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Environmental Pollution

Effectiveness of the use of diatoms in the filtration of waterborne bacteria

Efectividad del uso de diatomeas en la filtración de bacterias de transmisión hídrica

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Abstract

Bacterial transmission to humans can be carried by water and cause enteric diseases, so the objective of this paper is to evaluate the effectiveness of the use of diatoms in the filtration of waterborne bacteria. The study was carried out at Universidad Nacional de Chimborazo, Ecuador; from October 2019 to March 2020, combining culture techniques on blood agar and MacConkey agar, colony quantification and filtration versus filter time and length. 120 dilutions samples of commercial strains of *Escherichia coli* and *Staphylococcus aureus* were used to contaminate the water in such a way that the efficiency of filtration in diatoms of different origins could be observed. The results were contrasted with the ranges established by the World Health Organization and positive and negative controls were carried out on the culture media and water. The diatoms of Guayaquil were the ones that induced a better filtration of the water compared to the diatoms of Palmira. When applying the filter bed of 10 cm of diatoms, a growth of 86 CFU/100ml was obtained in 24 hours, while when the amount of the filter was increased to 20 cm, a decrease in the bacterial load of the water was observed by 21 CFU/100ml in 10 hours. According to the range established by the WHO, bacterial growth decreased, which indicates that diatom filters have the ability to retain bacteria. For this reason, it is presumed that, when combined with additional materials such as activated carbon, their filtering potential would increase.

Keywords: Diatoms, filter, culture, filter bed.

Resumen

La transmisión bacteriana al ser humano puede vehiculizarse por el agua y ocasionar enfermedades entéricas, por lo que el objetivo de la presente investigación es evaluar la efectividad del uso de diatomeas en la filtración de bacterias hidrotransmisibles. El estudio fue realizado en la Universidad Nacional de Chimborazo, Ecuador, desde octubre de 2019 a marzo de 2020, combinando técnicas de cultivo en agar sangre y agar MacConkey; cuantificación de colonias y filtración frente a tiempo y longitud de filtro. Se utilizaron 120 muestras de diluciones de cepas comerciales de *Escherichia coli* y *Staphylococcus aureus* para contaminar el agua de tal forma que se pudiera evidenciar la eficacia de la filtración en diatomeas de distinta procedencia. Los resultados fueron contrastados con los rangos establecidos por la Organización Mundial de la Salud y se realizaron controles positivos y negativos de los medios de cultivo y agua. Las diatomeas de Guayaquil fueron las que indujeron a una mejor filtración del agua frente a las diatomeas de Palmira. Al aplicar el lecho filtrante de 10 cm de diatomeas, se obtuvo un crecimiento de 86 UFC/100ml en 24 horas, mientras que al aumentarse la cantidad del filtro a 20 cm se observó un descenso de la carga bacteriana del agua en 21 UFC/100ml en 10 horas. De acuerdo al rango establecido por la OMS el crecimiento bacteriano disminuyó, lo que indica que los filtros de diatomeas tienen la capacidad de retener bacterias. Por esto, se presume que, al combinarlas con materiales adicionales como carbón activado, su potencial filtrante se incrementaría.

Palabras clave: Diatomeas, filtro, cultivo, lecho filtrante.

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1 Introduction

Various microbial waterborne outbreaks have been documented around the world due to different factors such as fecal contamination, which is a perfect vehicle until finding a host (Araujo et al., 1997; Rodríguez Miranda et al., 2016). There are examples even in industrialized countries such as the United States, where different cases (Rodríguez Gutiérrez et al., 2015; Lösch and Merino, 2016; Centros para el Control y la Prevención de Enfermedades, 2020; WHO, 2020) of bacterial outbreaks of *Escherichia coli*, *Campylobacter jejuni* and *Legionella* .spp., have been observed, as published by the journal *Quimica Viva* (Córdoba et al., 2010). However, the most significant microbial wasteborne outbreak occurred in Milwaukee, United States; the coccidium *Cryptosporidium parvum* infected about 403 000 people, causing serious complications to immunosupressed patients (Arora and Arora, 2009; Del Coco et al., 2009).

According to the World Health Organization (WHO), at least 2 000 million people worldwide are supplied with drinking water contaminated by feces, and 159 million depend on surface water that is highly likely to be contaminated. In addition, contaminated water is reported to transmit diseases such as diarrhea (Godoy et al., 2011; Cabezas Sánchez, 2018), colera (González et al., 2011; Zelada-Valdés et al., 2015), dysentery (González-Ramírez et al., 2020), typhoid fever (Peranovich, 2019) and poliomyelitis (Cué Brugueras, 2000), causing more than 502 000 deaths from diarrhea annually (WHO, 2019).

These infections usually occur because bacteria can use water as a vehicle until they reach a new host (Ferrari and Torres, 1998; Rodríguez et al., 2015; Hernández et al., 2017; Palomino-Camargo et al., 2018), in addition to the fact that these microorganisms can remain in perfect conditions and survive for weeks to months in water (Marin et al., 2020). Factors such as faulty pipe installations, damaged taps, repairs without the necessary safety measures, containers in poor condition or with poor hygienic-sanitary care and lack of proper maintenance of these facilities are potential reasons for the contamination of drinking water for human consumption (Duran and Torres, 2006), creating the environment for microorganisms to entry and multiply, causing various pathologies (Grupo de Tratamiento de Aguas Residuales Universidad de Sevilla, 2020).

In Ecuador, Palacios (2013), analyzed the condition of drinking water in a Cotopaxi region, and mentioned that fecal coliform contamination reached a prevalence of 35%, representing a major public health problem, being a source of infection for people with these water sources. In addition, several studies have been carried out in different areas of Chimborazo, which have demonstrated the water pollution of both agricultural irrigation and human consumption. In addition, it was found that the rural communities of the region are the most affected due to the lack of quality water supply since water is available directly from pipes without the corresponding previous treatment (Lara and Martínez, 2019; Tipán and Martínez, 2019). Water is an environment conducive to the proliferation and transport of bacteria, thus being mobilized from one geographical point to another, assuming contamination risk to every living being involved in this cycle.

Considering that it is possible to eliminate most of the microorganisms present in drinking water by using filters (Chulluncuy, 2011; WHO, 2012; Ríos-Tobón et al., 2017), its application is expected to significantly reduce the bacterial load of this resource, thanks to the retention of impurities and microorganisms in it. This post-filtration water has to have a low or no bacterial charge (according to WHO range) to be considered as consumable by humans. The decrease in bacteria in water shows the effectiveness of filters. However, as several materials are required for the development of commercial filters, research on affordable and easy-to-find materials has increased for the manufacture of filters to be able to obtain clean water efficiently and at a low cost (Leal, 2014). Several authors have studied the action of various materials that function as filtering beds, whether sand (Gil et al., 2002; Blacio and Palacios, 2011), charcoal (Silupú et al., 2017; Marín Velásquez et al., 2019), diatoms (Valencia, 2014), etc., and that be highly effective and cost-effective.

The aim of this paper is to verify waterborne bacteria after using diatom filtering beds from the coastal region of Guayaquil and the Andean region of Palmira, located in Ecuador, in order to compare and demonstrate the filtration and purification capacity of water against bacteria that may be carried on it.

2 Materials and Methods

This research has a quantitative and cross-sectional approach, with an exploratory-descriptive level and an experimental design. The population is constituted by the bacteria found in the water analyzed, based on the pollution risk ranges established by WHO (WHO, 2006), which was carried out with the intermediate risk range. The sample was determined based on the relationship between the filter bed height (centimeters) and the water filtration time (hours).

Data of the positive water control were included as well as positive filter control; results that were similar to each others were done by triplicate, excluding data whose controls indicate contamination, especially negative water control and negative filter control. The study did not require a bioethics permit because it was not conducted in humans.

Due to the experimental design of the research, filters with diatoms (*Bacillariophyceae sensu lato*) of different geographical origin (commercial diatoms from Guayaquil and Palmira) were gradually tested against bacteria of different sizes, *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) of commercial crops, bacteria with wide water distribution (Robert Pullés, 2014), and were evaluated after filtration in bacteriological culture media, Tryptic soy agar (TSA) as enrichment medium and specific (5% ram blood agar) and differential media (MacConkey agar). The culture media was prepared according to the specifications indicated by the HIMEDIA® commercial house. Filters were found to be free of contaminants that could alter the results.

To contaminate water, 1/10 dilutions were performed to obtain the desired intermediate range dilution of contamination, 10^{-11} dilution for *S. aureus*, whereas 10^{-13} for *E. coli*. To this, 9 ml of sterile physiological solution and 1 ml of broth containing the contaminating bacteria were added.

Once the dilutions of water contaminated with bacteria were obtained at a known concentration, diatoms were used in the filters. Two assemblies were used, the first with Guayaquil diatoms and the second with Palmira diatoms. The number of diatoms used as a filter bed was 10 cm, 15 cm and 20 cm for each source, respectively; in both cases, filtration time intervals were 30 minutes, 3 hours, 10 hours and 24 hours. During each set time, the culture was performed for the verification of bacterial load.

A glass holder (Figure 1) was used to make the filter, in which the diatoms were introduced separately according to their origin and filter height (10 cm, 15 cm, and 20 cm). The edges were covered with metal mesh and their base was fixed to a tap to control the filtration performed. At the other end of the filter, a drum was arranged which contained the contaminated water for filtering, which was then passed into a sterile flask in which the filtered material was collected for subsequent culture and bacterial quantification.

Figure 1. Diatoms were placed on a glass support which was connected to a faucet at one end and to a drum at the other.

3 Results

Of the 120 samples analyzed, it was found that the difference is minimal between the two classes of diatoms. However, filters made with diatoms from Guayaquil showed a greater filtering capacity compared to those of Palmira. Note in Table 1 that 2 filters were used; Guayaquil filter was the first one and Palmira filter was the second.

As for filter 1, using 10 cm of diatoms has a minimum filtration, yielding a growth of 86 CFU/100ml at 10 hours of waiting while using a diatom distance of 15 and 20 cm a growth of 77 CFU/100ml was obtained at 3 hours and 80 CFU/100ml at 24 hours, respectively, in reference to the strain of *S. aureus*. Regarding *E. coli* strain, when using 10 cm of diatom, growth of 88 CFU/100ml was obtained at 30 minutes of waiting, while using 15 cm of diatom showed a growth of 53 CFU/100ml at 3 hours and 21 CFU/100ml at 10 hours with 20 cm of diatoms.

As for filter 2 (Palmira) using 10 cm of diatoms has a minimum filtration, yielding a growth of 92 CFU/100ml at 10 hours of waiting while using a diatom distance of 15 and 20 cm provided a growth of 94 CFU/100ml at 3 hours and 65 CFU/100ml at 24 hours of waiting for *S. aureus* strain. As for *E. coli*, using 10 cm of diatom showed a growth of 95 CFU/100ml at 30 minutes of waiting while using 15 cm of diatom obtained a growth of 96 CFU/100ml at 3 hours and 65 CFU/100ml at 10 hours using 20 cm of diatoms.

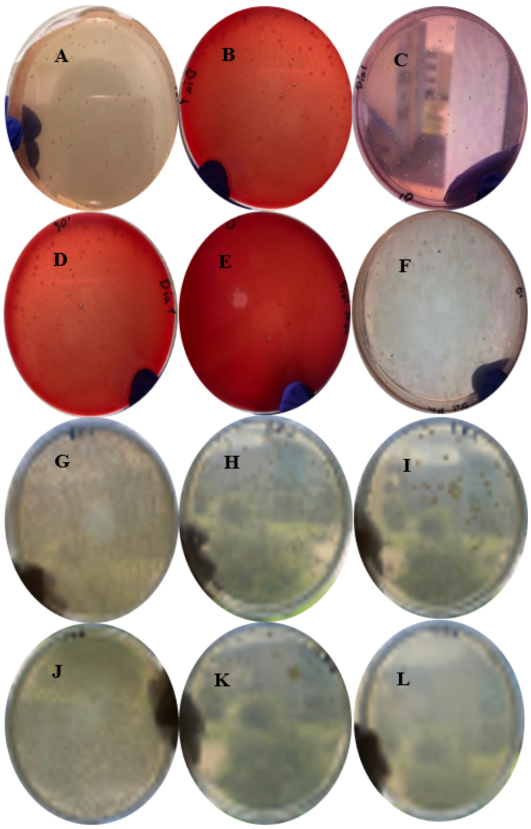

Figure 2. When using filter 2 (Palmira), of 10 cm, **A)** growth was of 95 CFU/100ml with respect to *E. coli* over a period of 30 minutes (low filtration). **B)** The growth of *S. aureus* was 74 CFU/100ml at 3 hours when using the 15 cm filter number 2, **C)** the growth of *E. coli* was 74 CFU/100ml in 0 hours. **D)** *S. aureus* growth was 108 CFU/100ml at 30 minutes. The use of filter 2 with 20 cm in length yielded the following results: **E)** *S. aureus* growth of 24 CFU/100ml at 0 hours. **F)** *E. coli* growth of 125 CFU/100ml at 0 hours. **G, H, I)** The pattern of consecutive dilutions for *E. coli* is observed until a dilution of 10^{-13}. **J, K, L)** Consecutive dilutions of *S. aureus* to a concentration of 10^{-11}.

Table 1. Comparison between diatom fiters from Guayaquil and Palmira

Measure from the filtering bed		0 hours		30 minutes		3 hours		10 hours		24 hours		
		CFU/100ml										
		S. aureus	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*	*Pm*
Filter 1	10 cm	15	24	94	88	49	57	86	80	84	99	67.6
Filter 2		65	82	95	95	74	40	92	85	65	87	78.0
Filter 1	15 cm	77	82	80	90	77	53	27	44	27	40	59.7
Filter 2		80	74	88	92	94	96	67	71	85	89	83.6
Filter 1	20 cm	85	54	58	49	99	43	74	21	80	37	60.0
Filter 2		24	86	32	81	83	96	59	61	65	83	67.0

CFU/100ml: Forming units of colonies present in 100 milliliters of solution. Pm: Average growth per filter and measure. Filter 1: From Guayaquil. Filter 2: From Palmira.

Table 2 shows the results of the water filtration using Guayaquil diatoms as a filter bed. It may be noted that diatom growth varies at different degrees. When using 10 cm of diatoms, growth was 94 CFU/100ml at 30 minutes, then the growth decreased to 86 CFU/100ml at 10 hours and then increased again to 99 CFU/100ml at 24 hours. The growth was similar with 15 cm of diatoms, being 80 CFU/100ml at 30 minutes, 77 CFU/100ml at 3 hours and then it decreased to 27 CFU/100ml at 24 hours. On the other hand, the growth was lower with 20 cm of diatoms; 49 CFU/100ml at 30 minutes, 21 CFU/100ml at 10 hours and 37 CFU/100ml at 24 hours.

Table 3 shows the results of the water filtration using Palmira diatoms as a filter bed. When using 10 cm of diatoms, growth was 95 CFU/100ml at 30 minutes while that growth was reduced at 85 CFU/100ml at 10 hours and 87 CFU/100ml at 24 hours. With 15 cm of diatoms the growth was 88 CFU/100ml at 30 minutes, 96 CFU/100ml at 3 hours and the growth decreased to 85 CFU/100ml at 24 hours. On the other hand, there was a lower growth with 20 cm of diatoms, 32 CFU/100ml at 30 minutes, 59 CFU/100ml at 10 hours and 53 CFU/100ml at 24 hours.

4 Discussion

The filters reduce the contamination present in water, whether these contaminants are macroscopic elements or microorganisms such as bacteria (Apella and Araujo, 2000). Materials that have been widely used in the manufacturing industries have varied as new techniques and research emerge, favoring the purification of water, such as dust, coal, and others. As a result, the increase in the use of diatoms for multiple purposes related to water filtration has been observed in recent years (Colín-García et al., 2013). In a research carried out by Valencia (2014), it is mentioned that diatoms have a wide range of uses, among which seawater filtration and removal of the saline load. For this reason, it is considered that the purifying capacity of diatom is used when creating water filters, which facilitate the retention of contaminants such as dust and reduce the presence of bacteria and parasites.

Diatoms have multiple benefits when it comes to innovating and researching. Colín-García et al. (2013), mention the use of diatoms as a source of silica (SiO_2), which has many technological applications such as “drug release, photovoltaic cells, and high-performance ceramic materials”. Likewise, Nájera-Arce et al. (2018), refer to the antimicrobial activity of diatoms in such a way that extracts obtained from species belonging to Bacillariophyceae s.l., have bactericidal qualities against common Enterobacteria such as *E. coli, S. aureus, Pseudomona.sp*, among others; therefore, diatoms, when applied to the water filtration for human consumption have a filter and an antimicrobial action.

By aggregating the results and contrasting diatoms from Guayaquil and Palmira, it was possible to show at first sight that the results obtained by filtration with diatoms from Guayaquil showed a higher filtering capacity (on average 77.03 CFU/100ml) unlike the diatoms from Palmira (on average 114.7

CFU/100ml) in *S. aureus* strain and *E. coli* strain. The filtration and retention effectiveness of bacteria increased as the number of diatoms used in the filter bed increased, with purification being higher when using a length of 20 centimeters of diatoms, followed by 15 centimeters and finally the lowest filter capacity when using 10 centimeters of diatoms.

Table 2. Water filtering with filter bed using diatoms from Guayaquil

FILTER 1	0 hours		30 minutes		3 hours		10 hours		24 hours	
	CFU/100ml									
	S. aureus	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*
10 cm	15	24	94	88	49	57	86	80	84	99
15 cm	77	82	80	90	77	53	27	44	27	40
20 cm	85	54	58	49	99	43	74	21	80	37

CFU/100ml: Forming units of colonies present in 100 milliliters of solution.

Table 3. Water filtering with filter bed using diatoms from Palmira

FILTER 2	0 hours		30 minutes		3 hours		10 hours		24 hours	
	CFU/100ml									
	S. aureus	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*
10 cm	65	82	95	95	74	40	92	85	65	87
15 cm	80	74	88	92	94	96	67	71	85	89
20 cm	24	86	32	81	83	96	59	61	65	83

CFU/100ml: Forming units of colonies present in 100 milliliters of solution.

These results differ from those obtained by Caballero and Zuni (2017), who in their research on the effectiveness of water filters using diatoms and chitosan obtained effectiveness with diatoms from the Andean region located at approximately 3000 meters. Using the filter they developed in their research, it was possible to reduce the bacterial load of an *E. coli* strain from 48 to 5 CFU/100ml, while in this research a significant improvement in filtration was obtained using diatoms from the coastal region (low geographic altitude), reducing the presence of the strain used from 100 CFU/100ml to 77.03 CFU/100ml. The data obtained show that diatoms alone have the ability to retain bacteria and decrease the bacterial load present in contaminated water (Caballero and Zuni, 2017). According to the study carried out by Pereira et al. (2017), the activated charcoal obtained from rice husks acts satisfactorily in the purification of water. However, the elimination of microorganisms present is not guaranteed. Therefore, it is necessary to study the combination of diatoms with activated carbon in the same filter in order to evaluate their behavior.

Pérez-Vidal et al. (2016), mention that the use of commercial filters, whose composition is better and more complex, is more effective in terms of water filtration, because the different materials arranged along the filter bed meet a percentage of water filtration, so if a layer of diatoms were added, bacterial filtration would increase and a more favorable result would be obtained (Calizaya-Anco et al., 2013).

5 Conclusions

When comparing diatoms from Guayaquil and Palmira as filter beds, it can be verified that diatoms from Guayaquil are the ones that yielded the best data.

S. aureus is used to obtain varying concentrations to contaminate water and pass it through the filter for filter bed verification. However, there is a growth of no less than 15 CFU/100ml at hour 0 in filter 1 with 10 cm with diatom from Guayaquil.

Water contamination with *E. coli* bacilli is performed to obtain the varying concentrations to contaminate water and pass it through the filter for filter bed verification. There is a growth of no less than 21 CFU/100ml at 10 hours in filter 1 with 20 cm of diatom from Guayaquil.

The filtration time is not too important on the purification of water because contamination remained the same even with the variation of time.

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