Research Journal of Microbiology

ISSN 1816-4935

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Microbiology

ISSN 1816-4935
DOI: 10.3923/jm.2020.82.89

Research Article
Physicochemical and Mycological Examination of Groundwater (Well Water) in Rumuosi Community, Rivers State, Nigeria

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Abstract

Background and Objective: Groundwater constitutes an important source of water for drinking but mycological contamination through anthropogenic activities and infiltration of run-off into the groundwater makes the water unsafe for drinking and other purposes. This research was aimed at investigating the physicochemical and mycological qualities of groundwater in the Rumuosi community, Rivers State. **Materials and Methods:** A total of 30 water samples collected from 10 different wells and analyzed using standard microbiological methods. Generally, there were differences ($p \leq 0.05$) in the physicochemical properties tested across the various well sampled except pH. **Results:** All physicochemical parameters were within the FEPA limit, except elevated iron level (0.70 ± 0.01) recorded for one well. There was also difference ($p \leq 0.05$) in the total heterotrophic fungal count ranging from $1.0 \pm 0.00 \times 10^4$ SFU mL^{-1} to $3.35 \pm 0.21 \times 10^4$ SFU mL^{-1} in WW9 and WW1 respectively. A total of 16 fungal isolates belonging to 6 genera were identified and they include *Acremonium* spp., *Aspergillus* spp., *Candida* spp., *Fusarium* spp., *Penicillium* and *Phialosphora* spp. *Candida* spp. had the highest (43.75%) occurrence while *Fusarium* spp., *Acremonium* spp. and *Phialosphora* spp. had the least (6.25% each). **Conclusion:** The presence of these potential pathogenic fungi in groundwater poses a serious public health risk. Monitoring and treatment of groundwater before consumption and use for other relevant purposes is advocated.

Key words: Physicochemical properties, mycological examination, groundwater, anthropogenic activities, fungal contamination, well water, public health risk

Citation: C.E.I. Nwankwo, P.N. Barika, N.P. Akani and O.N. Amadi, 2020. Physicochemical and mycological examination of groundwater (well water) in Rumuosi community, Rivers State, Nigeria. Res. J. Microbiol., 15: 82-89.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Groundwater is the water located beneath the earth's surface in soil pore and the fractures of rock formation[1]. Groundwater constitutes an important source of water for many Nigerians; for drinking, agriculture and domestic use[2]. The use of groundwater has increased significantly in recent decades due to its widespread occurrence and perceived overall good quality. With 982 km^3 of groundwater accessed yearly, it is the most exploited raw material[3].

About 60% of groundwater withdrawn worldwide is used for agriculture, more than half of groundwater is withdrawn for domestic use and 25-40% is used as drinking water[4]. Globally, about 38% is used for irrigation[5].

Although considered safe from contaminants due to its location, studies show that groundwater is grossly contaminated[5-11]. These studies report a myriad of contaminants ranging from metals to microorganisms. The contamination of groundwater has increased due to anthropogenic activities matched by population increase[12]. Groundwater contains a broad spectrum of microorganisms, similar in diversity to those found in the surface soils and waters[10]. These microbes encompass bacteria, fungi and protozoa.

Fecal contamination of groundwater is a serious public health issue and may explain the presence of these microbes in groundwater[13-16]. Fecal contamination of water has been confirmed by the presence of indicators organisms[13,17]. Traditionally, fecal indicator bacteria (FID) were used for centuries though with shortfalls, such as lack of host specificity[17]. The presence of indicator organisms, though no confirmation of pathogenic organisms provide a lead into further investigations for pathogens. Such studies have reported the presence of pathogenic organisms in groundwater including bacteria, viruses and protozoa[18]. These organisms have been implicated for various endemic waterborne illnesses, with diarrhea prevalent[19].

Relatively, few studies have investigated the fungal contamination of groundwater as fungal contaminants were least anticipated[8]. However, numerous fungi species such as *Penicillium*, *Cladosporium*, *Aspergillus*, *Phialosphora* and *Acremonium* are present in groundwater sources[6,8,20].

Fungi can colonize oligotrophic environments as they can harness nutrients from rare sources like air, water or their host substrate[21]. To minimize nutrient uptake, filamentous fungi form mats of fine hyphae in water[22]. Fungi also produce secondary metabolites that can cause much harm to humans. Fungal infections are becoming of increasing concern due to the increasing numbers of immunocompromised patients and those with other risk factors. Further, the secondary metabolites produced by some species such as mycotoxins can lead to deterioration in the organoleptic properties of water, leaving it unfit for use[23,24].

In many developing countries, groundwater pollution from agricultural run-off has been highlighted[25]. Studies have reported groundwater contamination from both point and non-point sources[26]. Of great concern, is the introduction of chemicals from fertilizers such as nitrate and other nutrients into groundwater sources. Further, wastes from animal farming have been blamed for releasing nutrients and other herbicides into groundwater[27]. These have also deteriorated the physicochemical parameters of groundwater such as temperature, pH, chlorides, total dissolved solids, biochemical oxygen demand, turbidity, salinity as well as certain metals.

With clean water listed at number 6 among the sustainable development goals[28], this research was aimed at determining the physicochemical and mycological quality of groundwater in the Rumuosi community. Specifically, we sought to ascertain the quality of Well water samples from Rumuosi, Rivers State, Nigeria.

MATERIALS AND METHODS

Description of the study area: The study was conducted in the Rumuosi community in the Obio-Akpor Local Government area with coordinates 7.07520°E to 7.07810°E and 4.84290°N to 4.8460 0°N (Fig. 1). This community is strategic, approximately 15 km from Port Harcourt, the Rivers State capital. Rumuosi is in the Obio-Akpor local government area and has a population slightly above fifty thousand[29]. Endowed appreciably with surface and groundwater resources, the indigenes are mostly farmers and fishermen[29]. Further, the majority of the residents here depend on wells to meet their daily water needs. A visit to this community leaves a lot of questions as to the quality of the water harnessed from these wells, based on numerous factors such as poor siting.

Sample collection: A total of 30 water samples were collected aseptically using sterile bottles. All samples were properly labeled and transported in an ice pack bag to the laboratory for Physicochemical and mycological analyses. All samples were collected in the peak of the wet season; between

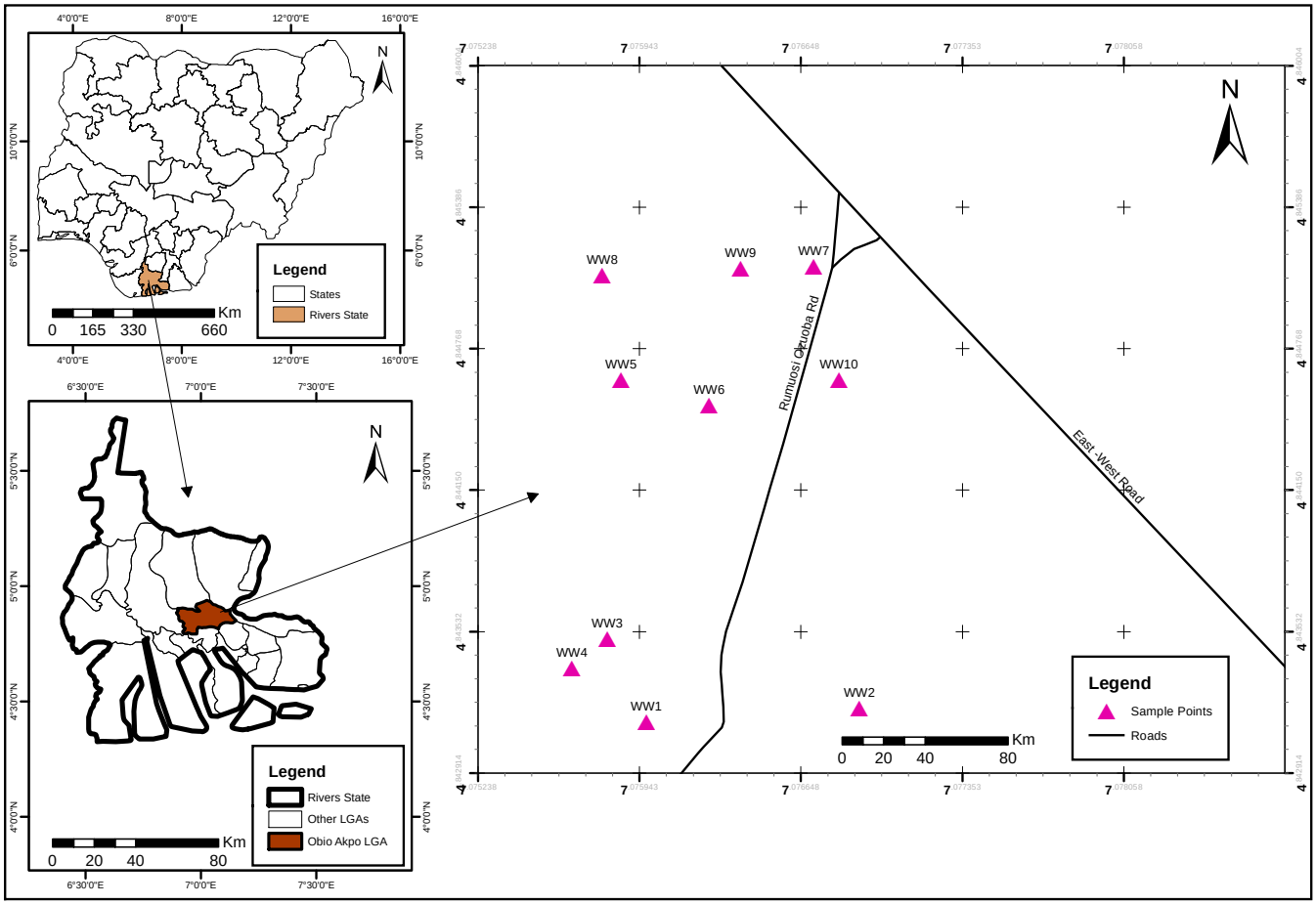

Fig. 1: Map of Rumuosi Community, showing the sampling locations
Source: Ministry of Land and Survey, Rivers State, Nigeria

May and July, 2019. All samples were aseptically transported to the Microbiology Laboratory, Rivers State University for analyses.

Determination of the physicochemical parameters and heavy metal: Six physicochemical properties were determined. The pH was determined using a calibrated pH meter. The turbidity was determined by using the Hach DR2010 spectrophotometer at a wavelength of 370 mn. The zinc content was determined by the titration method; the iron content was determined by using a spectrophotometer[30] at 415 nm. Also, the Biochemical oxygen demand was determined by using the Winkler method[30]:

$$\text{BOD}(\text{mg mL}^{-1}) = \frac{\text{DO1} - \text{DO2}}{\text{Df}} \quad (1)$$

where, DO1 is the initial dissolved oxygen at the time of dilution, DO2 is the final dissolved oxygen after 5 days and Df is the dilution factor.

Fungal enumeration: A ten-fold serial dilution was conducted on the water samples and an aliquot from a dilution (10^{-1}) was plated onto Sabouraud dextrose agar (SDA) plate and incubated at ambient temperature (25-27°C) for 3-5 days. Discrete spores were subcultured onto fresh Sabouraud dextrose agar plate and the isolates preserved in agar slant in bijou bottles[31,32].

Identification of the fungal Isolates: The morphological characteristics such as the shape and color of the isolate were used for primary identification. The isolates were further identified after staining with lactophenol cotton

blue and examined with ×40 objective lens which reveals the structure of the hyphae and the arrangement of the spores[33].

Data analysis: Statistical Package for Social Sciences (SPSS) version 22 was used to analyze the data obtained from the study. Analysis of Variance (ANOVA) was used to test for significance ($p \geq 0.05$) and where differences existed Duncan Multiple Range Test was used to separate the means.

RESULTS

The result of the physicochemical properties as presented in Table 1 revealed that pH ranged from 4.25±0.07 to 7.01±2.84 in WW9 and WW2; Temperature ranged from 27.00±0.00 to 28.75±0.35°C in WW9 and WW6; Turbidity ranged from 0.03±0.00 to 0.41±0.01 NTU in WW10 and WW2 respectively. Similarly, Biochemical Oxygen Demand ranged from 1.53±0.01 to 2.60±0.08 mg L^{-1} in WW6 and WW9; Iron ranged from 0.03±0.01 to 0.70±0.01 mg L^{-1} in WW1 and WW9; Zinc ranged from 0.01±0.01 to 2.37±0.02 mg L^{-1} in WW1 and WW9, respectively. Generally, there was a difference ($p<0.05$) in the physicochemical properties of the well water samples except for pH that showed no difference ($p>0.05$) (Table 2).

The result of the fungal population in the well as revealed in Fig. 2 shows that the Total Heterotrophic fungal count ranged from 1.0±0.00 to 3.35±0.21 ×10^4 SFU mL^{-1} in WW9 and WW1, respectively.

Table 1: Variation in Physicochemical properties of the well water samples during the study period

	Physicochemical properties					
Stations	pH	Temperature (°C)	Turbidity (NTU)	BOD (mg L^{-1})	Iron (mg L^{-1})	Zinc (mg L^{-1})
WW 1	4.90±1.27^{a}	27.25±0.35^{a}	0.22±0.01^{d}	1.64±0.01^{b}	0.03±0.00^{a}	0.01±0.00^{a}
WW 2	7.01±2.84^{a}	27.50±0.71^{a}	0.41±0.01^{f}	1.93±0.01^{d}	.23±0.00^{c}	0.29±0.00^{d}
WW 3	4.50±0.42^{a}	27.00±0.00^{a}	0.03±0.003^{a}	1.98±0.01^{d}	0.55±0.00^{e}	0.38±0.02^{e}
WW 4	6.80±0.28^{a}	27.65±0.49^{a}	0.23±0.007^{d}	1.86±0.03^{c}	0.28±0.01^{d}	0.57±0.007^{f}
WW 5	7.60±0.57^{a}	27.00±0.00^{a}	0.05±0.01^{ab}	1.57±0.02^{a}	0.05±0.01^{b}	0.36±0.00^{e}
WW 6	6.75±3.89^{a}	28.75±0.35^{b}	0.07±0.01^{b}	1.53±0.01^{a}	0.68±0.01^{h}	0.06±0.01^{b}
WW 7	4.25±0.35^{a}	28.00±0.00^{ab}	0.12±0.01^{c}	2.29±0.01^{e}	0.58±0.01^{f}	0.23±0.01^{c}
WW 8	5.30±0.42^{a}	27.50±0.71^{a}	0.23±0.01^{d}	2.59±0.01^{f}	0.29±0.01^{d}	0.65±0.01^{g}
WW 9	4.25±0.07^{a}	27.00±0.00^{a}	0.05±0.01^{ab}	2.60±0.08^{f}	0.70±0.00^{i}	2.37±0.02^{h}
WW 10	6.15±0.07^{a}	27.25±0.35^{a}	0.03±0.00^{a}	1.93±0.01^{d}	0.63±0.01^{g}	0.25±0.01^{c}
FEPA LIMIT	6-9	27	≥1.0	≥10	0.05-0.3	5.0

*Means with the same superscript along the columns are not significantly different ($p \geq 0.05$), BOD: Biochemical oxygen demand, WW: Well water, FEPA: Federal environmental protection agency

Table 2: ANOVA Table showing the level of significance of physicochemical properties of well water samples across the stations

Parameters		Sum of squares	Df	Mean square	F	Significant
pH * Stations	Between groups (Combined)	28.495	9	3.166	1.233	0.372
	Within groups	25.680	10	2.568		
	Total	54.175	19			
Temperature * Stations	Between groups (Combined)	5.418	9	0.602	3.716	0.026
	Within groups	1.620	10	0.162		
	Total	7.038	19			
Turbidity (NTU) * Stations	Between groups (Combined)	0.287	9	0.032	585.483	0.000
	Within groups	0.001	10	0.000		
	Total	0.287	19			
BOD (mg L^{-1}) * Stations	Between groups (Combined)	2.728	9	0.303	374.167	0.000
	Within groups	0.008	10	0.001		
	Total	2.736	19			
Iron (mg L^{-1}) * Stations	Between groups (Combined)	1.192	9	0.132	3777.671	0.000
	Within groups	0.000	10	0.000		
	Total	1.192	19			
Zinc (mg L^{-1}) * Stations	Between groups (Combined)	8.333	9	0.926	7712.629	0.000
	Within groups	0.001	10	0.000		
	Total	8.334	19			

*: Indicate the relationships between the main effects and variables, DF: Degrees of freedom

Table 3: Characterization of fungal isolates from various well water sampled

Isolates	Colonial Characteristics	Microscopy	Probable organism
1	White fluffy color aerial mycelium on a reverse surface	Phialides are cylindrical, with a small collarette	*Fusarium* spp.
2	White to cream color and smooth reverse	Budding to subspherical to ovoid blasto-conidia	*Candida* spp.
3	Yellow-green surface taming black with the formation of numerous black dots with creamy cracked reverse	The appearance of Dark brown furring head on white mycelium	*Aspergillus* spp.
4	Characteristic blue-grey coloration with a fanjet of white	Long awl-shaped phialides producing cylindrical one-celled conidia mostly in slimy heads	*Penicillium* spp.
5	Colonies are usually slow-growing, often compel pink pigmentation with creamy white surface	Possesses hyaline, septate hyphae which are typically fine and narrow	*Acremonium* spp.
6	Slow growing suede-like with radial furrow lustily whitish-grey	Cordial often occur in balls, hyaline and thin-walled	*Phialosphora* spp.

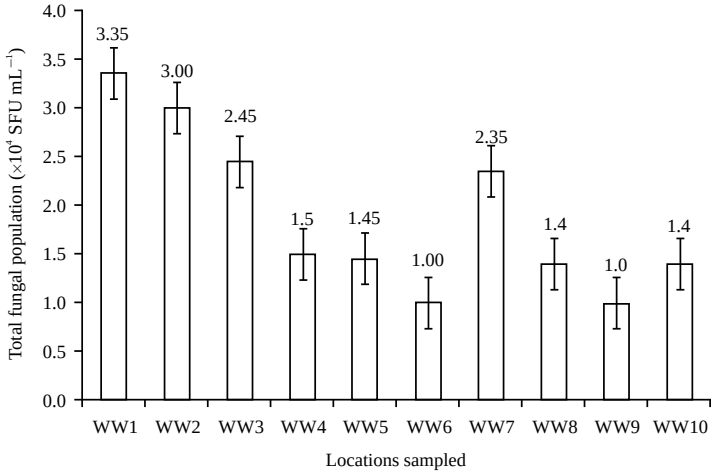

Fig. 2: Variation of the fungal population in the various wells sampled during the study period
WW: Well water at sites 1-10

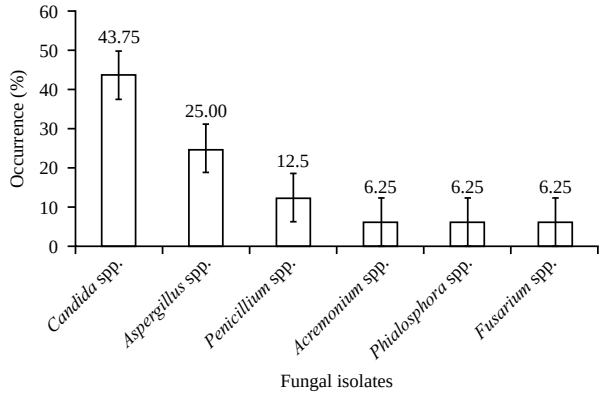

Fig. 3: Occurrence of the fungal isolates in the well water samples

A total of 16 fungal isolates belonging to 6 genera were isolated from the well and they include *Fusarium* spp., *Candida* spp., *Aspergillus* spp., *Penicillium*, *Acremonium* and *Phialosphora* spp. as revealed in Table 3.

The study revealed that *Candida* spp. has the highest percent occurrence (43.75%) and *Fusarium* spp., *Acremonium* spp. and *Phialosphora* spp had the least percentage occurrence (6.25%) as shown in Fig. 3.

DISCUSSION

This study reports heavy fungal contaminations of the wells studied which could pose severe public health challenges. There were variations in the physicochemical properties analyzed between the wells. Generally, there were differences ($\leq$0.05) observed except for pH. All the physicochemical parameters fall within the FEPA limit and similar to the report by Agbalagba *et al.*[34], except in one of the wells that recorded a high concentration of iron. Iron, although an important cofactor, could pose public health threat at elevated levels[35,36]. This high concentration of iron could be due to industrialization[37]. Other sources of elevated concentrations of iron could be agriculture through excess use of fertilizers and pesticides, as well as animal waste especially feeds from poultry farms. Further introduction of iron could be from domestic sources due to poor siting of wells[38]. Adeogun *et al.*[39] suggest that depth could also explain the

introduction of iron into underground water sources like well. The present study was conducted in the Rumuosi community where wells are usually hand-dug due to the nearness of the water table to the soil surface. The rather shallow nature of these wells, coupled with poor siting could explain the elevated levels of iron recorded. Our findings agree with previous studies also reporting elevated levels of iron from similar sources[37,39-42].

The fall within the acceptable limit of all other measured metals should not be celebrated as this is not a confirmation that all is well with samples studied. These low levels could be explained by seasonal variation of these parameters[7]. The wet season ensures an observable increase in the underground water table. An increase in the water level may dilute the available metal constituents to levels that seem within a safe limit. A study of these wells in the dry season could aid in determining the true status of these wells in terms of metal contamination.

The prevalence of these fungi in groundwater could be due to their ability to tolerate oligotrophic environments thereby making some of the pathogenic species able to colonize the domestic water system which is typically low in nutrient[24]. The pH range recorded appear favorable for fungal proliferation[23]. This could further explain the heavy fungal contamination in the wells tested.

Groundwater contamination has been reported higher in the wet season than dry[7,26]. This has been explained by high water volume due to increased precipitation leading to increased floatation. The present research was conducted in the peak of the wet season and the high floatation could be responsible for the high fungal population in the wells tested. Further, the poor siting of the wells considering the prevailing environmental practices could also be blamed for the high fungal population. For instance, septic tanks and other toilet facilities may be sited within worrisome distances from these wells. Further, open defecation is still rampant in these areas and has been implicated for contamination of groundwater[38]. Poor agricultural practices may also have contributed to the fungal contamination of the underground waters in this community. This is especially true because the indigenes are mostly farmers[29] and all these factors may be aided by increased floatation in the wet season[7].

Although there is no known permissible limit set for fungi in water[23,43], the results of this study present a remarkable fungal load. This load is sufficient to cause fungal infections in users of these wells. Fungi have been implicated for numerous health challenges in men ranging from infections to allergic challenges. Oliveira *et al.*[23] reported that fungi in water could deteriorate the organoleptic properties, cause pipeline blockages, produce mycotoxins and be pathogenic or allergenic.

The high contamination of these wells which serve as a source of drinking water for this community could be due to poor maintenance culture, pH condition of the well water, unhygienic handling as well as natural activities such as rainfall. Flooding resulting from heavy rainfall may sometimes cover the wells and thus introduce contaminants such as nutrients and microorganisms like fungi[44].

The fungi we reported in this study agree with previous studies[8,23,26,43,45]. These fungi are ubiquitous and widely distributed in nature and several types of research have implicated waterborne fungi to be of public health concern[22,23]. They have been known to cause diseases like Aspergillosis, leading to severe respiratory infections and mostly transmitted through water sources[46]. *Candida* species, can be found in 70% healthy individuals and considered as an opportunistic pathogen causing infections of the mouth, digestive tract and skin especially in immunocompromised individuals[47]. *Fusarium* spp. causes a lot of superficial infections (Keratitis) and *Penicillium* causes some system infections.

The presence of these fungal species poses a great threat to public health in the Rumuosi, Rivers State, Nigeria. Some of these species have been shown with adaptive features making them resistant to common disinfection procedures and so persist even after treatment[8,23]. Ameen *et al.*[8] showed that the spores of certain fungal species are not easily destroyed from chlorination and boiling. Their study mentioned the spores of *Aspergillus* and *Penicillium* species which were also present in these studies. This presents further worries as there is minimal to no treatment of these well waters studied before use.

CONCLUSION

Our study reports gross contamination of the underground water in Rumuosi, Rivers State, Nigeria. The presences of heavy metals, as well as appreciable fungal load, represent severe public health concerns. These metals and fungi species reported have been linked to diseases of humans and should not be present in water. Environmental practices including open defecation, poor agricultural practices, poor siting of underground water wells among others, have been blamed for this contamination. The need for improved environmental practices, sanitary conditions as well as water treatment procedures cannot be over mentioned in the interest of public health.

SIGNIFICANCE STATEMENT

This study has discovered fungal contamination in groundwater. Groundwater is seen as protected from contaminants due to its location. Our study is the first study to report fungal contamination of groundwater in Rumuosi, Rivers State, Nigeria. This study, therefore, presents a lead to further studies into fungal health challenges related to the use of groundwater such as itches associated with the use of well water for bathing.

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