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Improve The Microbiological Quality of Groundwater Around Bonoloyo Cemetery by Boiling

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Abstract: *Public cemeteries are considered to be a potential source of groundwater pathogen contamination. Many settlements in Indonesia are located near cemeteries and rely on groundwater for consumption. However, there is no information available on whether the simple method of boiling can improve the microbiological quality of groundwater around cemeteries. Therefore, this experimental study aimed to determine the effectiveness of boiling in improving the microbiological quality of groundwater around Bonoloyo Cemetery. The study had two treatments: groundwater without boiling (control) and groundwater that had been boiled. Samples were collected from two points (S1 and S2) inside the cemetery and three points (S3, S4, and S5) outside the cemetery. The microbiological quality of groundwater was analyzed using the MPN method for each treatment. Samples that produced gas in positive tubes were inoculated on eosin methylene blue (EMB) media. The results of the study showed that S1, S2, S3, and S5 had total coliform levels that exceeded the normal threshold for water. Fecal coliform was found in S3 and S5. However, after boiling, there was an average decrease in total coliforms in samples S1, S3, S4, and S5. There was also no growth of E. coli in samples S3 and S5. The decrease in MPN values in the samples indicates that boiling can improve the microbiological quality of groundwater at cemetery Bonoloyo Surakarta.*

Keywords: *MPN, Groundwater, Bonoloyo, Coliform, Boiling, Cemeteries.*

1. INTRODUCTION

Groundwater is the main source of clean water for people around the world. About a third of the world's population uses groundwater as a source of drinking water. On the other hand, water can also be a vector for the transmission of pathogenic microorganisms and potentially dangerous chemical compounds. The primary route for contamination by microorganisms into the human body is through the ingestion of drinking water (Ricolfi *et al.*, 2020). Therefore, it is necessary to monitor water quality continuously by evaluating vulnerability to pollution risks. This pollution is characterized by the presence of coliform bacteria in the water.

Microbiological water quality indicators generally consist of groups of microorganisms that can enter the water through feces. Currently, fecal indicator bacteria (FIB) such as *Escherichia coli* (*E. coli*) can be used to detect contamination in water quality management because the method is simple and cost-effective (Wen *et al.*, 2020). Based on Minister of Health Regulation No. 492/MENKES/PER/IV/2010, the mandatory parameters for determining the quality of drinking water in microbiology are total Coliform and *Escherichia coli* bacteria. In general, the presence of fecal indicator bacteria (FIB) in groundwater is closely related to sanitation, such as the construction of wells and septic tanks that do not meet the requirements (Syafarida *et al.*, 2022) (Genter *et al.*, 2021).

Public burial grounds (cemeteries) can also be predicted as a source of pathogen

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contamination for groundwater. Cemetery has the potential to store pathogens that cause *tuberculosis, diabetes mellitus, cholera, toxoplasma, Clostridia*, and *heterotrophic bacteria* (Abia *et al.*, 2019). This contamination is caused by the decomposition of corpses which produces by-products as nutrients for microorganisms (bacteria and viruses) that can live after being released into the environment (Vaezihir & Mohammadi, 2016).

The Surakarta City Government manages several cemeteries and the Bonoloyo cemetery is the largest, namely 28 ha. Administratively, Bonoloyo Cemetery is located on Jalan Sumpah Pemuda, Kadipiro, Banjarsari District, Surakarta City, Central Java 57136. Geographically, Bonoloyo cemetery is located between south latitude (7°32'09.5"S - 7°32'24.2") and longitude east (110°49'37.9" – 110°49'30.3"). The land contour at cemetery Bonoloyo varies between 112-143 meters above sea level (observation results). With land contours like this, leachate can flow which carries contaminants to lower locations.

Bonoloyo cemetery is situated amidst residential areas and is bordered by Jalan Bromo Raya on the northwest side, Jalan Gunung Slamet on the east side, and Jalan Sumpah Pemuda on the south side. The southern side is the farthest position from the tomb. There are 23 blocks in Bonoloyo cemetery, each with different land contours. Generally, the eastern block has a higher contour than the western block. This position has the potential to cause groundwater contamination due to the flow of leachate resulting from the decomposition process. Although most cemeteries in Indonesia are located close to residential areas, the research regarding the quality of groundwater around cemeteries is limited to date.

Several countries have researched potential groundwater pollution caused by cemeteries, but the same is not true for Indonesia where such research is still limited. In Tabriz, Iran, a study revealed increased concentrations of calcium, magnesium, sulfate, nitrate, and fluoride ions, as well as microbial colonies in aquifer samples, indicating potential groundwater pollution from cemeteries. The main factors that increase the risk of contamination are shallow groundwater levels, high aquifer permeability, and high levels of burial (Vaezihir & Mohammadi, 2016).

Various efforts can be made to improve water quality, namely boiling, filtration (Eka Naftalina *et al.*, 2021), disinfection by ozonation (Nabih *et al.*, 2023), and environmentally friendly phytoremediation technology (Suandy *et al.*, 2021). Research conducted by (Handayani *et al.*, 2023) has indicated that boiling river water has a significant impact on the presence of *E.coli* bacteria and other harmful pathogens. The study found that boiling water was effective in destroying *E.coli* and other potentially dangerous microorganisms, highlighting the importance of this process in maintaining the quality of river water.

Several residents around Bonoloyo cemetery still use groundwater for consumption and other household activities (observation results). Therefore, this research aims to determine if boiling can improve the microbiological quality of groundwater in the Bonoloyo cemetery area. Boiling is an affordable and simple water treatment option that is widely used in the community. According to research (Nabiilah *et al.*, 2021) boiling water at its boiling temperature is an effective method to kill *E. coli* bacteria. This finding can potentially be used as valuable information for residents and serve as a basis for managing water resources in the vicinity of Bonoloyo Cemetery, Surakarta.

2. RESEARCH METHOD

This research is an experimental study with two treatments, namely groundwater without boiling (control) and with boiling. Sampling was carried out at the Bonoloyo Public Cemetery, Surakarta. Then the water quality test analysis was carried out at the Biology Laboratory, Faculty of Teacher Training and Education, Campus I, Muhammadiyah University, Surakarta. The research will take place in September-December 2023.

Sampling was carried out using a purposive sampling method from five sample points. Two points come from inside the cemetery (S1, S2) and outside the cemetery (S3, S4, S5) (Figure 1). The specified source of water for sampling purposes is well water that is not stored in a reservoir. To collect samples, a pump well is used, and 2 liters

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of water are collected in a sterilized jerry can. Sampling and laboratory testing are conducted on the same day and must be completed within 24 hours of collecting the sample.

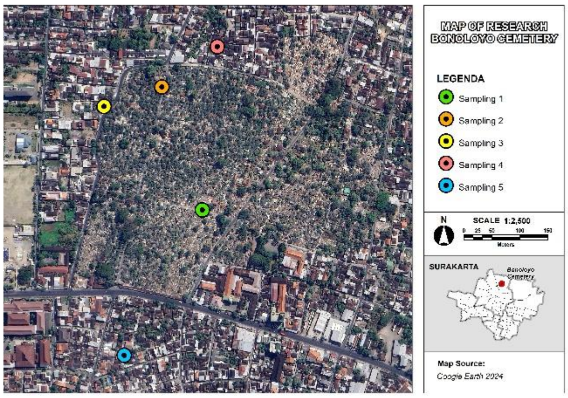

Figure 1. Bonoloyo Cemeteries map of research

To ensure that groundwater samples are sterilized, they should be subjected to boiling for 4-5 minutes. Quality testing of water utilizing the *Most Probable Number* (MPN) methodology is a multi-stage procedure that includes a presumptive test, a confirmatory test, and a complete test. The presumptive test is considered positive if gas formation occurs in the *lactose broth* (LB) medium. Gas formation in the brilliant *green lactose bile broth* (BGLBB) medium confirms the confirmation test. The complete test is deemed positive if colonies on EMBA (*Eosin Methylene Blue Agar*) media appear metallic green. Additionally, various parameters such as chemical parameters (e.g. pH) and physical parameters (e.g. TDS, temperature, smell, and taste) are evaluated.

Water quality data based on the number of coliform bacteria is compared with the standards set by the Republic of Indonesia's Minister of Health in 2017. The total coliform quality standard value should be 50/100 ml of sample, and *E. coli* must not be present in any 100 ml sample. This comparison was conducted to demonstrate that boiling groundwater at Bonoloyo cemetery, Surakarta at a temperature of 4-5 minutes can improve its microbiological quality

3. RESULTS AND DISCUSSION

Groundwater characteristics based on microbiological, chemical, and physical parameters are shown in Table 1.

Table 1. Data on groundwater characteristics before and after boiling.

Para meters	units	Standard	Sampling point									
			S 1		S 2		S 3		S 4		S 5	
			S1.I	S1.II	S2.I	S2.II	S3.I	S3. II	S4.I	S4.II	S5.I	S5.II
Total coliforms	CFU/100 ml	50	≥1898	4	271	271	≥1898	46	0	0	438	10
Fecal coliforms/ *E. coli*	CFU/100 ml	0	-	-	-	-	+	-	-	-	+	-
pH	mg/l	6.5-8.5	7.1	8.2	7.3	8.0	7.4	8.3	7.5	8.1	7.7	8.5
TDS	mg/l	1000	262	186	243	224	211	193	328	329	324	353
Temperature	°C	air temperature ± 3	33	30	31	31	29	32	29	33	30	32
Smell		There isn't any	There isn't any	There isn't any	No There is	There isn't any	There isn't any	There isn't any	There isn't any	There isn't any	There isn't any	There isn't any
Flavor		There isn't any	There isn't any	There isn't any	No There is	There isn't any	There isn't any	There isn't any	There isn't any	There isn't any	There isn't any	There isn't any

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According to the MPN (Most Probable Number) test, the presence of coliforms can be determined by observing the formation of bubbles in the Durham tube during the presumptive test stage (see Figure 2). The results of the test show that the microbiological quality of groundwater in all samples, except S4, exceeded the standard limit for total coliforms in water. The total coliform value ranged from 271 to ≥1898, which is higher than the maximum limit of 50/100 ml (as specified in Minister of Health Regulation Number 32 of 2017 regarding Environmental Health Quality Standards and Water Health Requirements for Sanitation Hygiene Purposes).

Table 2. Sampling Location Data

Sampling point	**1**	**2**	**3**	**4**	**5**
Latitude	7°32'12.5"	7°32'12.4"	7°32'28.1"	7°32'10.2"	7°32'13.6"
Longitude	110°49'28.9"	110°49'29.0"	110°49'26.7"	110°49'32.1"	110°49'25.5"
Altitude (masl)	109	107	101	100	100
Well depth (m)	15	15	12-13	10-11	10
Distance between septic tank and well (m)	3	-	10	3-4	15
Distance between cemetery and well (m)	0	0	134	80	31

During the complete test stage, positive results were obtained on S3 and S5. Positive results are indicated by the appearance of metallic green colonies on EMBA media (Figure 4). Contamination of well water with fecal coliform bacteria (*E. coli*) suggests that human and animal waste may contain disease-causing organisms such as viruses or bacteria (Awuy *et al*., 2018). Seepage is one of the factors causing contamination, which occurs when water seeps into the well due to poor construction that is not watertight or deep enough (Hamzar *et al*., 2021).

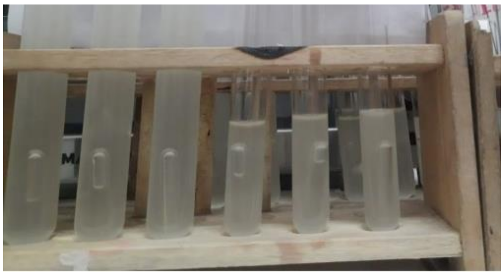

Figure 2. Positive tube at presumptive test stage

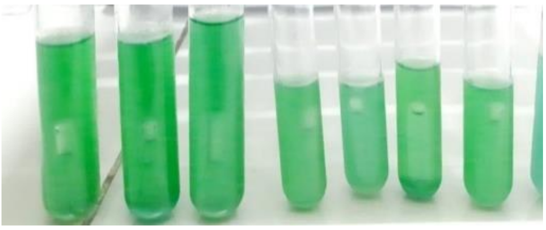

Figure 3. Positive tube on confirmative test

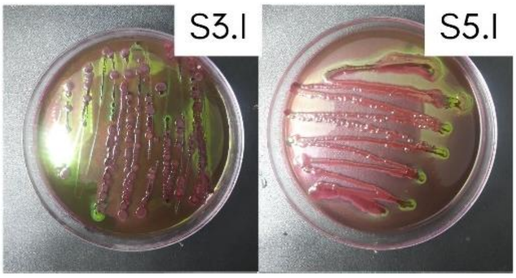

Figure 4. Complete test (control)

The wells at the five points have varying depths of 10 to 15 meters. According to Minister of Health Regulation Number 32 of 2017, the depth of these wells meets the requirements for shallow wells, which should range from 4 to 10 meters (Table 2). According to (Al-Hashimi *et al*., 2021) it has been found that groundwater, whether

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in shallow or deep aquifers, is never completely sterile. Factors such as the construction of shallow wells, land topography, unhygienic practices, and leakages from septic tanks can act as vectors for the spread of bacteria (Achmad *et al*., 2020).

Aside from the depth of the well, other factors could affect its water quality. One of these is the distance between the well and the possible sources of contamination which is often overlooked. It's also important to note that the presence of coliforms may be caused by the well's proximity to the cemetery. Based on previous research, there were several bacteria isolated from well water around the cemetery, including *Proteus spp., Vibrio spp., Klebsiella spp., Bacillus spp., Staphylococcus spp., Escherichia coli, Pseudomonas spp. and Glycomyces spp*. (Ozabor *et al*., 2022). The proximity of wells to cemeteries can lead to the seepage of rotting leachate that contains high levels of organic, inorganic, biological, and toxic metals into underground aquifers. The presence of coliform organisms and bacteria in groundwater can cause microbiological pollution, which can pose a serious threat to human health. Such pollutants require immediate attention to prevent the outbreak of pathogenic diseases. Microbiological contaminants are naturally present in the environment, including in the intestines of humans, warm-blooded animals, and plants. These microorganisms can cause various diseases, including dysentery and typhoid fever (Al-Hashimi *et al*. 2021).

Public burial grounds, also known as cemeteries, are prone to high levels of contamination due to the migration of leachate resulting from the decomposition process. An adult human body weighing 70 kg can produce 40 liters of leachate. In other words, every 1 kg of body weight can produce 0.4 to 0.6 liters of leachate. If the leachate is not retained by vegetation, it can infiltrate and cause eutrophication. It takes about 15 to 25 years for a human body to completely decompose. During the decomposition process, the body produces leachate consisting of water, protein, fat, mineral salts, and carbohydrates (Kadaoui *et al*., 2019).

According to the data collected, the eastern part of the Bonoloyo burial ground has a higher contour than the western part (109 masl > 107 masl). The height outside the cemetery remains relatively the same, ranging from 100-101 meters above sea level. The Bonoloyo cemetery is divided into old and new burial blocks. The land contours suggest that leachate resulting from decomposition could potentially migrate to lower areas (Gómez *et al*., 2022). The results of this decomposition have the potential to pollute groundwater around the cemetery and can have a negative impact on the health of residents around the cemetery.

The distance between the resident's well and the Bonoloyo cemetery varies significantly depending on the location. Point S3 is 134 meters away from the cemetery, while Point S4 is 80 meters away and Point S5 is only 31 meters away. One of the three points located outside the cemetery is too close to the cemetery, as shown in (Table 2). Although there is still a risk of contamination from the cemetery, the risk can be minimized if the cemetery is managed correctly, taking into consideration appropriate spacing, soil conditions and drainage.

So far, there have been no regulations set by the government regarding the minimum distance between the cemetery and the well. Based on research (Gómez *et al*., 2022) in Ecuador, the law requires that a cemetery must be located at a distance of at least 200 meters from any water source. However, according to the World Health Organization (WHO), the minimum distance permitted between a cemetery and a water source should be 250 meters. Additionally, the burial site must be at least 30 meters away from streams or other springs and at least 10 meters away from water channels in the field.

This study found that the presence of fecal coliforms in water sources was affected by the proximity of the location to the source of contaminants, which in this case were septic tanks. Specifically, the pump well and toilet were situated near each other, just 3 meters apart, while locations S3 and S5 were further away, at distances of 10 and 15 meters, respectively. It is worth noting that as per the Minister of Health Regulation Number 3 of 2014 on Community-Based Total Sanitation, a minimum distance of 10 meters is required between a well and a septic tank, while a minimum distance of 1.5 meters is necessary between a septic tank and a toilet

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building. Based on research (Mulyaningrum *et al*., 2021) Pipe leaks that occur often cause pollutants such as coliform bacteria due to seepage from septic tanks which are less than 10 meters from the pipe into the water.

Microbiological parameters after boiling

So far, many people think that clean water is only based on physical characteristics, such as clear, odorless and tasteless. The clear appearance of groundwater occurs because the soil layer is a natural filter for water (Edith *et al*., 2020). However, physical characteristics do not fully guarantee that the water is safe for human consumption. Most of the residents around the cemetery area still use groundwater for household activities, such as washing and bathing (S4 and S5), there is even one resident (S3) who still uses groundwater for consumption by boiling.

Boiling can be considered one of the simplest and most affordable water treatments for the community. To improve the microbiological quality of water, it is necessary to boil it to a boiling temperature so that it can kill microbes in the water (Nabiilah *et al*., 2021). By theory, water boiled at a temperature of 70°C and 100°C will kill pathogenic germs, especially *E. coli* (Handayani *et al*., 2023). The data obtained from this research is used as a reference to determine the success of the boiling method to improve the microbiological quality of Bonoloyo cemetery groundwater.

After boiling, the microbiological quality of the water improved on average, which was indicated by a decrease in total coliforms in samples S1, S3, S4, and S5. However, in sample S2, there was no reduction in total coliforms after boiling, as shown in (Table 1). Previous research has found that boiling water does not eliminate *E. coli* bacteria. Although boiling can reduce *E. coli* levels, it can still be detected in drinking water (Imtiyaz *et al*., 2021).

All samples had negative results on the complete test with EMBA media. Control treatment samples that were positive for containing *E. Coli* (S3 and S5) showed negative results after the boiling process was carried out at a boiling temperature for 4-5 minutes (Figure 5). This can prove that the boiling process can kill *E. Coli* bacteria. In line with research (Nabiilah *et al*., 2021) bacterial colonies at a temperature of 50°C found 52 colonies of *E. Coli* bacteria. Meanwhile, at 60°C, 70°C, 80°C, no bacterial colony growth was found.

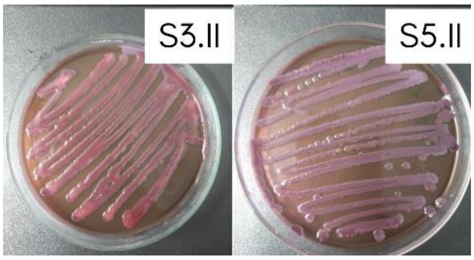

Figure 5. Complete test on EMBA media (boil)

Physico-Chemical Parameters of Ground Water

Good water quality must meet physical parameter test standards such as odorless, tasteless, colorless, clear or not cloudy, normal temperature, and does not contain solids or Total Dissolve Solids/low TDS levels. The results of the smell and taste parameter tests carried out by direct observation through the senses (organoleptic method) showed results that met standard standards, namely odorless and tasteless. According to (Rohmawati & Kustomo, 2020) the appearance of changes in the smell and taste of water can be caused by the presence of decaying organic matter, chemical compounds, the presence of algae and other aquatic plants and animals that enter as contaminants in the water sample.

Total Dissolved Solids or "Dissolved Solids" refers to any minerals, salts, metals, cations, or anions dissolved in water. This includes anything in water other than pure water molecules (H2O) and solid waste. Solid waste is particles/substances that are insoluble and do not remain in water, such as wood grain and others. These solids come from organic materials such as leaves, mud, plankton, industrial waste, and dirt. Other sources come from runoff from urban areas, road salt used on roads during the winter, and fertilizers and pesticides used on lawns and livestock. Apart from that, Dissolved Solids also come from inorganic materials such as rocks and air which may contain calcium bicarbonate, nitrogen, phosphorus, iron, sulfur, and other minerals. Most of these materials form salts,

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which are compounds that contain both metals and nonmetals. Salt usually dissolves in water to form ions. Ions are particles that have a positive or negative charge (Setianto & Murjainah, 2019).

Based on research and laboratory tests, it is known that TDS (amount of dissolved substances) shows varying results with a range of 286-353, this data shows values that are below the government standard (1000 mg/l), so it can be said to still be within normal limits. The materials contained in groundwater, both organic and inorganic materials, are still in the good category.

The temperature in springs can be influenced by several factors, namely, season, weather and measurement time, element content and land cover vegetation in the research area (Setianto & Murjainah, 2019). The results of temperature measurements in the Bonoloyo cemetery area show varying results due to differences in location which influence temperature differences. Temperature measurements in the Bonoloyo cemetery area obtained average temperature data ranging from 29-33 °C. According to (Rohmawati & Kustomo, 2020) good water temperature should not be too hot or cool, the temperature is comparable to air, which is around 28°C. This aims to prevent the dissolution of chemical substances that are dangerous to health, stop biochemical reactions, and inhibit the growth of pathogenic microorganisms.

The pH measurement results showed a different range between the control treatment and the treatment after boiling. The control treatment had a pH range of 7.1-7.7. Meanwhile, the boiling treatment showed a pH range of 8.0-8.5. According to the standards set by the government, this range can be said to have passed the water quality standard test, namely 6.5 – 8.5.

This research shows that the pH of water can change after the boiling method is carried out. According to (Bajgai, 2016)The impact of boiling water not only increases alkalinity and reduces the oxidation-reduction potential, but also reduces the level of reactive oxygen species in the body and increases the long-term possibility that drinking boiled water can prevent diseases associated with oxidative stress.

4. CONCLUSION

After conducting observations and laboratory tests on five water samples from Bonoloyo Cemetery Surakarta, by Ministry of Health regulations, it has been concluded that the boiling method is effective in improving the microbiological quality of groundwater. The average increase in microbiological quality of water is characterized by a decrease in total coliforms in samples 1, 3, 4, and 5, and no growth of *E. coli* bacteria in samples 3 and 5. The presence of fecal coliforms (*E. coli*) in well water can be caused by several factors, including the distance between the septic tank and the well, the distance from the cemetery, and the depth of the well.

Water quality can be assessed based on its physico-chemical properties. When water is boiled, its pH value can increase, but even with the increase, the pH remains within a safe range of 6.5-8.5. The TDS test results indicate that the water is safe for consumption, as the values obtained are below the safe threshold of 1000 mg/l. The TDS value falls within the range of 286-353 mg/l. Additionally, the organoleptic test results show that the water is odorless and tasteless.

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