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IDENTIFICATION OF ESCHERICHIA COLI AND ORGANIC SUBSTANCES BASED ON THE DISTANCE FROM THE DUG WELL TO THE SEP-TIC TANK

Gendhis Brilyan Nissa & Peni Pujiastuti*

Chemical Analysis Study Program, Faculty of Engineering, Universitas Setia Budi, Indonesia

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*Corresponding author: penipujiastuti@setiabudi.ac.id

Abstract

As many as 70% of drinking water sources in Indonesia are contaminated with feces which can cause the spread of diarrheal diseases. There were 1,177 cases of diarrhea spread across Colomadu District. In Pucung Village, Colomadu District, complaints about well water being colored, smelly, tasteful, slimy, and people often experiencing diarrhea, especially those located less than 10 m from the septic tank. In accordance with Minister of Health Regulation Number 32 of 2017 concerning Water Hygiene, the E. coli content is 0 CFU/100 ml and the maximum organic material content is 10 mg/l. The E. coli analysis method uses Total Plate Count (TPC) on agar plates according to SNI 2897:2008. Research needs to be carried out to ensure the quality of well water regarding the content of E. coli and organic substances. Determination of organic substance levels was carried out using the permanganometric titration method according to SNI 06-6989.22-2004. This research uses the TPC method to determine water quality and is included in qualitative research which focuses on case studies. Based on the research, the results showed that the values of organic substances and E. coli in sample I were 17.20 mg/l and 1.6 x 10^9 CFU/100 ml, sample II was 11.88 mg/l and 5.0 x 10^5 CFU / 100 ml, while sample III was 6.88 mg/l and 2.3 x 10^4 CFU/100 ml. So, the quality of the well water in the Pucung Village studied did not meet the requirements of Minister of Health Regulation Number 32 of 2017.

***Keywords**: Well Water; Septic Tank; Escherichia Coli; Organic Substances; TPC*

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INTRODUCTION

According to the United Nations International Children's Emergency Fund (UNICEF) Indonesia, drinking water sources in Indonesia have been polluted by human waste. 70% of 20,000 household drinking water sources are contaminated with human waste, causing the spread of diarrheal disease which is the main cause of death for children under five (Lating, 2017). One source of clean water used by people in Indonesia is well water (Sandi, 2021). Around 45% of people in Indonesia use wells as a means of clean water and 75% of the people use dug wells (Khomariyatika & Pawenang, 2011).

The source of clean water that is widely used by people in rural areas is dug wells, because making dug wells does not require a lot of money and is very easy to obtain (Khomariyatika & Pawenang, 2011) However, dug wells have a high risk of pollution. Dug well water comes from underground, so there is a possibility of a decrease in water quality in the dug well. Of course, you need to be aware of the possibility of contamination by pathogenic bacteria such as E. coli.

E. coli one of the inhabitants of the body. The presence of *E. coli* in water or food can cause diarrhea and other digestive disorders. According to PERMENKES No. 32 of 2017, the *E. coli* content in clean water for sanitation hygiene is 0 CFU/100 ml. So that dug well water used as a source of sanitation hygiene is not allowed to contain *E. coli* which will cause various diseases. The dug well itself is easily contaminated by *E. coli* at a distance of less than 10m, through seepage originating from human waste. Therefore, it is best to make a well at a distance of more than 10 meters so that the well water is protected from various kinds of pollution. *E. coli* it self is an indicator of water quality. The lower the *E. coli* content, the better the water quality.

Quality standards are needed that can protect human health and the environment. Therefore, by referring to PERMENKES No. 32 of 2017 we can maintain quality standard values that are in accordance with the classification. So that it can reduce the risks arising from the quality of the water. Apart from that, well water pollution due to septic tanks can also affect the amount of organic substances contained.

The high levels of organic substances in well water are influenced by several factors, namely the cleanliness of the environment around the well, the condition of the well, and septic tank seepage due to the layout of the well close to the latrine. PERMENKES No. 32 of 2017 limits the organic substance content in

water for sanitation hygiene to a maximum level of 10 mg/l. If a well is contaminated by *E. coli* and has a high organic substance content caused by the septic tank, it is possible that people who consume water from the well will experience various kinds of diseases, one of which is diarrhea. So, the aim of this research is to ensure the quality of well water in Pucung Village, Colomadu District, Karanganyar Regency regarding the content of *E. coli* and organic substances as required in PERMENKES No. 32 of 2017.

RESEARCH METHODS

The research was carried out in May 2023, in the Microbiology laboratory and wastewater analysis laboratory at Setia Budi University.

The equipments used were Pyrex glassware; ICA C-Mag hot plates; Ohaus analytical balance; colony counter Stuart scientific; Duran Steriplan petri dishes; All American autoclave; Memmert incubator, refrigerator, cool box, spirit burner, meter, boiling stone; ICA C-Mag hot plate.

The materials used were dug well water samples; distilled water; Merck PCA powder; Merck BPW powder; 70% alcohol, Sulfuric acid (H_2SO_4) 8 N Merck which is free of organic substances; $KMnO_4$ pa 0.1 N; Oxalic acid pa 0.1 N.

The research population was 3 dug water wells close to a septic tank and located in Pucung Village, Colomadu District, Karanganyar Regency, Central Java. The sampling location points were selected using convenience sampling. Samples were taken by grab sampling from dug wells that had physical characteristics of septic tank contamination such as smell, color, taste and slimy, a distance of <10m and >10m from the septic tank.

E. coli was analyzed by using Total Plate Count (TPC) in agar plates according to SNI 2897:2008. Determination of organic substance levels was carried out using the permanganometric titration method according to SNI 06-6989.22-2004.

Data analysis

Determination of *E. coli* (TPC) Analysis (SNI 2897:2008)

$$Colonies = number\ of\ colonies\ on\ the\ plate\ x\ 1/fdilution$$

Determination of Organic Substance Analysis (SNI 06-6989.22-2004)

$$KMnO4 = \frac{[(10+a)b-(10\ x\ c)1\ x\ 31{,}6\ x\ 1000}{d} xf$$

where:

a: volume of $KMnO_4$ 0.01N

b: actual $KMnO_4$ normality

c: normality of oxalic acid

d: sample volume

f: sample dilution factor

Well water quality is determined by comparing the analysis results with appropriate water quality standards for hygiene and sanitation PERMENKES No. 32 of 2017, especially on *E. coli* parameters and organic substance content.

RESULTS AND DISCUSSION

E. coli

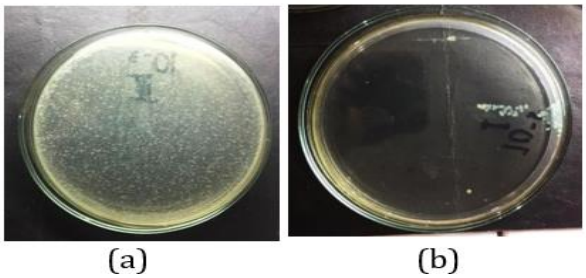
Samples were taken representatively and aseptically in dug wells at a distance of 1m, 5m, 11m from the septic tank. TPC testing was carried out by diluting the sample, to reduce the number of microorganism populations. Colonies that grow in petri dishes will accumulate, making it difficult to calculate the number of colonies (Mursalim, 2018). As shown in table 1, dilutions were carried out at concentrations of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}. Next, they were planted using PCA media which was poured in 15-20 ml and left until solid, then incubated at a temperature of 34^0C – 36^0C for 48 hours, then calculations were carried out. The number of *E. coli* bacterial colonies was different in each sample. The complete number of *E. coli* colonies in each dug well water sample is presented in table 1.

Table 1. Number of *E. coli* Colonies

Distance (m)	Dilution						
	Petri	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Number of Colonies (CFU/100ml)
1	A	tbud	tbud	tbud	tbud	162	1600000000
	B	tbud	tbud	tbud	tbud	157	
5	A	tbud	35	13	8	0	500000
	B	tbud	64	10	5	0	
11	A	20	4	3	0	0	23000
	B	25	5	3	0	0	

Information:
tbud :Too many to count (>250)
Yellow: data that falls within the calculation range (25-250)

Calculation of total plate numbers of microorganisms selected from petri dishes with colony numbers between 25-250 as shown in table 1 in yellow. It s since agar media with high (>250 colonies) and low (<25 colonies) numbers as in Figure 1 are not statistically valid to count (Mursalim, 2018).



(a) (b)

Figure 1. TBUD colonies (a) (>250) & (b) <25

Meanwhile, the number of colonies in samples 1m apart is presented in Figure 2.

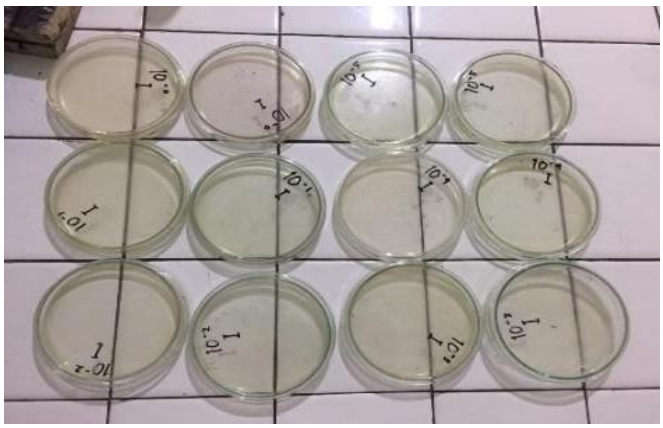


Figure 2. Colonies on a petri dish

Based on table 1 and figure 2, the research results show that the three well water samples at a distance of 1m, 5m and 11m from the first to the fifth replication showed the growth of *E. coli* colonies on the cup. In well water samples at a distance of 1m from the septic tank, the *E. coli* colonies included in the calculation at the fifth dilution (10^{-5}) were 1.6 x 10^{9} CFU/100ml. In well water samples at a distance of 5m from the septic tank, the *E. coli* colonies included in the calculation in the second dilution (10^{-2}) were 5.0 x 10^{5} CFU/100ml. Meanwhile, in well water samples at a distance of 11m from the septic tank, the *E. coli* colonies included in the calculation in the first dilution (10^{-1}) were 2.3 x 10^{4} CFU/100 ml. There is an increase in the number of *E. coli* colonies in well water adjacent to the septic tank.

The closer the septic tank is to the dug well, the more the number of *E. coli* colonies increases. The presence of *E. coli* in well water is due to infiltration from septic tanks which are less than 10m away from the well. In line with research by Korniasih & Sumarya (2021), 5 dug wells and 5 drilled wells located less than 10 m from septic tanks in Gianyar Regency, had an average *E. coli* content that did not meet quality standards, respectively, at 18.8 MPN/100 ml and 9.8 MPN/100 ml.

Research by Awuy *et al*., (2018) *E. coli* in wells 12 m from the septic tank was 198 MPN/100 ml and at a distance of 6 m was >1600 MPN/100 ml. It is since the dug well is shallow and located close to the septic tank, causing large amounts of water to seep into the dug well. In SNI 03-2398-2002, septic tanks and wells must be more than 10 m apart. The septic tank working system collects human waste, which will settle at the bottom of the tank.

Sewage slowly seeps into the ground and water pollution occurs if there is a well close to the septic tank (Afifah, 2019). Moreover, if the construction is not appropriate, the septic tank will not be able to filter feces and will have an impact on the quality of the surrounding water (Achmad *et al*., 2020).

Organic Substances

The organic substances in the samples are presented in table 2 below:

Table 2. Content of organic substances

Well distance (m)	Organic substances (mg/l)		% RPD	Average (mg/l)
	Simplo	Duplo		
1	17.20	17.20	0 %	17.20
5	12.20	11.57	5.28 %	11.88
11	7.19	6.57	9.06 %	6.88

Well water contains quite high levels of organic substances, the highest in well water samples located 1m from the septic tank. It can be seen in table 2 that the closer you are to the septic tank, the organic substance content increases. Well water samples that are more than 10m from the septic tank are much smaller than those close to the septic tank. It is in line with research by Ningrum (2018) that samples of well water 4m from the septic tank contained organic substances of 11.3-11.5 mg/L. Munfiah (2013) found that well water samples less than 10m away contained organic substances of 1.67-14.85 mg/L. This well water has been contaminated with human feces.

According to Nurjanah & Kusumadewi (2017) if a clean water source contains a high content of organic substances, it can be concluded that the water contains pollutants, one of which is human feces. Septic tanks have the function of storing human waste. The main components of human feces include 75% water and solid substances which are organic substances. These organic substances consist of 25-54% biomassa bacteria, 2-25% protein, 25% carbohydrates and indigestible substances, and 2-15% fat (Nurjanah & Kusumadewi, 2017). Organic substances are pollutants that are easily degraded by microorganisms, one of which is *E. coli* (Pujiastuti, 2024).

Comparison of the content of *E. coli* and organic substances with the Quality Standards of PERMENKES No. 32 of 2017

Well water is clean water that is used by the community for sanitation hygiene purposes to meet their daily

needs. It is necessary to pay attention to the mandatory quality parameters regarding the quality standards of PERMENKES No. 32 of 2017, concerning environmental health quality standards and water health requirements for sanitation hygiene purposes, swimming pools, solus per aqua, and public baths. The quality of the *E. coli* content and organic substances as mandatory parameters in this research is compared with the quality standards of PERMENKES No. 32 of 2017 presented in table 3. The *E. coli* content in the three samples exceeds the quality standards. Meanwhile, the content of organic substances in well water samples within a distance of less than 10 m exceeds the quality standard, while those within a distance of more than 10 m meet the quality standard.

Table 3. Comparison of Quality Standards

Well Distance (m)	*E. coli*		Organic Substances	
	Quality standards (CFU/100ml)	Sample (CFU/100ml)	Quality standards (mg/l)	Sample (mg/l)
1	0	1.6 x 109	10	17.20
5	0	5.0 x 105	10	11.88
11	0	2.3 x 104	10	6.88

The presence of *E. coli* in the water indicates that the water is not suitable for consumption (Thani *et al*., 2016), so it can cause various water-borne infectious diseases such as diarrhea. Water contaminated with *E. coli* can cause digestive-related diseases including diarrhea, cholera, polyomelistis etc. *E. coli* must be watched out for because it has the ability to be resistant to several types of microbes (Awuy *et al*., 2018).

CONCLUSION

The well water samples at a distance of 1m, 5m and 11m that were studied contained *E. coli* and organic substances that exceeded the quality standards of PERMENKES No. 32 of 2017. The value of organic substances and*E. coli* in sample I was 17.20 mg/l and 1.6 x 10^9 CFU/100 ml, sample II was 11.88 mg/l and 5.0 x 10^5 CFU/100 ml, while sample III was 6. 88 mg/l and 2.3 x 10^4 CFU/100 ml. This well water has been contaminated with *E. coli* and organic substances so it is not suitable for sanitation hygiene purposes. The closer the well is to the septic tank, the greater the content of *E. coli* and organic substances in the well water.

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