

## Microbiological properties of both drinking and domestic waters in Çorum

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### Abstract

Drinking water is of vital importance for all living things and regular monitoring of water quality is of great importance for public health. In this study, we aimed to examine some microbiological characteristics and to be evaluated in terms of the health of drinking water and wells used in Çorum. In Çorum province, once a month from ten sources-wells and water tanks used for consumption and five days a week from the central stopcock water relating to 40 locations; samples were taken in observance with the hygiene precept to 250 mL private bacteriological sterile drinking water cruet with 10% sodium thiosulfate solution. In winter, spring and summer seasons, total 1234 samples taken from sources-well, water tanks and central city tap water between January and July were used. A total of 66 samples taken from resources and wells used for consumption in Çorum proved to have seasonal changes, and some increment and diminish in bacteria rate are defined. According to the “Regulation on Waters for Human Consumption”; Chloride value: 0.2-0.5 mg/L (photometric method) was detected in the value range, and at the end daily control and checking, 1168 samples were gathered from the water samples among January and July in Çorum province. As a result of the information determined, there is no negative and contradictory data.

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### 1. Introduction

It is declared that nearly 80% (about 4.8 billion) of the world’s population inhabit in domain where incident human water either safety or biodiversity threats overrun 75th percentile (Vorosmarty et al., 2010). Water quality across the world tends to deteriorate significantly (WWAP 2009). As such, it is very important have dependable information of

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water quality on the river for water resource management from the regional to universal scale (Huang et al., 2014).

Water quality is influenced by a combination of natural factors (e.g., rains, temperature, bedrock, land, estate) and anthropogenic factors (e.g., agricultural application, domestic wastewater/industrial effect) (Ouyang et al., 2006; Baker, 2003; Li et al., 2013).

The quality of drinking water should be acceptable for human consumption. Water quality depends on the composition of water affected by natural continuity and human activities. Also, for water quality is described water parameters (microbiological, physical and chemical), and human health is at risk if values overrun admissible limits (Akter et al., 2016; BIS 2012; WHO 2012).

Inefficient access to adequate and safe drinking water is causes billions of people diarrhoea disease and of approximately 900,000 deaths per year (Kayser et al., 2015; Clasen et al., 2014). This has directly effects on public health, and the impacts are most remarkable on children under-five (Kayser et al., 2015; Haller et al., 2007; Hunter et al., 2010).

Contamination of pathogens via dirty water is a major cause of illness global. It has been forecasted that more than half of gastrointestinal diseases are caused by contaminated drinking water (Shaw et al., 2015; Hunter, 1997), and 4% of all deaths worldwide are due to grimy drinking water and low sanitation (Shaw et al., 2015; Prüss et al., 2002). In advanced nations water quality evaluations and treatment services have been acquainted to decrease microbial contamination, resulting in a important reduction in drinking water-related illnesses and deaths. Water treatment mostly contains the reduction of organics and other contaminants via coagulation and sedimentation, decomposition of any residuary solids via filtration, and eventually sterilization via ultraviolet (UV) radiation or chemical oxidants. The addition of chemical oxidants such as monochloramine and chlorine is the very widespread method of drinking water disinfection (Shaw et al., 2015; EPA 1999).

The microorganism causing pollution identified in the stool is a member of the microflora in the human and animal intestinal tracts. It must also be present in water sample if fecal contamination is available (Luyt et al., 2012; Tandlich and Muller, 2008). The microorganism indicator for the detection of waterborne pathogens must meet some criteria: if the pathogen is

present, it should be present in the water sample. If the pathogen is absent, it should not be present in the water sample; finally, the indicator microorganism must have similar characteristics to that pathogen (Luyt et al., 2012; Genthe and Franck, 1999).

A obvious relationship has been declared between the concentration of *E. coli* in a certain water sample and the possibility of gastroenteritis symptoms in humans exposed to the water through drinking (Luyt et al., 2012; Pruss, 1998). Hence, *E. coli* must fulfill the this criteria for an indicator organism (Luyt et al., 2012). Coliform bacteria are pathogenic, and their presence in the water indicates contamination with the feces, and therefore it's a potential threat. Since diseases such as cholera, dysentery and typhoid are the cause of intestinal infection, the presence of coliform bacteria in the water may indicate that the bacteria causing the specified diseases are present in that water. So these bacteria are indicators of such a danger. *Clostridium* species acquire ATP by phosphorylation only at the substrate level, which lacks the respiratory chain. There are many mechanisms in these organisms that provide energy for anaerobic digestion and are formed by *C. perfringens*, a gaseous gangrene disease (Samsunlu, 2017).

The study is first and original in terms of the evaluation of the microbiological parameters of Çorum. In this study, we aimed to examine some microbiological characteristics of drinking and domestic water used in Çorum province and evaluate them from the health point of view.

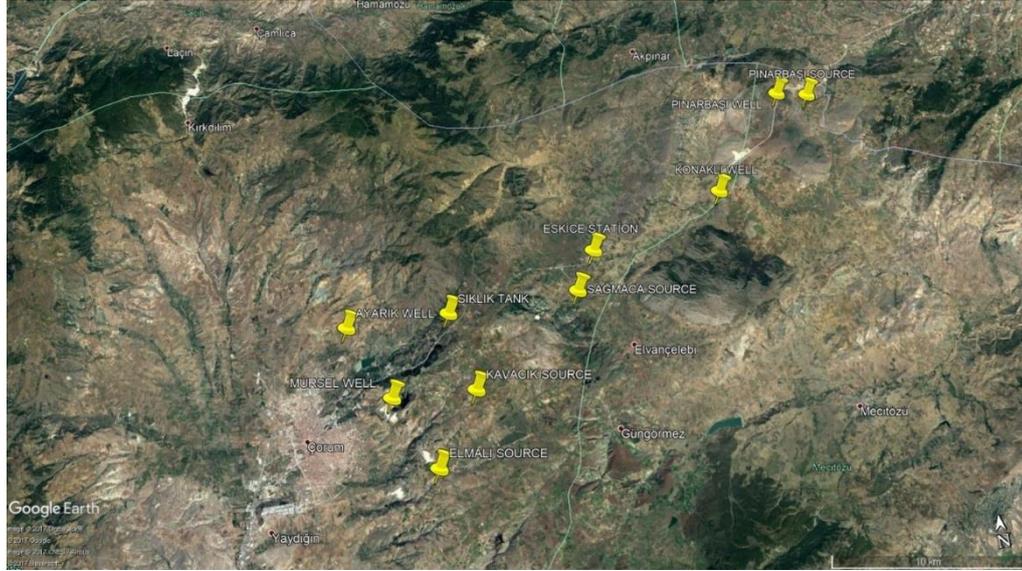
## **2. Materials and Methods**

Standards related to drinking water in our country, "The Regulation on Waters for Human Consumption" rearranged within the framework of European harmonisation legislation was published in the Official Gazette dated 20 October 2016 and numbered 29863. Based on this regulation; In Çorum province (center), once a month from 10 sources-wells and water tanks used for consumption and five days a week from the central stopcock water belonging to 40 locations (for example: schools, parks, mosques, etc.); samples were taken in suitable with the hygiene rule to 250 mL special bacteriological sterile drinking water bottles with 10% sodium thiosulfate solution. In winter, spring and summer seasons, total 1234 samples taken from sources-wells, water tanks and central provincial tap water between January and July were used. Resources, wells, stations and tanks were routinely checked monthly. These; Pınarbaşı

(well), Pınarbaşı (source), Konaklı (well), Sağmaca (source), Elmalı (source), Kavacık (source), Mürsel (source), Ayarık (well), Eskice (station), Sıklık (tank) (Figure 1). There are 8 tanks where daily routine control is performed. This is a total of 40 locations with five different endpoints of the 8 tanks. These tanks; Akkent, Ayarık, Bağcılar, Bahabey, Çamlık, Kale, Melikgazi and Nadık (photometric method) (Figure 2). Images from all locations are created using the Google Earth Pro program. Coordinates of endpoints of all tanks can be obtained from the 1<sup>st</sup> register if requested (Table 1).

**Table 1.** Latitude and longitude of sources-wells and tanks

Sources-Wells and Tanks	Latitude	Longitude
Pınarbaşı well	40.680239	35.289949
Pınarbaşı source	40.677296	35.314425
Konaklı well	40.631342	35.236364
Sağmaca source	40.593427	35.131958
Elmalı source	40.520783	35.027552
Kavacık source	40.553970	35.057642
Mürsel source	40.557053	35.005191
Ayarık well	40.594646	34.982869
Eskice station	40.612227	35.147155
Sıklık tank	40.592923	35.048109
Ayarık tank	40.586426	34.982597
Çamlık tank	40.556197	34.975061
Bahabey tank	40.556629	34.974697
Akkent tank	40.530988	34.890307
Nadık tank	40.552773	34.987481
Kale tank	40.531153	34.978864
Bağcılar tank	40.522415	34.955798
Melikgazi tank	40.559610	35.000202



**Figure 1.** Sources, wells, station and tank facilities in Çorum that were controlled monthly.



**Figure 2.** Tanks in Çorum that were controlled daily.

108 samples were gathered between the environmental health staff of Çorum provincial Health Directorate between January-July by within the program jointly determined. Microbiological parametric values in the "Regulation on Waters for Human Consumption" number/250 mL for *E. coli*, Coliform, Enterococci and number/50 mL for *C. perfringens* are taken into consideration (Table 2).

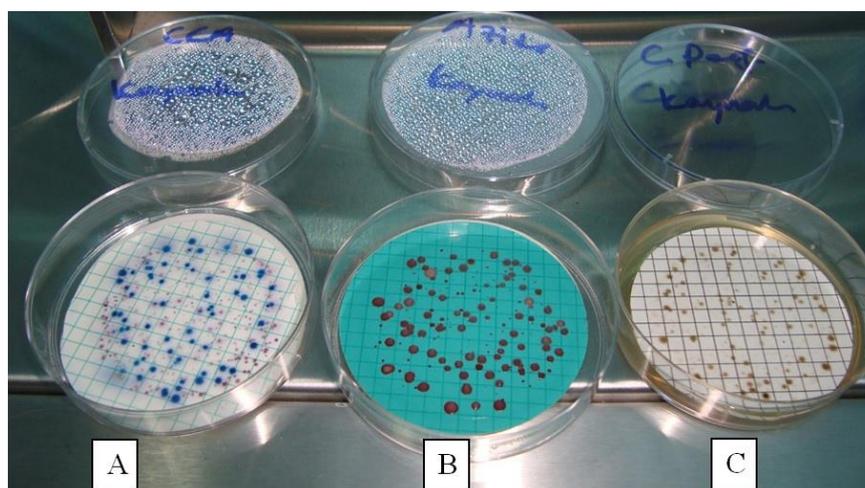
**Table 2.** Microbiological parameters for drinking and usage water based on the "Regulation of Waters for Human Consumption."

Parameter	Parametric value number / mL
<i>Escherichia coli</i>	0/250 mL
Enterococci bacteria	0/250 mL
Coliform bacteria	0/250 mL
<i>Clostridium perfringens</i>	0/50 mL

Counting of *E. coli*, Coliform, Enterococci and *C. perfringens* bacteria was done by membrane filtration method. For *E. coli* bacteria Standard: TS EN ISO 9308-1, from Coliform Standard: TS EN ISO 9308-1, for Enterococci Standard: TS EN ISO 7899-2 and *C. perfringens* bacteria; Standard: TS 8020 EN 26461-2 are taken into consideration. For *E. coli* bacteria CCA (Chromogenic Coliform Agar) medium, for Coliform CCA medium, for Enterococci Bile Aesculin Azide and for *C. perfringens* bacteria TSC Agar (Tryptose Sulfite Cycloserine Agar) medium was used (Table 3).

**Table 3.** Microbiological analysis and properties

Microorganism	Min. °C	Opt. °C	Max. °C	Media	Standards
<i>Escherichia coli</i>	8	37	47	CCA	TS EN ISO 9308-1
Coliform bacteria	-5	28	40	CCA	TS EN ISO 9308-1
Enterococci bacteria	8	37	47	Bile Aesculin Azide	TS EN ISO 7899-2
<i>Clostridium perfringens</i>	15	37	50	TSC Agar	TS 8020 EN 26461-2



**Figure 3.** Examples of positive results a-*E. Coli*, Coliform, b-Enterococci and c-*C. perfringens*.

Water coming from Sağmaca (source), Konaklı (well), Pınarbaşı (well) and Pınarbaşı (source) is transferred to Eskice station; Water coming from Elmalı and Kavacık sources is transferred to Kale tank; The water coming from the Mürsel and Eskice stations is transferred to the Melikgazi tank, and also the water coming from the Eskice stations is transferred to the Ayarık tank and supplied to the network by chlorination. The water from Akkent, Bağcılar, Bahabey, Çamlık and Nadık tank is collected from Çomar, Hatap and Yenihayat Dams, which are treated and transferred from Treatment Plant in Çorum. For Free Chlorine; Photometric Method use high-quality access filters with LEDs as a light source without moving any part by using powder reagent in the transparent sampling chamber (Lovibond MD 100).

Each stage of the research were performed under sterile conditions, and all analyzes were carried out in a sterile cabinet in the laboratory. Analyzes also provided filtration of microorganisms through membrane filtration technique (MFT) through a vacuum system on the filter paper. The funnels included in the MFT system are preferred disposable and sterilized at 121°C for 15 minutes in each autoclave after each use.

For the Assay of *Escherichia coli* and Coliform Bacteria; CCA agar supplemented medium was used. It was activated by adding 3 mL of sterile purified water to the ready-made media which is prepared dry and can be kept in this room for about two years at room temperature and darkness. Prior to the addition of the water sample to be analyzed, filter paper produced from Cellulose Acetate and having a pore size of 0.45 µm was added to capture the bacteria on the MFT system. After passing the filtered water sample, the filter paper flame was taken into the petri dish containing medium with the help of a sterile tong which was passed through the flame. All water samples in petri dishes were stored at  $36 \pm 2$  °C for 24 h with appropriate labelling. *E. coli* positive if the breeding colonies were transformed into dark blue or purple color after 24 hours; Coliform group bacteria were evaluated as positive if they had turned red or pink color. If there are other colonies other than the characteristic colonies, or if there is no reproduction, the water sample is clean regarding *E. coli* and Coliform. The report was prepared according to the result (Figure 3a).

For the identification of Enterococci bacteria; Bile Aesculin Azide agar supplemented medium was used. It was activated by adding 3mL of sterile purified water to the ready-made media which is prepared dry and can be kept in this room for about two years at room temperature and darkness. Prior to the addition of the water sample to be analyzed, filter paper

produced from Cellulose Acetate and having a pore size of 0.45  $\mu\text{m}$  was added to capture the bacteria on the MFT system. After passing the filtered water sample, the filter paper flame was taken into the petri dish containing medium with the help of a sterile tong which was passed through the flame. All water samples in petri dishes were stored at  $36 \pm 2$   $^{\circ}\text{C}$  for 48 h with appropriate labeling. After 48 hours, colonies transformed into red, pink or reddish brown colonies in the Enterococci bacteria group were evaluated as positive. If there are other columns other than the characteristic columns, or if there is no reproduction, the water sample is clean regarding Enterococci bacteria. The report was prepared according to the result (Figure 3b).

*Clostridium perfringens* bacteria is a bacterial species that reproduces in the anaerobic environment, and unique methods are required for the recycling. For *Clostridium perfringens* Bacteria Detection; TSC agar was used in combination with the *C. perfringens* selective admixture. Weighing 7.8 g of TSC Agar to prepare 200 mL of medium, we put it in 200 mL of pure water and solution with a magnetic stirrer with fish. After autoclaving at  $121^{\circ}\text{C}$  for 15 min, 0.6 mL of the prepared *C. perfringens* selective adjuvant was added via sterile automatic pipette. The prepared medium was distributed to 25 petri dish with automatic pipette aid as 8 mL. Prepared media were stored at +4 in ready to use. Prior to the addition of the water sample to be analyzed, filter paper produced from Cellulose Acetate and having a pore size of 0.2  $\mu\text{m}$  was added to capture bacteria on the system of the MFT system. After passing the filtered water sample, the filter paper flame was taken into the petri dish containing medium with the help of a sterile tong that was passed through the flame. The anaerobic bacteria is incubated with anaerobic test stain and anaerobic chemical in a specially locked package together with petri jar for reproduction. All water samples in petri dishes were stored at  $36 \pm 2$   $^{\circ}\text{C}$  for 24 h with appropriate labeling. *Clostridium perfringens* was considered positive if the breeding colonies at the end of 24 hours were transformed to black color or yellow in the UV-light ( $\sim 366$  nm). If there are other columns other than the characteristic colonies, or if there is no reproduction, the water sample is clean concerning *Clostridium perfringens*. The report was prepared according to the result (Figure 3c).

### 3. Results and Discussion

A total of 66 samples taken from sources and wells used for consumption in Çorum proved to have seasonal changes, and some rises and reduces in bacteria rate are defined. According to the “Regulation on Waters for Human Consumption”; Chloride value: 0.2-0.5 mg/L was to set in the value range, and as a result of daily control and inspection, 1168 samples were collected from the water samples between January and July in Çorum province. The results of the analysis of the 108 stopcock water samples taken routinely within the program determined together with the environmental health teams of Çorum provincial Health Directorate are given (Table 4).

**Table 4.** Total number of analyzes between January and July

	January	February	March	April	May	June	July	Total
<i>Escherichia coli</i>	176	160	176	160	168	160	168	1168
Enterococci	176	160	176	160	168	160	168	1168
Coliform	176	160	176	160	168	160	168	1168
<i>C. perfringens</i>	0	0	176	32	24	160	168	560
Public health	48	36	64	39	84	52	68	391
Source-Well	24	24	40	40	40	40	40	248
<b>Total</b>	600	540	808	591	652	732	780	4703

The colony number of *E. coli*, Coliform, Enterococci and *C. perfringens* bacteria were determined at the ratio of min: 0 to the max: 4 in the Ayarık well in June and July. In Kavacık and Elmalı sources there were no bacterial species in January. An increase of min: 0 to the max: 83 was observed in *E. coli*, Coliform, Enterococci and *C. perfringens* bacterial counts between April and July depending on temperature increase. According to the results of the Konaklı wells; only Coliform group bacteria were determined as min: 0 to the max: 4 between February and April. The highest bacterial counts in Pınarbaşı (source) were identified as *E. coli* at max: 120 in June, whereas *E. coli*, Coliform, Enterococci and *C. perfringens* bacteria were observed at the rate of min: 0 to the max: 60 during the following months. In the Pınarbaşı (wells), there is no variation depending on the temperature between January and

July. *E. coli*, Coliform, Enterococci and *C. perfringens* bacteria were observed in the ratio of min: 0 to the max: 66. *C. perfringens* or Enterococci group bacteria were not found in the between January and July. The highest bacterial count was identified as *E. coli* at 96 max in June, while max: 20 at min: 0 during the other month. There is no variability in the Mürsel (source) due to the temperature between January and July; *E. coli*, Coliform, Enterococci and *C. perfringens* bacteria were observed in the ratio of min: 0 to the max: 60. Coliform group bacterium was detected only at min: 0 and the max: 4 in July, although it is estimated that it is due to the high-temperature increase in the Eskice station and Sıklık tanks.

The chlorine values given to the tanks according to the monthly temperature value are indicated on the table (Table 5). In these months chlorine values of all endpoints are between 0.2/0.5 mg/L. As a result of the sample analyzes from all *E. coli*, Coliform, Enterococci and *C. perfringens* tap water samples obtained no data was found to be in compliance with the regulation.

**Table 5.** Temperature and tank chlorine values between January and July

Months	Temperature	Tank Chlorine (between)
January	-11/13°C	0.4/0.5mg/L
February	-11/19°C	0.4/0.6mg/L
March	-4/22°C	0.4/0.6mg/L
April	-3/28°C	0.4/0.5mg/L
May	2/28°C	0.4/0.5mg/L
June	6/35°C	0.4/0.9mg/L
July	9/41°C	0.4/0.9mg/L

#### 4. Conclusion

All microbiological analyzes for the newly established Laboratory and Scada Center in Çorum province as of January 2017 have been reported as the first and new data in accordance with Çorum Municipality Water and Sewerage Directorate. Microbiological studies carried out in large cities have started to be carried out regularly in Çorum province, which is an essential step in Çorum province in terms of environment and health. As Çorum

Municipality Laboratory and Scada Center, it is aimed to make better water quality and control by adding new analysis studies without slowing down the work done.

There was no comprehensive study in the literature. It is clear that this study, prepared for the detection and prevention of microorganisms, which negatively affecting the quality of life, will an example to many province and it will fill a significant gap in this area in terms of its application.

In line with these goals, all the newly established Scada system for drinking water used for consumption in Çorum province was controlled and supervised.

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