Heritage and Sustainable Development ISSN 2712-0554
Vol. 2, No. 2, July 2020, pp.100-107
https://doi.org/10.37868/hsd.v2i2.46

The effect of active charcoal filter on viability of bacteria isolated from the tap water in Sarajevo

Merjema Ikanović[1], Mevlan Iseni[1**], Muhamed Adilović[1], Altijana Hromić-Jahjefendić[1*]**
[1] Genetics and Bioengineering, International University of Sarajevo

*Corresponding author: ahromic@ius.edu.ba
**The authors have contributed equally to the study

Abstract

Clean water is essential to our existence and problems might arise when it becomes contaminated with different pathogens, which might pose a threat to human health. Tap water is generally considered drinkable since it passes different forms of disinfection during processing. Some households have additional disinfection procedures, the most common one being the usage of charcoal filters, in order to further clean the tap water from both undesirable solvents and microorganisms. In the first independent study of this kind, we have tested tap water for bacteria from five different locations in Sarajevo, and we have tested the efficiency of charcoal filter in trapping of bacteria. According to regulations in Bosnia and Herzegovina, there should be 1 colony forming unit (CFU) per 50ul of water sample, which was satisfied in only one location from Sarajevo, while one had significantly higher levels (6.7, p val. 0.0148). Overall, the charcoal filter has decreased the number of bacteria in the water, with the exception of one sample.

Keywords: Tap Water in Sarajevo; Tap Water Contamination; Bacteria; Charcoal Filter

1. Introduction

1.1. Water

Water comprises 75% of the human body and makes about half of the volume of every cell [1]. Roles of water range from being a building material, nutrients carrier, reactant, lubricant, reaction medium and solvent [2]. Due to these and our regular daily need for water, it is crucial for drinking water to be clean of pathogens, yet lack of clean water is one of the biggest challenges humanity will have to face in the future [3].

Besides developing countries where the lack of tap water may be apparent, many other regions of the world are familiar with the tap water for more than a century [4]. Even though it is mostly considered as a drinking water, it may not always be the case, which is why certain purification and sterilization methods can be implemented in order to make the water potable [5]. Millions of microorganisms inhabit the water and they can be harmful or harmless [6]. The presence of such organisms in water may be due to water systems that sometimes host bacteria or due to contamination of tap water sources [4].

1.2. Pathogens

Pathogens are microorganisms which can cause the disease. Many microbial inhabitants are present in our organisms but are in fact beneficial, and some might cause trouble only in cases of weakened immune systems or penetration into sterile parts of the human body. On the other hand, devoted pathogens do not depend on injury or weakened immune system because they have mechanisms which enable them to overcome biochemical and cellular in order to proliferate [7].

Pathogenic organisms are of five main types: viruses, bacteria, fungi, protozoa, and worms [8]. The focus of this article is on bacteria. As pathogens get transmitted, they penetrate into their host and multiply until infected host starts to show symptoms, including flu, fever, ineffective cough and others [9]. Some organisms that may be found in tap water are *Escherichia coli*, *Vibrio cholerae, Vibrio parahaemolyticus*, *Salmonella enterica* and *Shigella*. Diseases developing from these bacterial agents range from Cholera, Gastroenteritis, Typhoid fever, severe salmonellosis, bacillary dysentery or shigellosis, Acute diarrhea and gastroenteritis [6].

The concerning issue of today is whether the maintenance of water supply systems is appropriate, since it can result in an outburst of waterborne diseases because there are over 500 pathogens in tap water that could pose a potential risk [10]. Bacterial growth and interactions in tap water are regulated by many factors, including: type and concentration of available organic and inorganic nutrients, type and concentration of residual disinfectant, presence of predators, such as protozoa and invertebrates, environmental conditions, such as water temperature, and the spatial location of microorganisms [7].

1.3. E. coli

Apart from the general focus on bacteria, this study focuses more specifically on *E. coli*. It is a Gram-negative bacterium which belongs to the group of facultative pathogens and is mostly present in feces and colon. What distinguishes it from two usual intestinal pathogens Salmonella and Shigella is its property of being lactose fermenter [8]. Most of *E. coli* strains are usually harmless but in cases of water or food contamination some serotypes can cause of food poisoning, including O157:H7 and Shiga toxin-producing E. *coli* [7], [11].

Diarrhea and abdominal pain are the main symptoms of bowel infection but it may lead to even more serious cases such as kidney failure, dehydration or even bloody diarrhea [12], [13]. Not all people react same to invasion of pathogens and those who have bigger tendency to be affected by *E. coli*-related illness are people with low stomach acid, weakened immune system, young children and older people. Immune system of these categories of people may struggle to fight bacteria due to various reasons but most people make a full recovery within a week. However, there is a risk of developing hemolytic uremic syndrome (HUS) among young children and older people which can cause anemia, ischemia or kidney failure and even though the percentage of risk is around 10%, it may be fatal in 3% to 5% of patients [14].

1.4. Water treatment

Human and animal waste are main sources of *E. coli* which may end up in the sources of drinking water during snow melts or rainfalls [15]. In order to provide drinkable water, tap water goes through different processes to get rid of pathogenic bacteria and viruses. This may be done with help of chemicals or radiation but public water treatment systems mostly use chemicals. Through chlorination many microorganisms in water get killed but not all pathogens are affected, which is the reason why for some water treatment facilities use ultra violet radiation or additional filtering in order to totally remove all pathogenic bacteria and viruses from drinking water [9].

1.5. Active charcoal filter

In order to cope with water that is not completely clean, many household water treatments were established, including: coagulation, flocculation, and sedimentation; filtration; chemical disinfection; and disinfection by heat, ultraviolet radiation, or solar radiation [16]. All of these treatments have some kind of limitations which can be overcome by combining more of them in order to increase effectiveness [17].

In this study, we are focusing on active charcoal filters which are commercially available and can be used in households. The active charcoal filter will absorb some of the pathogens, though the problem might occur when the charcoal rapidly develops a biofilm [17].

1.6. Aim of the study

The aim of this study is to test for the presence of bacteria in tap water from different locations in Sarajevo and to see whether it is according to a standard. Additionally, the aim is to see how effective is the charcoal filter in cleaning the water from bacteria. To our knowledge, this is the first independent study of this kind in Sarajevo area.

2. Materials and methods

All processes that required sterile environment were done in laminar flow hood (Scala, Germany). All procedures were done three times (sample collection, colony enumeration, and gram staining). The data was analyzed using Microsoft Excel.

2.1. Sample collection

Five tap water samples were collected three times from five different locations in Sarajevo: Koševsko brdo (43.866136/18.404271), Nedžarići (43.844799/18.342110), Otoka (43.852597/18.365266), Hladivode (43.878334/18.451547) and Ilidža (43.821749/18.309133). The samples were collected in sterile tubes to prevent any additional contamination.

2.2. LB agar plates and LB medium preparation

We have used a protocol that was adapted from Sambrook & Russell for the preparation of Luria Bertani medium: for 1 liter of liquid LB medium we mixed 10 g of tryptone, 5 g of yeast extract and 10 g of NaCl with distilled water in autoclave bottle. Additionally, for solid LB agar plates we have also added 20 g of agar. After preparing solutions, we have mixed them by shaking in order to avoid caramelization of powder in the autoclave. The pH of both liquid and solid LB media was adjusted to 7 prior to autoclaving. After the autoclave, the solution containing agar was left to cool to 55° C, poured into sterile petri dishes, left to cool at room temperature, covered with parafilm and stored at 4°.

2.3. Inoculation of agar plates

Shortly after collecting all the samples, we pipetted 50 μl of water from each sample and spread it on agar plates which were then labelled and incubated at 37° C for 24 hours before enumeration. The same procedure was done the second time, after the samples were filtrated through the charcoal filter.

2.4. Colony forming units enumeration

For colony forming units (CFU) enumeration, the standard plate count method is used. In standard plate count colonies of bacteria can be seen with the naked eye and can be easily counted. The same counting method was used for both tap water and filtered water. For the analysis of the CFU enumeration, z-test has been performed against the maximum allowed 1 CFU per sample.

2.5. Filtration of the water

Samples were filtrated using the commercially available household filter 'Britta'. 15 ml of each sample was filtrated with Britta filter and filtrated water was collected into the sterile tubes. This sample is later spread over the agar plate.

2.6. Preparation for Gram staining

After bacterial culture enumeration on agar plates, bacterial colonies were transferred to liquid LB medium by precise picking with inoculating loop in order to avoid the touching of solidified agar.

Three different gram staining solutions were produced using a protocol from Cornell university (http://compost.css.cornell.edu/preparing.html). Crystal violet is prepared by dissolving 2g of crystal violet in 20 ml of 95% ethanol. To the first solution, 80 ml of 1% Ammonium Oxalate is added. The solution was filtered and stored in the fridge. For gram iodine, 1 g of Iodine and 3 g of Potassium Iodide is added to 300 ml distilled water. This solution is stored in an amber bottle in the fridge. Safranin is prepared by adding 2.5 g of safranin to 10 ml of 95% ethanol. Also, 100 ml of distilled water is added. The solution was filtered and stored in the fridge.

2.7. Gram staining procedure

This procedure allows the differentiation between Gram-positive and Gram-negative bacteria using crystal violet-iodine complex and safranine. The cell wall of Gram-positive bacteria absorbs crystal violet and gram iodine complex after the treatment with ethanol, decolorization, and look purple, while Gram-negative bacteria after this procedure appear pink.

The protocol for this method is adapted from Cornell university [18]. Bacteria were taken from the liquid LB medium with the inoculation loop and smeared on the slide. The sample is dried using a Bunsen burner and exposed to three gram staining solutions separately. Firstly, to crystal violet with waiting time of 20s and cleaning with distilled water for 2s. Then to gram iodine with waiting time of 60s and slow washing with 95% ethanol. Finally, the sample was exposed to safranine with waiting time of 20s and cleaning with distilled water for 2s.

3. Results

3.1. Colony forming units enumeration

CFU from each location and from each sample, as well as their means are shown in table 1. The highest average number of CFU is in Ilidža, followed by Koševo, Nežarići, Hladivode, and Otoka. It is noticeable that sample 2 has the smallest number of CFU compared to other samples. During its collection, there were major reparations of public water supply in Sarajevo and there was additional chlorination performed.

Table 1. CFU from all locations/samples

Locations	Sample 1	Sample 2	Sample 3	Average
Nedžarići	11	2	7	6.67
Otoka	2	0	0	0.67
Ilidža	60	0	25	28.33
Hladivode	5	0	2	2.33
Koševo	46	0	20	22.00

Fig. 1 shows the average CFU per each of the five locations in Sarajevo, compared to the allowed health standard in Bosnia and Herzegovina, according to drinking water quality standards found in "Pravilnik o zdravstvenoj ispravnosti vode za piće" [19]. The allowed number of CFU is 20 per 1ml, or 1 per 50ul, the amount used in our samples. Out of five locations, only the average value of one (Otoka) is below the allowed threshold, while the second one (Hladivode) is relatively close to the limit. Other three locations are well above the limit, with Nedžarići having a significantly higher CFU compared to the maximum allowed number (p value 0.0148), while Ilidža and Koševo are near the threshold of significance level (p values 0.0581 and 0.0574, respectively).

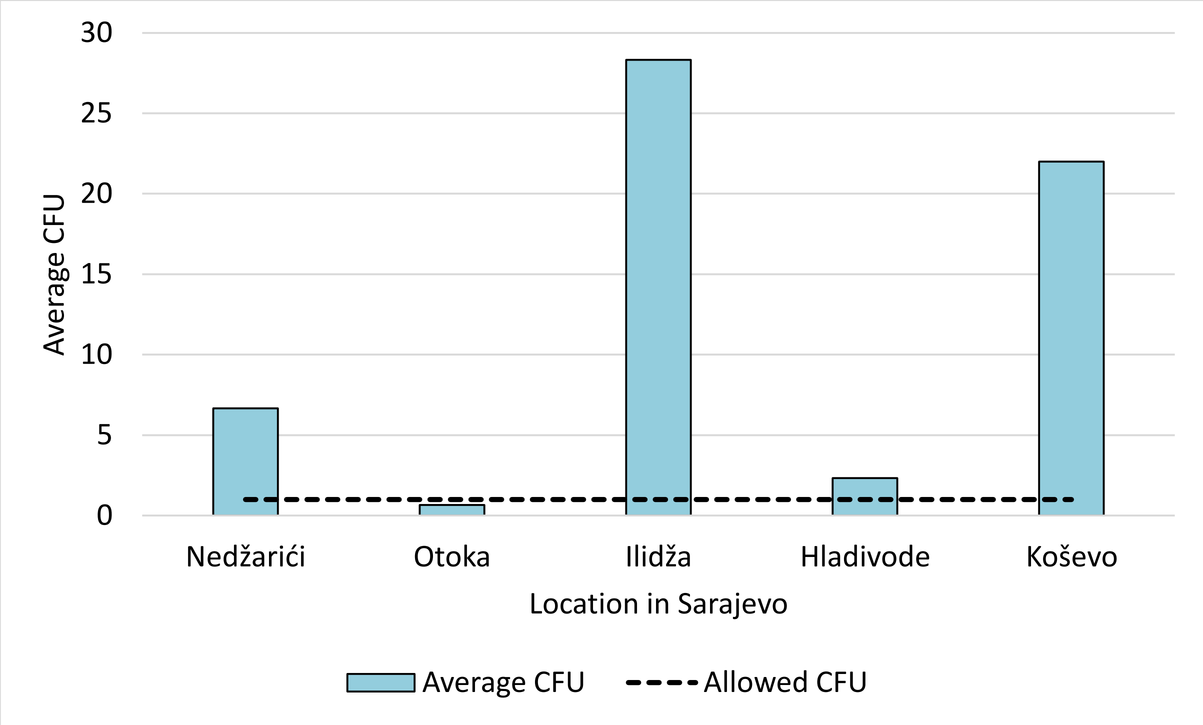

Figure 1. Average CFU compared to the standard CFU

3.2. Gram staining

Fig. 2 shows the difference in the types of bacteria present across different locations in Sarajevo. Samples from all locations contained gram-negative bacteria, while all except Otoka contained gram-positive bacteria. Otoka is the only location which contained only gram-negative bacteria.

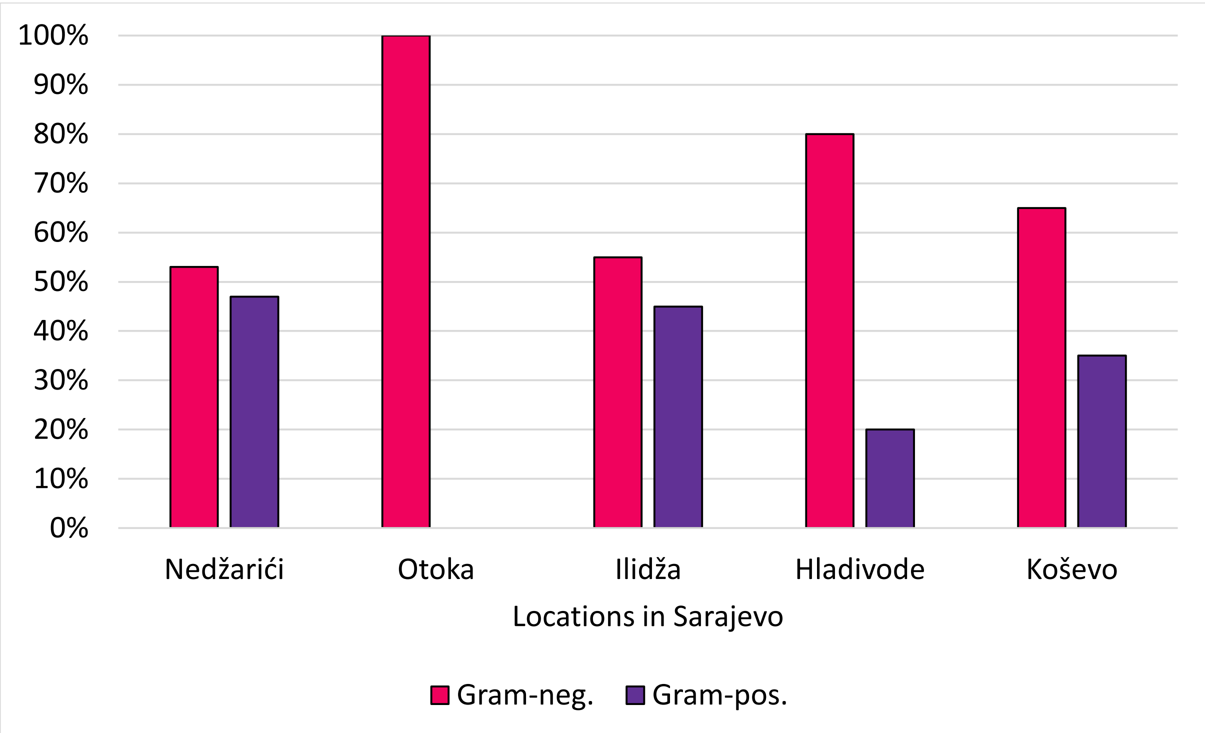

Figure 2. Percentage of gram-negative and gram-positive bacteria in samples

3.3. Filtration

Fig. 3 shows the mean values of CFU from 5 locations in Sarajevo compared to their filtered counterparts. It is noticeable that using the Britta filter decreased the number of CFU in 4 out of five samples. In one case, Nedžarići, there were more CFU in filtered water, compared to the regular water.

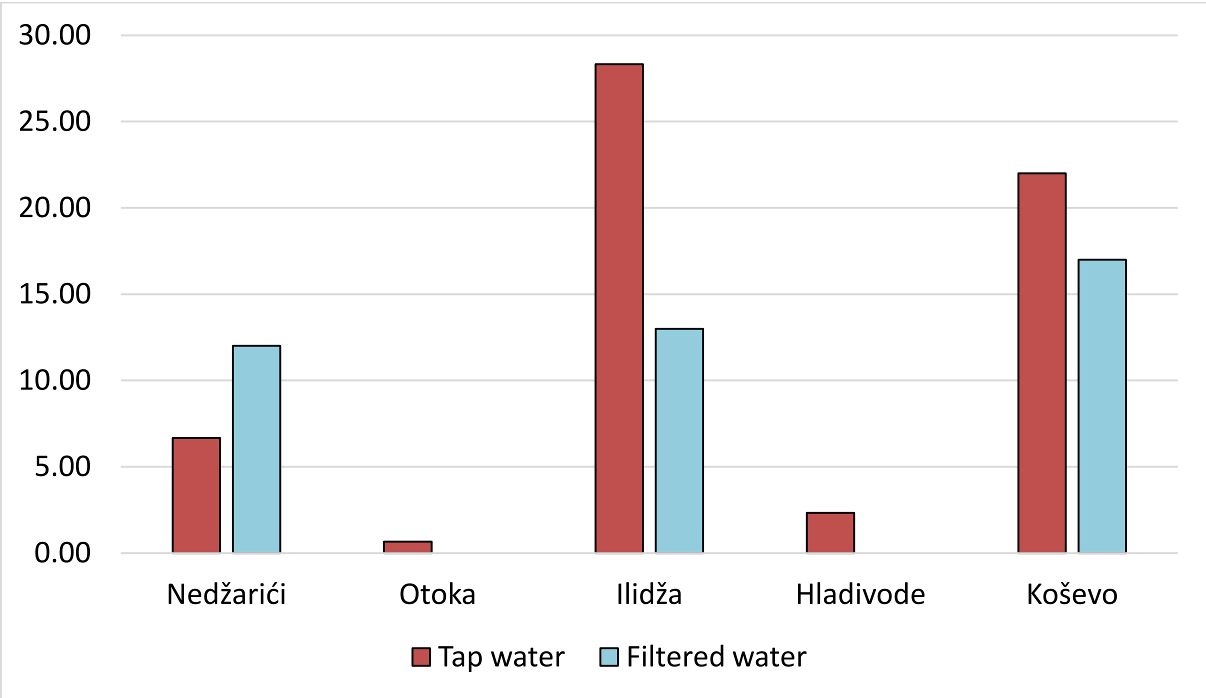

Figure 3. Comparison of CFU for regular and filtered tap water

4. Discussion and Conclusion

Our results show that there is a large difference in the microbiological water quality from different locations in Sarajevo. The allowed limit the number of CFU is 1 per 50ul of water sample (or 20 CFU per 1ml of water sample). Overall, only one location satisfied the allowed limit for the number of CFU per sample of water – Otoka (0.67), and it is closely followed by Hladivode (2.33). Other locations – Nedžarići, Koševo, and Ilidža – show substantially higher levels of CFU – 6.67, 22, and 28.33, respectively – which is well above the allowed limit. Further analysis is needed to conclude whether different water sources or damaged water supply system is the reason for higher contamination in some locations. If the integrity of the water supply system is decreased in certain parts, then the contamination is most likely coming from the soil through the cracks.

Water samples have been collected three separate times and the absence of bacterial cells during the second sample collection is noticeable, with the exception of sample from Nedžarići (2 CFU per 50ul). During this time, KJKP VIK Sarajevo (water utility company in Sarajevo) implemented repairs in water supply system and added more chlorine to the water which may be the cause for the absence of bacteria. Repairs on water supply system are common in Sarajevo, which is the reason why we have opted to use the data and we have not replaced it with additional measurements when the repairs are not being conducted. Since repairs are common, we believe that this data represents a more accurate and more common scenario for the tap water quality in Sarajevo.

In terms of the type of bacteria present in water samples across Sarajevo, gram-negative, including the *E. coli*, were the dominant ones (70.6% average across all location), while gram-positive bacteria were less present (29.4% average across all locations). Some of these bacteria were morphologically different than the *E. coli*, and additional analysis is required in order to understand which type do they belong to.

In general, commercially available charcoal filter can help decrease the number of CFU in tap water. After the filtration, two water samples were in accordance with the health regulations of Bosnia and Herzegovina (Otoka – 0 CFU, and Hladivode – 0 CFU), while three others were still considerably above the allowed threshold (Nedžarići – 12 CFU, Ilidža – 13 CFU, Koševo – 17 CFU). Additional analysis might be conducted on water samples in order to measure the levels of chlorine and study their correlation to the levels of bacteria present.

Our data suggest that, while the charcoal filter may help reduce the number of bacteria in tap water, chemical disinfection of public water supply with chlorine (combined with additional filtration) is not always enough to have desirable health standards since some locations in Sarajevo have significantly higher bacteria levels than the maximum recommended health standards. The quality of the water supply system is most likely an important factor to consider, as well as sources of water for different locations, which is why additional measurements (on additional locations) as well as the analysis of chlorine levels might bring further insights into both the water quality and the integrity of the public water supply system of Sarajevo.

References

[1] "The Water in You: Water and the Human Body." https://www.usgs.gov/special-topic/water-science-school/science/water-you-water-and-human-body?qt-science_center_objects=0#qt-science_center_objects (accessed Aug. 22, 2020).

[2] E. Jéquier and F. Constant, "Water as an essential nutrient: the physiological basis of hydration," *Eur J Clin Nutr*, vol. 64, no. 2, pp. 115–123, Feb. 2010, doi: 10.1038/ejcn.2009.111.

[3] E. Brini, C. J. Fennell, M. Fernandez-Serra, B. Hribar-Lee, M. Lukšič, and K. A. Dill, "How Water's Properties Are Encoded in Its Molecular Structure and Energies," *Chem. Rev.*, vol. 117, no. 19, pp. 12385–12414, Oct. 2017, doi: 10.1021/acs.chemrev.7b00259.

[4] "Water supply and sanitation services in modern Europe: developments in 19th-20th centuries," *ResearchGate*. https://www.researchgate.net/publication/319623260_Water_supply_and_sanitation_services_in_modern_Europe_developments_in_19th-20th_centuries (accessed Aug. 22, 2020).

[5] V. B. Nesfield, "A chemical method of sterilizing water without affecting its potability," *Public Health*, vol. 15, pp. 601–603, Oct. 1902, doi: 10.1016/S0033-3506(02)80142-1.

[6] J. P. S. Cabral, "Water Microbiology. Bacterial Pathogens and Water," *Int J Environ Res Public Health*, vol. 7, no. 10, pp. 3657–3703, Oct. 2010, doi: 10.3390/ijerph7103657.

[7] J. M. Willey, L. Sherwood, and C. J. Woolverton, *Prescott's Microbiology*. McGraw-Hill, 2011.

[8] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter, *Molecular Biology of the Cell*, 4th ed. Garland Science, 2002.

[9] P. K. Pandey, P. H. Kass, M. L. Soupir, S. Biswas, and V. P. Singh, "Contamination of water resources by pathogenic bacteria," *AMB Express*, vol. 4, p. 51, Jun. 2014, doi: 10.1186/s13568-014-0051-x.

[10] N. J. Ashbolt, "Microbial Contamination of Drinking Water and Human Health from Community Water Systems," *Curr Environ Health Rep*, vol. 2, no. 1, pp. 95–106, 2015, doi: 10.1007/s40572-014-0037-5.

[11] "E. coli (Escherichia coli) | E. coli | CDC," Feb. 26, 2020. https://www.cdc.gov/ecoli/index.html (accessed Aug. 22, 2020).

[12] R. J. Cahill, C. J. Foltz, J. G. Fox, C. A. Dangler, F. Powrie, and D. B. Schauer, "Inflammatory bowel disease: an immunity-mediated condition triggered by bacterial infection with Helicobacter hepaticus.," *Infection and Immunity*, vol. 65, no. 8, pp. 3126–3131, Aug. 1997.

[13] R. C. Spiller, "Role of infection in irritable bowel syndrome," *J Gastroenterol*, vol. 42, no. 17, pp. 41–47, Jan. 2007, doi: 10.1007/s00535-006-1925-8.

[14] "E. coli infection: Symptoms, causes, and treatment," Dec. 11, 2017. https://www.medicalnewstoday.com/articles/68511 (accessed Aug. 22, 2020).

[15] O. US EPA, “National Primary Drinking Water Regulations,” *US EPA*, Nov. 30, 2015. https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations (accessed Aug. 22, 2020).
[16] D. Lantagne and T. Yates, “Household Water Treatment and Cholera Control,” *J Infect Dis*, vol. 218, no. suppl_3, pp. S147–S153, Oct. 2018, doi: 10.1093/infdis/jiy488.
[17] V. Agrawal and R. Bhalwar, “Household Water Purification: Low-Cost Interventions,” *Med J Armed Forces India*, vol. 65, no. 3, pp. 260–263, Jul. 2009, doi: 10.1016/S0377-1237(09)80019-1.
[18] “Preparing Slides of Microorganisms.” http://compost.css.cornell.edu/preparing.html (accessed Aug. 22, 2020).
[19] “FSA,” *Agencija za sigurnost hrane Bosne i Hercegovine*. http://extwprlegs1.fao.org/docs/pdf/bih148927.pdf (accessed Aug. 22, 2020).