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Proximity Matters: Spatial Gradient of Bacterial Pollution in Groundwater in the Vicinity of Al Amodi Cemetery in Dhamar City, Yemen

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Abstract

Public cemeteries are potential sources of contamination by groundwater pathogens. Many settlements in Yemen are located near cemeteries, and rely on groundwater for consumption. However, no information is available on the effect of cemeteries on groundwater quality in Yemen. Therefore, this study, which is the first of its kind in Yemen, aimed to assess the bacteriological and physiochemical quality of groundwater from four wells in proximity to the Al Amody cemetery in Dhamar city, Yemen, with a focus on evaluating potential contamination risks to local communities relying on untreated well water. Water samples were analyzed for total aerobic heterotrophic bacteria (HPC), total coliforms, *E. coli*, fecal indicators, and a range of pathogenic bacteria, along with physicochemical parameters, including pH, temperature, total dissolved solids (TDS), and electrical conductivity (EC). Results revealed severe microbial contamination in wells closest to the cemetery, particularly Well 1 (5 m from the site), where HPC and multiple bacterial indicators—including total coliforms, fecal coliforms, *E. coli* , *Salmonella* spp., *Vibrio cholerae* , *Proteus* spp., and *Pseudomonas aeruginosa* —were present at "too numerous to count" (TNTC) levels, far exceeding WHO and Yemen standards. Wells 2 (147 m distance) and 3 (323 m distance) showed moderate contamination, with *E. coli* detected at 55 CFU/mL and 17 CFU/mL, respectively, whereas all bacterial groups were absent in the control well (Well 4, 1,169 m away). Physicochemical analysis indicated TDS and EC levels below the Yemen standard limit (1500 mg/L), although two wells exceeded the WHO guideline (1000 mg/L); pH (7.6–8.3) and temperature (20–28 Â °C) remained within acceptable ranges. These findings confirm that groundwater near cemeteries in areas with permeable soils and high water tables is highly vulnerable to fecal and pathogenic contamination, posing significant public health risks.

Article Info

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Groundwater contamination, Cemetery leachate, Fecal coliforms, *E. coli*, Waterborne pathogens.

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1. INTRODUCTION

Although cemeteries are sacred sites where decomposing bodies are permanently deposited, relatively little attention has been paid to the possibility that they are a source of water contamination [1]. Assessing groundwater microbial contamination in the vicinity of cemeteries is critical for safeguarding public health and environmental quality, particularly in regions facing water scarcity and sanitation challenges, such as Yemen. Groundwater serves as a primary source of drinking water in many parts of Yemen, including the Dhamar Governorate, where the ongoing impact of conflict has severely disrupted water, sanitation, and hygiene (WASH) infrastructure, leading to deteriorated microbial water quality and increased incidence of waterborne diseases such as cholera [2].

Most existing cemeteries worldwide are established without adequate consideration of their potential environmental and public health risks [3]. Globally, cemeteries have been recognized as potential sources of groundwater contamination owing to the leaching of organic matter and nutrients from decomposing bodies, which can alter the physicochemical and microbiological characteristics

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of aquifers [4–8]. Generally, aquifers, whether shallow or deep, as illustrated in (Fig. 1), are influenced by land-use and occupation patterns [9]. These human activities can alter both the volume of water the aquifers are able to store and the quality of the groundwater they contain [10].

Cemeteries have the potential to harbor pathogens that cause tuberculosis, cholera, toxoplasma, Clostridia, and heterotrophic bacteria [11]. This contamination is caused by the decomposition of buried bodies, which involves biological, physical, and chemical processes that produce by-products as nutrients for microorganisms (bacteria and viruses) that can survive after being released into the environment [12, 13].

During decomposition, cemeteries generate a liquid known as leachate, which percolates through the soil layers and can infiltrate groundwater systems. Although certain soil types possess the capacity to filter out bacteria, viruses, and chemicals, their effectiveness can be overwhelmed, resulting in significant microbial and chemical contamination of the groundwater [14–16]. Leachate migration poses a potential health hazard to communities that rely on groundwater near cemeteries. Empirical studies have shown elevated levels of microbial and chemical contaminants, such as nitrates, heavy metals, and pathogenic bacteria, in groundwater proximate to cemeteries, posing significant health risks and rendering water unsafe for consumption [4, 6, 17]. However, the extent of such contamination is influenced by local hydrogeological conditions, soil properties, and cemetery management practices, which can either mitigate or exacerbate contaminant migration to the groundwater [5, 17].

In the context of Yemen, and specifically the Dhamar Governorate, groundwater is an unplanned settlement where access to piped water is limited. This ongoing conflict has further compromised water, sanitation, and hygiene (WASH) infrastructure, exacerbating the risk of waterborne diseases such as cholera [2]. Cemeteries in Dhamar are often situated near boreholes used for domestic water supply; however, there is a notable lack of studies investigating the impact of cemetery-derived contamination on groundwater quality in this region.

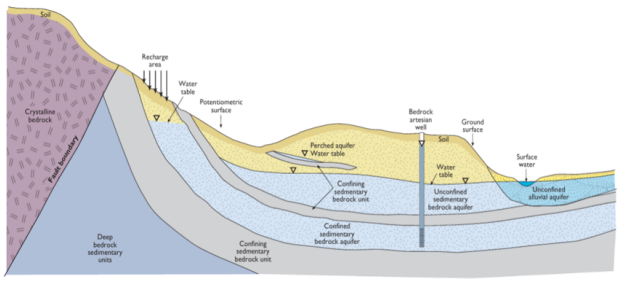


Figure 1. The main types of aquifers, Barkmann *et al.*, [18]

Given these concerns and the paucity of region-specific data, this study aims to comprehensively assess microbial contamination in four groundwater sources near the Al Amody Cemetery in Dhamar City, Dhamar Governorate. By providing updated, context-specific evidence, this study seeks to inform public health policies, urban planning, and sustainable WASH interventions in Yemen's challenging socio-environmental landscape. Ultimately, this work will contribute to raising environmental awareness among urban residents and decision makers regarding the implications of cemetery proximity on groundwater quality and community health.

2. MATERIALS AND METHODS

2.1. Description of the study area

This study was conducted in Dhamar City, located at geographical coordinates: Northing 14°33'0" and Easting 44°24'6", Dhamar Governorate, Yemen. The city is located approximately 100 km south of Sana'a (the capital city of Yemen). The annual rainfall is 407.8 mm, the maximum rainfall was 148.2 mm in June, and the minimum was 88.2 mm in January. The average monthly temperature ranged from 21.4ºC in December to 27.7ºC in June [19]. Al Amody cemetery, which is located within this city and has been operating since 1969 and continues to accept burials, is the largest among the cemeteries in Dhamar City and covers an area of approximately 14.8 ha (Fig.2).

The Dhamar area has moderate weather, and the average maximum annual temperature measured during the period 1999-2015 was approximately 24 ℃ [20]. Only 10% of the total rainfall contributes to groundwater recharge, and the remainder is surface runoff [21]. The geology of the study area consists of Quaternary alluvium, Tertiary, and Quaternary volcanic formations, including rhyolite, tuff, and ignimbrite ash flows, with occasional granite intrusions. These sediments, primarily composed of gravel and sand, exhibit primary permeability and rest on solid rock formations that act as barriers, limiting groundwater infiltration through them [22, 23].

2.2. Water samples collection

Permission to access and collect groundwater samples from boreholes located in privately owned houses within the study area was granted verbally by all landlords who preferred to remain anonymous. Groundwater samples for microbial quality evaluation were collected over a two-month period from November to December 2024 (Table 1) from the four wells to characterize the microbiological and physicochemical parameters of the water (Fig. 3).

At each well, the outlet pipe of the boreholes was swabbed with cotton wool soaked in 70% ethanol, and water samples were collected after flushing for 4 to 5 min to purge stagnant water and ensure representative sam-

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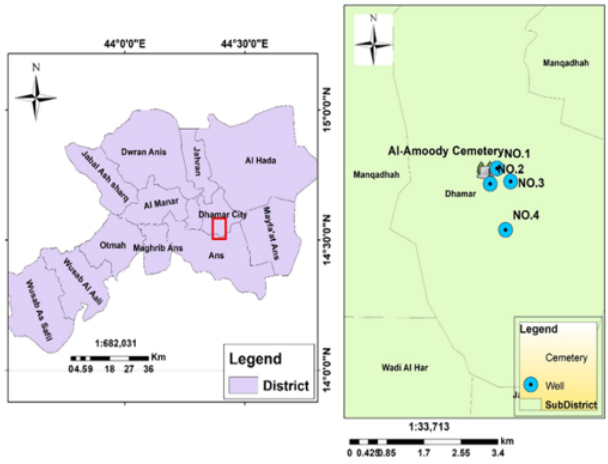


Figure 2. Map showing the location of Al Amody cemetery in Dhamar city, Yemen

pling of aquifer water, following standard groundwater sampling protocols.

Table 1. Sampling location data

Well number	Latitude	Longitude	Well depth (m)	Distance from cemetery (m)
1	14°7.91'N	44°51.96'E	20	5
2	14°57.28'N	44°46.94'E	192	147
3	14°58.98'N	44°2.13'E	220	323
4 (control)	14°25.91'N	44°58.31'E	170	1,169

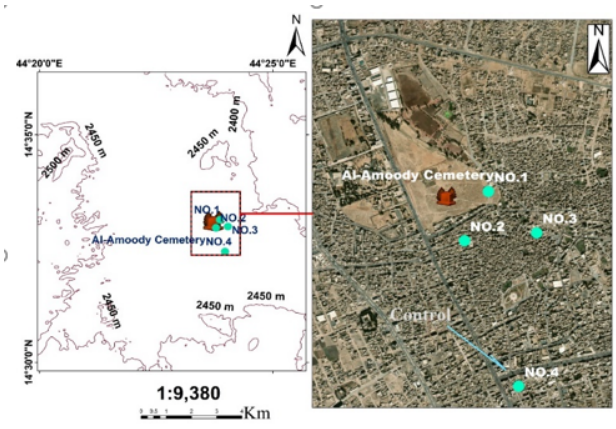


Figure 3. Map showing sites of sample collection within the cemetery.

For the determination of parameters other than *in situ* parameters, the microbial content was collected with pre-cleaned 500 ml polyethylene bottles. At each sampling point, the bottles were rinsed with the groundwater sample to condition the container before filling. All samples were collected in triplicate, labeled properly, and stored in ice-cooled containers at 0– 4 ℃. The samples were immediately taken to the Local Water & Sanitation Corporation Laboratory in Dhamar City, and analyses were performed within 6 h of sample collection to ensure sample integrity [24].

2.3. Microbial analysis

Water samples were collected in sterile 500 ml glass bottles from each monitoring well for bacterial analysis (total coliform and fecal coliform) using the APHA standard procedure [24]. The numbers of total and fecal coliforms were determined by the membrane filter technique using mEndo agar and mFC agar, respectively. Positive tubes were streaked on Eosin Methylene Blue (EMB) agar plates. The MF of fecal streptococci was determined using the Slanetz and Bartley Medium at 45℃ for 48 h [24]. Total heterotrophic bacterial (THB) counts were enumerated using Plate Count Agar (PCA)-(Hi-Media) [25]. The samples were analyzed for pathogenic indicator organisms, including *E. coli, Pseudomonas aeruginosa*, fecal enterococci, *Clostridium perfringens*, *Enterobacter*, *Salmonella*, *Shigella*, *Vibrio*, *Proteus* bacteria using different selective media [24]. Isolates from the examined water samples were subjected to biochemical characterization using Bergey's Manual of Systematic Bacteriology [26].

2.4. Physiochemical analysis

In situ parameters, including electrical conductivity (EC), total dissolved solids (TDS), and hydrogen ion concentration (pH), were determined with the aid of an electrochemical meter (HQ430d flexi/HACH/USA) for both EC and TDS and a pH meter (HQ411d PH/mV, HACH/USA) for pH, along with temperature, and then compared with the guidelines admitted by the Yemen standards and the World Health Organization [27].

2.5. Statistical analysis

Data analysis was carried out using Pearson's correlation to establish the correlation of parameters and significance difference between analyzed parameters to evaluate the relationship between samples from wells and the distance between groundwater wells and cemeteries. One-way ANOVA was used to establish significant differences set at p,0.05 among analyzed parameters. All data analyses were performed using the SPSS software version 27 (IBM Corp.,2020).

3. RESULTS AND DISCUSSION

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3.1. Microbial Analysis

Groundwater has served as a vital source of drinking water for many decades and continues to be essential for this purpose [28], despite the impairment of its quality in some localities by different human activities. Consequently, monitoring groundwater quality has become indispensable for ensuring sustainable water resource management. The microbiological and physiochemical parameters assessed in this study align with standard monitoring guidelines and are widely recognized as key indicators for evaluating the potential impacts of cemeteries on groundwater quality [29, 30].

The bacterial contamination profile of groundwater from four wells near the Al Amody cemetery site showed a clear gradient of significant microbial presence related to proximity and possible contamination sources, raising concerns regarding water safety for local populations consuming untreated water. The total aerobic heterotrophic bacterial plate count (HPC) shown in Table 2 reached too high to count (TNTC) levels in well 1, with wells 2 and 3 showing elevated counts of 27 CFU/mL and 150 CFU/mL, respectively, far exceeding the commonly cited safe threshold of 25 CFU/mL for drinking water according to the Yemen Standards and WHO [27], as elevated heterotrophic bacteria often reflect the presence of pathogenic microorganisms or organic pollution [31], and confirming that groundwater in this area is not suitable for drinking without treatment [27].

Table 2. Indicator bacteria detected in groundwater samples near Al Amody cemetery, Dhamar city, Yemen.

Well No	Bacterial count (CFU/100 ml)			
	Heterotrophic bacteria	Total coliforms	Faecal coliforms	*E. coli*
1	TNTC*	TNTC	TNTC	TNTC
2	1.5×10^{2}	5.2×10^{1}	0.2×10^{1}	5.5×10^{1}
3	2.7×10^{1}	0.7×10^{1}	ND*	1.7×10^{1}
4	ND	ND	ND	ND

TNTC: Too Numerous To Count.

ND: Not Detected.

The findings of this study revealed significant variations in the bacterial contamination levels among the four wells (Table 2). Well 1, which is 5 m from the cemetery, had extremely high contamination, with total coliforms, fecal coliforms, and *E. coli* counts exceeding the level classified as too numerous to count (TNTC), indicating severe microbial pollution. In comparison, well 2 (147m) and well 3 (323m away), exhibited moderate contamination levels; total coliforms were measured at 5.2 × 10^1 CFU/mL & 0.7 × 10^1 CFU/mL for well 2 &3, respectively, while faecal coliforms were present only in well 2 at 2.0 × 10^1 CFU/mL. Additionally, *E. coli* counts were recorded as 5.5 × 10^1 CFU/mL in well 2 and 1.7 × 10^1 CFU/mL in well 3. The total coliform levels in all wells, except Well 4, exceeded the permissible limits set by the World Health Organization [27] and Yemen Standards.

Fecal streptococci, *P. aeruginosa*, *Salmonella sp., V. cholera*, *P. vulgaris*, and *P. mirabilis* were all present in Well 1 at TNTC concentrations. *Enterobacter* was also detected at 5.7 × 10^1 CFU/100 mL (Table 3). Well 2 showed moderate contamination with *Salmonella* sp. at 0.4 × 10^1 CFU/100 mL, *Enterobacter* at 0.1 × 10^1 CFU/100 mL, and *P. vulgaris* at 5.8 × 10^1 CFU/100 mL. No pathogenic bacteria were detected in well 3. Several pathogens, including *Klebsiella* sp., *Shigella* sp., *C. perfringens* and *P.mirabilis*, were not detected in any well. Well 4 (1,169m away), which served as a control, showed no contamination. Statistical analysis showed a significant negative correlation $(r = -0.648)$ between bacterial growth and distance, highlighting a clear spatial pattern of microbial contamination likely influenced by the proximity to contamination sources and well characteristics, which diminishes with increased distance, contingent on local soil and hydrogeological conditions, emphasizing the importance of appropriate cemetery management and protective zoning [32].

Supporting these observations, Engelbrecht [33], identified significantly increased counts of indicator bacteria such as fecal coliforms and *E. coli* in South African cemetery wells relative to municipal control sites, with some wells exhibiting extraordinarily high microbial loads comparable to the “too numerous to count” (TNTC) levels noted herein. Reinforcing the link between microbial contamination and burial site proximity. 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 [34].

The increase in *E. coli* counts approached TNTC near the cemetery, suggesting contamination from decomposing human remains or sewage infiltration. The concurrent presence of fecal coliforms and fecal streptococci-indicators of recent and historical fecal pollution, respectively, further confirms this contamination source [27, 35]. Elevated counts of these indicators are frequently observed in shallow wells and regions with high water tables and permeable aquifers [36].

Dent and Knight [37] observed low-to-moderate levels of fecal coliforms and *P. aeruginosa* in groundwater at burial sites. Similarly, Pacheco *et al.* [38] identified significant microbial pollution, including total and fecal coliforms, fecal streptococci, and proteolytic bacteria near cemeteries with shallow water tables, reflecting the active infiltration of organic decomposition products into aquifers. Rodrigues and Pacheco [39] confirmed similar patterns in Portuguese cemeteries and reported strong statistical links between microbial contamination, burial

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Table 3. Other pathogenic bacteria detected in groundwater samples near Al Amody cemetery, Dhamar city, Yemen.

Well No. / Bacterial Isolates	Unit	1	2	3	4
Faecal streptococci	CFU/100 ml	TNTC	ND	ND	ND
Pseudomonas aeruginosa	CFU/100 ml	TNTC	ND	ND	ND
Pseudomonas mirabilis	CFU/100 ml	ND	ND	ND	ND
Clostridium perfringens	CFU/100 ml	ND	ND	ND	ND
Salmonella sp.	CFU/100 ml	TNTC	0.4×10^1	ND	ND
Shigella sp.	CFU/100 ml	ND	ND	ND	ND
Vibrio cholerae	CFU/100 ml	TNTC	ND	ND	ND
Enterobacter	CFU/100 ml	5.7×10^1	0.1×10^1	ND	ND
Klebsiella sp.	CFU/100 ml	ND	ND	ND	ND
Proteus vulgaris	CFU/100 ml	TNTC	5.8×10^1	ND	ND
Proteus mirabilis	CFU/100 ml	TNTC	ND	ND	ND

TNTC: Too Numerous To Count.

density, and local hydrogeological conditions.

Salmonella spp., *Enterobacter* spp., *V.cholerae, P. vulgaris*, and *P. mirabilis* are well-documented agents of waterborne diseases, including gastroenteritis, typhoid, and cholera, presenting clear public health concerns and underscoring the urgency of systematic monitoring and water treatment interventions [33, 38]. Based on previous studies, several bacteria were isolated from well water around the cemetery, including *Proteus spp., Vibrio spp., Klebsiella spp., Bacillus spp., Staphylococcus spp., E. coli, Pseudomonas spp. and Glycomyces spp.*[40].

In this study, the wells at the four points had depths of 20–220 m. Well 1 was the shallowest among the four wells studied in this area. This shallow depth, combined with the highly permeable gravel and sandy soils in the cemetery region, contributes to an increased vulnerability to microbial contamination by facilitating the rapid infiltration and transport of contaminants from surface sources, including cemeteries leachate. The statistical analysis of One-way ANOVA indicated that there was a statistically significant ($p<0.05$) relationship between TC, *E. coli*, HPC, and groundwater borehole depth. Groundwater, whether from shallow or deep aquifers with permeable soils, is rarely free of microorganisms, and contamination is influenced by well construction, land topography, poor sanitation, and septic tank leakage [41, 42].

This observation aligns with international research demonstrating that hydrogeological settings with shallow aquifers and permeable soils are particularly susceptible to cemetery-sourced microbial pollution [14, 38]. Moreover, elevated HPC counts in urban groundwater systems have been strongly linked to fecal contamination and organic matter infiltration, particularly in wet seasons, which enhances contaminant migration [43]. Environmental conditions substantially influence bacterial transport and survival. Soil permeability, aquifer depth, and proximity to burial sites modulate contaminant movement, as demonstrated by Fineza *et al.* [44]. Consistent with Saba *et al.* [45], this investigation found that wells within 250 m of cemeteries exhibited markedly higher contamination, likely due to the increased flushing of contaminants into groundwater during post-monsoon aquifer recharge.

Brennan *et al.* [46] further detailed how nutrients and organic compounds released through decomposition create favorable conditions for microbial proliferation deep in soils and aquifers near cemeteries. The decomposition of human bodies releases gases, such as carbon dioxide, ammonia, and bacteria, that enter the soil and reach groundwater. Water percolates through cemeteries carrying human remains, and the variety of hydrogeology and geology found at various cemetery sites affects the movement of these dissolved elements into the environment [47].

3.2. Physiochemical Analysis

The total dissolved solids include all minerals, salts, metals, cations, and anions dissolved in water, representing all substances present in water, apart from pure water molecules (H_2O) and insoluble solid waste. comprises inorganic salts (principally calcium, magnesium, potassium, sodium, bicarbonate, chlorides, and sulfates) and small amounts of organic matter that are dissolved in water. TDS in drinking water originates from natural sources, sewage, urban runoff, and industrial wastewater [27].

The combined assessment of TDS and EC in the groundwater near the Al Amody Cemetery revealed a clear pattern of inorganic contamination that correlated strongly with proximity to the burial site, highlighting the influence of cemetery leachate on groundwater quality.

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Table 4. Some physiochemical characters of the tested wells

Well #	Depth (m)	Distance (m)	pH	EC (μS/cm)	TDS (mg/L)	Temperature (°C)
1	20	5	7.6	1,826	1,186	20
2	192	147	7.7	1,822	1,184	24
3	220	323	7.8	598	389	25
4	170	1,169	8.3	821	533	28

**TDS: Total Dissolved Solids*

The TDS values in the four wells ranged from 389 to 1,186 mg/l, while the EC values varied from 598 to 1,826 µS/cm (Table 4). All measured values fall below the Yemen standard of 1,500 mg/L, thus classifying the water as compliant from a regulatory standpoint. Two of the four wells (wells 1 and 2) exceeded the WHO guideline value of 1,000 mg/L, which is recommended for palatability and to prevent potential aesthetic and operational issues in water supply systems [27].

The highest levels of TDS and EC were recorded in Well 1 and decreased progressively with distance, with the lowest values found in the control well (Well 4). This spatial gradient strongly implies that cemetery-derived leachate is the primary source of dissolved ions.

Although TDS itself is not a direct health hazard at these concentrations, high levels can affect the taste, odor, and color of water, thereby reducing its acceptability to consumers. As humans decompose, they release significant quantities of inorganic compoundsincluding ammonium, phosphate, and chlorideinto the surrounding soil and groundwater [33, 47, 48]. The co-occurrence of elevated TDS/EC with severe microbial contamination (e.g., *E. coli, Salmonella, Proteus*) in the same wells suggests a complex pollution scenario involving both organic and inorganic inputs from decomposing matter (Fig.4 a and b).

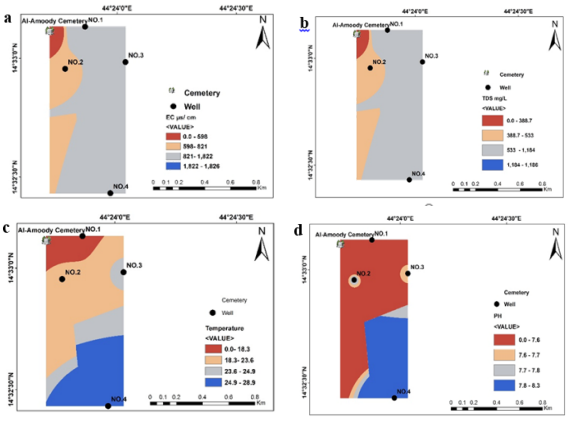


Figure 4. (a) EC values, (b) TDS, (c) Temperature and (d) pH of the groundwater samples near Al Amody

Statistical analysis showed a strong positive correlation between TDS and EC, significant at the 0.01 level ($p = 0.001$), indicating that these parameters are closely linked (Table 5).

Table 5. Pearson's correlation coefficient matrix for physicochemical parameters in the tested wells

	pH	EC	TDS	Temp.
pH	1			
EC	-0.644	1		
TDS	-0.644	0.001**	1	
Temp.	0.860	-0.538	-0.538	1

***. Correlation is significant at the 0.01 level (2-tailed). Temp.: Temperature.*

Temperature can be influenced by several factors, including season, weather, measurement time, element content, and land cover vegetation in the research area. The results of temperature measurements in the Al Amody cemetery area showed varying results owing to differences in location, which influence temperature differences. The data ranged from to 20-28℃ (Table 4), which falls within the typical ambient temperature spectrum for shallow and deep groundwater systems in tropical and subtropical regions, where minimal insulation from surface fluctuations allows for moderate thermal variation. According to Rohmawati & Kustomo [49], a good water temperature should not be too hot or cool, and the temperature is comparable to that of air, which is approximately 28℃. This aimed to prevent the dissolution of chemical substances that are dangerous to health, stop biochemical reactions, and inhibit the growth of pathogenic microorganisms (Fig. 4c). Temperature was not significantly correlated with pH, TDS, or EC (Table 5).

The pH measurement results showed a pH range of 7.6-8.3 (Table, 4) which falls within the acceptable limits set by the Yemen standards (6.5-8.5). The observed alkalinity, particularly in Well 4 (control well), located 1,169 m from the cemetery, may have played a beneficial role in water quality.

As noted by Bajgai [50], slightly alkaline water can contribute to a lower oxidation-reduction potential (ORP)

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and reduce the concentration of reactive oxygen species (ROS), which are associated with cellular oxidative stress. Over the long term, the consumption of such water may offer protective effects against certain chronic diseases linked to oxidative damage. This suggests that the natural alkalinity of groundwater, especially in uncontaminated aquifers such as that of Well 4, could have potential health-promoting properties. However, these findings should be interpreted with caution. Although the favorable pH and alkalinity in the control well reflect good physicochemical stability, the same pH range in contaminated wells (1–3) does not mitigate the severe microbial hazards present. A slightly alkaline environment can support the survival and proliferation of certain pathogenic bacteria, including *Salmonella* spp. and *Proteus* spp., both of which were detected at high concentrations in wells near the cemetery (Fig. 4d). Statistical analysis showed no significant correlation between the pH and TDS or EC (Table 5).

In summary, the strong correlation between TDS and EC, along with their spatial distribution, provides compelling evidence that groundwater near the Al Amody cemetery is chemically affected by cemetery leachate. When considered alongside severe bacteriological contamination, these findings underscore that water, especially from wells within 150 m of the site, is unsafe for human consumption without treatment. However, it is important to note that this study did not capture short-term effects of specific rainfall events on contaminant release. Future research should investigate these episodic dynamics to enhance our understanding of how rainfall influences groundwater contamination near cemeteries.

4. CONCLUSION

This study demonstrates that groundwater quality near the Al Amody cemetery in Yemen is severely compromised by microbial contamination, with a clear correlation to proximity to the cemetery, implicating leachate from decomposing organic matter as a primary contamination source. These findings confirm that shallow and deep groundwater in areas with permeable soil and high-water tables is highly vulnerable to contamination from cemeteries, posing serious public health risks to communities using untreated water. Urgent measures, including sanitary buffer zones, regular water quality monitoring, and public health education, are essential to prevent waterborne disease outbreaks.

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