rojas, B., Cascante, L., Gutiérrez, O., and Montero Campos, V. (2011). Hallazgo de la bacteria Helicobacter pylori en agua de consumo humano y su relación con la incidencia de cáncer gástrico en Costa Rica. *Tecnologia En Marcha*, *24*(3), 3-14.

Chowdhury, S. (2012). Heterotrophic bacteria in drinking water distribution system: A review. *Environmental Monitoring and Assessment, 184,* 6087-6137. https://doi.org/10.1007/s10661-011-2407-x

Engstrand, L. (2001). Helicobacter in water and waterborne routes of transmission. *Symposium Series* (*Society for Applied Microbiology*), *30*, 80-84.

Erzin, Y., Koksal, V., Altun, S., Dobrucali, A., Aslan, M., Erdamar, S., Dirican, A., and Kocazeybek, B. (2006). Prevalence of *Helicobacter pylori vacA, cagA, cagE, iceA, babA2* genotypes and correlation with clinical outcome in Turkish patients with dyspepsia. *Helicobacter, 11*(6), 574-580. https://doi.org/10.1111/j.1523-5378.2006.00461.x

Fernández-Delgado, M., Contreras, M., García-Amado, M. A., Michelangeli, F., and Suárez, P. (2008). Evidencias de la transmisión acuática de *Helicobacter pylori*. *Interciencia, 33*(6), 412-417. http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S0378-18442008000600005

Fernández, J., Cruz, A., and Benavides, E. (2006). Remoción de materia orgánica por filtración en múltiples etapas. *Ingeniería Hoy*, *24*, 20-27.

Galvis, G., Teun Visscher, J., and Lloyd, B. (1992). Multi-stage surface water treatment for community water supply in Colombia. *Waterlines*, *10*(3), 26-29, https://doi,org/10.3362/0262-8104.1992.008

Galvis, G. (1999). *Development and evaluation of multistage filtration plants: An innovative, robust and efficient water treatment technology* [Doctoral thesis, Univertsity of Surrey]. https://openresearch.surrey.ac.uk/esploro/outputs/doctoral/Development-and-evaluation-of-multistage-filtration/99513881902346

Galvis, G., Latorre, J., and Visscher, J. T. (1999). *Filtración en múltiples etapas: tecnología innovativa para el tratamiento de agua*. Artes Gráficas de Univalle. https://www.ircwash.org/sites/default/files/255.9-99FI-17025.pdf

Gião, M. S., Azevedo, N. F., Wilks, S. A., Vieira, M. J., and Keevil, C. W. (2008). Persistence of *Helicobacter pylori* in heterotrophic drinking-water biofilms. *Applied and Environmental Microbiology*, *74*(19), 5898-5904. https://doi.org/10.1128%2FAEM.00827-08

Goh, K., Chan, W., Shiota, S., and Yamaoka, Y. (2011). Epidemiology of *Helicobacter pylori* infection and public health implications. *Helicobacter*, *16*, 1-9. https://doi.org/10.1111/j.1523-5378.2011.00874.x

Haig, S. (2014). *Characterising the functional ecology of slow sand filters through environmental genomics* [Doctoral thesis, University of Glasgow]. https://theses.gla.ac.uk/5523/1/2014HaigPhD.pdf

Haig, S. J., Collins, G., Davies, R. L., Dorea, C. C., and Quince, C. (2011). Biological aspects of slow sand filtration: Past, present and future. *Water Science and Technology: Water Supply, 11*(4), 468-472. https://doi.org/10.2166/ws.2011.076

Hooi, J. K. Y., Lai, W. Y., Ng, W. K., Suen, M. M. Y., Underwood, F. E., Tanyingoh, D., Malfertheiner, P., Graham, D. Y., Wong, V. W. S., Wu, J. C. Y., Chan, F. K. L., Sung, J. J. Y., Kaplan, G. G., and Ng, S. C. (2017). Global prevalence of *Helicobacter pylori* infection: Systematic review and meta-analysis. *Gastroenterology*, *153*(2), 420-429. https://doi.org/10.1053/j.gastro.2017.04.022

Hulten, K., Han, S. W., Enroth, H., Klein, P. D., Opekun, A. R., Gilman, R. H., Evans, D. G., Engstrand, L., Graham, D. Y., and El-Zaatari, F. A. K. (1996). *Helicobacter pylori* in the drinking water in Peru. *Gastroenterology*, *110*(4), 1031-1035. https://doi.org/10.1053/gast.1996.v110.pm8612990

Ministerio de Vivienda Ciudad y Territorio (2020). *Informe Nacional de Calidad del Agua para Consumo Humano INCA 2019*. https://www.minvivienda.gov.co/sites/default/files/documentos/informe-calidad-de-agua-2019.pdf

Moncayo, J. I., Santacruz, J. J., Álvarez, A. L., Franco, B., López, M. A., Ángel, A., Gallego, M. L., and Serrano, H. (2006). Comparación de métodos diagnósticos en la infección por *Helicobacter pylori* en Quindío, Colombia. *Colombia Medica*, *37*(3), 203-212. https://www.redalyc.org/articulo.oa?id=28337306

Montero-Campos, V. (2019). *Helicobacter pylori* en Costa Rica, más de una década de investigaciones. *Revista Tecnología En Marcha*, *32*, 94-103. https://doi.org/10.18845/tm.v32i9.4636

Moreno, Y., Piqueres, P., Alonso, J. L., Jiménez, A., González, A., F. M. (2007). Survival and viability of *Helicobacter pylori* after inoculation into chlorinated drinking water. *Water Research*, *41*(15), 3490-3496. https://doi.org/10.1016/j.watres.2007.05.020

Mushila, C. N., Ochieng, G. M., Otieno, F. A. O., Shitote, S. M., and Sitters, C. W. (2016). Hydraulic design to optimize the treatment capacity of multi-stage filtration units. *Physics and Chemistry of the Earth*, *92*, 85-91. https://doi.org/10.1016/j.pce.2015.10.015

Nkwonta, O., and Ochieng, G. (2009). Roughing filter for water pre-treatment technology in developing countries: A review. *International Journal of Physical Sciences, 4*(9), 455-463). https://www,researchgate,net/publication/237827490_Roughing_filter_for_water_pre-treatment_technology_in_developing_countries_A_review

Ochieng, G. M. M., Otieno, F. A. O., Ogada, T. P. M., Shitote, S. M., and Menzwa, D. M. (2004). Performance of multistage filtration using different filter media against conventional water treatment systems. *Water SA*, *30*(3), 361-367. https://doi.org/10.4314/wsa.v30i3.5085

Oliver, J. D. (2005). The viable but nonculturable state in bacteria. *Journal of Microbiology, 43*, 93-100.

Ordóñez, J. F. (2015). *Evaluación de la calidad microbiológica de las fuentes de abastecimiento del acueducto rural El Saladito – Timbío – Cauca* [Undergraduate thesis, Universidad del Cauca]. http://repositorio.unicauca.edu.co:8080/xmlui/handle/}123456789/6862

Pardo, C., De Vries, E., Buitrago, L., and Gamboa, Ó. (2017). *Atlas de mortalidad por cáncer en Colombia*. https://www.cancer.gov.co/ATLAS_de_Mortalidad_por_cancer_en_Colombia.pdf

Posso, D. (2012). *Análisis de la operación y mantenimiento de la filtración en gravas de flujo ascendentes a escala real* [Undergraduate thesis, Universidad del Valle]. https://hdl.handle.net/10893/7675

Premoli, G., González, A., Millán-mendoza, B., Percoco, T., and Vielma, A. (2004). Diagnóstico de *Helicobacter pylori* mediante la reacción en cadena de la polimerasa. *Revista Cubana de Medicina Tropical*, *56*(2), 85-90. http://scielo.sld.cu/pdf/mtr/v56n2/mtr01204.pdf

Raman, A., Paramasivam, R., Heijnen, H. A., Visscher, J. (1992). *Filtracion lenta en arena : tratamiento de agua para comunidades : planeacion, diseno, construccion, operacion y mantenimiento*. https://es.ircwash.org/resources/filtracion-lenta-en-arena-tratamiento-de-agua-para-comunidades-planeacion-diseno

Ramos, A. R., and Sánchez, R. S. (2009), Contribución de Latinoamérica al estudio del *Helicobacter pylori*. *Acta Gastroenterologica Latinoamericana, 39*(3), 197-218. https://www.redalyc.org/exportarcita.oa?id=199317345011

Samra, Z. Q., Javaid, U., Ghafoor, S., Batool, A., Dar, N., and Athar, M. A. (2011). PCR assay targeting virulence genes of *Helicobacter pylori* isolated from drinking water and clinical samples in Lahore metropolitan, Pakistan. *Journal of Water and Health*, *9*(1), 208-216. https://doi.org/10.2166/wh.2010.169

Sánchez, L. D., Sánchez, A., Galvis, G., and Latorre, J. (2007). *Filtración en Múltiples Etapas*. IRC. https://es.ircwash.org/resources/filtraci%C3%B3n-en-m%C3%BAltiples-etapas

Sánchez, L. D. (2017). Determinación del factor de adherencia en filtros de grava de flujo ascendente en capas. *Ingeniería Y Competitividad*, *19*(2), 121-130. https://doi.org/10.25100/iyc.v19i2.5299

Santiago, P. (2016). *Transmisión de Helicobacter pylori a través del agua: estudio de la presencia del patógeno e identificación de formas viables mediante técnicas moleculares* [Doctoral thesis, Universidad Politécnica de Valencia]. https://doi.org/10.4995/Thesis/10251/75086

Vega Serrano, H. A. (2013). *Evaluación del sistema de filtración en múltiples etapas FiME en tanques plásticos con pre-sedimentación y retro-lavado en la hacienda Majavita* (*Socorro, Santander*) [Master's thesis, Universidad de Manizales]. https://ridum.umanizales.edu.co/xmlui/bitstream/handle/20.500.12746/151/402_Vega_Serrano_Haimar_Ariel_2013_Documento.pdf

Vesga, F. (2018). *Detección y viabilidad de Helicobacter pylori en aguas crudas y potables en tres plantas de potabilización en la ciudad de Bogotá* [Doctoral thesis, Pontificia Universidad Javeriana, Universidad Politécnica de Barcelona]. https://doi.org/10.11144/Javeriana.10554.41877

Watson, C. L., Owen, R. J., Said, B., Lai, S., Lee, J. V., Surman-Lee, S., and Nichols, G. (2004). Detection of *Helicobacter pylori* by PCR but not culture in water and biofilm samples from drinking water distribution systems in England. *Journal of Applied Microbiology*, *97*(4), 690-698. https://doi.org/10.1111/j.1365-2672.2004.02360.x

Wegelin, M. (1996). *Surface water treatment by roughing filters: A design, construction and operation manual*. SKAT. https://www.ircwash.org/resources/surface-water-treatment-roughing-filters-design-construction-and-operation-manual

World Health Organization (WHO) (2017). *Guidelines for drinking-water quality: fourth edition incorporating the first addendum*. WHO. https://www.who.int/publications/m/item/guidelines-for-drinking-water-quality-4th-ed.-incorporating-the-1st-addendum-(chapters)

Yamaoka, Y., Kodama, T., Gutiérrez, O., Kim, J. G., Kashima, K., and Graham, D. Y. (1999). Relationship between *Helicobacter priori* iceA, cagA, and vacA status and clinical outcome: Studies in four different countries. *Journal of Clinical Microbiology*, *37*(7), 2274-2279. https://doi.org/10.1128/jcm.37.7.2274-2279.1999