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Assessment of the Vulnerability of Groundwater to Biological Contamination in the Khartoum State, Sudan

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ABSTRACT

This study aims to determine how vulnerable groundwater in Khartoum is to contamination. For this purpose, the DRASTIC Index idea was used. A descriptive cross-sectional analytical analysis is designed in this study. A total of 279 boreholes were sampled from a total of 1015 boreholes (27.5 percent). The following criteria were utilized to define the DRASTIC Index: depth, net recharge, aquifer media, soil texture, terrain, video media, and soil conductivity. Standard bacteriological test methodologies were used for groundwater. The biological data from the 279 boreholes revealed that total coliform, thermo-tolerant coliform, and E. coli were found in 34.4 percent, 18.6 percent, and 0.36 percent of the boreholes, respectively. Bacteriological contamination is common in Sharge Elnile, although only a few cases have been reported in Khartoum. According to the study, the bulk of boreholes in Khartoum State were built without any criteria. Many sources of contamination were discovered within a radius of less than 120 meters, which was deemed to violate Khartoum State's Environmental Health Law of 2002. For this reason, bacteriological contamination is common in Sharge Elnile, although only a few cases have been reported in Khartoum.

INTRODUCTION

Because of the spatial heterogeneity of aquifers and the many physical processes and chemical reactions that occur in the soil, unsaturated, and saturated zones, managing groundwater contamination is a complex undertaking. Because of this intricacy, a contaminant's physical condition or chemical form may vary, resulting in a change in the degree of contamination in an aquifer (Massimo & Civita 2010)

For potable drinking water, the majority of people in underdeveloped countries rely on untreated groundwater supplies. The rising demand for water from these sources has created concerns that some groundwater sources may not be as safe as they appear, putting people's lives at risk. Boreholes (tube wells) that are often rig-drilled >20 meters deep and potentially tap deeper aquifers; and shallow wells (hand-dug wells) that are typically dug 20 meters deep and often tap unconfined aquifers (Macdonald & Davies 2000).

Water is a basic necessity of life, an inevitable resource available underneath the surface and in soil pores, not the only constituent of life. Groundwater is a vital foundation of drinkable freshwater on the planet, and it theaters a key part in human survival and growth. Groundwater pollution is an important environmental hazard from various sources and is costly to human health. For example, nitrate groundwater pollution may result in health issues such as (blue baby syndrome), cancer risks), and high drinking water treatment costs (Schneider & Lechevallier 2017).

Boreholes and protected shallow wells are commonly associated with a few possible concerns in Sudan. For starters, their proximity to township latrines exposes them to infectious adulteration. Second, their proximity to the use points (houses) necessitates laborious transportation from the beginning until the end of water storage within the households. Water in storage facilities in houses can have a poorer microbiological quality than water at the beginning, implying that contamination occurs before consumption, during collection, transit, storage, and drawing from containers (Schneider & Lechevallier 2017). As a result, Pollution may limit the water's potential health advantages. Rather than closeness to toilet facilities, poor borehole features, such as hygienic seals, can be water quality risk factors and cause microbial contamination of groundwater sources (Wright et al. 2004). The studies showed that the presence of the virus (COVID-19) in the stool had been significantly reported in the literature. The presence of stool in sewage drains leading to groundwater contamination can be an emerging threat to water pollution and could lead to the spread of COVID-19 (Cronin et al. 2006).

Freshwater testing methods aren't done regularly. This is a third possible issue. It is exceedingly expensive to freshen up contaminated groundwater and might take an extended recovery period. Furthermore, groundwater observation is both time-wasting and expensive, making it impossible to accurately quantify the topographical extent of pollution on a regional basis. As a result, identifying the longitudinal dissemination of regions in danger of pollution and zones susceptible to pollution is the best strategy for managing groundwater pollution (Huo et al. 2021, Mimi & Assi 2009). Danger and susceptibility charts are effective for allocating imperfect observing efforts toward the most critical locations. In these places, a significant effort is necessary to avoid or ameliorate the environmental impact of human activity (Lahr & Kooistra 2010, Thapinta & Hudak 2003).

The map of where the resource is vulnerable to pollution from surface operations is often produced as part of a groundwater vulnerability assessment. Vulnerability evaluations identify areas that need more research, protection, and monitoring. Vulnerability evaluations are also effective instructional tools for improving public knowledge about the importance of groundwater protection, which is a constant requirement (Almasri 2008). Groundwater vulnerability maps are essential for protecting groundwater properties and assessing the possibility of improved growth in food techniques and landscape use affecting water quality (Rivera & Bouchard 2005). The rationale behind groundwater potential modeling is that certain geographical areas seem more susceptible to underground pollutants than others (Babiker et al. 2005). An evaluation of shallow groundwater quality in the Beni Mellal region ranges from medium to poor, with 14% and 20% of samples rated as poor and bad water quality, respectively (Gogu & Dassargues 2000).

Most underdeveloped countries, like Sudan, continue to focus their research on groundwater contamination on the outcome of actual solutions to filter intake water (Barakat et al. 2020). Nevertheless, little effort has been made to demystify underground pollution from a specific hydrogeology and sociological perspective. This allows professionals to create efficient groundwater protection methods from perceived pollution and review the method to scale up pathogenically infested groundwater bases. As a result, this study was carried out to determine the state of Khartoum's groundwater's vulnerability to contamination.

MATERIALS AND METHODS

Study Area and Climate

Geographically, the study area is approximately 28.165 square kilometers and is located within longitudes 31.5-34 East and latitude 15-16 North. The River Nile Region borders it on the north and east, the Northern Region on the north,

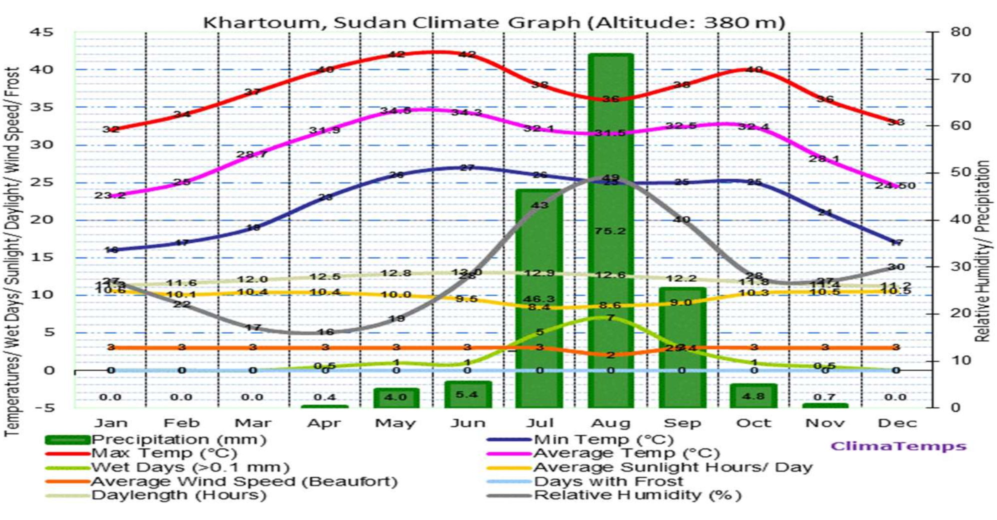

Fig. 1: Graph showing Khartoum climate in metric units.

Table 1: Online calculator to determine the sample size.

Determine Sample Size	
Confidence Level:	95%
Confidence Interval:	5
Population:	1015
Sample size needed:	279

and the states of Kassala, Gedaref, and Gezira on the east and south-east.

The population of Khartoum state is projected to be around five million people, according to the 2008 population data representing different parts they engaged in economic activities like agriculture, workers, and officers.

The annual average temperature in Sudan's Khartoum state is 29.9 degrees Celsius (85.8 degrees Fahrenheit). The average monthly temperature varies by only 11.3 degrees Celsius (20.3 degrees Fahrenheit), which is a small variation. The average diurnal temperature variation/range is 15.1 degrees Celsius (27.2 degrees Fahrenheit). With an average temperature of 34.5 degrees Celsius, May is the warmest time (very, very hot) (94.1 degrees Fahrenheit). With an average temperature of 23.2 degrees Celsius, the coolest month (January) is also the hottest (73.76 degrees Fahrenheit.) Fig. 1.

Sampling Techniques

The sample size for the study area was determined by using a online calculator (Table 1)

Biological Tests for Water

Water samples were collected in sterile bottles for bacteriological testing. The samples' site, date, time, and location were carefully labeled. The water samples were then taken to the Ministry of Health's public health laboratory in Khartoum for analysis using the following tests: When positive results are known to be uncommon, presence-absence tests may be suitable. They are not measurable, and their title suggests that they indicate whether the required indication exists. In nations or situations where contamination is frequent, such results are of little utility, and the goal of the study is to assess the grade of pollution rather than to signal its occurrence. As a result, occurrence-absenteeism assays are not advised for use in the study of surface water and raw small-scale community resources in places where there may be concerns with maintenance and upkeep.

Before deciding whether or not to apply the presence-absence test to analyze a water source, the test's results should be compared to those obtained using a well-known, quantitative method of analysis. Both procedures should be used to evaluate approximately 100 samples (Pruss-Ustun and WHO 2008). When evaluating a 50 mL sample, the medium employed for the presence-absence test for coliform bacteria was lauryl tryptose sulfate broth, which was double strength.

- The dehydrated lauryl tryptose sulfate broth was dissolved in water in stages without being heated.
- 50 mL of the medium to be poured into 250-300 mL screw-cap glass dilution bottles. It's vital to have a fermentation tube.
- The samples were autoclaved for 12 min at 121 degrees Celsius, with a total time in the autoclave of no more than 30 min.
- After autoclaving, the pH of the medium was determined; it should be 6.8.

Presumptive Test

- The sample was mixed carefully by inverting the sample bottle sometimes.
- 50 mL of the sample was added to the dilution bottle.
- Samples were incubated at 35°C and examined after 48 h.
- A positive result is indicated by the production of gas and turbidity and is regarded as a positive presumptive test (WHO 1997).

Confirmative Test

- Each gas-positive presumptive tube was infected with an inverted Durham tube into a tube containing 5ml of vivid green bile broth.
- All tubes were incubated for 24 hours at 44-45 degrees Celsius to identify fecal coliform. The presence of negative tubes was observed, and the results were recorded. Gas output and turbidity appearance were used to identify positive tubes.

E. coli Test (Completed Test)

A loop of vivid green positive tubes was inoculated into 5ml peptone water and incubated at 44-45 degrees for 24 h before adding a drop of Kovac's reagent (0.2-0.3ml). The surface culture's intense red color showed a positive indole test. E. coli is the only coliform bacteria capable of generating indole from tryptophan-containing media at 44-45 degrees Celsius.

Confirmative *E. coli* Test

EMB media were incubated where *E.coli* created green metallic cheen (WHO 1997) .Two commonly employed methods of testing water are the membrane filtration technique for its sensitivity and the MPN method due to its accuracy and applicability.

Membrane Filtration

- A membrane filter filters a 100 mL water sample or a diluted sample in this approach. The membrane was then cultivated on a pad of sterile selective broth containing lactose and an indicator with the coliform organisms on it.
- The number of coliform colonies was counted after incubation. The estimated number of E. coli in a 100-milliliter water sample

Multiple Tube/Most Probable Number (MPN)

- One 50 mL sample, five 10 mL samples, five 1 mL samples, and five 0.1 mL samples were inoculated into an appropriate broth to determine the (MPN) in water samples.
- When coliforms were found, a Durham tube captured any gas produced.
- Following incubation, the number of samples generating a positive reaction (coliforms, turbidity, acid, and gas generation in the case of coliforms) is counted, and the most likely number of bacteria in the original sample is calculated using published MPN tables (Pruss-Ustun and WHO 2008).
- In this scenario, the 50 mL sample and all 10 mL samples, four 1 mL samples, and one 0.1 mL sample are positive.

Membrane Filtration Methods

Bacteria were collected using membrane filters with porosities ranging from 0.22 to 0.45 nm and a diameter of 47 mm. Membrane filters were incubated in solid media, while pads were immersed in liquid media or enrichment broth as an MPN system (WHO 2003).

Method: The filtration unit and suction device were assembled according to membrane instructions by antiseptic blunted-ended forceps. Antiseptic membrane filter grid-side. The unit was reassembled with the highest on the filter base (positioned centrally). The taster of water was mixed thoroughly, upsetting the flask many times.

Testing the Water to Make Sure That it is Safe to Drink

Although bacteriological testing of water is done regularly, it is not necessary to do biological testing on drinking water. It's difficult to culture many lab viruses that cause water-borne diseases. They might also last longer in water than bacterial fecal indicator species.

Test water samples have been inoculated into tissue cultures and then examined for panel growth or an exact cytopathic impact, although these procedures are limited to specialized laboratories. To get around these issues, it's been suggested that bacteriophages may operate as virus indicators, highlighting water that could be infected with viruses. Observing drinking water for viruses is not a common practice. Maybe this is an area where molecular genetics techniques such as PCR could be useful. However, caution is suggested when analyzing the results of such tests.

The existence of virus nucleic acid in a specimen does not necessarily imply the existence of infectious virus particles. The European Community has established a set of guidelines for the concentration of virus particles in water sources (Evans 2004). The first case for COVID-19 also experienced diarrhea before pneumonia was examined by Providence Regional Medical Center Everett in Washington. In many studies, authors reported that infectious/carrier visions could exist in human feces (Pritchard et al. 2010, Quilliam et al. 2020). Also, this virus can sustain in feces for up to 33 days. Afterward, the carrier has a negative test for respiratory viral ''RNA'' (Xu et al. 2020). This is an alarming threat because most gastrointestinal diseases are caused by contaminated water.

RESULTS

Table 2 shows that the boreholes were mostly owned by private individuals (81.4 percent), and no metering system was discovered. Nearly half of the boreholes were linked to other systems, 78.5 percent of the borehole samples were drilled, and the rest were driven or bored.

According to our findings, the capacity of water sources exceeded the maximum daily demand by 88.2% (not considering the losses of the non-accounted-for). It was discovered that 71 percent of the boreholes were encased in bedrock. At the same time, 59.9% were found filling annular spaces with grout or bentonite surrounding them, 50.9 percent had adequate sealing, and 54.8 percent had casing extensions at least 30 cm above the slab.

Table 3 shows total coliform bacteria were found in 34.4 percent of boreholes as positive results, 54.2 percent of samples showed positive results in thermotolerant coliform, 92.3 percent for Presumptive *E. coli* test, and 37.5 percent for Confirmative E. coli test, according to bacteriological analysis of groundwater samples.

Table 4 shows no significant association between the type of construction of the borehole and the presence of bacteria. The study discovered that total coliform was present in 97 out of 279 boreholes, thermotolerant bacteria in 52 boreholes, presumptive *E. coli* was positive in 48 boreholes, and the confirmative E. coli test yielded 18 positive results.

Table 2: Characteristic of sources of groundwater in Khartoum State.

Variables	Categories	Frequency	Percent %
Ownership of boreholes	Publicly	52	18.6
	Privately	227	81.4
Metering of services	Metered	2	.7
	Not metered	277	99.3
Number of customers	Less than 250	14	5.0
	251-500	18	6.5
	501-750	21	7.5
	751 -1000	11	3.9
	More than 1000	72	25.8
	Unknown	143	51.3
Interconnection of the system with neighboring systems	Interconnected	116	41.6
	Not interconnected	162	58.4
Groundwater source capacity	Equal / exceed	246	88.2
	Not equal / exceed	33	11.8
Borehole encased into bedrock.	Yes	198	71
	No	81	29.0
Filling the annular space around the borehole casing with grout or bentonite clay	Filled	167	59.9
	Not filled	112	40.1
Sealing of the borehole at the surface	Properly sealed	142	50.9
	Not properly sealed	137	49.1
Extension of the casing for at least 30 cm above the borehole slab	Extended	154	55.2
	Not Extended	125	44.8

Table 3: Results of the bacterial tests of groundwater samples.

Types of bacterial tests	Results	Frequency	Percentage %
Coliform test	+	96	35.8
	_	172	64.2
Thermo-tolerant coliform test	+	52	54.2
	_	44	45.8
Presumptive *E. coli* test	+	48	92.3
	_	4	7.7
Confirmative *E. coli* test	+	18	37.5
	_	30	62.5

Table 4: Relationship between the type of construction of the borehole and the presence of bacteria.

Types of Testing / Types of Construction	Coliform test (n = 279)		Thermotolerant coliform test (n = 97)		Presumptive E.coli test (n = 52)		Confirmative E.coli test (n = 48)	
	+	-	+	-	+	-	+	-
Dug	16(36.4)	28(63.6)	8(50)	8(50)	7(87.5)	1(12.5)	3(42.9)	4(57.1)
Drilled	74(33.8)	145(66.2)	39(52.7)	35(47.3)	37(94.9)	2(5.1)	13(35.1)	24(64.9)
Bored	0 (00.0)	2(100)	0	0	0	0	0	0
Driven	7(50)	7(50)	5(71.4)	2(28.6)	4(80)	1(20)	2(50)	2(50)
Total	97(34.8)	182(65.2)	52(53.6)	45(46.4)	48(92.3)	4(7.7)	18(37.5)	30(62.5)
p. value	0.451		0.606		0.430		0.802	

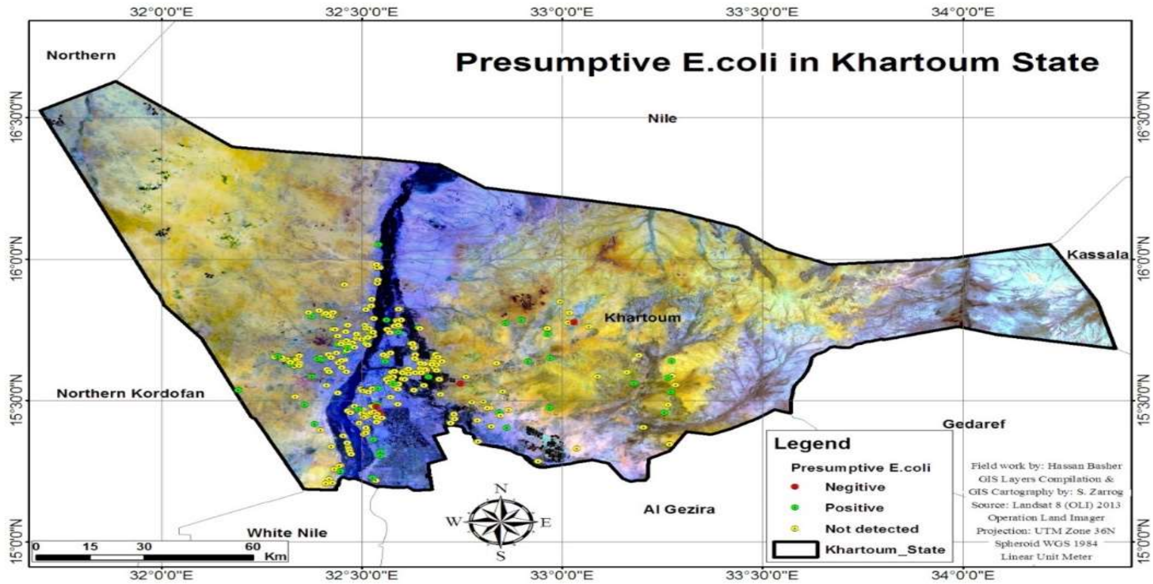

Fig. 2: The presence of presumptive *E. coli* in groundwater in Khartoum State.

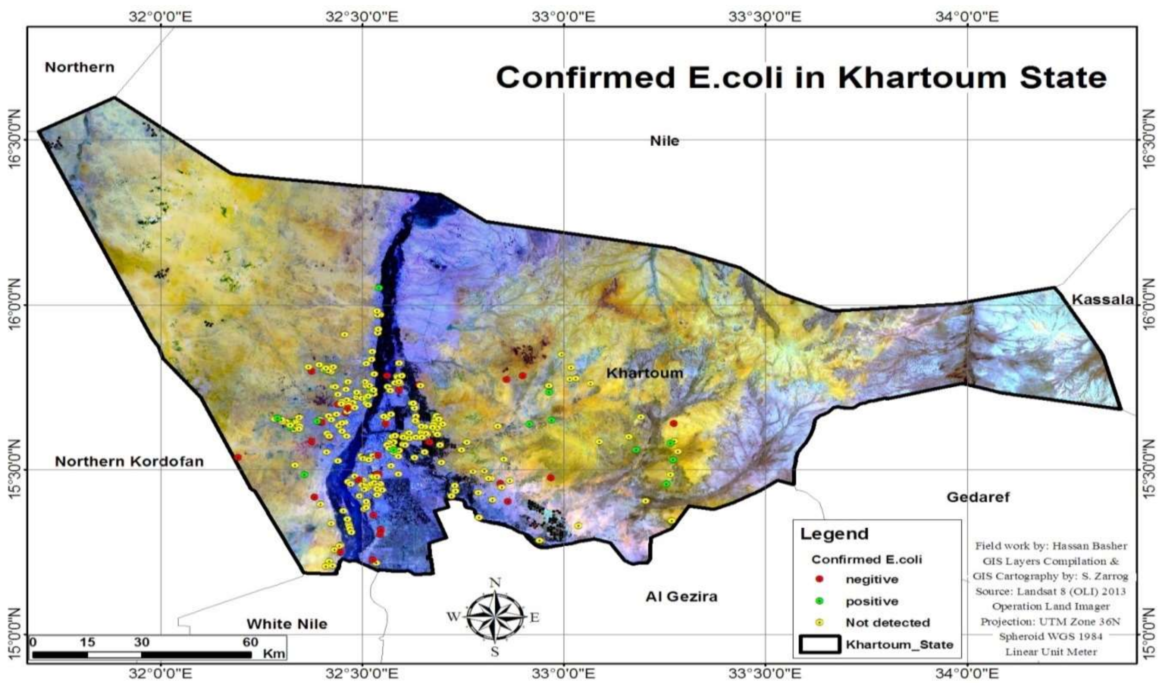

Fig. 3: The presence of confirmed *E. coli* in groundwater in Khartoum State.

The quality of Khartoum State groundwater was assessed using bacteriological and chemical tests. It was discovered that total coliform was present in 96 out of 279 boreholes, thermotolerant bacteria in 52 boreholes, and presumptive E. coli was positive in 48 boreholes (Fig. 2). The confirmative E. coli test yielded 18 positive results, indicating that the boreholes were contaminated due to the current environment being surrounded by contaminants (Fig. 3).

DISCUSSION

All parts of the base soil and substrate are equally powerful in pollution mitigation when it comes to the security of groundwater from contaminants. Groundwater pollution is most commonly associated with what we refer to as free, unconfined springs, especially when the air circulation zone isn't thick and the free surface of the groundwater sow. Still, it can also occur in semi-bound springs. The holding layers are thick and permeable in general.

A pollutant column is frequently discharged into the aquifer due to the contaminant. Pollutants are dispersed over a larger region as a result of water movement and distribution inside the aquifer. The approaching boundary, the emission edge, can collide with surface water, such as leaks and streams or groundwater wells, putting human and wildlife's water supplies at risk. A hydrological transport or groundwater model can analyze the vertical emission movement, known as the vertical emission interface. Soil features, in situ geology, hydrogeology, hydrology, and the nature of pollutants may all be included in a groundwater pollution analysis. Physical, inorganic, chemical, organic, bacteriostatic, and radioactive characteristics are all present in groundwater contaminants. Essentially, many of the same chemicals are found in polluted surface water. Polluted groundwater contains these as well, though their relevance varies (Ling et al. 2019).

Dr. McDonough said it was important to understand what caused high DOC concentrations in groundwater. "An increase in groundwater DOC concentration impacts the ability and cost to make groundwater drinkable," she said. For example, we projected a 16 percent increase in annual household water costs in some parts of the United States because of rising water treatment costs due to the need to implement additional water treatment measures to remove increased DOC concentrations. The decrease in groundwater quality and substantial increase in water treatment costs will also compound existing constraints on groundwater resources, including availability (Owens et al. 2019).

The limit of the water sources exceeded the largest everyday interest by 88.2 percent, according to our investigation (considering unaccounted misfortunes). Lack of cleanliness, incorrect drainage, and unprotected groundwater sources may make groundwater resources more vulnerable to microbial pollution. According to our findings, 71 percent of the wells were shrouded in shale, while 59.9% occupied the annular space with gypsum or betonies around it, 50.9 percent had an acceptable fitting, and 54.8 percent of the wells had a packaging augmentation of around 30 cm over the chunk. Another study on groundwater quality in rural North Central Nigeria found that poor management of groundwater resources can lead to E. coli and Enterobacter aerogenes pollution (McDonough et al. 2020).

This was evident in the investigation's aftermath, which showed a link between the well's development and the presence of germs. The bacteriological and compound tests were used to determine the nature of groundwater in Khartoum State. It was discovered that all E. coli forms were present in 96 (35.8%) of the 279 wells, while heat-safe microbes were found in 52 (54.2%). The putative E. coli was positive in 48 (92.3%) of the wells, and the corroborative test for E. coli yielded 18 (37.5%) positive results, implying that Usher 2007.91. This indicates that the boreholes were contaminated either due to the current environment's existence of contaminants or due to the boreholes' construction (Sojobi 2016). Len Ritter said loading of contaminants to surface waters, groundwater, sediments, and drinking water occurs via two primary routes, point-source pollution, and non-point-source pollution. Point-source pollution originates from discrete sources whose inputs into aquatic systems can often be defined spatially explicitly. Non-point-source pollution, in contrast, originates from poorly defined, diffuse sources that typically occur over broad geographical scales (McDonald et al. 2016).

Five of the six groundwater samples examined for fecal coliform exceeded the drinking water requirements, with concentrations as high as 13,000 per 100 mL (Ritter et al. 2002), discovered microbial contamination in ten of the 24 wells tested due to total coliform, E. coli, and enterococci. Enterococci had the highest volume and identification frequency, with enterococci pollution found in 40-48 percent of the wells. Although bacteriological testing of water is routine, organic testing of drinking water is less usual. Various infections that cause aquatic illnesses are difficult to purify in a laboratory setting. They may also survive longer in water than trash bacterial life forms. The study found that the amount of drinking water produced by the Khartoum State Water Corporation (KSWC) in Khartoum ranges between 1,500,000 and 1,700,000 $m^3.day^{-1}$, with 50% coming from groundwater (1015 producing boreholes). The private sector owns most of the sampled boreholes (81.4%), although the law assigns KSWC responsibility for all water sources. This was obvious in the construction methods, which included dug, drilling, driven, and bored wells with no metering system for all services (99.3%). According to the study, 41.6 percent of boreholes had linkage with neighboring systems, implying that contaminants could be transferred through the distribution network. The extension of the casing at least 30 cm above the borehole slab and the design of encased boreholes into the bedrock and filling the annular area around the borehole played a part in protecting boreholes from contamination.

In theory, locations with a low slope are more sensitive to groundwater pollution Because water can collect for extended periods, increased penetration and, thus, pollutant migration are possible (Yolcubal et al. 2016). Groundwater contamination is a risk when water sources are located in low-lying wet areas, partly because reservoirs are likely to be in close physical contact with surface water in such areas. Our research found a link between E. coli bacteria and borehole casing, as well as between E. coli bacteria and boreholes encased in bedrock or unweathered subterranean rock strata. Boreholes that aren't protected against vandalism and accidents can become contaminated with bacteria (there's a link between the presence of *E. coli* and borehole protection). This agrees with a study conducted by Syampadzi, using E. Coli parameters as a representation of pollution risk where the results exceed 85% of total samples that a value exceeds the standard of quality >0 MPN/100 mL (Rahman 2008). Pathogen concentrations in groundwater samples can vary significantly, so time-spaced samples might not accurately represent the microbial biota present in groundwater (Nurroh et al. 2020).

Specific diagnostic information assessments and applying the DRASTIC approach alone will not be enough to pinpoint the causes of drinking water pollution. Observational evidence on the position of water sources vs. latrines, low-lying wet regions, slope features, and knowledge of the local hydrogeological environment provided additional information to advise the optimum siting of water sources and sanitation services. This agrees with Robins (2007).

The presence of thermotolerant bacteria and the suitability of the cooling system demonstrated a significant association between the presence of thermotolerant bacteria and the appropriateness of the cooling system. The safety distance between the source of contamination and the boreholes protects the groundwater from contamination. Khartoum state legislation keeps the distance between the source of contamination and the boreholes in the range of not less than 120m. The results revealed that 161 boreholes were within the range of 120 m and less to the source of contamination, and there was a strong relationship between total coliform bacteria presence and the distance of 120 m and less.

Although disinfectants (chlorine, ozone, and ultraviolet) are used to protect groundwater supplies from contamination, only ten boreholes employed disinfectants (chlorine, ozone, and ultraviolet) without employing disputed online monitoring.

CONCLUSION

According to the report, to protect subterranean aquifers from pollution, land use, solid and liquid waste disposal techniques must be restricted, and general restrictions on land use and liquid waste disposal must be implemented.

Inoculating test water samples in tissue cultures and then examining plaque growth or the specific effect of cell pathology has been done. Still, these tests are only available in specialized laboratories. To get around these issues, it's been hypothesized that phages could operate as virus indicators, recognizing water that's probable to be infected.

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