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COMPARISON OF STERILIZATION TIMES IN MEMBRANE FILTER DEVICES WITH MICROBIAL SUSPENSIONS AT DIFFERENT CONCENTRATIONS.

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ABSTRACT

Introduction
Sterilization of the membrane filter device used in water microbiology analysis is very important.

Aim of the study
To compare the efficacy of sterilization by burning with alcohol and flaming with a Bunsen burner.

Material and methods
Bacterial suspensions were prepared at different concentrations and for each concentration, each analyst studied 10 culture media for each time periods. The contaminated membrane filter funnels with the prepared samples were sterilized by flaming for 10, 20, 40 and 60 seconds. Then, the membrane filter was placed and 100 ml of sterile distilled water was filtered. Membrane filter was placed on appropriate growth medium and it was incubated. The same procedure was also carried out by burning with alcohol.

Results
At 10^2 concentration, bacterial growth was not observed. At 10^3 concentration, bacterial growth was observed 6,66% of growth medium at 10 seconds. At 10^4 concentration, bacterial growth was observed 30% of growth medium at 10 seconds and 23,3% of growth medium at 20 seconds. At 10^5, 10^6 and 10^7 concentrations, bacterial growth was observed in different percentages at all the time periods. Bacterial growth was not observed in sterilization using alcohol burning technique at all the time periods.

Conclusions
Effective sterilization can be achieved by flame with Bunsen burner with low microbial concentration suspensions and low time periods. At high concentration suspensions, membrane filter device can be effectively sterilized by burning with alcohol.

Key words: sterilization, sanitation, *E.coli, Pseudomonas aeruginosa,* membrane filter device, water quality.

INTRODUCTION

Water is an indispensable material for the life of people and other living things (1- 4). About 70% of the human body consist of water (5). ¾ of the world is covered with waters; but the amount of fresh water that can be used is 3% of the total amount of water on earth (2, 6, 7). Although the fresh water on the world is limited, the quality of water is adversely affected in order to meet the vital and economic needs such as increasing population, urbanization and industrialization (2, 6). It is stated that as in many countries of the world in the coming years, there will be water shortages in our country as well (3).
Water pollution is the change of the physical, chemical and biological properties of water quality so as to limit the use of water. The causes of water pollution are population growth, urbanization, industrialization, pesticides and fertilizers (3, 8-10).
In terms of public health, the ideal drinking and use water should not contain pathogenic microorganisms (11, 12). According to the Guidelines for Drinking-water Quality published by the World Health Organization (WHO), *E.coli* or coliform bacteria should not be present in 100 ml of drinking water (13). In addition to this, according to "TS 266" and Turkish Public Health Authority's "İnsani Tüketim Amaçlı Sular Hakkındaki Yönetmelik", 100 ml of drinking-use water should not contain *E.coli* and coliform bacteria, 250 ml of drinking water and spring water should not contain *Pseudomonas aeruginosa* (14, 15).
The microbiology analysis are based on the determination of the presence of undesirable microorganisms in the water or the presence of the specified amounts. For this purpose membrane filtration method is used. In this method, it is possible to filter the water and to keep the bacteria on the filter by using different µm diameter membrane filter. After that, the membrane filter is moved to the selective medium and allowed to incubate at the appropriate temperature and time. After incubation, the colonies on the membrane filter surface are counted and evaluated (7, 11, 12).
Sanitation of membrane filter device parts is very important. Sanitation can be performed by different methods. These are flaming with a Bunsen burner, burn with alcohol, autoclaving and the use of disposable sterile funnels (16).
In our work; it is aimed to show whether sterilization using flame with Bunsen burner technique or using alcohol incineration technique at different times by using bacterial suspensions of different concentrations is effective.

MATERIALS AND METHODS

Contaminated water with a certain number of microorganisms was used in the study by using Standard methods of TS EN ISO 16266 (17) and ISO 9308-1:2014(E) (18). 0,5 Mc-farland (Liofilchem) was used to prepare a sample with a certain number of microorganisms. In the study, *E.coli* and *Pseudomonas aeruginosa* bacteria were used. Three different analysts joined the study and contaminated samples were prepared at different concentrations. For each concentration (10^2,10^3,10^4,10^5,10^6,10^7), each analyst studied 10 culture media.
For *E.coli*, 10^2, 10^4, 10^6 and for *Pseudomonas aeruginosa* 10^3, 10^5, 10^7 concentrations were prepared. From the prepared samples, 100 ml of contaminated water was filtered from each funnel of the membrane filter device (Sartorius, 6-manifold) without filter. The membrane filter device was sterilized by flaming with Bunsen burner at different times (10, 20, 40, 60 seconds).

After cooling, 0,45 µm diameter membrane filter was placed and 100 ml of sterile distilled water was filtered. Membrane filter was placed on appropriate growth medium and it was incubated at the appropriate temperature and time. After incubation, the membrane filters were examined and we tried to establish a significant relationship between sterilization times.
The same procedure was also carried out for each concentration by burning with alcohol.

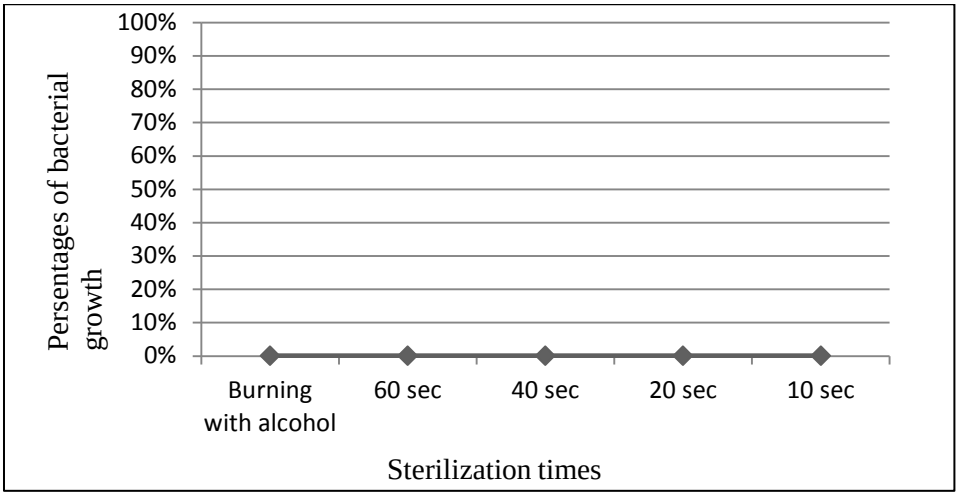
RESULTS

The results of three different analysts for each time period at different concentrations are given in Table-1. A total of 30 studies were performed for each concentration. The analysts worked at 6 different concentrations in 4 different time periods.

Table 1. The results of study at different concentrations.

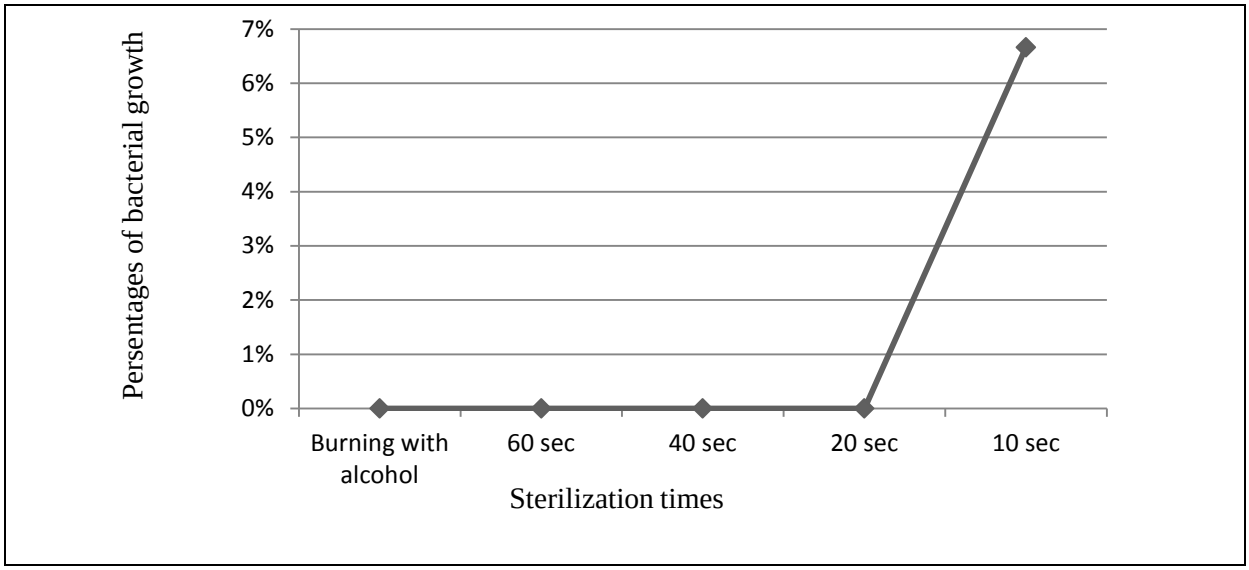
Concentration	Time				
	10 sec	20 sec	40 sec	60 sec	Burning with alcohol
10^2 (*E.coli*)	0	0	0	0	0
10^3 (*Ps. aeruginosa*)	2	0	0	0	0
10^4 (*E.coli*)	9	7	0	0	0
10^5 (*Ps. aeruginosa*)	16	12	8	7	0
10^6 (*E.coli*)	26	20	13	10	0
10^7 (*Ps. aeruginosa*)	30	29	28	28	0

At 10^2 concentration;
Bacterial growth was not observed in all time periods (10 sec, 20 sec, 40 sec, 60 sec). Bacterial growth was not observed in all of the 30 growth medium studied by burning with alcohol. Percentages of bacterial growth in cultures at determined times at 10^2 concentration is shown in Graph 1.



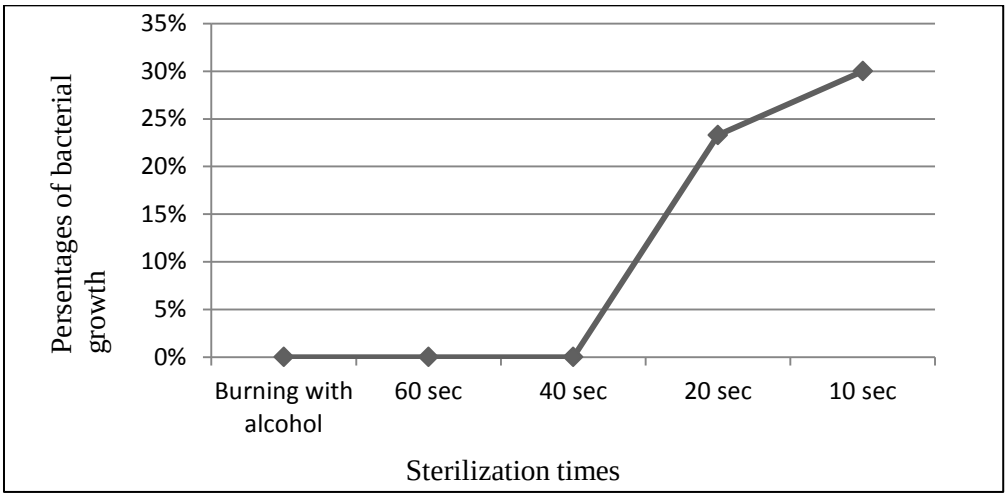
Graph 1. Percentages of bacterial growth at 10^2 concentration.

At 10^3 concentration;
Bacterial growth was observed in 2 growth medium in 10 seconds (2/30= %6.66). In other time periods (20 sec, 40 sec, 60 sec) bacterial growth was not observed. Bacterial growth was not observed in all of the 30 growth medium studied by burning with alcohol. Percentages of bacterial growth in cultures at determined times at 10^3 concentration is shown in Graph 2.



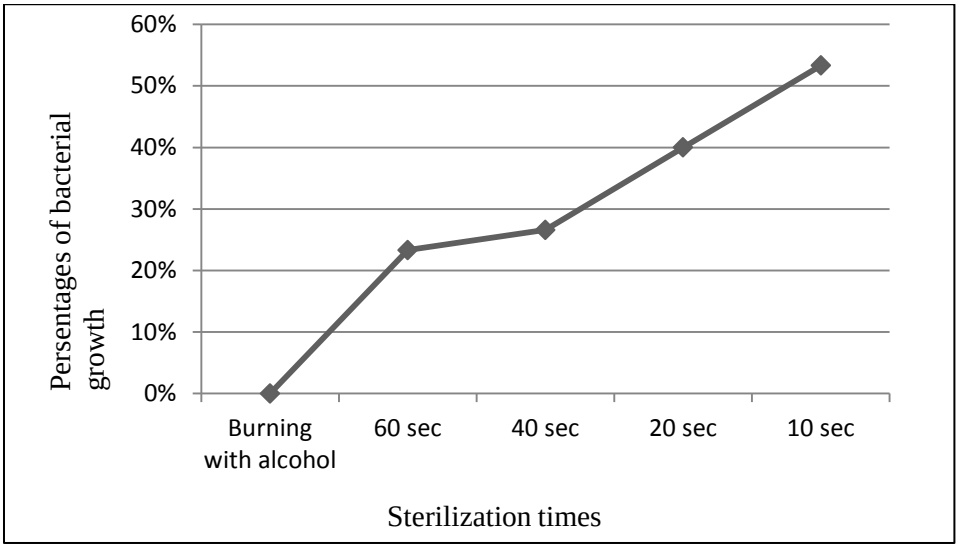
Graph 2. Percentages of bacterial growth at 10^3 concentration.

At 10^4 concentration;
Bacterial growth was observed in 9 growth medium in 10 seconds (9/30= % 30) and 7 growth medium in 20 seconds (7/30= % 23.3). In other time periods (40 sec, 60 sec) bacterial growth was not observed. Bacterial growth was not observed in all of the 30 growth medium studied by burning with alcohol. Percentages of bacterial growth in cultures at determined times at 10^4 concentration is shown in Graph 3.



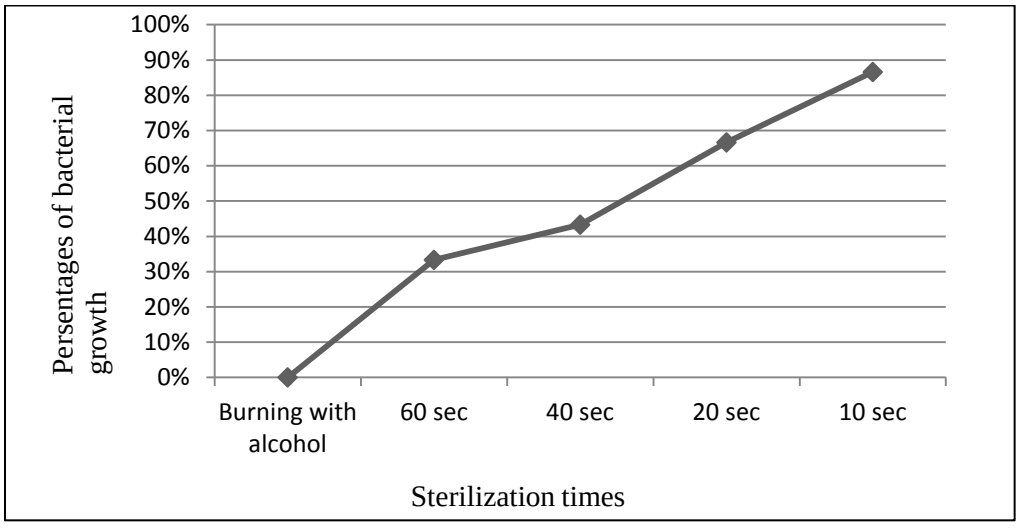
Graph 3. Percentages of bacterial growth at 10^4 concentration.

At 10^5 concentration;
Bacterial growth was observed in 16 growth medium in 10 seconds (16/30= %53.3), 12 growth medium in 20 seconds (12/30= %40), 8 growth medium in 40 seconds (8/30= %26.6) and 7 growth medium in 60 seconds (7/30= %23.3). Bacterial growth was not observed in all of the 30 growth medium studied by burning with alcohol. Percentages of bacterial growth in cultures at determined times at 10^5 concentration is shown in Graph 4.



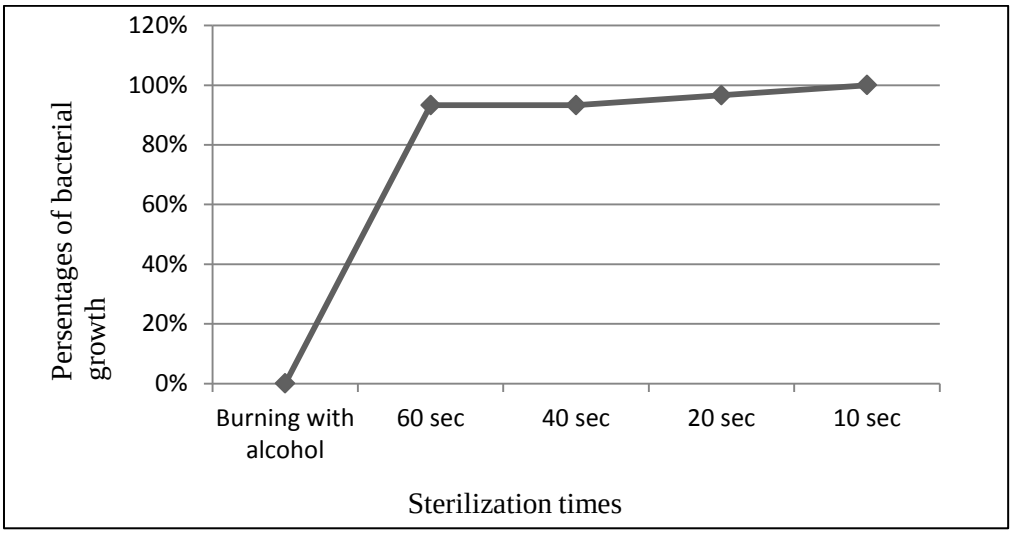
Graph 4. Percentages of bacterial growth at 10^5 concentration.

At 10^6 concentration;
Bacterial growth was observed in 26 growth medium in 10 seconds (26/30= %86.6), 20 growth medium in 20 seconds (20/30= %66.6), 13 growth medium in 40 seconds (13/30= %43.3) and 10 growth medium in 60 seconds (10/30= %33.3). Bacterial growth was not observed in all of the 30 growth medium studied by burning with alcohol. Percentages of bacterial growth in cultures at determined times at 10^6 concentration is shown in Graph 5.



Graph 5. Percentages of bacterial growth at 10^6 concentration.

At 10^7 concentration;
Bacterial growth was observed in 30 growth medium in 10 seconds (30/30= %100), 29 growth medium in 20 seconds (29/30= %96.6), 28 growth medium in 40 seconds (28/30= %93.3) and 28 growth medium in 60 seconds (28/30= %93.3). Bacterial growth was not observed in all of the 30 growth medium studied by burning with alcohol. Percentages of bacterial growth in cultures at determined times at 10^7 concentration is shown in Graph 6.



Graph 6. Percentages of bacterial growth at 10^7 concentration.

DISCUSSION

The process of cleaning or killing all microorganisms in an environment or material with all kinds of live, active and spore forms is called sterilization. Sterilization is done by physical and chemical methods according to types of materials used and to be sterilized. In laboratory, sterilization is usually preferred by physical methods (19).
Physical methods are heat methods, filtration methods and sterilization with radiation. Heat method is also divided into dry heat methods and moist heat methods. Flame with Bunsen burner method used at the sterilization of membrane filtre device is based on the type of dry heat methods (19).
Dry heat sterilization is advantageous in that it effectively kills all forms of microorganisms, easy and cheap to apply, reliable, has no toxic and carcinogenic effects and has few application errors (19).
The sterilization by burning with alcohol is disadvantageous form the material injury and form the damage to the membrane filter device.

CONCLUSIONS

In our work; it has been found that effective sterilization can be achieved by alcohol burning, in which the membrane filter device can be effectively sterilized in low microbial concentration suspensions and in low times with flame, and in the case of suspensions with high microbial concentration, by flaming with Bunsen burner is not achieved for up to 1 minute.
It is recommended to sterilize the membrane filter device with alcohol after working for water which is thought to contain bacteria at high concentration.

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