

Research Article

Detection of Biofilm Layer in Water Plumbing and Determination of its Effect on Water Quality

Sihhi Tesisatlarda Biyofilm Tabakası Tespiti ve Su Kalitesine Etkisinin Belirlenmesi

Nurullah Said Yeken*, A. Çağlan Günel

Gazi University, Graduate School of Natural and Applied Sciences, Environmental Sciences
Department, Ankara/Türkiye

nurullahsaid.yeken@gazi.edu.tr (<https://orcid.org/0000-0003-2888-2976>)

caglangunal@gazi.edu.tr (<https://orcid.org/0000-0002-9072-543X>)

Received Date: 15.09.2022, Accepted Date: 08.11.2022

DOI: 10.31807/tjwsm.1175836

Abstract

In this study, biofilm formation on the plumbing, the presence of pathogenic microorganisms in biofilms and their effects on water quality were examined by membrane filtration with dehydrated medium method in 22 different buildings in a facility's water network. A relation with biofilm formation and water quality deterioration with the age of the plumbing and the pipe material was also studied. The results were expressed as colony forming units (CFU). According to the results of the galvanized metal pipes, the average colony count was determined as >200 CFU/250 mL, average pathogen count was calculated as 107 CFU/250 mL and the highest deterioration in the water quality were observed. For the Polyethylene (PE) pipes, the average colony count was found as >200 CFU/250 mL and the average pathogen count was found as 145 CFU/250 mL. No physical and chemical changes in water quality were observed. For the Polypropylene Random Copolymer (PPRC) pipes, neither physical/chemical change in water quality nor pathogenic growth were observed. The total colony count was found as 34 CFU/250 mL. Biofilm formation was detected at 15 points in the network. *Pseudomonas aeruginosa* was the most common detected pathogens in plumbing as 12 points from 22 buildings. The highest colony formation was *Escherichia coli*, which was detected in four of 22 plumbing as 600 CFU/250 mL colonies. It has been observed that more accumulations occurred in galvanized metal pipe surfaces, and microbiological growth was higher than PE and PPRC pipes.

Keywords: plumbing, water quality, biofilm, pathogen microorganisms

Öz

Çalışmamızda, bir işletmeye ait içme suyu şebekesine bağlı 22 binanın sıhhi tesisatlarında biyofilm oluşumu ve biyofilm içindeki patojenlerin varlıkları membran filtrasyon ve hazır kurutulmuş besiyeri yöntemiyle incelenmiş, su kalitesine etkileri tespit edilmiştir. Biyofilm oluşumu ve su kalitesindeki bozulma ile sıhhi tesisat yaşı ve boru malzemesi arasındaki ilişki de ayrıca incelenmiştir. Sonuçlar koloni oluşturan birim (CFU) olarak ifade edilmiştir. Yapılan analiz sonuçlarına göre galvaniz metal

*Corresponding author.

tesisatlarda ortalama koloni sayısı >200 CFU/250 mL, ortalama patojen sayısı 107 CFU/250 mL olarak tespit edilmiştir ve su kalitesindeki en yoğun bozulma görülmüştür. Polietilen (PE) tesisatlarda ortalama koloni sayısı >200 CFU/250 mL, ortalama patojen sayısı 145 CFU/250 mL olarak ölçülmüştür. Fiziksel ve kimyasal açıdan su kalitesinde değişim görülmemiştir. Polipropilen Rastgele Kopolimer (PPRC) tesisatlarda su kalitesinde değişim görülmemiş, ortalama koloni sayısı 34 CFU/250 mL olup patojen çoğalması gözlemlenmemiştir. Tesisatlarda 15 noktada biyofilm oluşumu tespit edilmiştir. En çok tespit edilen patojen tür 22 noktanın 12'sinde görülen *Pseudomonas aeruginosa* olmuştur. En fazla koloni oluşumu ise 22 noktanın 4'ünde tespit edilen ve toplamda 600 CFU/250 mL'nin üzerinde koloni sayımı yapılan *Escherichia coli* olmuştur. Galvaniz metal tesisatlarda daha fazla birikim olduğu, bu yüzeyler üzerinde mikrobiyolojik çoğalmanın PE ve PPRC'ye göre daha fazla olduğu görülmüştür.

Anahtar sözcükler: sıhhi tesisat, su kalitesi, biyofilm, patojen mikroorganizmalar

Introduction

Access to safe drinking water is a fundamental human right and a vital component of an effective health protection policy. Some physical, chemical and microbiological water parameters can affect the aesthetic parameters of water (such as appearance, odor, taste etc.) and the quality and acceptability of the water for the consumer (World Health Organization [WHO], 2017). In order to provide high quality water that complies with international standards and regulations, it is necessary to have an integrated water production and distribution process. This process starts from the water source, continues with the transmission lines, drinking water treatment plants, water distribution structures and finally ends with the plumbing of the end users. The most important step of this process in terms of having a direct effect on the end user is the indoor water plumbing and the water tanks.

The quality of the tap water may deteriorate due to inappropriate plumbing installations, wrong material selection, reverse and cross connections in plumbing, unmaintained and uncleaned domestic water tanks. In such cases the color, odor and taste of the water may change, the concentrations of heavy metals (depending on the pipe material like iron, manganese, copper, zinc, lead etc.) may increase and pathogenic microorganisms may be encountered in the water (WHO, 2017; Gray, 2008). Microorganisms can always be found in drinking water systems no matter how strict precautions and treatment processes are applied in the production and distribution stages of water. Both chemical and biological quality changes can occur in drinking water supplied to the network (Boe-Hansen, 2001). Factors such as the amount of organic nutrients in the water, initial bacterial concentration, water age in the network, hydraulic effects, pipe and connection materials, pipe corrosion, sediment accumulation on the pipes affect the bacterial growth in pipelines (Acehan, 2007). High alkalinity values promote the accumulation on the pipes and suitable

environment for bacterial growth (Küçükgül et al., 2004). Most of the naturally occurring microorganisms in the water network do not pose a risk to human health (Skjevraak et al., 2004). However, when the microorganisms multiply in the water and start to form colonies, they can cause some consumer complaints such as taste, odor or appearance problems (Türetgen, 2005). These bacterial colonies are called as biofilms that adhere to a living or inanimate surface and spread in a gel-like layer of polymeric structure that they produce (Costerton et al., 1995). The biofilm formed in drinking water systems is mainly composed of bacteria and exopolymeric substances (EPS) secreted by bacteria. EPS ensures the coexistence of bacteria and acts as a barrier that protects microorganisms against environmental stresses and external factors (Freeman & Lock, 1995). Especially, water networks and tanks that are not cleaned and disinfected become a suitable environment for bacteria to form a biofilm (LeChevallier et al., 1988). Microorganisms are constantly multiplying in a biofilm layer. Microorganisms can detach from the biofilm by the flow rate and mix with the water. Those colonies are immediately replaced in the biofilms. Depending on the flow rate of the water, a single cell can break away from the biofilm layer, as well as a cell cluster with a diameter of 500 µm (Telgmann et al., 2004). Some pathogenic microorganisms can also develop and multiply in biofilms (Liu et al., 2016; Zhang et al., 2009; Boe-Hansen, 2001). With the detachment from the biofilm, the microorganisms can pass into the aquatic environment and finally into the human body. As a result, pathogens in the biofilms cause a potential health risk for the consumers and needs to be carefully examined in terms of drinking water quality and human health.

Many studies and researches have been carried out on the microorganisms in the biofilm layers formed in domestic and industrial water systems with different water sources and pipeline materials. The materials used in the water distribution systems and plumbing affect the biofilm formation. Biofilm can form in all types of materials, but each pipe material creates different conditions for microorganisms to attach, form biofilm and multiply. Pietrzyk et al. (2017) found that galvanized steel is particularly susceptible to the adhesion of microorganisms. Niquette et al. (2000) stated that iron pipes support microbiological growth 10 to 45 times more than plastic pipes. Camper (2003) also stated that in the presence of high organic matter, there is much more bacterial growth in cast iron pipes than in other pipe materials. In the study conducted by Türetgen (2005), it was observed that a high rate of biofilm formation was observed on galvanized steel and copper surfaces, while less bacteria and EPS were found in plastic pipes. According to Keskin and Kahveci (2019), a thin biofilm layer was formed on the inner surface of polyethylene pipes under the same conditions, while a very thick biofilm layer was formed in iron pipes. The studies on the biofilms in water networks are generally lab-scale pilot systems, which are modeled in laboratory conditions. Analysis of existing biofilms and

microorganisms in actively operated water networks is also necessary in terms of detecting and minimizing current risks for consumers. There is no universal directive or guide on when and how often samples should be taken from plumbing for microbiological monitoring. However, monthly, 3-month, 6-month and annual samples can be taken for *Pseudomonas aeruginosa* monitoring (Hong et al., 2017). Since each region has different water qualities, environmental conditions, characteristics, water treatment, distribution and plumbing systems, the biofilm formations and pathogen microorganism compositions should be analyzed and investigated separately.

There are several ways to detect bacterial growth in a sample. However, membrane filtration is one of the most effective methods that can be used in the analysis of samples with volumes such as 100 mL - 250 mL, especially in the analysis of samples containing very few microorganisms in potable water. Since the entire sample is filtered, all microorganisms in it can be easily counted without the need for any estimation or escalation. (Akpınar et.al., 2019; Sartorius, 2014).

In order to detect the biofilm layer formation in water plumbing and determine its effect on water quality according to the pipe material types and plumbing ages, this paper examines bacterial colony count on pipe surfaces in an operating water network via membrane filtration method. To achieve this, a separate water network is used rather than the main water supply network of Ankara but both networks supply water from the same water source. It is aimed that the outcomes of our study can give an idea for the buildings using similar water plumbing throughout the Ankara Province. In this study, the effects of plumbing age, galvanized metal, polyethylene (PE) and polypropylene random copolymer (PPRC) pipe types used in plumbing on biofilm formation, presence of pathogenic microorganisms and also the quality of water are examined individually. To observe biofilm formation and the presence of pathogens, the indicator microorganisms are determined. In the plumbing; total colony, total coliform, *Pseudomonas aeruginosa*, *Salmonella* spp., *Staphylococcus aureus*, *Enterococci* and *Escherichia coli* (*E. coli*) species are studied together with physical and chemical analysis of water. The aim of this study are to (1) detect the biofilm formation and pathogenic microorganisms in biofilms, to (2) observe the highest colony formation on the pipe surfaces and to (3) record the highest deterioration in the water quality according to the pipe materials and plumbing ages in the selected drinking water network in Ankara Province.

Materials and Methods

Study Area and Sampling

The selected water network belongs to a facility in Ankara Province. The facility has totally 70 buildings inside and serves nearly 20.000 people. It has its own water treatment plant, wastewater treatment plant and water distribution network apart from the Ankara Province. This network was selected as a model for Ankara Province's similar settlements. The facility gets raw water directly from the Çamlidere Dam. The treatment plant of the facility has the five units: Coagulation, flocculation, sedimentation, filtration, and disinfection as the other conventional treatment systems. Galvanized metal, PE and PPRC pipes are used in the water distribution network and plumbing systems in the buildings. PE pipes are used in raw water and some main lines, and also at the outlet of the treatment plant. Treated water from the wastewater treatment plant is used as irrigation water in the facility.

In the study, 22 buildings of different ages and pipe materials out of 70 buildings in the network have been analyzed. Samples were collected from the raw water at inlet of the treatment plant, the effluent treated water at the outlet of the water treatment plant and from 20 different plumbing in the water network. The sampling points were selected according to pipe types, plumbing ages, and locations.

The plumbing system in a building in the network is shown in Figure 1.

The distribution of the 22 buildings and layout of the network are represented in Figure 2.

Plumbing systems were evaluated in three categories: 1) Sampling points, 2) pipe types and 3) age category, given in Table 1.

Figure 1

Indoor Plumbing System of a Building

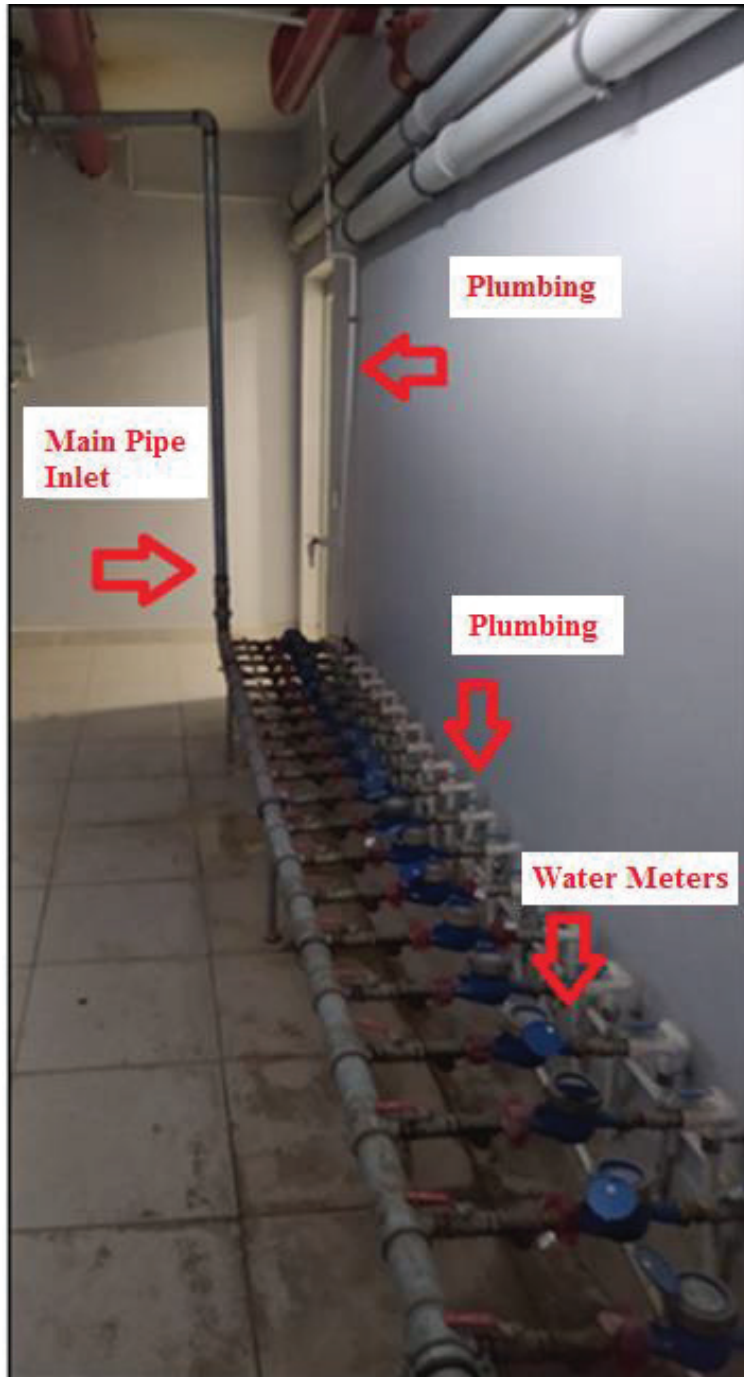
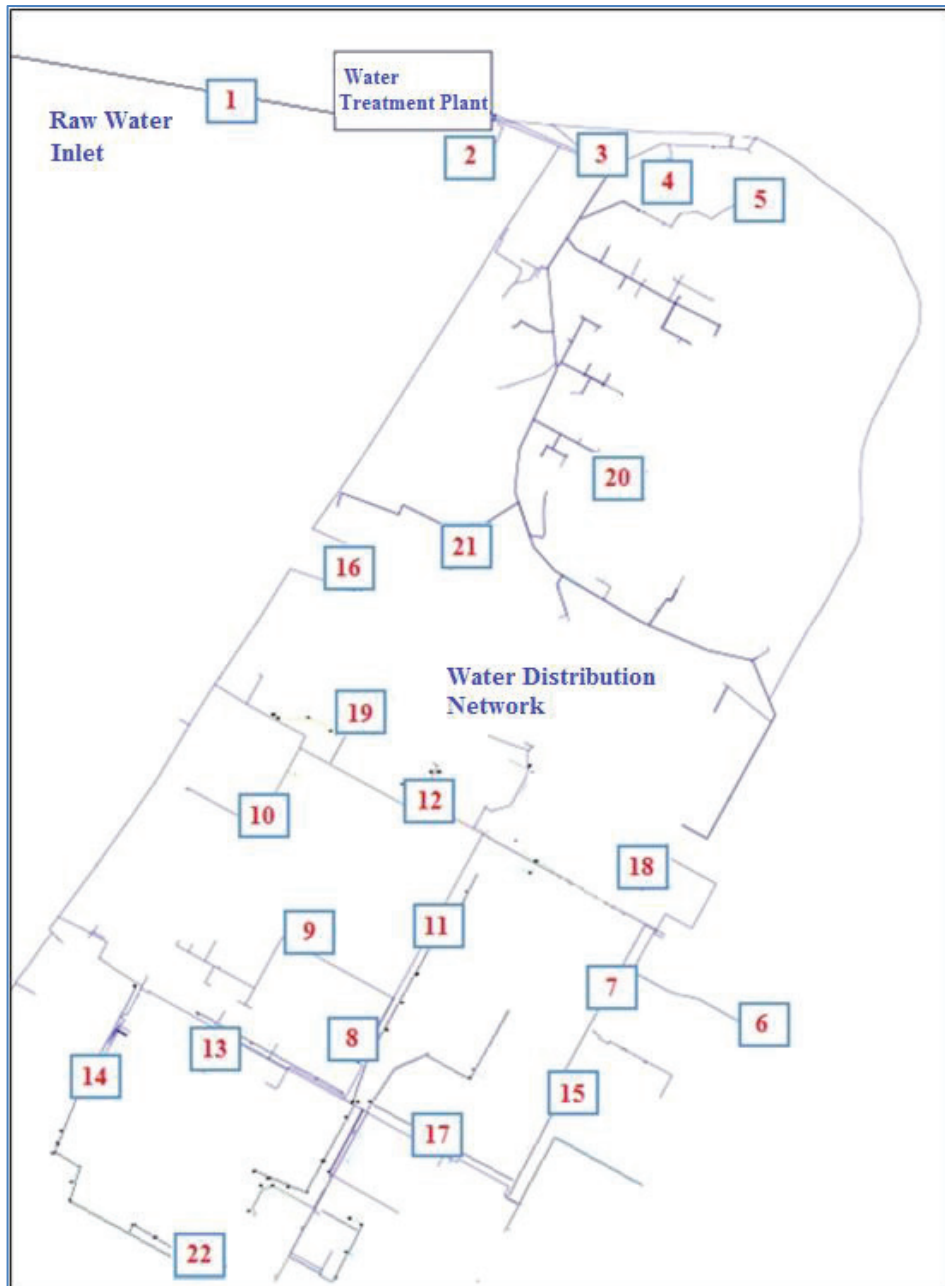


Figure 2

Layout of the Network and Buildings



Note. Due to the security restrictions, images of the study area cannot be given.

Table 1

Sampling Points and Their Characteristics

Building No	Pipe Types	Water Usage	Sampling Points	Plumbing Age Category
1	PE	Raw Water	Sample Tap	III***
2	PE	Potable	Sample Tap	II**
3	PPRC	Potable	Plumbing	II
4	PPRC	Potable	Plumbing	II
5	Galvanized	Potable	Plumbing	III
6	PE	Potable	Inlet Collector	II
7	PE	Potable	Inlet Collector	II
8	PPRC	Potable	Plumbing	I*
9	Galvanized	Operational	Plumbing	III
10	Galvanized	Potable	Inlet Collector	III
11	PPRC	Potable	Inlet Collector	I
12	Galvanized	Potable	Plumbing	III
13	Galvanized	Potable	Plumbing	II
14	Galvanized	Potable	Plumbing	II
15	Galvanized	Potable	Plumbing	I
16	Galvanized	Potable	Inlet Collector	II
17	Galvanized	Potable	Inlet Collector	II
18	Galvanized	Potable	Inlet Collector	I
19	PPRC	Potable	Plumbing	I
20	Galvanized	Potable	Inlet Collector	III
21	Galvanized	Potable	Plumbing	III
22	PPRC	Potable	Plumbing	I

* the age of plumbing in the new buildings is between 0 and 5 years.

** the age of the plumbing in the middle-aged buildings is between 6 and 15 years.

*** the age of plumbing in the old buildings is over 15 years.

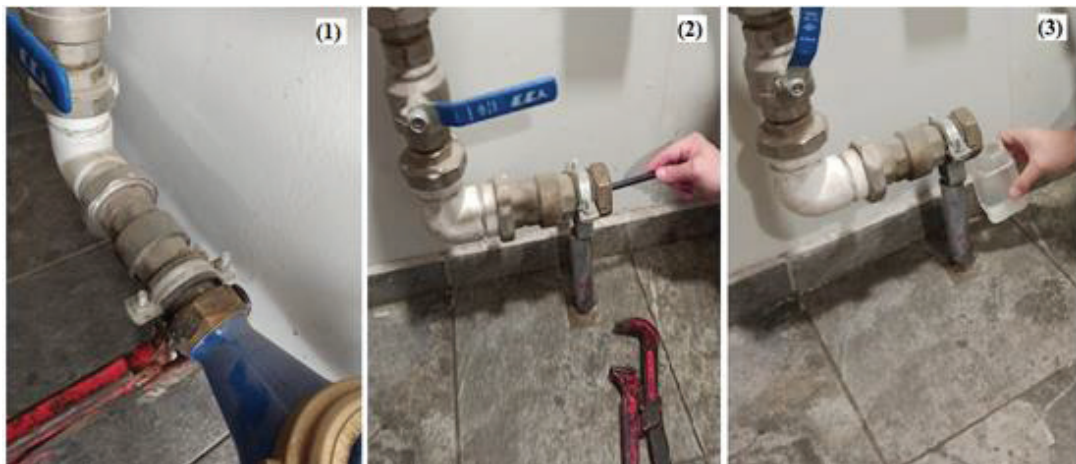
For the number 1, sample was taken from the raw water inlet. For the number 2, sample was taken from the effluent treated water. For the numbers between 3 and 22, samples were collected from the different points on the water distribution network in the buildings. In this study, a simple random sample was taken from each point annually. Total hardness, total alkalinity, iron, turbidity, pH, odor and total organic carbon load were also examined in terms of microbial growth stimulation.

At each sampling point, sample was firstly taken from tap water flowing in order to determine the current water quality. These samples were called as "Before". After disassembling the pipes, the accumulations on the pipe surface were scraped by a sterilized rod and the water sample was taken by providing the transition of the substances on the pipe surfaces into the water phase. These samples were named as "After". In this way, the effect of those accumulations on the physical, chemical and bacteriological quality of the water was examined and biofilm formations were studied.

The method of taking the samples from the pipes is shown in Figure 3.

Figure 3

Water Sampling Method



Note. (1) Disassembling the tap/water meter, (2) Scraping the inner surface of the pipes with a sterile rod, (3) Sampling from the flowing water.

Water samples containing the detached layers were transferred into the laboratory for the microbiological analysis on the same day under. Furthermore, in order to determine changes in physical and chemical characteristics of the water, additional control samples were taken and analyzed in the laboratory. The microbiological samples were incubated according to the incubation duration and temperatures. The results were preserved on the filter papers.

Microbiological Analysis

In this study, dehydrated nutrient sets developed by Sartorius Company were used as the medium, referred as “Nutrient Pad Set (NPS)”. It consists of media and membrane filter sets, each of which is impregnated with an absorbent pad by the manufacturer, then dehydrated and placed in sterile Petri dishes (Sartorius, 2014).

Membrane filtration method was used in the study for the detection of pathogens in the biofilm layer. Sterile sample containers (with Sodium Thiosulfate), vacuum pump, vacuum hose, glass vacuum flask, stainless steel filter holder, filter body, dosing syringe, stainless steel forceps and 0.2 nm pore size membrane filters were used in the membrane filtration process. In order to observe bacterial growth on filter papers, samples were placed in the incubator.

Different media, incubation durations and temperatures were used for the examined microbial species. “Yeast NPS” for total colony (germ count) detection, “Chromocult NPS” for total coliform detection, “Endo NPS” for *E. coli* detection, “Cetrimide NPS” for *Pseudomonas aeruginosa* detection, “Bismuth Sulfite NPS” for *Salmonella* spp. detection, “Chapman NPS” for *Staphylococcus aureus* detection and “Azide NPS” for *Enterococci* detection were used. The incubation conditions of the media and species are given in Table 2:

Table 2

Species and Incubation Conditions

Media Name (NPS)	Species	Incubation Temperature (°C)	Incubation Duration (h)
Yeast	Total Colony	37	48
Chromocult	Total Coliform	36	24
ENDO	<i>E. coli</i>	36	24
Cetrimide	<i>Pseudomonas aeruginosa</i>	42	48
Bismuth Sulfide	<i>Salmonella</i> spp.	36	48
Chapman	<i>Staphylococcus aureus</i>	30 - 35	72
Azide	<i>Enterococci</i>	36	48

When calculating the colonies formed in the medium in the microbiological evaluations, it is accepted that each colony consists of a single microorganism. Therefore, the result can be expressed as colony forming units (CFU). When the number of aerobic microorganisms that can grow at a certain temperature and hour is expressed as “n CFU/ 250 mL”, the results of the microbiological analyzes were calculated as follows:

- If $n = 0$, result = 0 CFU/ 250 mL
- If $1 < n < 200$, result = $n \text{ CFU} / 250 \text{ mL}$
- If $n > 200$, result = 200 CFU / 250 mL

Physical and Chemical Analysis

Total hardness, total alkalinity, iron, turbidity, pH and odor parameters were analyzed in "Before" and "After" samples taken from the water network. Also, total organic carbon (TOC) was measured in the water samples. Total hardness was determined by titrimetric method according to EPA130.2. The alkalinity analysis was performed using the titrimetric method according to ISO 9963. Iron analysis was carried out in accordance with ISO 6332-1998, DIN 38406 E1-1 using the LCK 521 Kit on a spectrophotometer device (Hach Lange DR 6000). Turbidity analyzes were performed on a turbidimeter device (Hach Lange 2100N) according to ISO 7027. pH analyzes were performed using a pH meter (Hach Sension1) according to TS 3263 and ISO 10523. TOC were analyzed in accordance with TS 8195 EN 1484 with a TOC analyzer (Shimadzu TOC -L) operating with combustion catalytic oxidation method at 680°C.

Results

Raw Water Analysis Results

TOC results of the raw water are given in Table 3.

TOC values for Çamlıdere Dam water correspond to A2 category (water that becomes drinkable after physical treatment, chemical treatment and disinfection).

Table 3

Raw Water TOC Values

Parameter	Unit	Limit Values			Method	Raw Water Source	Sample Date	
		A1	A2	A3			May 2020	April 2021
Total Organic Carbon	mg/L	4	4.7	10	TS 8195 EN 1484	Çamlıdere Dam	7.41	4.04

Note. A1, A2, A3 are categories in Turkish Regulation on the Quality and Purification of Drinking Water Supply.

The alkalinity and hardness values of raw water are given in Table 4:

Table 4

Raw Water Alkalinity and Hardness Values

Parameters	Results
P Alkalinity (CaCO ₃)	0 mg/L
M Alkalinity (CaCO ₃)	75 mg/L
Total Hardness (CaCO ₃)	78 mg/L

The physical and chemical analysis results of raw water were given in Table 5. The results for Çamlıdere Dam water also correspond to A2 category.

Table 5

Physical and Chemical Water Quality of Raw Water

Parameters	Unit	Result
Odor		Rusty
Total Hardness	mg/L CaCO ₃	78
Total Alkalinity	mg/L	75
Iron	µg/L	361
Turbidity	NTU	10.28
pH		7.93

Microbiological analyzes were carried out in raw water and pathogen species were examined via membrane filtration method. The samples of raw water were diluted 50% in microbiological analyzes, so the number of colonies counted in the medium was multiplied by two. The result of the microbiological analysis of raw water were stated in Table 6.

Table 6

Raw Water Microbiological Analysis Results

Sample Name	Media (NPS)	Species	Dilution Coefficient	Result (CFU/250 mL)
1. Point (Raw Water)	Yeast	Total Colony	0.5	200
	Chromocult	Total Coliform	0.5	120
	Azide	<i>Enterococci</i>	0.5	10
	Cetrimide	<i>Pseudomonas aeruginosa</i>	0.5	14
	Chapman	<i>Staphylococcus aureus</i>	0.5	200
	Endo	<i>E. coli</i>	0.5	112
	Bismuth Sulfide	<i>Salmonella</i> spp.	0.5	4

Distribution Network (Plumbing) Analysis Results

Although TOC values in water distribution network changed periodically, the average values were above 3 mg/L. In the period of May 2021, samples were taken from the raw water, the treatment plant effluent, sample point 6 (as the middle point of distribution network), sample point 22 (as the last point of distribution network) and the TOC values were examined. The results were stated in Table 7.

Table 7

TOC Values in the Water Network

Sample Period	Results (mg/L)			
	Raw Water	Treated Water Effluent	Plumbing No.6 (from network)	Plumbing No.22 (from network)
May 2021	4.37	3.35	3.66	3.45

Samples were taken as “Before” and “After” at each point in the plumbing and the physical, chemical and microbiological quality changes were examined. Physical and chemical analysis results of “Before” and “After” water samples were given in Table 8. Microbiological results were given in Tables 9 and 10.

Table 8
 Physical and Chemical Analysis Results of “Before” and “After” Water Samples

Plumbing No	Pipe Material	Plumbing Age	TOC mg/L	Odor		Turbidity (NTU)		pH		Hardness (mg/L CaCO ₃)		T. Alkalinity (mg/L)		Iron (µg/L)		Deterioration in Water Quality ²
				"Before" Sample	"After" Sample	"Before" Sample	"After" Sample	"Before" Sample	"After" Sample	"Before" Sample	"After" Sample	"Before" Sample	"After" Sample	"Before" Sample	"After" Sample	
1	PE	III	4.37	Rusty	Rusty	10.28	10.28	7.93	7.93	78	78	75	75	361	361	- ³
2	PE	II	3.35	Normal	Normal	0.1	0.1	7.4	7.41	79	78	55	58	11	27	A
3	PPRC	II	-	Normal	Normal	0.09	0.12	7.45	7.45	86	86	70	75	10	20	A
4	PPRC	II	-	Normal	Normal	0.1	0.1	7.55	7.54	89	89	58	57	10	20	A
5	Galvanized	III	-	Normal	Normal	0.1	0.59	7.5	7.51	79	80	55	72	10	57	B
6	PE	II	3.66	Normal	Normal	0.44	0.78	7.55	7.53	77	77	57	66	36	40	A
7	PE	II	-	Normal	Normal	0.4	0.62	7.54	7.55	86	78	57	64	20	28	A
8	PPRC	I	-	Normal	Normal	0.1	0.42	7.5	7.51	79	80	58	70	10	26	A
9	Galvanized	III	-	Normal	Rusty	0.1	0.73	7.5	7.51	79	78	58	70	10	151	B
10	Galvanized	III	-	Normal	Rusty	0.21	1.78	7.55	7.55	80	77	64	66	24	539	D
11	PPRC	I	-	Normal	Normal	0.3	0.33	7.5	7.51	79	79	60	65	14	16	A
12	Galvanized	III	-	Normal	Rusty	0.44	5.28	7.55	7.55	77	76	59	58	46	656	D
13	Galvanized	II	-	Normal	Normal	0.4	1.29	7.5	7.51	76	78	50	51	30	134	C
14	Galvanized	II	-	Normal	Normal	0.21	1.23	7.55	7.55	78	78	63	70	26	151	C
15	Galvanized	I	-	Normal	Normal	0.21	0.32	7.5	7.51	78	80	63	63	10	13	A
16	Galvanized	II	-	Normal	Normal	0.24	0.43	7.5	7.51	76	78	58	64	26	43	A
17	Galvanized	II	-	Normal	Normal	0.4	0.7	7.55	7.51	78	78	57	57	10	510	D
18	Galvanized	I	-	Normal	Normal	0.44	0.43	7.55	7.55	76	78	58	60	26	26	A
19	PPRC	I	-	Normal	Normal	0.5	0.57	7.51	7.51	78	76	57	58	60	60	A
20	Galvanized	III	-	Normal	Rusty	0.64	6.98	7.55	7.51	80	80	64	66	10	2126	D
21	Galvanized	III	-	Normal	Rusty	0.2	1.52	7.5	7.51	76	78	50	49	11	200	D
22	PPRC	I	3.45	Normal	Normal	0.09	0.12	7.5	7.51	78	80	63	64	10	11	A

Note 1. I: New buildings (plumbing age: 0 - 5 years), II: Middle-aged buildings (plumbing age: 6 - 15 years), III: old buildings (plumbing age: over 15 years).

Note 2. Level A: No change in water quality, Level B: Deterioration did not exceed the regulation limit values, Level C: Deterioration with one parameters exceeding the limit values, Level D: Too much deterioration in water quality and at least two parameters exceed the limit values.

Note 3. Raw water (untreated) sample. Before and After values are same.

Table 9
 Microbiological Analysis Results of “Before” Water Samples

Sampled Plumbing No	Pipe Material	Plumbing Age ¹	Number of Counted Colonies (CFU/250 mL)												
			Total Colony	Other Species ²	Total Coliform	Other Species ³	Enterococci	<i>Pseudo. aer.</i>	Other <i>Pseudo.</i> ⁴	<i>Staphyl. aur.</i>	<i>E. coli</i>	Other Species ⁵	<i>Salmonella</i> spp.		
1	PE	III	0	0	0	0	0	0	0	0	0	0	0	0	0
2	PE	II	0	0	0	0	0	0	0	0	0	0	0	0	0
3	PPRC	II	0	0	0	0	0	0	0	0	0	0	0	0	0
4	PPRC	II	0	0	0	0	0	0	0	0	0	0	0	0	0
5	Galvanized	III	0	0	0	0	0	0	0	0	0	0	0	0	0
6	PE	II	0	0	0	0	0	0	0	0	0	0	0	0	0
7	PE	II	0	0	0	0	0	0	0	0	0	0	0	0	0
8	PPRC	I	0	0	0	0	0	0	0	0	0	0	0	0	0
9	Galvanized	III	0	0	0	0	0	0	0	0	0	0	0	0	0
10	Galvanized	III	0	0	0	0	0	0	0	0	0	0	0	0	0
11	PPRC	I	0	0	0	0	0	0	0	0	0	0	0	0	0
12	Galvanized	III	0	0	0	0	0	0	0	0	0	0	0	0	0
13	Galvanized	II	0	0	0	0	0	0	0	0	0	0	0	0	0
14	Galvanized	II	0	0	0	0	0	0	0	0	0	0	0	0	0
15	Galvanized	I	0	0	0	0	0	0	0	0	0	0	0	0	0
16	Galvanized	II	0	0	0	0	0	0	0	0	0	0	0	0	0
17	Galvanized	II	0	0	0	0	0	0	0	0	0	0	0	0	0
18	Galvanized	I	0	0	0	0	0	0	0	0	0	0	0	0	0
19	PPRC	I	0	0	0	0	0	0	0	0	0	0	0	0	0
20	Galvanized	III	0	0	0	0	0	0	0	0	0	0	0	0	0
21	Galvanized	III	0	0	0	0	0	0	0	0	0	0	0	0	0
22	PPRC	I	0	0	0	0	0	0	0	0	0	0	0	0	0

NOTE 1. I: New buildings (plumbing age: 0 - 5 years), II: Middle-aged buildings (plumbing age: 6 - 15 years), III: old buildings (plumbing age: over 15 years).

NOTE 2. Shows other microorganisms formed on Yeast NPS medium rather than total colony bacteria.

NOTE 3. Shows other microorganisms formed on Chromocult NPS medium rather than total coliform bacteria.

NOTE 4. Shows other *Pseudomonas* species that do not show fluorescence under a UV lamp with a wavelength of 365 nm.

NOTE 5. Shows other coliforms formed on Endo NPS medium rather than *E. coli*.

Table 10
 Microbiological analysis results of “After” water samples

Sampled Plumbing No	Pipe Material	Plumbing Age ¹	Deterioration in Water Quality ²	Number of Counted Colonies (CFU/250 mL)				Other Species ⁴	Total Coliform	Other Species ³	Total Colony	Number of Counted Colonies (CFU/250 mL)					
				Enterococci	<i>Pseudo. aer.</i>	<i>Pseudo. aer.</i>	<i>Staphyl. aur.</i>					<i>E. coli</i>	Other Species ⁶	Other <i>Salmonella</i> spp.			
1	PE	III	-	0	14	200	10	120	0	200	0	200	200	112	0	4	
2	PE	II	A	0	0	0	0	0	0	0	0	0	0	0	0	0	
3	PPRC	II	A	0	0	0	0	0	0	0	0	0	0	0	0	0	
4	PPRC	II	A	0	0	0	0	0	0	0	0	0	0	0	0	0	
5	Galvanized	III	B	13	0	0	0	0	0	0	0	0	0	0	0	0	
6	PE	II	A	200	0	0	200	0	0	0	0	32	0	0	200	0	
7	PE	II	A	3	0	0	0	0	0	0	0	2	0	0	0	0	
8	PPRC	I	A	197	0	0	0	0	0	0	0	0	0	0	0	0	
9	Galvanized	III	B	200	0	0	0	0	0	0	2	0	7	0	0	0	
10	Galvanized	III	D	200	0	200	0	200	0	0	7	0	12	200	0	0	
11	PPRC	I	A	0	0	0	0	0	0	0	0	0	0	0	0	0	
12	Galvanized	III	D	200	0	0	0	0	0	0	2	0	1	1	1	0	
13	Galvanized	II	C	200	200	0	0	0	0	1	0	1	0	0	0	0	
14	Galvanized	II	C	200	54	0	0	0	0	1	0	7	0	0	0	0	
15	Galvanized	I	A	200	0	200	0	200	0	0	0	0	0	200	0	0	
16	Galvanized	II	A	29	200	1	0	1	6	1	0	0	0	0	0	0	
17	Galvanized	II	D	38	2	0	0	1	2	1	11	0	0	0	0	0	
18	Galvanized	I	A	200	0	0	0	0	0	0	0	0	0	0	0	0	
19	PPRC	I	A	4	0	0	0	0	0	0	0	0	0	0	0	0	
20	Galvanized	III	D	200	0	0	0	0	0	1	0	0	0	0	0	0	
21	Galvanized	III	D	200	0	200	0	200	0	2	2	13	200	0	0	0	
22	PPRC	I	A	0	0	0	0	0	0	0	0	0	0	0	0	0	

NOTE 1. I: New buildings (plumbing age: 0 - 5 years), II: Middle-aged buildings (plumbing age: 6 - 15 years), III: old buildings (plumbing age: over 15 years).

NOTE 2. Level A: No change in water quality, Level B: Deterioration did not exceed the regulation limit values, Level C: Deterioration with one parameters exceeding the limit values, Level D: Too much deterioration in water quality and at least two parameters exceed the limit values

NOTE 3. Shows other microorganisms formed on Yeast NPS medium rather than total colony bacteria.

NOTE 4. Shows other microorganisms formed on Chromocult NPS medium rather than total coliform bacteria.

NOTE 5. Shows other *Pseudomonas* species that do not show fluorescence under a UV lamp with a wavelength of 365 nm.

NOTE 6. Shows other coliforms formed on Endo NPS medium rather than *E. coli*.

Results According to the Pipe Materials

The “Before” and “After” analyzes were also compared according to the pipe materials. In microbiological analysis, the averages of total colony and total pathogen numbers were taken. The age of the plumbing and the degree of deterioration in water quality were also considered during the calculations. The results and average values of the all samples with respect to galvanized metal, PE and PPRC pipe materials were given in Tables 11, 12 and 13 as follows:

Table 11

Results of the “After” Samples for the “Galvanized Metal” Plumbing

Sampled Plumbing No	Pipe Material	Plumbing Age	Deterioration in Water Quality	Total Colony Count (CFU/250 mL)	Total Pathogen Count (CFU/250mL)
5	Galvanized Metal	III	B	13	1
9	Galvanized Metal	III	B	>200	9
10	Galvanized Metal	III	D	>200	419
12	Galvanized Metal	III	D	>200	4
13	Galvanized Metal	II	C	>200	2
14	Galvanized Metal	II	C	>200	8
15	Galvanized Metal	I	A	>200	400
16	Galvanized Metal	II	A	>200	8
17	Galvanized Metal	II	D	40	15
18	Galvanized Metal	I	A	>200	0
20	Galvanized Metal	III	D	>200	1
21	Galvanized Metal	III	D	>200	417
AVERAGE		II	C	>200	107

Table 12

Results of the “After” Samples for the “PE” Plumbing

Sampled Plumbing No	Pipe Material	Plumbing Age	Deterioration in Water Quality	Total Colony Count (CFU/250 mL)	Total Pathogen Count (CFU/250mL)
2	PE	II	A	0	0
6	PE ¹	II	A	>200	433
7	PE	II	A	3	2
AVERAGE		II	A	>200	145

Note 1. Infiltration from the pipe leakage into the water network has been detected.

Table 13

Results of the “After” Samples for the “PPRC” Plumbing

Sampled Plumbing No	Pipe Material	Plumbing Age	Deterioration in Water Quality	Total Colony Count (CFU/250 mL)	Total Pathogen Count (CFU/250mL)
3	PPRC	II	A	0	0
4	PPRC	II	A	0	0
8	PPRC ¹	I	A	197	0
11	PPRC	I	A	0	0
19	PPRC	I	A	4	0
22	PPRC	I	A	0	0
AVERAGE		I	A	34	0

Note 1. The sample was taken from a tap in the toilets.

Discussion and Conclusion

In this study, the pipe materials and the age of the plumbing were associated with biofilm formation, presence of pathogenic microorganisms and deterioration in water quality. The results obtained have been helpful in terms of realizing the effect of biofilms on water quality, factors affecting biofilm formation on plumbing in Ankara city conditions, detection of biofilms and pathogenic microorganism via dehydrated NPS media. It was concluded that biofilm formations could be detected in indoor plumbing by using total colony count on Yeast NPS medium. At 15 points in the network, biofilm formation was detected by this way. It has also been found that once biofilms detached from pipe surfaces and mix with water, they are deteriorating the quality of treated tap water. The necessity of examining the presence of pathogenic microorganisms in the network after the initial detection of biofilm formation was pointed out.

Inappropriate Plumbing Conditions

In this study, it was observed that the inlet of the pipes left opened during the replacement of broken pipes at one point. When the new or repaired pipes are put into service directly without cleaning and flushing, pathogens and other microorganisms from the outer environment (like soil) can enter the water network and quickly form a biofilm layer on suitable surfaces. Microbiological water qualities may deteriorate even the disinfection is applied to the water network. So one of the

causes of the bacterial growth and biofilm formation in the network was the inappropriate pipe replacement works.

At sample point 6, biofilm layers and pathogen microorganisms resulting from pipe leakages and infiltrations from the environment was detected. At sample point 8, it is considered that since the sample is taken from the toilets, the microorganisms in the environment might reach the pipe surface with the effect of negative pressure. At the sample point 18, it was revealed that the treated irrigation water was connected to the drinking water collector with a valve and a cross-connection in the network was formed. Although it was a new building, a biofilm layer formed on the pipe surface due to that cross connection. Pathogenic microorganisms may enter the clean water networks due to cross connections and may form biofilms on pipe surfaces.

Effect of Pipe Material on Water Quality

When the results are evaluated according to the pipe types, in the 12 buildings with galvanized metal plumbing, it was observed that the physical, chemical and microbiological parameters of the waters in “Before” samples complied with the “TS 266 Water Intended for Human Consumption Standard” limit values. However, in the “After” samples taken by scraping the pipe surfaces, it was observed that the physical and chemical qualities of the water samples deteriorated significantly (Level C and D). Iron and turbidity parameters exceeded the TS 266 standard values at 7 points. In addition, the average total colony was counted as >200 CFU/250 mL in those 12 galvanized plumbing, and the average number of different types of pathogens was calculated as 107 CFU/250 mL.

In the PE plumbing, also the physical, chemical and microbiological parameters of the waters in “Before” samples complied with the TS 266 standard. For the “After” samples, the average total colony count of the PE plumbing was >200 CFU/250 mL and the average pathogen colony was 145 CFU/250 mL. Only one point in PE pipes, a bacterial growth was observed which was resulting from the infiltration caused by a pipe leakage detected at sample point 6. Therefore, intense bacterial growth was detected at this point. Different types of pathogens can enter the water plumbing due to such pipe leaks from the soil with backpressure and maintain their existence by forming a biofilm on the pipe surface. No significant change was observed in the physical and chemical qualities of the "Before" and "After" water samples (Level A).

PPRC plumbing, on the other hand, were counted as the least microbial growth and water quality deterioration with respect to the other pipe types. The physical, chemical and microbiological parameters of the waters in “Before” samples

complied with the TS 266 standard. For the “After” samples, the average total colony count was 34 CFU/250 mL, and no pathogenic species were found at those six plumbing. Only at sample point 8, total colony formation was observed. At that point, the water sample was taken from a PPRC type in the toilets, so it was considered as the source of microorganisms. No significant change was observed in the physical and chemical qualities of the "Before" and "After" samples (Level A).

More accumulations and scums occurred in galvanized metal pipes, and microbiological growth on this residue was higher when compared with PE and PPRC pipes. Especially at the network age of II. and III. category, more bacterial growth and deterioration in water quality were detected in galvanized metal pipes. In the buildings with the same plumbing age, biofilms formed more in galvanized metal pipes according to the total colony count results. It was concluded that biofilm formation deteriorates the physical, chemical and microbiological quality of water. In general, although total colony detection and biofilm formation were observed in PE and PPRC pipes, the colony number and diversity of pathogen species in galvanized metal pipes was much higher. These results were corresponded to the previous studies of Niquette et al. (2000), Camper (2003), Türetgen (2005) and Keskin and Kahveci (2019).

Effect of TOC on Biofilms

TOC values were found as 4.37 mg/L in raw water and as 3.35 mg/L, 3.66 mg/L, 3.45 mg/L in the water network respectively. There was no carbon removal unit (ozonation, membrane filters, etc.) in the water treatment plant. For this reason, the organic carbon in the raw water was reaching to the water network with partial removal and bringing an organic load to water. The amount of organic matter in the network was an important source for the biofilm formation and bacterial growth. Organic carbon should be removed in treatment plants in order to limit the bacterial growth and biofilm formation in the water networks.

Effect of Pipe Age on Water Quality

The plumbing age of total 70 buildings in the study area was mainly II. category (between 6-15 years) and plumbing materials were galvanized metal. Similarly, the plumbing age of 22 points selected within the scope of this study was same as II. category and pipe types were galvanized metal generally.

When the results are evaluated according to the plumbing ages, it has been determined that in the old plumbing, which are in the third category in terms of building age, the accumulations on the pipe surfaces were denser than the second and first category plumbing. Over the years, the accumulation on the pipe surfaces

increases for the hard and high-alkalinity waters because of the minerals inside, which forms a suitable environment for the microorganisms to attach the surface and form biofilms. Galvanized metal plumbing was used in the second and third category building ages in the facility. Since PE and PPRC materials are used in networks recently, they were found only in buildings with first and second category network age in the study area. Biofilm and pathogen formation in PE and PPRC pipes in I. and II. category buildings were lower than galvanized metal pipes. The highest water quality deterioration and the most intense corrosion was observed in galvanized metal pipes in III. category buildings.

Detected Pathogens in Plumbing

The results were also evaluated in terms of the detected pathogens. Total colony formation was detected in 16 of the 22 plumbing examined, and pathogenic microorganism colonies was observed in 13 of these points. *Pseudomonas aeruginosa*, a chlorine-resistant pathogen, can escape the final chlorination and disinfection processes in the treatment plants and survive in biofilms in the water networks. In this study, *Pseudomonas aeruginosa* was the most detected pathogens, which was counted at 11 of the 22 plumbing on drinking water network. Percival et al. (1998) isolated also *Pseudomonas* sp. from stainless steel plumbing and Critchley et al. (2003) isolated from copper plumbing. The highest colony formation was *E. coli*, which was detected in 4 of 22 plumbing and counted over 600 CFU/250 mL in total. *Enterococci* are relatively resistant to chlorine and chemicals. Therefore, the remaining cells after disinfection at the treatment plant can multiply again in biofilms on the pipe surfaces. *E. coli* and *Staphylococci* are not as resistant to chlorine and are usually removed by disinfection. It is considered that *Enterococci*, *Staphylococci* and *E. coli* entered the network from the soil with backpressure. Those pathogens can enter the water network from soil or outer environment due to the leakages or pipe repairing works. *Salmonella* species are not resistant to chlorine, and no growth was detected in the network.

In conclusion, it has been observed that more accumulations occurred in galvanized metal pipe surfaces, and microbiological growth was higher in galvanized metal pipes than PE and PPRC pipes. The highest water quality deterioration and biofilm formation was observed in galvanized metal pipes in III. category buildings (plumbing age over 15 years). More aged galvanized metal plumbing promotes more biofilm formation. *Pseudomonas aeruginosa* was the most common detected pathogens in plumbing. The highest colony formation was *Escherichia coli*. High total colony counts detected on Yeast NPS medium during the study showed the presence of biofilms on the pipe surfaces. It is considered that the total colony analysis on Yeast NPS medium with membrane filtration method can be used to

make a preliminary assessment for biofilm formations on the plumbing. In cases where the total colony results exceed the TS 266 standard limit values, additional pathogen type analysis can be performed in the plumbing. Biofilms in drinking water distribution networks are an important issue that should be carefully examined in terms of human health due to the pathogenic microorganisms they contain and their negative effects on drinking water quality. This study and methodology can be applied to water networks of different cities in order to reveal the plumbing situation and their effect on water quality. Another study for the water tanks in the water networks can be carried out to detect the biofilm formation and pathogen microorganisms in similar way. In addition, a plumbing water quality risk analysis method can be developed with the results obtained in this study in order to increase the awareness of the consumers about the effect of plumbing on water quality and to minimize the possible health problems related to them.

References

- Acehan, G. (2007). *İçme Sularının Mikrobiyolojik Kirlenme Potansiyelinin İncelenmesi*, (Publication No.) [Master's thesis, Çukurova University].
<https://tez.yok.gov.tr/UlusalTezMerkezi/tezDetay.jsp?id=fxXvSTuGxU7RNdT4Q1HeIg&no=usQZiM-JgwFtThOYgd21w>
- Akpınar, M., Ataman, P., Bağder, E.S., Halkman, A. K., Halkman, B., Halkman, H., Koçaker, N., Kolcuoğlu, G., Ömeroğlu, Y.P., Özçelik, F., Sağdaş, Ö. E., Sacar, B. Ç., Yıldırım, G., & Yılmaz, A. (2019). Gıda Mikrobiyolojisi 08. Sayım Yöntemleri (A. K. Halkman, Ed.). Başak Matbaacılık ve Tanıtım Hizmetleri Ltd. ISBN: 978-605-245-683-5
- Boe-Hansen, R. (2001). *Microbial growth in drinking water distribution systems*. Environment & Resources DTU. Technical University of Denmark.
https://backend.orbit.dtu.dk/ws/portalfiles/portal/127447176/MR2001_075_1_.pdf
- Camper, A.K., Brastrup, K., Sandvig, A., Clement, J., Spencer, C., & Capuzzi, A.J. (2003). Effect of distribution system materials on bacterial regrowth. *Journal of American Water Works Association*, 95(7), 107-121. <https://doi.org/10.1002/j.1551-8833.2003.tb10412.x>
- Critchley, M.M., Cromar, N.J., McClure, N.C., & Fallowfield, H.J. (2003), The influence of the chemical composition of drinking water on cuprosolvency by biofilm bacteria, *Journal of Applied Microbiology*, 94(3), 501-507. <https://doi.org/10.1046/j.1365-2672.2003.01857.x>
- Costerton, J.W., Lewandowski, Z., Caldwell, D.E., Korber, D.R., & Lappin-Scott, H.M. (1995). Microbial biofilms. *Annual Review of Microbiology*, 49, 711-745.
<https://doi.org/10.1146/annurev.mi.49.100195.003431>
- Freeman, C. & Lock, M.A. (1995). The biofilm polysaccharide matrix: A buffer against changing organic carbon supply? *Limnology Oceanography*, 40(2), 273- 278.
<https://doi.org/10.4319/lo.1995.40.2.0273>
- Gray, N.F. (2008), *Drinking Water Quality: Problems and Solutions* (2nd ed.) Cambridge University Press.
- Hong W., Emilie B., Michele P., Anne K. C., Vincent R. H., & Amy P. (2017). Methodological approaches for monitoring opportunistic pathogens in premise plumbing: A review. *Water Research*, 117, 68-86. <https://doi.org/10.1016/j.watres.2017.03.046>
- Keskin, N.O., & Kahveci, E.F. (2019). Polietilen ve Demir Boru Sistemlerinde Oluşan Mikrobiyel Biyofilmlerin Karakterizasyonu. *Fırat Üniversitesi Fen Bilimleri Dergisi*, 31(1), 1-8.
<https://dergipark.org.tr/tr/download/article-file/796738>
- Küçükgül, E.Y., & Özdağlar, D. (2004). İçme Suyunda Agresivitenin Saptanması ve Şebekede Korozyonun Önlenmesi. *Dokuz Eylül Üniversitesi Mühendislik Fakültesi Fen ve Mühendislik Dergisi*, 6(3), 19-39. <https://dergipark.org.tr/tr/pub/deumffmd/issue/40874/493497>

- LeChevallier, M., Cawthon, C.D., & Lee, R.G. (1988). Factors promoting survival of bacteria in chlorinated water supplies. *American Society for Microbiology Journals Applied Environmental Microbiology*, 54(3), 649-654. <https://doi.org/10.1128/aem.54.3.649-654.1988>
- Liu, S., Gunawan, C., Barraud, N., Rice, A.S., Harry, J.E., & Amal, R. (2016). Understanding, monitoring, and controlling biofilm growth in drinking water distribution systems. *Environmental Science and Technology*, 50(17), 8954–8976. <https://doi.org/10.1021/acs.est.6b00835>
- Niquette, P., Servais, P., & Savoie, R. (2000). Impacts of pipe materials on densities of fixed bacterial biomass in a drinking water distribution system. *Water Research*, 34(6), 1952–1956. [https://doi.org/10.1016/S0043-1354\(99\)00307-3](https://doi.org/10.1016/S0043-1354(99)00307-3)
- Percival S.L, Knapp J.S, Edyvean R.G.J, Wales D.S, Biofilms, mains water and stainless steel, *Water Research*, Volume 32, Issue 7, 1998, Pages 2187-2201. [https://doi.org/10.1016/S0043-1354\(97\)00415-6](https://doi.org/10.1016/S0043-1354(97)00415-6)
- Pietrzyk, A., & Papciak, D. (2017). The influence of water treatment technology on the process of biofilm formation on the selected installation materials. *Journal of Civil Engineering, Environment and Architecture*, 64(2), 131–142. <http://dx.doi.org/10.7862/rb.2017.87>
- Sartorius (2020/10/31). *Microbiological testing of foods, beverages, drinking water and pharmaceuticals*. <https://www.sartorius.com/download/459058/broch-microbiological-testing-sm-4017-e-data.pdf>
- Skjevrak, I., Lund, V., Ormerod, K., Due, A. & Herikstad, H. (2004). Biofilm in water pipelines; a potential source for off-flavours in the drinking water. *Water Science & Technology*, 49(9), 211–217. <https://doi.org/10.2166/wst.2004.0573>
- Telgmann, U., Horn, H., & Morgenroth, E. (2004). Influence of growth history on sloughing and erosion from biofilm. *Water Research*, 38(17), 3671-3684. <https://doi.org/10.1016/j.watres.2004.05.020>
- Turkish Standards Institution, (2005). TS 266 Water Intended for Human Consumption Standard (İnsani Tüketim Amaçlı Sular) <https://intweb.tse.org.tr/Standard/Standard/Standard.aspx?081118051115108051104119110104055047105102120088111043113104073082080080071077100076119105103072>
- Türetgen, İ. (2005). *Su Sistemlerinde Mikrobiyal Biyofilm Oluşumunun İncelenmesi* (Publication No.) [Doctoral dissertation, İstanbul Üniversitesi Fen Bilimleri Enstitüsü]. Database or Archive Name yazılacak
- World Health Organization. (2017/10/16). *Guidelines for drinking-water quality: Fourth Edition incorporating the first addendum*. <https://www.who.int/publications/i/item/9789241549950>
- Zhang, Y., Love, N. & Edwards, M. (2009). Nitrification in drinking water systems. *Critical Reviews in Environmental Science and Technology*, 39(3), 153–208. <https://doi.org/10.1080/10643380701631739>
-

**Extended Turkish Abstract
(Genişletilmiş Türkçe Özet)**

Sihhi Tesisatlarda Biyofilm Tabakası Tespiti ve Su Kalitesine Etkisinin Belirlenmesi

Arıtma tesislerinde arıtılan içme suyunun kalitesini bozan ve insan sağlığı açısından risk teşkil eden hususlardan birisi de su dağıtım sistemlerinde oluşan biyofilm tabakası ve içeriğindeki patojen mikroorganizmalardır. Bu çalışmada bir işletmedeki binaların sıhhi tesisatlarında biyofilm varlığı, biyofilmlerdeki patojen mikroorganizmaların tespiti ve biyofilmlerin içme sularının fiziksel, kimyasal ve mikrobiyolojik kalitesine etkisi araştırılmıştır. Mikrobiyolojik incelemelerde biyofilm varlığı ve patojenlerin tespiti için membran filtrasyon yöntemi ve hazır kurutulmuş besiyerleri kullanılmıştır. “Nutrient Pad Set (NKS)” olarak da adlandırılan bu sistem, üretici tarafından her biri absorban pede emdirildikten sonra kurutulup, steril olarak petri kutularına yerleştirilmiş besiyerleri ve membran filtre setlerinden oluşmaktadır. Çalışmada ağırlıklı olarak indikatör mikroorganizmalar incelenmiştir. Türlerin tespitinde hazır kurutulmuş besiyeri kullanıldığı için üretici firma tarafından besiyeri temini yapılabilen Toplam Koloni, Toplam Koliform, Enterokoklar, *Pseudomonas aeruginosa*, *Salmonella* spp., *Staphylococcus aureus* ve *E. coli* türleri çalışmaya dahil edilmiştir. Biyofilm ve içerdiği mikroorganizmalar ile ilgili yapılan çalışmalarda yaygınlıkla laboratuvarında kontrollü ortamda boru üzerinde biyofilm oluşumu gözlenirken, bu çalışmada doğrudan canlı ve aktif bir şebeke üzerinde var olan biyofilmler ve içerikleri incelenmiştir. Numune noktalarındaki su kalitesinin analizi yapılırken barajlardan gelen ham sudan başlanarak içme suyu arıtma tesisi çıkışı ve iç tesisatın son noktasında su kalitesindeki değişim incelenmiştir. Demir, toplam sertlik, toplam alkalinite, pH ve bulanıklık değerleri ile suların görüntü ve kokusu, musluktan akan su (öncesi) ve boru yüzeyi kazındıktan sonra akan su (sonrası) olmak üzere değerlendirilmiş ve su kalitesine yönelik fiziksel ve kimyasal etkiler de analiz edilmiştir.

Sihhi tesisatlarda kullanılan boru türü ve tesisat yaşı ile biyofilm oluşumu, patojen mikroorganizma varlığı ve su kalitesindeki değişimler ilişkilendirilmiştir. Elde edilen sonuçlar, ülkemiz şartlarında içme suyu şebekelerinde gelişen biyofilm tabakaları içinde mevcut olabilecek patojen mikroorganizma türleri ve bu türlerin hazır kurutulmuş besiyerleriyle tespit edilebilmesi açısından yol gösterici olmuştur. Özellikle Yeast besiyeri kullanılarak iç tesisatta biyofilm oluşumlarının tespit edilebildiği gözlemlenmiştir. Ayrıca biyofilmlerin bozulup suya karıştığında arıtılmış şebeke suyu kalitesini değiştirdiği de tespit edilmiştir. Biyofilm oluşumunun tespit edilmesinin akabinde şebekede patojen mikroorganizma varlığının incelenmesinin gerekliliği de ortaya çıkmıştır.

Şebekede boru patlakları, çevreden infiltrasyonlar, çapraz bağlantılar, ters basınç, boru yenileme çalışmalarından kaynaklı kirlenmeler vb. dış etmenlerden kaynaklı biyofilm ve patojen mikroorganizma varlığı da gözlemlenmiştir. İncelenen 22 tesisatın 16’sında toplam koloni oluşumu tespit edilmiş olup bu noktaların 13’ünde de patojen mikroorganizma çoğalması görülmüştür.

Yapılan analiz sonuçlarına göre galvaniz metal tesisatların ortalama şebeke yaşı kategorisi II (6 - 15 yıl arası), suyun fiziksel ve kimyasal kalitesindeki bozulma derecesi C seviyesi (su kalitesinde bozulma var, en fazla bir parametrede sınır değer aşılmış), ortalama toplam koloni sayısı >200 CFU/250 mL, ortalama patojen sayısı 107 CFU/250 mL olarak tespit edilmiştir. PE tesisatlarda fiziksel ve kimyasal açıdan su kalitesinde değişim görülmemiş, ortalama şebeke yaşı kategorisi II (6 - 15 yıl arası), ortalama toplam koloni sayısı >200 CFU/250 mL, ortalama patojen sayısı 145 CFU/250 mL olarak ölçülmüştür. PE tesisatlardaki patojen sayısının yüksekliğinin nedeni, şebekede meydana gelen boru patlağından kaynaklı şebeke suyuna çevresel su sızıntısı (infiltrasyon) olarak tespit

edilmiştir. PPRC tesisatlarda da su kalitesinde değişim görülmemiş, ortalama şebeke yaşı kategorisi I (0 - 5 yıl arası) ortalama toplam koloni sayısı 34 CFU/250 mL olarak ölçülmüştür. PPRC tesisatlarda herhangi bir patojen mikroorganizma çoğalması gözlemlenmemiştir.

Patojen türleri açısından değerlendirildiğinde çalışma yapılan şebekede *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *E. coli*, Toplam Koloni, Toplam Koliform ve Enterokok türleri farklı sayılarda tespit edilmiştir. Ham suda tespit edilen *Salmonella* spp. türüne ise artırılmış şebeke suyunda rastlanmamıştır. Bina içi şebekelerde numune alınan tesisatlarda en çok tespit edilen patojen tür 12 noktada koloni oluşturan *Pseudomonas aeruginosa* olmuştur. En fazla koloni oluşumu ise 4 noktada tespit edilen ve toplamda 600 CFU/250 mL'nin üzerinde koloni sayımı yapılan *E. coli* olmuştur.

Tesisatlar boru türüne göre incelendiğinde galvaniz metal tesisatlarda daha fazla tortu ve birikim olduğu, bu tortu üzerinde mikrobiyolojik çoğalmanın diğer boru türlerine göre (PE ve PPRC) daha fazla olduğu görülmüştür. Özellikle şebeke yaşı II. ve III. kategori olan binaların tesisatlarında galvaniz metal borularda daha fazla çoğalma ve su kalitesinde bozulma tespit edilmiştir. Tesisat yaşı aynı olan binalarda galvaniz metal tesisatlarda daha hızlı biyofilm oluşmakta ve boru yüzeyinde mikroorganizma çoğalmaktadır. Biyofilm oluşumunun suyun fiziksel, kimyasal ve mikrobiyolojik kalitesini bozduğu tespit edilmiştir. III. kategori olan eski binaların galvaniz metal tesisatlarında yoğun korozyon nedeniyle boru bağlantı noktalarının boruya kaydığı gözlemlenmiştir. Bu tür korozyona uğramış galvaniz borularda bağlantı yerinden boru sökülmeğe çalışıldığı zaman boru tamamen kopabilmekte ve deforme olabilmektedir. Bu nedenle bazı noktalarda korozyona uğrayan boru sökülememiş ve boru yüzeyi gözlemlenememiştir. Çalışmada PE ve PPRC borularda da toplam koloni tespiti ve biyofilm oluşumu gözlemlense de galvaniz metal borularda patojen tür sayısı ve çeşitliliği diğer boru türlerine göre daha fazla tespit edilmiştir.

Bina yaşı açısından değerlendirildiğinde I. ve II. kategori binalarda PE ve PPRC borularda biyofilm ve patojen oluşumu galvaniz metal tesisatlara göre daha düşüktür. En yüksek kirlilik en yoğun korozyonun da gözlemlendiği galvaniz metal tesisatlarda ve III. kategori binalarda tespit edilmiştir.

Şebeke suyunda toplam organik karbon (TOK) değerleri dönemsel olarak değişmekle birlikte 3 mg/L'nin altına düşmemektedir. TOK değerleri ham suda 4.37 mg/L ve su şebekesinde sırasıyla 3.35 mg/L, 3.66 mg/L, 3.45 mg/L olarak tespit edilmiştir. İçme suyu arıtma tesisinde organik karbon için bir giderim ünitesi (granüler aktif karbon, ozonlama, membran filtre vs.) bulunmamaktadır. Bu nedenle ham sudaki organik karbon kısmi bir giderimle şebekeye ulaşmakta ve şebeke suyuna organik yük getirmektedir. Şebekedeki organik madde miktarı mikroorganizmaların biyofilm oluşturmaları ve çoğalması için önemli bir kaynak oluşturmaktadır. Arıtma tesislerinde organik karbonun giderilmesi gerekmektedir.

Çalışma sırasında Yeast besiyeri üzerinde tespit edilen yüksek toplam koloni sayıları, tesisat borularının iç yüzeyinde biyofilm varlığının göstergesidir. Boru yüzeylerinden alınan numunelerde toplam koloni sayısı analizinin, ön değerlendirme yapmak ve burada biyofilm oluşumunu gözlemlemek için kullanılabileceği değerlendirilmektedir.

İçme suyu dağıtım şebekelerindeki biyofilmler, içerdikleri patojen mikroorganizmalar ve içme suyuna olumsuz etkileri nedeniyle insan sağlığı açısından dikkatle araştırılması gereken bir konudur. Farklı su kaynaklarına, farklı arıtma tesisleri ve su dağıtım sistemlerine göre içme suyu şebekelerinde biyofilm oluşumunun ve patojen varlığının incelenmesi gerekmektedir. Bu konudaki teknik bilgi ve farkındalık düzeyleri yetersiz olan tüketicilerin, olumsuz bina içi tesisat koşulları nedeniyle yaşanabilecek su kalite problemlerinden ve buna bağlı gelişecek sağlık sorunlardan etkilenmeleri kaçınılmazdır.