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Reducing *E. coli* and *Coliform* in clean water using chlorine diffuser combined with white sand

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Abstract

Background: Water intended for hygiene, sanitation, and drinking must be free of physical, chemical, and microbiological pollutants. The most frequent microbiological contaminants in water are *E. coli* and *Coliform* bacteria. Chlorination, which uses chlorine compounds, is a widely used technique to eliminate germs from water. This process is regarded as effective and safe for sanitation, disinfection, and consumption.
Purpose: To analyze differences in the reduction of *E. coli* and *Coliform* bacteria in water using the *chlorine diffuser* method combined with white sand.
Method: A quasi-experimental method and a pretest posttest design, carried out at a boarding house x in Gonilan Kartasura village. The research sample was taken by purposive sampling. The sample criteria are water at the research location, namely Gonilan village which classified as densely populated. Research data was collected using observation techniques using observation sheets with three folds and three divisions of time span, namely 30 minutes, 45 minutes and 60 minutes. The data collected was then analyzed using the anova method to determine the average differences in group samples.
Results: The application of a *chlorine diffuser* combined with white sand can reduce levels of *E. coli* bacteria. In the Anova test, the p-value scores were obtained at 0.045 and 0.031, thus the alternative hypothesis was accepted, namely that there was a decrease in *E. coli and Coliform* bacteria using the *chlorine diffuser* method combined with white sand. The research results in the 60th minute of the experiment went from 1 to 0. Meanwhile, there was no significant decrease in *Coliform* bacteria. Initially the *Coliform* level was 68.3, then after 60 minutes of experimentation it only decreased to 63.6.
Conclusion: Based on the research carried out, it was concluded that there was a significant reduction in the levels of *E. coli* and *Coliform* bacteria using the *chlorine diffuser* combined with white sand with a higher dose of chlorine used.

Keywords: *Chlorine Diffuser; Coliform; E. Coli; Water.*

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INTRODUCTION

Water is a renewable natural resource, but evidence shows that groundwater availability cannot increase. Water is essential for human life, as many activities cannot occur without it. It also serves as a medium for the growth of microorganisms, and areas with water often contain various organisms, including the bacteria Escherichia coli (*E. coli*) (Winarni & Puspitasari, 2013). Water is necessary for daily activities related to hygiene and sanitation, and waterborne diseases can contribute to the spread of dangerous and harmful infections (Jesika & Hilal, 2017). Communities source clean water from rainwater, rivers, swamps, lakes, seas, and groundwater. One common source of groundwater is dug wells, which are frequently used for drinking water and typically have a depth of around 20 meters (Sasongko, Widyastuti, & Priyono, 2014).

According to the 2016 Indonesian health profile, 75.88% of households in Central Java had access to drinking water, leaving 24.2% without adequate water sources. Despite this, the primary water sources for household needs in Central Java are still dug wells and drilled wells, which remain the top choice at 44.86% (Afifah, 2019; Central Bureau of Statistics of Central Java, 2024).

Environmental health quality standards for water facilities used in hygiene and sanitation consider biological, physical, and chemical parameters, including both additional and mandatory criteria. According to the Environmental Health Quality Standards outlined in the Regulation of the Minister of Health of the Republic of Indonesia Number 2 of 2023, which implements Government Regulation Number 66 of 2014 on Environmental Health, specific requirements are set. For biological parameters, the acceptable standard for Coliform bacteria is 0 CFU/100 ml, the same standard applied to *E. coli*. Since *E. coli* is a subset of *Coliform* bacteria, its presence in water can lead to digestive diseases (Sholikhah & Yulianto, 2019; Ministry of Health of the Republic of Indonesia, 2023).

Coliform bacteria are often used as an indicator of water contamination by pathogens. *E. coli*, a rod-shaped gram-negative bacterium measuring 1.0-1.5 µm x 2.0-6.0 µm, can be either motile (with flagella) or non-motile and can grow in both aerobic and anaerobic conditions. It can also survive in low-nutrient environments. Biochemically, *E. coli* can produce indole and fails to ferment citrate, with urease tests yielding negative results.

The issue of access to clean drinking water directly impacts public health. Communities with poor water quality face a higher risk of digestive diseases. In Kartasura, the number of diarrhea cases reached 5,238, increasing significantly to 7,422 in 2022, followed by a slight decrease to 7,209 in 2023. This indicates that the water quality in Kartasura remains low, as digestive issues persist at a high level (Central Bureau of Statistics of Central Java, 2024).

One method to reduce germs in water is through the use of chemicals like chlorine, which is affordable, easy to use, and effective. Chlorine also provides disinfectant protection for several hours after application (Suyanto, Indraswati, Hendrarinata, Jayadi, & Nugroho, 2022). However, chemical use can have negative environmental and health effects, such as ozone depletion, global warming, and environmental pollution. For human health, it can harm the liver, kidneys, digestive system, immune system, and nervous system (Rahmadevy, El Jannah, Syahri, Wahyuni, Ani, 2022).

The chlorination method, using chlorine compounds, is employed to eliminate germs in water. Chlorine can also be used to make drinking water safe and for sanitation and disinfection purposes. The chlorine diffuser is a practical technology used to treat water and prevent bacterial contamination, particularly indicated by total coliform bacteria, which can cause digestive problems. The chlorine diffuser method gradually releases chlorine into the water, improving water quality standards by reducing bacterial contamination. It does so without causing unpleasant odors and can be safely used by the public (Patmawati & Sukmawati, 2020).

The use of a chlorine diffuser is crucial for microbiological control, but after installing a quality chlorine diffuser, some water improved while other samples remained unchanged. This suggests that the effectiveness of the chlorine diffuser requires further research (Sholikhah et al., 2019).

Fine white sand, also known as silica sand, contains silica, with a content of about 59.20% (Dewa & Pasaribu, 2020). In addition, it includes calcium carbonate, which is derived from the mineral calcium (Karbeka, Manimoy, & Abolasinga, 2021). White sand also contains compounds like SO3 (Sulfur Trioxide),

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CaO (Calcium Oxide), AL2O3 (Aluminum Oxide), and Fe2O3 (Iron (III) Oxide). White sand was selected to be used in combination with the chlorine diffuser because its mineral content is believed to enhance the water purification process by removing bacteria that can cause disease.

In this study, a chlorine diffuser was used in combination with white sand, with an evaluation of its contents. However, the chlorine dose applied will be higher than the commonly used levels. Typical doses are 3.5 mg/l and 4 mg/l with a reaction time of 60 minutes (Patmawati et al., 2020). In this research, a dose of 200 mg per 20 liters, or 10 mg/l, will be used with the same reaction time of 60 minutes, but periodic measurements will be taken at 30, 45, and 60 minutes.

RESEARCH METHOD

A quasi-experimental method with a pretest-posttest design, carried out at a boarding house x in Gonilan Kartasura village, a densely populated area. Purposive sampling was used to select samples. Data were collected through observation. The research instruments included three categories: field instruments (a chlorine diffuser and observation sheets), laboratory instruments (tools for testing total *Coliform* and *E. coli* content, MPN coli tables, stationery, calculators, and computers), and reporting instruments (water source observation sheets, secondary data, approval sheets, stationery, computers, and cameras).

The materials required to construct a chlorine diffuser included soldering equipment, a saw, scissors, chlorine, and adhesive. The diffuser was built from a 2-inch PVC pipe (30 cm long), a ¾-inch PVC pipe (20 cm long), two 2-inch PVC caps, and two ¾-inch PVC end caps. The technical procedure for water filtration using a chlorine diffuser involves the following steps: First, open the diffuser and fill it with a 1:1 mixture of chlorine and sand. Securely seal the diffuser after filling (Kuswanto, Yuniarno, & Hastawati, 2021).

Next, prepare the water sample from the designated source and place the chlorine diffuser in a container, submerging it to a depth of approximately 20 cm from the base. Leave the diffuser submerged for a designated time based on the water condition (30, 45, or 60 minutes). Finally, take the water sample and test it in a laboratory.

Each water sample was 20 liters, with a chlorine dose of 200 mg/20 liters or 0.02 g/20 liters. Examinations were conducted on samples treated for 30, 45, and 60 minutes, with each sample tested three times (labeled repetition I, II, and III). The chlorine diffuser's design in this study used a 2-inch PVC pipe (30 cm in length) as the outer structure and a ¾-inch PVC pipe (20 cm in length) inside, filled with chlorine and white sand. The ¾-inch pipe contained 170.76 grams of white sand, while the 2-inch pipe held 938.18 grams.

The procedure went through this flow: *Sample Collection*; Samples are collected in the morning by filling sterilized glass bottles with water, *Preparation of Presumptive Media*; Lactose Broth is measured based on the required concentration (13g/L), then mixed with distilled water and heated until fully dissolved. Nine test tubes are prepared and divided into 3 labeled groups, with each group containing 3 tubes. Each tube is then filled with 5 ml of LB media solution and sterilized, *Inoculation*; Each test tube, already containing 5 ml of LB media, is inoculated with 10 ml of sample. A Durham tube is carefully placed in an inverted position within each tube, ensuring no air bubbles are trapped inside. The test tubes are then capped with a cotton plug wrapped in gauze and incubated at 37°C for 24 hours, *Data Analysis*; Data is processed by recording the tubes that are positive for BGLB media. Results are displayed in table format with MPN values converted into CFU specifically for *E. coli* calculation, using 0.37 and 0.9 as regression coefficients. The formula is as follows: Log (CFU) = 0.37 + 0.9 x log (MPN)

Data processing was carried out at the Microbiology Laboratory, Muhammadiyah University of Surakarta using filtration techniques to determine the levels of *E. coli* and *Coliform* bacteria in the water both before and after the application of the *chlorine diffuser* device, the filtration part of which is combined with white sand. The data obtained was analyzed using a parametric statistical method, namely Anova. In the following research, there are several sample groups with repetition, so the Anova test can be used to determine the difference in averages.

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RESEARCH RESULTS

Table 1. *E. coli* Bacteriological Examination

Time	Trial I	Trial II	Trial III	Average
Before	0	1	2	1
30 Minutes	1	1	2	>1
45 Minutes	0	0	0	0
60 Minutes	0	0	0	0

Table 1 shows that in the first test stage, 1 colony was found after 30 minutes, 0 colonies after 45 minutes, and 0 colonies after 60 minutes. In the second test stage, 1 colony was also found after 30 minutes, 0 colonies after 45 minutes, and 0 colonies after 60 minutes. In the third test stage, 2 colonies were found after 30 minutes, and 0 colonies were found after both 45 and 60 minutes.

Table 2. *Coliform* Bacteriological Examination

Time	Trial I	Trial II	Trial III	Average
Before	77	64	64	68.3
30 Minutes	43	52	55	50
45 Minutes	65	76	73	71.3
60 Minutes	70	68	53	63.6

Table 2 shows that in the first test stage, 43 *Coliforms* were found after 30 minutes, 65 *Coliforms* after 45 minutes, and 70 *Coliforms* after 60 minutes. In the second test stage, 52 *Coliforms* were found after 30 minutes, 76 *Coliforms* after 45 minutes, and 68 *Coliforms* after 60 minutes. In the third test stage, 55 *Coliforms* were found after 30 minutes, 73 *Coliforms* after 45 minutes, and 53 *Coliforms* after 60 minutes.

Table 3. Bivariate Analysis of *E. coli* and *Coliform* Bacteria Count

Variables	Results				
	Sum of Squares	Df	Mean Square	F	Sig.
***E. coli* bacteria count**					
Between Groups	4.250	3	1.417	4.250	.045
Within Groups	2.667	8	.333		
***Coliform* bacteria count**					
Between Groups	800.667	3	266.889	4.989	.031
Within Groups	428.000	8	53.500		

Table 3 shows the bivariate analysis of *E. coli* bacteria count, with a value of 0.045 for between and within-group comparison. Meanwhile, the *Coliform* bacteria count yielded a value of 0.031 for between and within-group comparison.

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DISCUSSION

One method to reduce *E. coli* and *Coliform* bacteria in water is the use of chlorine compounds, which are considered safe for disinfecting drinking water sources. Chlorine is applied using a chlorine diffuser, which diffuses the chlorine into the water. This method is effective at reducing bacterial contamination and improving water quality while remaining safe for public consumption (Patmawati et al., 2020). The higher the chlorine dose, the faster *E. coli* and *Coliform* bacteria are eliminated. The recommended chlorine dosage in a chlorine diffuser is between 3.5 mg/l and 4 mg/l. In this study, a higher dose of 200 mg/20 liters was used, equating to 10 mg per liter. The water, collected from a tap in a sterile bottle, was tested using a Compact Dry apparatus after a 24-hour incubation. Initial tests showed an average *E. coli* level of 1 and *Coliform* at 68.3. After treatment with the chlorine diffuser and white sand for 60 minutes, with checks at 30, 45, and 60 minutes, there was a significant reduction in bacteria. *E. coli* dropped from 1 to 0, and *Coliform* from 68.3 to 63.6. An ANOVA test confirmed this reduction as statistically significant, with significance scores of 0.045 for *E. coli* and 0.031 for *Coliform*, both below the 0.05 threshold.

However, the study also found that at 45 and 60 minutes, *Coliform* levels increased in some cases, suggesting the possible presence of protozoa in the water. *Coliform* bacteria can become more resistant when in contact with protozoa, especially if ingested. This indicates that the chlorine must also eliminate protozoa to prevent bacterial resurgence (King, Shotts Jr, Wooley, & Porter, 1988).

There is a strong correlation between the presence of *E. coli* and *Coliform* in water and the existence of pathogens (Ramadhani & Rezania, 2023). Poor sanitation often leads to the presence of these bacteria, which can cause diarrhea and other health problems, reducing public health quality (Putri & Wulandari, 2019). Both bacteria thrive in water and are known to cause disease, making them indicators of polluted water (Vikahadi, Wicaksono, Nugroho, Gomareuzzaman, & Prasetya, 2023).

E. coli is normally found in the human digestive tract, where it helps in digestion. However, excessive *E. coli* levels or its presence outside the digestive tract can cause serious health issues. Different strains of *E. coli* can lead to diseases like diarrhea (including bloody diarrhea), kidney failure, and severe stomach cramps (Ramadhani, Ngazizah, & Khasanah, 2021). The bacteria also cause hemolytic uremic syndrome and hemorrhagic colitis, and they spread quickly from human to human or from animals (Nurjanah, Cahyati & Windria, 2020). There are several harmful strains of *E. coli*, such as *enterotoxigenic E. coli*, which causes traveler's diarrhea, and *enteroaggregative E. coli*, which causes acute diarrhea (Leona, 2023).

Coliform bacteria are an indicator of water contamination, as their presence is linked to other pathogenic bacteria. Higher levels of *Coliform* indicate greater risks of disease. *Coliform* is often found together with *E. coli*, contributing to illnesses like diarrhea, kidney failure, and stomach cramps (Widyaningsih, Supriharyono, & Widyorini, 2016). Testing for *Coliform* is faster, cheaper, and simpler than testing for other pathogens, making it a reliable water quality indicator. Lower *Coliform* levels generally indicate better water quality. However, *Coliform* can also cause diseases like typhus, diarrhea, and dysentery (Kumalasari, Rhodiana, & Prihandiwati, 2018).

Both *E. coli* and *Coliform* reproduce asexually through methods such as binary fission, budding, and fragmentation. In favorable conditions, binary fission is the most common method, producing genetically identical offspring (Nisfiatul, 2017). Budding occurs when environmental conditions are less favorable, and fragmentation happens when bacteria break off from a parent cell to form new bacteria (Rini & Jamilatur, 2020).

The time required for chlorine to effectively reduce bacteria levels in water can vary depending on the chlorine dose and water temperature. At higher temperatures, around 180°C, a contact time of approximately 30 minutes is needed, but colder water requires longer contact times. Therefore, chlorine is typically added after water is transferred to a storage tank, allowing sufficient time for the chlorine to react before the water is consumed (Sofyan, 2018). An effective contact time for chlorine to significantly reduce or eliminate bacteria is around 60 minutes, using doses of approximately 3.5 mg/l to 4 mg/l (Patmawati et al., 2020). In other research, a contact time of 30 to 40 minutes at a dose of 160 ppm was tested, producing chlorine residues of 88.03 ppm and 97.5 ppm, effectively reducing Coliform levels by

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98.83%, leaving less than 2% of Coliform bacteria in the water. This highlights the importance of contact time, as a 15-minute duration is often insufficient for chlorine to fully react with the water, leaving many bacteria present. Bacterial levels drop significantly after 30 minutes, and longer contact times of 40 to 45 minutes yield even better results, with 40 minutes being considered optimal. Longer contact times increase chlorine's effectiveness in eliminating bacteria with the appropriate dosage (Busyairi, Dewi, & Widodo, 2016).

The chlorine diffuser is a clean water treatment technology that introduces chlorine to the water to eliminate *Coliform* bacteria. It is considered a strategic method to tackle water pollution and can be used by various communities. Using a double pot diffuser method, which involves two tubes in the device, the chlorine diffuser has been shown to improve water quality and reduce turbidity (Marwati, Rusminingsih, Mahayana, Suyasa, Aryana, & Yulianti, 2022). In this study, the optimal time required for treatment was four days. The chlorine diffuser can be applied in many different contexts, including hospitals (Chiemchaisri, Chiemchaisri, Dachsrijan, & Saengam, 2022), industrial settings such as factories (Hanum (2021), tourism areas (Marwati et al., 2022), and in daily life to improve water quality for general consumption (Terin, Freitas, Nasser Fava, & Sabogal-Paz, 2022). This versatility makes the chlorine diffuser highly adaptable to various situations and water conditions, making it a widely applicable solution.

CONCLUSION

Chlorine diffuser combined with white sand effectively reduced levels of *E. coli* and *Coliform* bacteria. This chlorine diffuser with white sand proved effective in decreasing *E. coli* and *Coliform* bacteria levels. However, it is important to note that the study was conducted on an experimental scale and over a relatively short duration.

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