Microbiological Study of *Escherichia Coli* Contamination in Dug Wells in Demak District, Central Java

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Abstract

Demak Regency is a peri-urban area in Java experiencing an increasing demand for clean water due to population growth and development. Groundwater is the primary source to meet this demand, although its quality is often threatened by pollution from household waste and unprotected septic tanks. This study aims to evaluate groundwater quality in Demak District with the Escherichia coli presence parameter in unconfined aquifers using a GIS-based spatial approach and IDW interpolation method. The geology of the study area includes sandstone, carbonate mudstone, and alluvium lithologies, with most regions having flat slopes. The hydrogeological potential of the study area shows diverse aquifer productivity, with groundwater levels ranging from 0.3–41 meters. The results of the groundwater quality analysis in the study area showed that 47% of the samples exceeded the safe limit for *Escherichia coli* by Ministry of Health Number 2 Year 2023. The shallow depth of the groundwater table and poor well construction allow the infiltration of contaminants from the land surface into the groundwater, especially from polluting sources around the well. This study provides an essential overview of groundwater quality in the Demak District, which can support sustainable groundwater management and improved sanitation to improve public health.

Keywords: contamination; *Escherichia coli*; groundwater quality; unconfined aquifer.

Introduction

Demak Regency is one of the peri-urban areas with high population density and intensive agricultural activities, potentially increasing the risk of contamination of groundwater sources (Alifya & Mardiansjah, 2021). Demak's geographical location directly adjacent to Semarang City as an economic center has led to a high population growth rate, reaching an average of 1.09% by 2023. Rapid residential and economic development resulted in a population density of 1,269 people/km² (BPS, 2024), which further increased the demand for clean water, especially from groundwater sources. Problems that are often encountered include groundwater owned by the community that does not meet the criteria for clean and safe water (Rizal & Asyfiradayati, 2024). Water is a vital resource for life, but when contaminated by biological pollutants such as Escherichia coli, viruses, and parasites, it can cause serious health impacts, especially in developing countries that have limited access to clean water and adequate sanitation (Kristanti et.al., 2022). In this case, *E. coli* is an important microbiological indicator for determining water quality, given that its presence often indicates the presence of fecal pollution that can

endanger human health (Rongre et al., 2018). *E. coli* can cause various diseases whose transmission involves contaminated water, such as diarrhea, typhoid, and dysentery, known as waterborne diseases (Priyanto, 2019). The effect of Escherichia coli bacteria can also cause death (Awuy, et, al., 2018). To find out whether the water used contains E-Coli bacteria is not easy, because its size is very small and invisible to the eye (Fauziah, 2021).

Hydrogeological conditions in the northern part of Demak have a shallower groundwater depth compared to the depth of water in the southern part of Demak (Putranto et al, 2021). The quality of groundwater in Demak Regency is uncertain, as many residents still rely on well water as their main source. Many of these wells are located close to pollutant sources, such as toilet waste, household waste, and leaking septic tanks. The non-optimal physical condition of these wells can affect the bacteriological quality of the well water. Previous studies have shown that the suboptimal physical condition of wells also greatly contributes to the decline in groundwater quality, which is an important concern for public health (Syafarida et al., 2022). The bacteriological quality of unqualified dug well water will be higher when compared to qualified dug wells (Dewi et al., 2019). Therefore, it is important to evaluate groundwater quality in community dug wells to provide relevant information for groundwater resource management and public health protection efforts.

Although there have been previous studies on groundwater quality in this area, there has been no specific research on microbiological parameters, such as the presence of *E. coli* Therefore, the use of a Geographic Information System (GIS) based spatial approach and Inverse Distance Weighting (IDW) method is essential to determine the concentration and distribution pattern of *E. coli*. This study aims to identify groundwater quality based on *E. coli* in community-owned unconfined aquifers that are expected to provide recommendations for sustainable groundwater management and microbiological pollution mitigation in Demak Regency.

Materials and Methods

The study area is in the northern part of Java Island, and most of the study area is directly adjacent to the Java Sea. The morphology of this area includes alluvial plains with elevations between 0 and 15 meters, while the southern part has structural hills that reach elevations of 15 to 325 meters. Geologically, this area belongs to the alluvial plains of Northern Java and is within the Bogor – North Serayu – Kendeng Anticlinorium Zone (van Bemmelen, 1949). The stratigraphy of this area is divided into six rock units arranged sequentially from the oldest to the youngest. The rock units are carbonate sandstone unit, carbonate mudstone, carbonate sandstone, carbonate sandstone, sandstone and carbonate mudstone, volcanic breccia unit, and alluvial sediment unit, which dominates almost the entire area of Demak Regency. The alluvial deposits, consisting of sand, gravel, and clay, have highly variable porosity. The flow of water in these alluvial deposits units is highly dependent on the proportion of each material.

Demak District has two cross-municipal groundwater basins, which are CAT Semarang-Demak and CAT Kudus, where the potential availability of groundwater reaches 1,249 million m^3/year (Rifai, 2022). There are two types of aquifers in this region, which are aquifers that flow through intergranular spaces, small productive fissured or nested aquifers, and rare groundwater areas. Intergranular flow aquifers fall into three main categories: high-productivity and widely distributed aquifers, widely distributed productive

aquifers, and widely distributed moderately productive aquifers. High productivity aquifers consist of alluvium material with high porosity and medium to high permeability, allowing significant water flow. These alluvium deposits, present in Demak District, can potentially increase the aquifer's ability to store and transport water.

The relationship between *E. coli* concentrations and aquifer characteristics is essential, as the rocks that make up an aquifer affect its porosity and permeability. Aquifers with high porosity allow for more significant water movement, potentially increasing the risk of contamination by *E. coli*.

The research method is divided into two main stages: collection and processing. The data collection phase was conducted from June 27 to July 3, 2024, and included secondary and primary data obtained directly from the research site through geological and hydrogeological mapping. Geological mapping provided information on the geology and land use data of the investigation area, while hydrogeological mapping provided data on the groundwater table, elevation, groundwater samples, and the results of insitu measurements, including temperature, pH, DHL and TDS. Groundwater samples were taken from unconfined aquifers (community dug wells) to ensure the accuracy of data related to water quality and *E. coli* distribution. Data related to groundwater quality sampling locations in the Demak District are presented in Table 1.

Table 1. Groundwater sample locations for each sub-district

No	Sub-district	Number of Sample Points	Sample Code
1	Karangawen	3	SG 3, SG 38, SG 39
2	Mranggen	5	SG 5, SG 10, SG 11, SG 22, SG 40
3	Guntur	2	SG 26, SG 27
4	Karangtengah	2	SG 9, SG 17
5	Sayung	2	SG 12, SG 23
6	Bonang	2	SG 36, SG 37
7	Demak	2	SG 15, SG 16
8	Wedung	1	SG 20
9	Mijen	1	SG 13
10	Wonosalam	5	SG 14, SG 25, SG 29, SG 30, SG 33
11	Karanganyar	1	SG 35
12	Gajah	1	SG 34
13	Dempet	2	SG 7, SG 32
14	Kebonagung	1	SG 8

Description: Code of Sample Containing *E. coli*

Microbiological testing was conducted at the Testing and Calibration Laboratory to analyze the *E. coli* content in the samples, by the APHA 9221-G standard, 23rd Edition 2017, using the Most Probable Number (APM) method. APM is a statistical method used to estimate the number of microorganisms, such as bacteria, in a sample (Sunarti et al., 2015). In this context, APM/100 ml indicates the number of *E. coli* measured in 100 milliliters of groundwater. The laboratory data obtained was then analyzed using GIS software by applying the IDW interpolation technique, a simple deterministic method that considers a point's value based on surrounding values. The data collected was then analyzed to identify the concentration and distribution pattern of bacteria and the relationship between geological and hydrogeological factors with the presence of these bacteria in the Demak District. The methodological steps applied, from data collection to interpretation of results, can be seen in Figure 1.
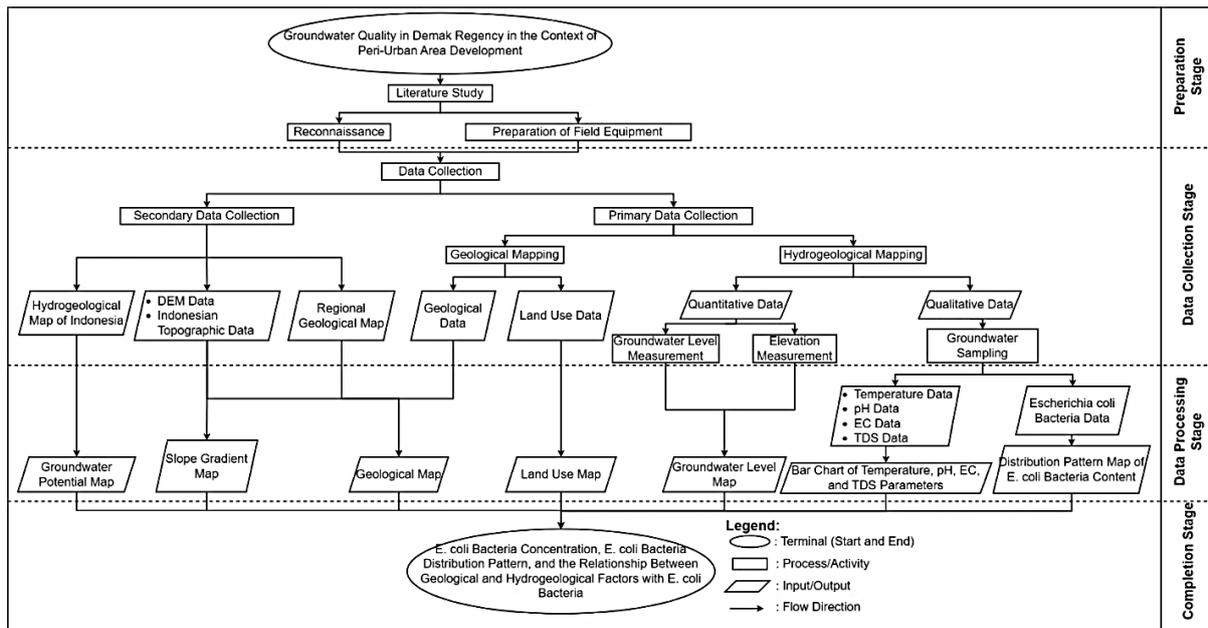
Figure 1. Research flow chart.

Results and Discussion

Groundwater Level Analysis of the Study Area

Based on the results of hydrogeological mapping in the study area, shows the dependence of the community on shallow groundwater sources for daily needs. Visualization of well location points and groundwater flow directions was carried out using GIS applications, which produced an interactive map of the Demak Regency area. The results of the survey of 30 wells showed that the groundwater table elevation varied between 0.24 to 41.17 m, with the highest groundwater table found in Banyumeneng Village, Mranggen Sub-district, and the lowest in Bungo Village, Wedung Sub-district. This difference in elevation causes groundwater flow to move from higher to lower areas, with the direction of groundwater flow in Demak Regency generally moving from south to north, following the topography of the area which slopes to the north (Figure 2).

Analysis of E. coli *Concentration in the Study Area*

The analysis was conducted on 30 dug well points in unconfined aquifers in the study area. The results of this test were compared with the quality standards set in the Minister of Health Regulation No. 2 of 2023. The results show that the concentration of *E. coli* in groundwater varies (Figure 3). The highest value reached 1400 APM/100 ml in sample SG 34. On the other hand, sample points such as SG 8, SG 9, SG 10, SG 14, SG 17, SG 25, SG 26, SG 27, SG 30, SG 33, SG 35, SG 36, SG 37, SG 38, SG 39, and SG 40 showed results of 0 APM/100 ml, indicating the absence of bacterial contamination at these locations. Meanwhile, other samples showed varying concentrations, such as SG 3 with 5 APM/100 ml, SG 5 with 2 APM/100 ml, SG 7 with 9 APM/100 ml, and SG 20, SG 29, and SG 32 with 450 APM/100 ml each.
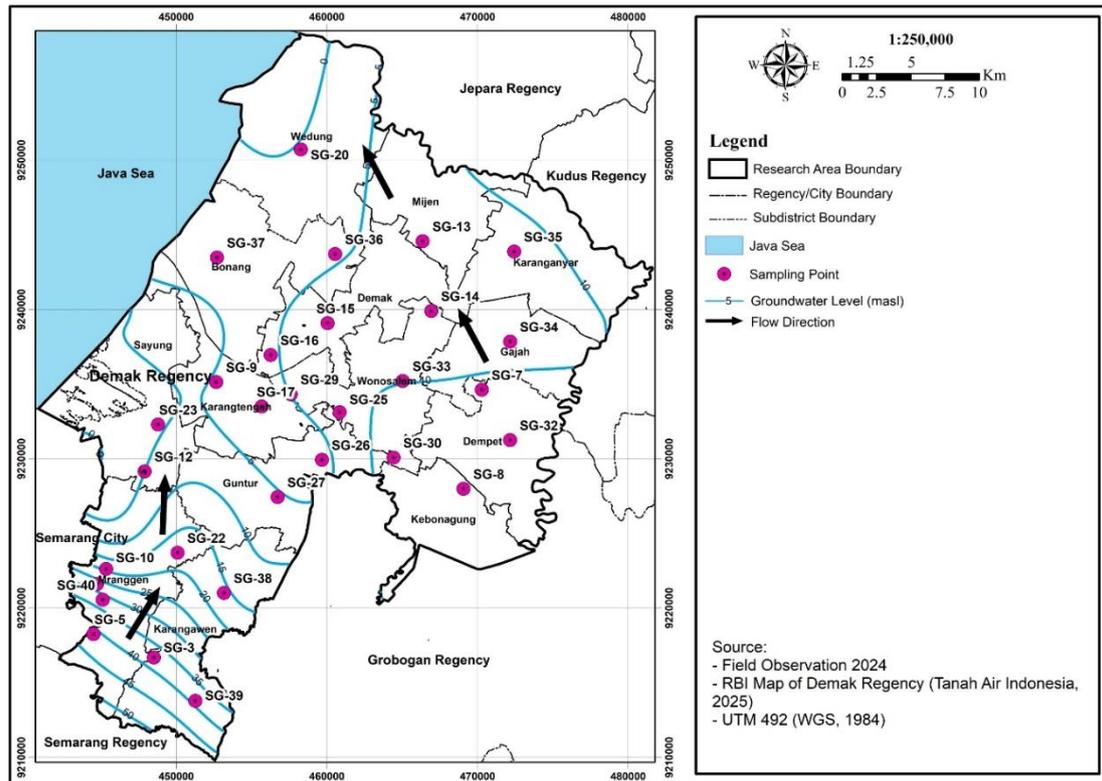
Figure 2. Groundwater table map of the study area.

Based on the quality standard for clean water, the concentration of *E. coli* in 100 ml should not be detected (Ministry of Health, 2023). However, the test results showed that 14 out of 30 sample points in Demak Regency exceeded the permissible limit, indicating potential groundwater pollution from urban activities. High concentrations of *E. coli* are caused by various factors, including the cleanliness of the environment around the well, cracks in the well wall or ring, and too close a proximity between the bathroom, toilet, or septic tank and the well. The physical condition of wells near sources of contamination, such as latrines, livestock pens, damp areas, or garbage dumps, as well as leaks in well construction that allow waste to flow through soil cracks, also increase the risk of contamination (Marsono, 2009). Previous research has also shown that groundwater pollution is often associated with poor sanitation practices and inadequate waste management, which can worsen groundwater quality in urban areas (Rifai, 2022).

Effect of Depth of Groundwater Table on E. coli *Content*

Based on Marlinda et al. (2019), the content of *E. coli* in groundwater is influenced by the variable depth of the groundwater table, where these bacteria tend to be found more often in shallow groundwater layers. This result aligns with the data shown in Figure 4, which shows that most of the groundwater samples containing *E. coli* have a water table depth of less than 15 meters. Data on the depth of the groundwater table in the study area can be seen in Table 3. The depth of the water table is measured vertically from the ground surface to the level where the groundwater is located. Shallow water table depths are more susceptible to bacterial contamination from the ground surface due to their proximity to potential pollution sources, such as septic tanks, domestic waste disposal sites, or livestock pens. The proximity of the water table to the surface allows bacteria and other contaminants from human and animal activities to enter more easily through infiltration or slow groundwater movement at that depth. The

increasing the depth of the water table can reduce *E. coli* concentrations, indicating that deeper soil layers can act as natural filters that reduce bacterial contamination (Marlinda et al., 2019).

Distribution Pattern of E. coli

The selection of sampling points was based on the distribution of land use, geological conditions, and population density. Analysis of *E. coli* concentrations was conducted to identify the bacteria levels at each sampling point and determine the factors influencing these results.

The mapping of the distribution pattern of groundwater quality based on the concentration of *E. coli* in Demak Regency, analyzed using the IDW method, can be seen in Figure 5. Areas with the highest concentrations, marked in red, were found in several sub-districts, such as the Wedung, Sayung, Wonosalam, Gajah, and Dempet sub-districts, at sample points SG 23, SG 20, SG 29, SG 34, and SG 32. The distribution pattern shows that areas around points with high concentrations tend to have more excellent bacterial content, while areas further away from points with high concentrations show a decrease in bacterial content, which is indicated by a gradation of color from red to dark green, where dark green indicates areas with low bacterial concentrations. The color sequence from dark green to red depicts a gradual increase in concentration.

E. coli in groundwater in residential areas can endanger human health, especially if the water is consumed without boiling. The bacteria can produce toxins in contaminated drinking water and cause diarrheal disease, one of the many diseases caused by the poor microbiological quality of drinking water (Sulistiyawati, 2019). Previous studies have shown that factors such as land use, population density, and geological conditions contribute significantly to groundwater contamination by *E. coli* (Marlinda et al., 2019).

Therefore, it is essential to ensure drinking water is free from bacterial contamination to prevent health risks.

Based on the research of Jung et al. (2014), microbial contamination of water often comes from human-associated feces (wastewater treatment plants, combined sewage overflows (CSOs), non-collective sewage systems), domestic animals (manure spreading, manure overflows).

Observations of 30 dug wells in Demak Regency show that the wells are in unconfined aquifers and are utilized by the community for various daily needs, such as bathing, cooking, drinking, washing clothes, washing household appliances, as well as watering plants and washing vehicles. The characteristics of the dug wells studied in relation to potential sources of pollutants in the vicinity, such as the distance between the well and the septic tank, the distance between the well and the source of waste, the condition of the well floor, as well as the distance between landfills and cattle pens, are described in Table 2. In the context of health, dug wells that meet standards must have certain characteristics, including strong well walls or rings, impermeable floors, adequate well lips, effective wastewater disposal channels, and well covers to avoid direct contamination (Mudatsir, 2020). The difference in *E. coli* content between wells SG 34 and SG 40, despite having similar environmental parameters, is likely influenced by several factors that determine the level of bacterial contamination. One such factor is the physical condition of the well in SG 34, potential leaks in the well walls or a poorly sealed cover may increase the risk of contaminant ingress from the surface. The closer proximity of SG 34 to septic tanks and cattle pens compared to SG 40 also contributes to greater potential for contamination. The cattle pens around SG 34 are larger in scale compared to those around SG 40, which could lead to higher levels of contamination.
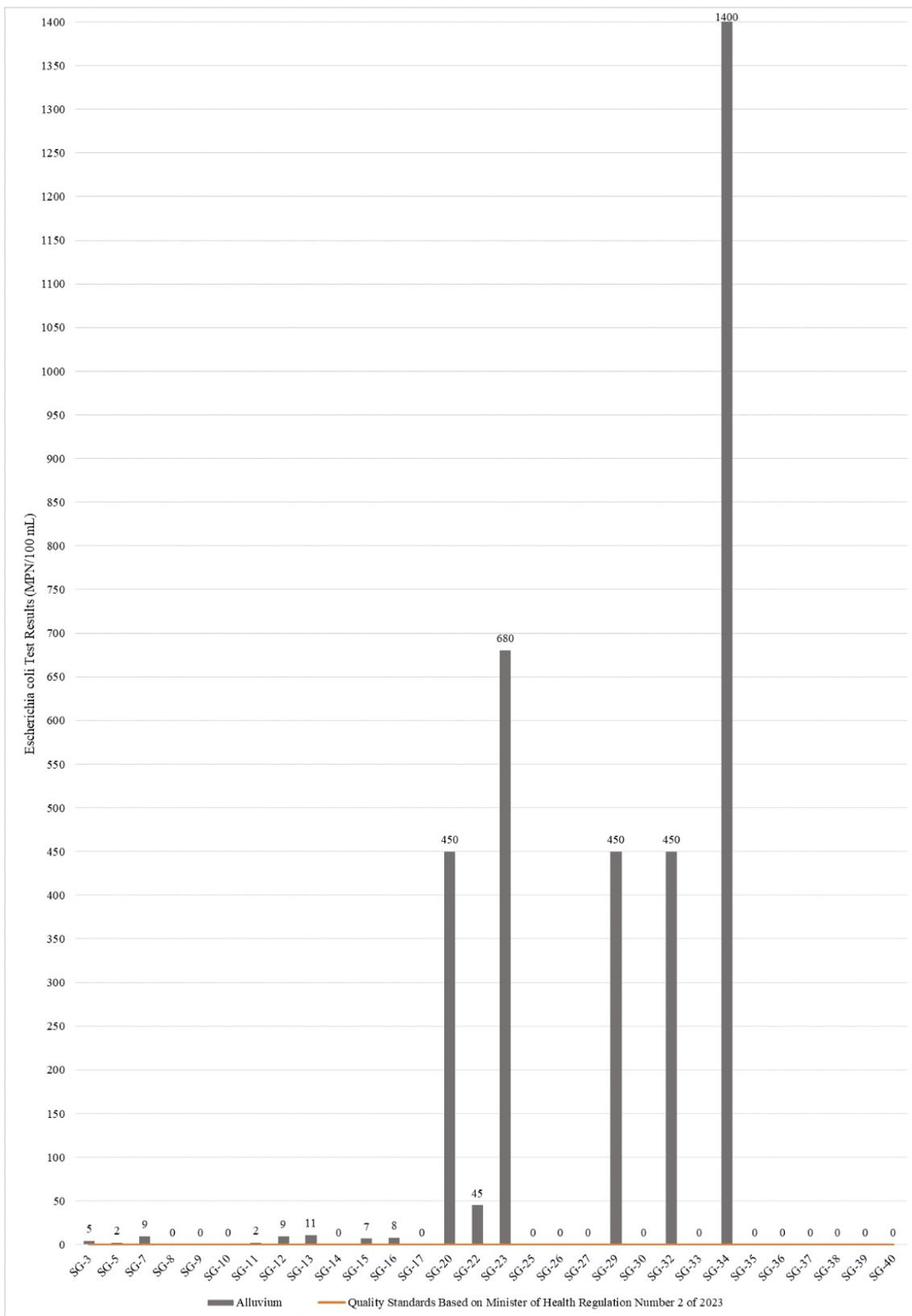
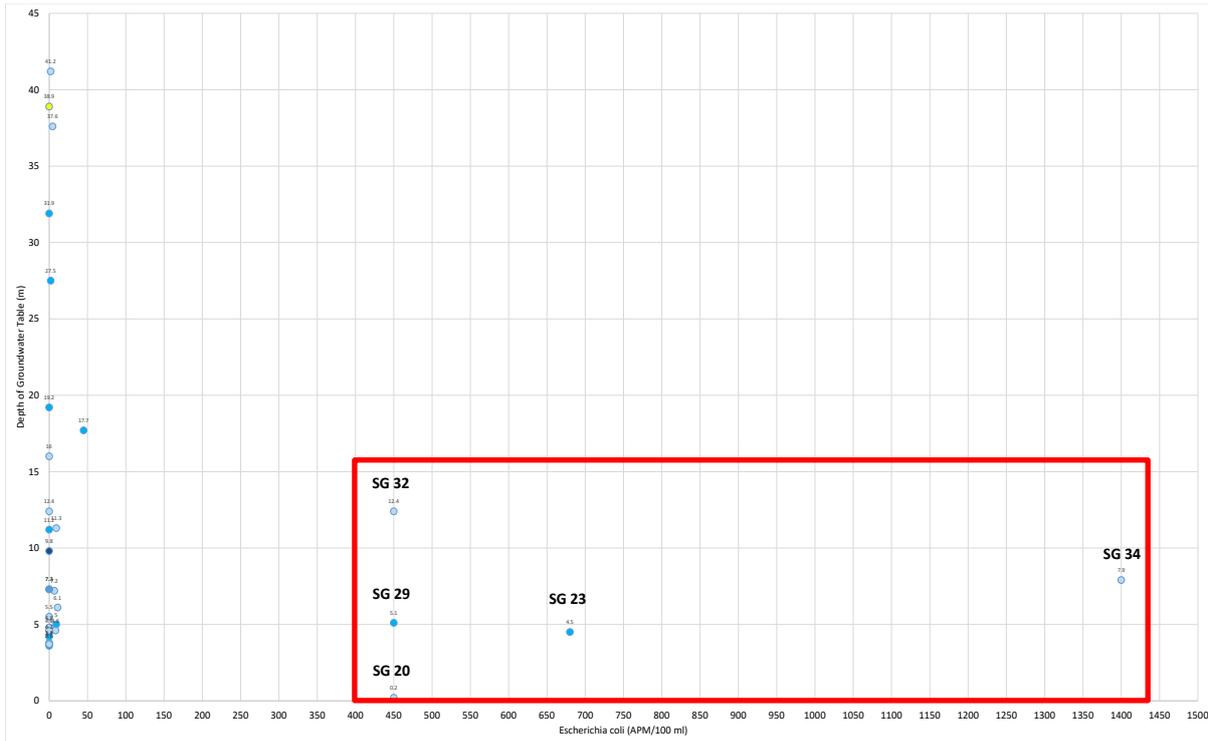**Figure 3**. Concentration of *E. coli* in Groundwater in Demak Regency (n=30)

Figure 4. The relations between *E. coli* concentration and Depth of Groundwater Table in the study area.
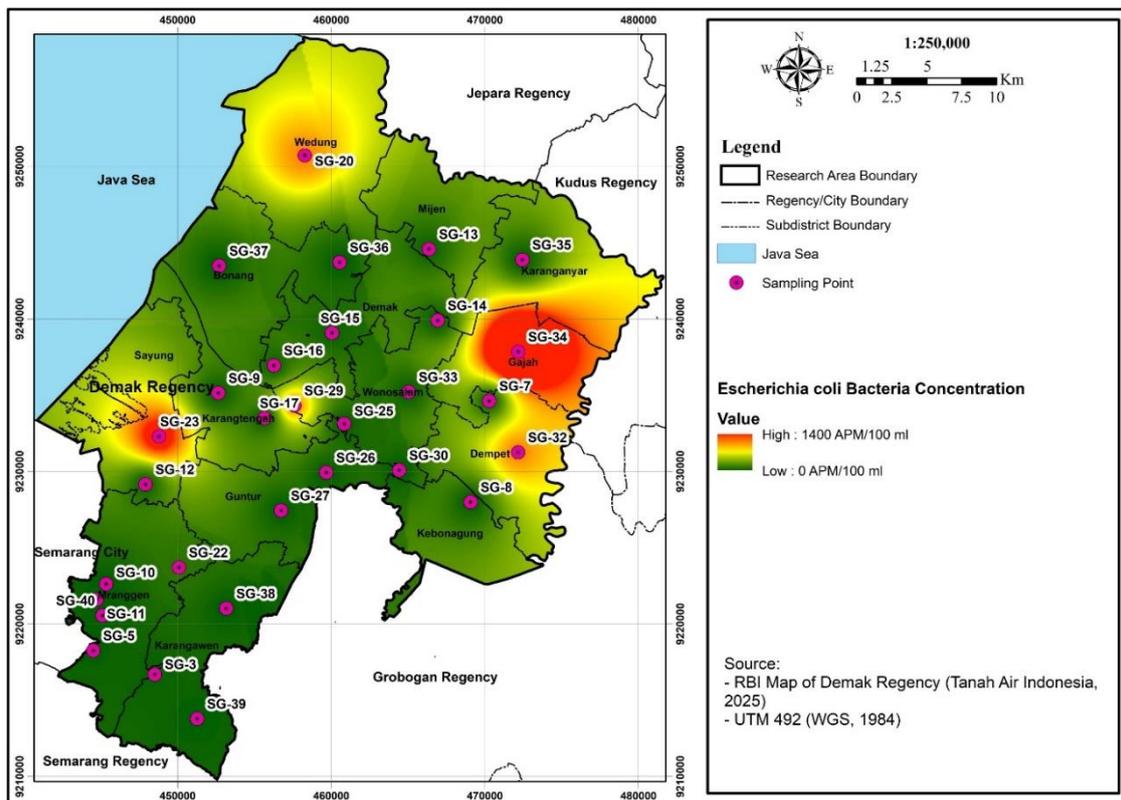

Figure 5. Map of the distribution pattern of *E. coli* in the study area.

There is a significant relationship between septic tank wastewater management systems and *E. coli* content which has an impact on the quality of dug well water (Achmad et al., 2020).

From the mapping results (Table 3), the SG 34 sampling point in the Gajah sub-district recorded the highest 1400 APM/100 ml concentration, indicating that this location was seriously polluted. Physical

observations showed that SG 34 did not meet well construction standards, such as the absence of strong walls, impermeable floor, and well cover. The well's proximity to septic tanks and livestock pens (less than 10 m) exacerbates the contamination, as the groundwater around the well is potentially exposed to fecal bacteria from both humans and animals. Sampling point SG 20, which recorded a bacteria concentration of 450 APM/100 ml, also showed a similar pattern. This well is located close to a garbage dump and chicken slaughterhouse, which are potential sources of bacteria and pollutants. Inadequate environmental hygiene conditions around this well facilitated the increase in the concentration of pathogenic microorganisms in the groundwater. On the other hand, sampling point SG 5 with a concentration of 2 APM/100 ml shows that the unsafe distance between the well and septic tank (less than 10 m) still has the potential to trigger contamination, albeit at a lower level. Several other sampling points also showed a significant correlation between the physical quality of the well and bacterial contamination. For example, sampling point SG 11 had high bacterial concentrations because household waste was discharged directly into the river, less than 10m from the well. This indicates that improper waste disposal practices worsen groundwater quality. Sampling point SG 29 had a contamination of 450 APM/100 ml, which could be attributed to the poor physical condition of the well, such as the absence of well walls and impermeable floor, making the groundwater vulnerable to exposure to bacteria from external sources.

Table 2. Observation results of sampling points in Demak Regency

No	Pollutant Source	Eligible	Not Eligible	Eligible (%)	Not Eligible (%)
1	Septic tank distance >10 m	21	9	70	30
2	Distance with sewage >10 m	19	11	63.33	36.67
3	Physical condition well	12	18	40	60
4	Distance to trash can >10 m	27	3	90	10
5	Distance with to livesrock pens >10 m	28	2	93.33	6.67

Table 3. Complete description of the condition of the observation well.

Sample Code	*E- coli* (APM/100 ml)	Lithology	Land Use	Depth of Groundwater Table (m)	Groundwater Potential
SG 3	4.5	Alluvium	Residential Area	37.6	Moderately productive aquifer, with wide distribution
SG 5	2	Alluvium	Residential Area	41.2	Moderately productive aquifer, with wide distribution
SG 7	9.3	Alluvium	Residential Area	11.3	Moderately productive aquifer, with wide distribution
SG 8	0	Alluvium	Residential Area	11.2	Productive aquifer with wide distribution
SG 9	0	Alluvium	Residential Area	4.8	Moderately productive aquifer, with wide distribution
SG 10	0	Alluvium	Residential Area	19.2	Productive aquifer with wide distribution
SG 11	2	Alluvium	Residential Area	27.5	Productive aquifer with wide distribution
SG 12	9.3	Alluvium	Residential Area	5	Productive aquifer with wide distribution
SG 13	11	Alluvium	Residential Area	6.1	Moderately productive aquifer, with wide distribution

Sample Code	*E- coli* (APM/100 ml)	Lithology	Land Use	Depth of Groundwater Table (m)	Groundwater Potential
SG 14	0	Alluvium	Residential Area	3.8	Moderately productive aquifer, with wide distribution
SG 15	6.8	Alluvium	Residential Area	7.2	Moderately productive aquifer, with wide distribution
SG 16	8.22	Alluvium	Residential Area	4.6	Moderately productive aquifer, with wide distribution
SG 17	0	Alluvium	Residential Area	3.6	Productive aquifer with wide distribution
SG 20	450	Alluvium	Residential Area	0.2	Moderately productive aquifer, with wide distribution
SG 22	45	Alluvium	Residential Area	17.7	Productive aquifer with wide distribution
SG 23	680	Alluvium	Residential Area	4.5	Productive aquifer with wide distribution
SG 25	0	Alluvium	Residential Area	7.3	Highly productive aquifer, with wide distribution
SG 26	0	Alluvium	Residential Area	4.2	Productive aquifer with wide distribution
SG 27	0	Alluvium	Residential Area	5.5	Moderately productive aquifer, with wide distribution
SG 29	450	Alluvium	Residential Area	5.1	Productive aquifer with wide distribution
SG 30	0	Alluvium	Residential Area	12.4	Highly productive aquifer, with wide distribution
SG 32	450	Alluvium	Residential Area	12.4	Moderately productive aquifer, with wide distribution
SG 33	0	Alluvium	Residential Area	9.8	Highly productive aquifer, with wide distribution
SG 34	1400	Alluvium	Residential Area	7.9	Moderately productive aquifer, with wide distribution
SG 35	0	Alluvium	Residential Area	7.3	Productive aquifer with wide distribution
SG 36	0	Alluvium	Residential Area	4.6	Moderately productive aquifer, with wide distribution
SG 37	0	Alluvium	Residential Area	3.7	Moderately productive aquifer, with wide distribution
SG 38	0	Alluvium	Residential Area	16	Moderately productive aquifer, with wide distribution
SG 39	0	Carbonate sandstone interbedded with siltstone	Residential Area	38.9	Groundwater scarce areas
SG 40	0	Alluvium	Residential Area	31.9	Productive aquifer with wide distribution

Overall, the results of this analysis show that the physical quality and location of wells have a significant impact on *E. coli* concentrations in groundwater. Wells with

inadequate construction and near pollutant sources, such as septic tanks, livestock pens, or landfills, tended to show high bacterial concentrations. According to Widiyanti (2019), also stated that contamination of well water by *E. Coli* bacteria is related to pollutant sources such as septic tanks, the distance between wells and pollutant sources, landfills, and inadequate sanitation facilities. In the study by Rahman et al (2021), it is also stated that it is important to emphasize water quality monitoring and adequate hygiene practices to improve public health outcomes. Therefore, improved sanitation infrastructure, improved well construction, and community education on well maintenance and waste management practices are necessary to maintain groundwater quality.

Distribution Point Analysis of E. coli *Concentration*

Based on the A-B cross-section conducted with an incision from point A to point B, as seen in Figure 6 and Figure 7, the lithology along the cross-section shows an alluvium layer. This incision cuts through three test wells: SG 25, SG 33, and SG 34. Test results showed that wells SG 25 and SG 33 did not contain *E. coli*, despite having shallow water table depths. This indicates that the existing conditions in wells SG 25 and SG 33 meet sanitary requirements. In addition, well SG 35 has a high Electrical Conductivity (DHL) value, generally containing more mineral ions, such as sodium, calcium, or magnesium. Increased levels of these ions can increase the osmotic pressure of the environment, which in turn creates stress on bacterial cells, inhibiting their cellular activity or even causing death. Inorganic ions or metals in high concentrations in water with high DHL can also have antimicrobial effects, directly inhibiting or limiting the growth of *E. coli* (Arivo & Annissatussholeha, 2017). In contrast, SG 34 showed a very high *E. coli* content, reaching 1400 APM/100 ml. This high bacterial content is most likely due to the groundwater table being less than 15 m deep and the existing condition of the well not meeting sanitary standards. The shallow depth of the water table and poor well construction allow for infiltration of contaminants from the land surface into the groundwater, especially from polluting sources in the vicinity of the well. The low DHL value in SG 34 indicates that this well contains a lesser number of dissolved ions, resulting in a lower osmotic pressure. These conditions create a more conducive environment for bacterial growth, allowing *E. coli* to survive and thrive better as they do not face inhibition from inhibitory ions or high osmotic stress.

The results of the A-B cross-section analysis show that the lithology of the area consists of alluvium layers with different well characteristics. Wells SG 25 and SG 33 were not contaminated with *E. coli*, indicating good sanitation standards. The high electrical conductivity (DHL) values in these two wells created high osmotic pressure, which inhibited bacterial growth. In contrast, well SG 34 had a very high *E. coli* content. This was influenced by the shallow water table, low DHL values and poor sanitary conditions, which allowed infiltration of contaminants from the soil surface. According to Murray et al. (2018), it is necessary to emphasize the education of dug well owners regarding testing and maintaining well quality for public health. This conclusion confirms the importance of maintaining sanitation, DHL values, and managing the depth of the groundwater table to prevent bacterial contamination.
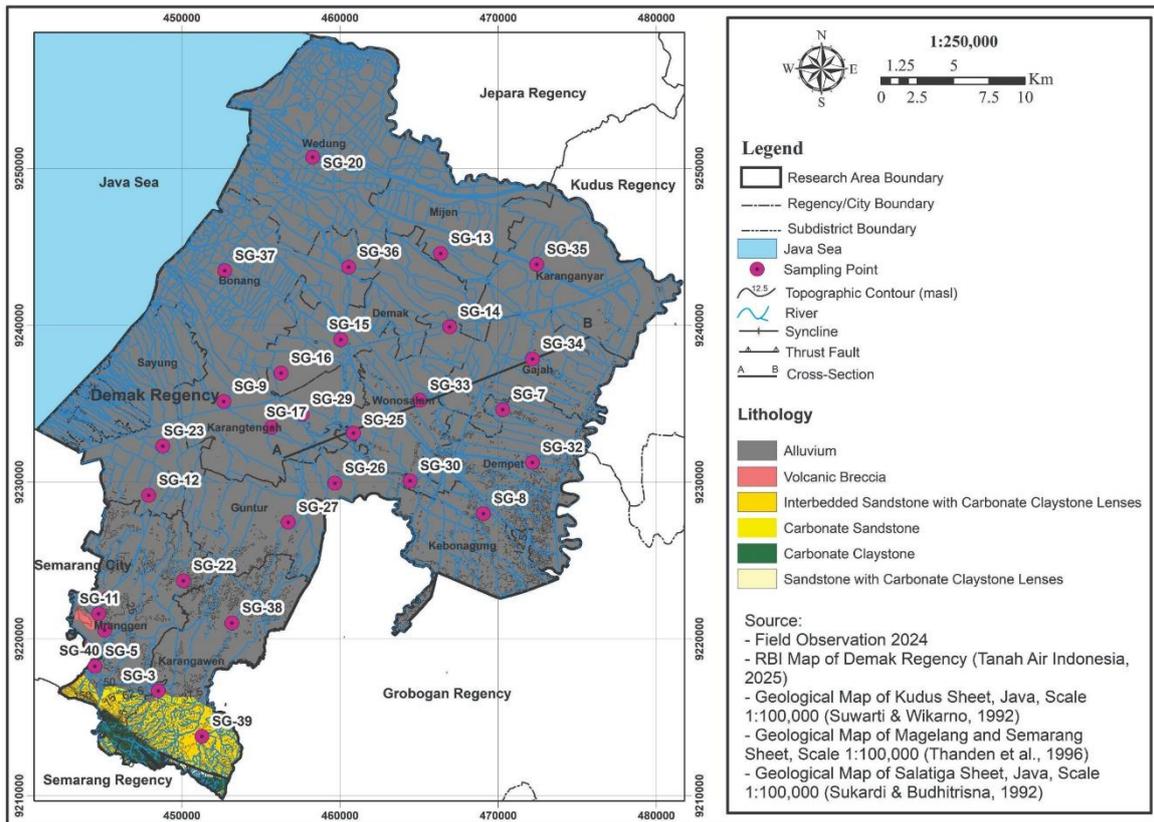
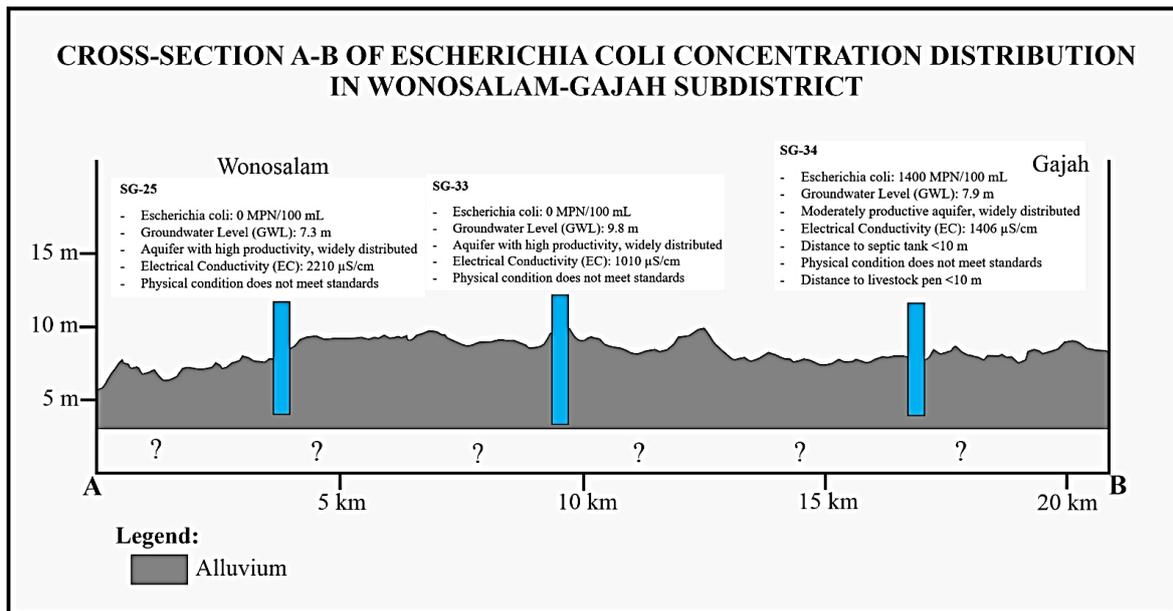**Figure 6**. Cross-sectional incision map of the study area.

Figure 7. Cross-section of *E. coli* distribution points in the study area.

Conclusion

Based on the results of the research conducted, it can be concluded that the study area has diverse geological conditions, including carbonate sandstone, carbonate mudstone, volcanic breccia, and alluvium. Groundwater potential in the area indicates the presence of aquifers with high productivity and wide distribution, as well as aquifers with medium to small local productivity. The groundwater table varies from 0.24 to 41.17 meters, with flow patterns leading from south to north. *E. coli* concentrations in the Demak area also varied, with the highest value reaching

1400 APM/100 ml in sample SG 34, while sample points SG 8 to SG 40 showed results of 0 APM/100 ml, indicating no contamination. Other samples showed varying concentrations, such as SG 3 with 5 APM/100 ml and SG 20 with 450 APM/100 ml. Most of the dug wells contaminated with *E. coli* have shallow groundwater tables and are in aquifers with extensive productivity, and the existing conditions of the wells that do not meet the requirements further exacerbate the level of contamination. These findings indicate the importance of sanitation management and groundwater quality monitoring to prevent bacterial contamination in the study area.

Acknowledgements

Author Contribution

Each team member had an important contribution to this research. Authors 2 and 5 conceived and designed the experiments, as well as conducted dug well water sampling and laboratory analysis to identify the presence of Escherichia coli. Authors 1, 3 and 4 analyzed the data and compiled the report supporting the conclusions of this study in Demak District, Central Java.

Conflict of Interest

The authors declare that there is no conflict of interest in this research.

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