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Research Article

Study of the Microbiological Quality of Groundwater Consumed by the Population of the Messassi District in the City of Yaoundé, Cameroon

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Abstract

The lack of an efficient drinking water management and distribution system across the territory of developing countries has forced populations to turn to other sources of water supply including wells and boreholes. The objectives of this study were to assess the microbiological quality of groundwater consumed by the population of the Messassi district in the city of Yaoundé. After a household survey to identify sources of drinking water supply, water samples were taken from the boreholes and wells most frequented by the population. The microorganisms were isolated after filtration of 100 ml of sample through a nitrocellulose membrane, with a microporosity of 0.22 µm. This membrane was then placed on the surface of the agar plates and incubated at 37 °C and 44 °C for 24 +/- 2 hours. After subculture on different media, pure bacterial isolates were identified by their cultural characteristics and commercialized biochemical galleries. Of the 101 households surveyed, 23 were supplied with borehole water and 21 with water from developed wells, i.e. 43.56% of households that consumed and used groundwater for their various needs when the other 56.44% households consumed tap water supplied by Cameroonian Water or mineral water. Of the 20 water samples that were taken in total, 04 came from boreholes, 07 from developed and regularly disinfected wells and 09 from developed and not regularly disinfected wells. The probable source of pollution was that of latrines located less than 15m from the wells (70%). The 04 water samples taken from the boreholes were all compliant with total flora (TF), total coliforms (TC) and fecal coliforms (FC). Of the 07 wells developed and disinfected regularly, 06 samples were non-compliant with TF while 05 were non-compliant with TC and FC. Concerning the samples from the 09 wells developed and not disinfected, they were non-compliant with both TF and TC and FC. Bacterial species such as *Salmonella arizonae* (40%), *E. Coli* (30%), *Proteus*

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mirabilis (20%) and *Salmonella choleraesuis* (10%) were isolated. The results of the current survey show risks of microbiological contamination among the drinking water samples studied and consumed by the population. Regular and proper disinfection of the boreholes and wells in the town of Messassi is necessary to ensure a better quality of life for the population that consumes groundwater.

Keywords

Groundwater, Messassi, Microorganisms, Contamination, Disinfection

1. Introduction

Water is undoubtedly one of the most precious goods for the survival of living beings in general and humans in particular. However, although vital, this precious liquid is not accessible to all in an equitable manner. According to the United Nations Children's Fund and the World Health Organization (WHO), in 2017, 2.1 billion people, or 30% of the world's population, still did not have access to domestic drinking water supply services and 4.4 billion, or 60%, did not have safely managed sanitation services [1]. Africa, for example, currently suffers from a lack of distribution and sanitation infrastructure that would allow populations to have access to drinking water [2]. This is what drives the inhabitants of areas not covered by an irrigation system to turn to other sources of water supply including groundwater from wells and boreholes, which represent more than 98% of the fresh water reserves on the planet [3]. Behind this alternative lies the problem of the quality of the water consumed because these practices can lead to the occurrence of several pathologies within the population. Indeed, the boreholes and wells developed by the populations are very often not carried out in accordance with standards such as respect for depth or proximity to potential sources of contamination such as latrines. [3]

Many studies have been conducted around the world and in Africa. In northern Morocco, a study carried out by Benajiba et al in 2013 showed significant contamination of the water table by microbial agents; out of 270 analyses of total coliforms, fecal coliforms (FC) and fecal streptococci (90 analyses for each group of bacteria), 100%, 96.67% and 92.23% respectively of the results were positive [4]. This same study stipulates that the concentrations of the microbial indicators monitored differ depending on the location of the wells in relation to the different sources of contamination [4]. More locally, a study was conducted in 2020 in some districts of the city of Yaoundé, Cameroon in its 7th district by MOUSSIMA et al, thus showing that contrary to the recommendations of the Cameroonian standard, the indicators of fecal pollution and bacteria of the genus *Salmonella* were isolated in the water analyzed [5]. Similarly, the work of Fokou et al., 2022 carried out in the locality of Don Bosco in the Mimboman district in the city of Yaounde stipulates that the borehole water consumed by the population of the said locality does not comply with WHO standards. The non-compliance of the samples analyzed here was 50% for total flora, 38.8% for total coliforms, 31.5% for fecal coliforms and 31.5% for fecal streptococci. [6]

Messassi being a peri-urban area of the city of Yaoundé, where the low coverage of the irrigation system with drinking water has pushed the populations to turn to the consumption of groundwater, it is important to control this water in order to ensure the microbiological quality and obviously to prevent any risk of contamination by identifying the potential sources of pollution or germs likely to be pathogenic there.

2. Materials and Methods

2.1. Description of the Study Site

The study was conducted in the Messassi district of the city of Yaoundé. It is a peri-urban locality of the city of Yaoundé. It is bordered to the north by the locality of Emana and to the south by the locality of Olembe. (See Figure 1).

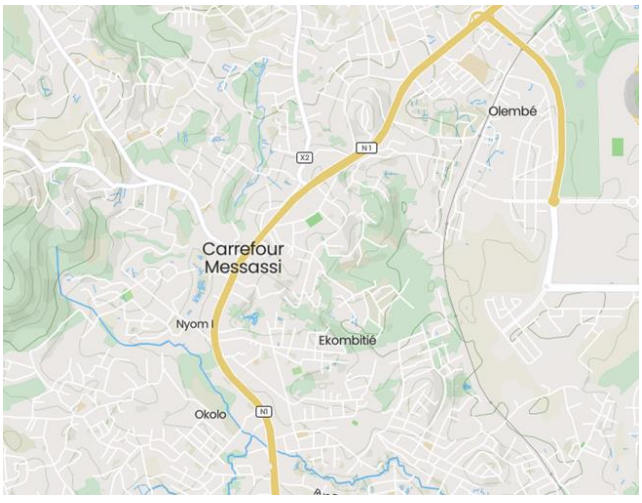

Figure 1. *Geographic map of the locality of MESSASSI [7].*

2.2. Household Survey Authorization

A research authorization No. 063/MP/J06-01/SP issued by the sub-prefect of the Yaoundé 1st district was obtained for field visits. Ethical clearance No. 3181CEI-Udo/05/2022/M was issued by the institutional ethics committee for human health research at the University of Douala and a consent form duly signed by each head of household who participated in the study was also obtained before inclusion in this study.

2.3. Household Recruitment Method and Choice of Water Sampling Sites

Households were randomly selected for the survey on the different sources of drinking water supply, storage containers and the time of water storage. The choice of boreholes and wells for water samples to be analyzed was based on the increase in its use by the population.

2.4. Collection and Transport of Samples

2.4.1. Collection

After ensuring that the sampling bottles were sterile, we labeled them. To ensure that the tap was clean at the water outlet, we may have cleaned and then torched the tap using a lighter. We then let the water run for about 30 seconds to 1 minute before carefully filling the sterile 500 ml bottle, closing it, storing it in a cooler containing accumulators and filling out the relevant information sheet. To collect water from the developed wells, it was necessary to disinfect the containers used to collect the water in the wells, then insert the sterile 500 ml bottle to collect the water to be analyzed.

2.4.2. Transport

The transport was carried out in strict compliance with the concept of triple packaging; once the samples were in the sterile vials, they were placed in plastic bags before being placed in the cooler and sent for analysis (at most 3 to 4 hours after collection) to the multidisciplinary laboratory of galenic pharmacy and pharmaceutical legislation of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé

2.5. Microbiological Analysis

Microbiological analyses aim to highlight the presence of germs based on the search and counting of these in the samples to be analyzed. The germs sought according to the WHO 2012 quality references are [8]

1) Total flora: < 10 germs / ml
2) Coliformes: 0 / 100 ml
3) *Escherichia coli*: 0 / 100 ml

2.5.1. Sample Concentration by Membrane Filtration

The inoculation method was almost the same for total coliforms, fecal coliforms, total germs. Only the culture media and incubation conditions differed [9, 10]. In order to count the number of microorganisms suspended in the water sample, a volume of 100 ml was filtered through a nitrocellulose membrane, with a microporosity of 0.22 µm. The filter was then placed on isolation medium, incubated at 37 ℃ for 24 to 48 h for total coliforms, total germs and 44 ℃ for 24 to 48 h for fecal coliforms [11, 12]. The number of colonies found was expressed as the mean Colony Forming Unit (CFU/mL) (NF S93-315, 2008) [9].

Sterile water for injection (WFI) purchased from a pharmacy was used as a control.

2.5.2. Identification of Bacteria

Identification was made after observation of the cultural characteristics on the different environments, then after the production of the control GRAMs, this was followed by subculturing on different specific environments. [13, 14]

1) Total germs or total flora: The inoculation was done in Tryptone Glucose Extra Agar (TGEA) which is a medium developed for the research and enumeration of microorganisms in water, milk and dairy products.
2) Total coliforms: Inoculation was done in hektoen agar and incubation was at 37 ℃ for 24 ±2 hours;
3) *Escherichia coli, salmonella: inoculation was done on MacConkey agar and incubation at 37 ℃ for 24 ± 2 hours.*

After carrying out the oxidase and catalase tests, pure bacterial isolates were identified from the commercialized biochemical galleries API 20E [8, 9].

2.6. Data Processing and Analysis

The data were collected, processed, and analyzed by Excel software (version 2010). The results were presented in the form of tables and graphs.

3. Results

3.1. Main Source of Drinking Water Supply

In order to obtain information on the habits of the population with regard to their water supply source, a survey was conducted on 101 households. Table 1 presents the results of the survey. Of the 101 households visited, 29 households consumed water from Camerounaise des eaux (CDE) and 28 mineral water, while 23 obtained water from boreholes and 21 from developed wells. That is a total of 44 (43.6%) households obtained water from groundwater. The most frequent source of contamination of the water points of these households was the proximity to latrines, i.e. less than 15m from

wells or boreholes.

Table 1. *Results of the survey on sources of drinking wate r supply.*

Household related information				
Household size	2-5 people	5-10 people	Greater than 10 people	
	63=62.37%	32=31.68%	6=5.94%	
Sources of direct drinking water (survey in 101 households)	drilling	Wells fitted out	CDE	Mineral water
	23	21	29	28
	22.8%	20.8%	28.7%	27.7%
Storage container	cans	Bucket	Autres	
	91=90.1%	10=9.9%	0	
Retention time	0-4 hours	4-12 h	More than 12 hours	
	11=10.9%	60=59.4%	22=21.8%	
Use of groundwater	Direct consumption:	Household	Hygiene	
	44=43.6%	101=100%	101=100%	
Abdominal pain	Yes regular	No	Rare	
Present after direct consumption: drinking water	22.7%	9.1%	68.2%	
Actions carried out (40 households concerned)	Plant extract infusions	Hospital/antibiotic	No action	
	20=50%	02=5%	18=45%	
Information related to drilling and wells sampled				
Distance from latrines	0-15 m	More than 15 m		
	14=70%	6=30%		
Types of water points at the 20 collection points	Wells fitted out	Drilling		
	16= 80%	4 = 20%		
Drilling depth	80-110 meters	10-15 meters	Unknown	
	2= 10%	4=20%	14=70%	
Disinfection of developed wells (16 wells concerned)	Regular disinfection	No Disinfection		
	7=43.75%	9=56.25%		

3.2. Total Flora Count (Total Germs) After Filtration

The microbiological analysis carried out showed us after counting that our water points contained high loads of bacteria. The total germs at 37 ℃ are bacteria of intestinal origin (human or animal). In order to see if the number of germs likely to be present in well and borehole waters complied with international standards, the colonies were counted at each well and borehole and expressed in CFU/ml. The study showed that: the samples from the E1, E3, E4, E6, E8, E10, and E12 wells were those with a greater number of colonies of total germs, i.e. a rate of > 300 CFU/mL, while borehole E19 was the one with the greatest number of total coliforms (Table 2).

Table 2. *Enumeration of colonies of the total flora after filtration in CFU/ml.*

Samples	**Number of colonies in CFU/ml**	**Samples**	**Number of colonies in CFU/ml**
E1	>300	E11	64

Samples	Number of colonies in CFU/ml	Samples	Number of colonies in CFU/ml
E2	100	E12	>300
E3	>300	E13	54
E4	>300	E14	9
E5	123	E15	3
E6	>300	E16	62
E7	150	E17	2
E8	>300	E18	0
E9	200	E19	8
E10	>300	E20	1

Legend: Wells fitted = E1 to E16; Drilling = E17 to E20

3.3. Total Flora Conformity After Cultivation

It is observed from Figure 1 that all samples (100%) from the boreholes were compliant with WHO standards (< 10 CFU / ml). On the other hand, 5 or 71.42% of the wells developed and disinfected, 9 or 100% of the wells developed not disinfected regularly were non-compliant with a number of colonies well above the standard (> 300 CFU / ml).

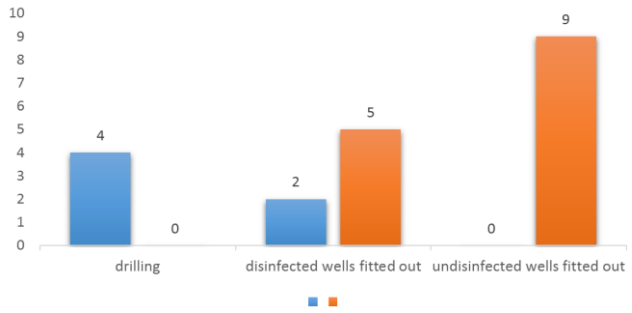

Figure 2. *Total flora conformity after cultivation.*

3.4. Enumeration of Total Coliforms, Fecal Coliforms After Culture

Unlike boreholes, the wells that were not regularly disinfected were all contaminated with total coliforms and fecal coliforms. In addition, 6 out of 7 samples from wells that were regularly disinfected were non-compliant with total coliforms, compared to 5 out of 7 for non-compliance with fecal coliforms.

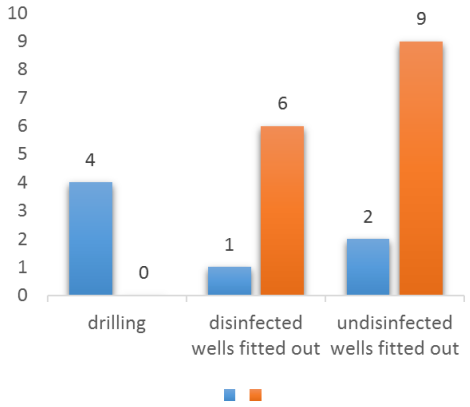

Figure 3. *Total coliforms compliance after culture.*

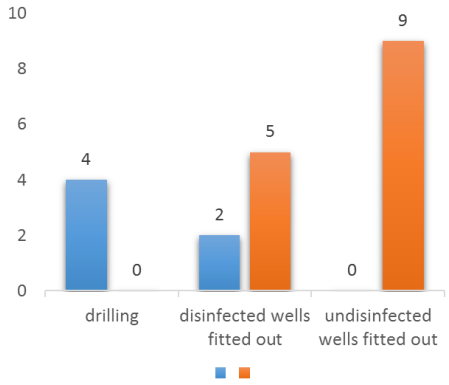

Figure 4. *Fecal coliform conformity after culture.*

3.5. Distribution of Germs Found According to the Number of Samples

The bacterial species isolated belonged to the family Enterobact eria. The most commonly isolated were: *Salmonella* arizonae (40%), *E. Coli* (30%), *Proteus mirabilis* (20%) and *Salmonella choleraesuis* (10%). This shows a clear predominance of the genus *Salmonella* (50%). (Figure 4)

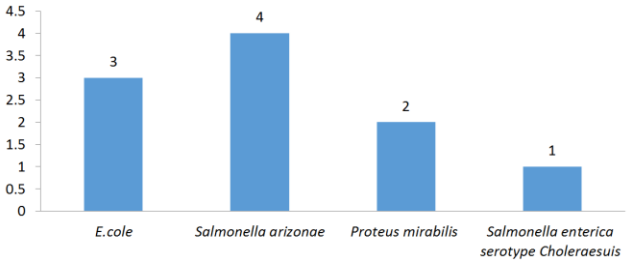

Figure 5. *Distribution of isolated germs.*

In our study, we identified a total of four different germs belonging to the enterobacteria family, the most isolated of which were *Salmonella arizonae* and *E. coli*.

4. Discussion

The problem related to the quality of groundwater from wells and boreholes and consumed by the populations of the locality of Messassi in the city of Yaoundé in Cameroon is of a major nature. Many studies have been conducted in the world on the one hand and in Africa in particular on this subject [14, 15]. The objective of this study was to evaluate the bacteriological quality of groundwater consumed by the populations of this locality. We were thus able to obtain various results almost similar to other studies carried out previously.

Indeed, following the various surveys carried out among the populations of this locality, we were able to identify that one of the main sources of water supply were wells and boreholes. Of the 20 samples taken, 16 (80%) came from developed wells while 4 (20%) were boreholes. These data are similar to those of Briand et al. obtained in 2009 during his study in Senegal which focused on the determinants of the choice of water supply for households in Dakar. [16] However, these authors were able to note a penchant of the populations for borehole water and this is due to the fact that public waters have characteristics that are not very appreciated such as bad color, undesirable taste, frequent cuts, low water pressure at the outlet of the tap. [16].

Following the various investigations carried out on the 16 developed wells, 7 (43.75%) were disinfected regularly while 9 (56.25%) were not. The bacteriological analysis of the well samples collected allowed us to note that 100% of the wells not regularly disinfected were contaminated by fecal coliforms while the wells regularly disinfected were contaminated at 71.42%. Despite the high percentages of contamination in the two groups of wells identified, regular sterilization of the well could have a positive impact on limiting wells contaminated by these FC and other pathogens [17]. The results obtained during this study were somewhat different from those of Kapembo *et al* in Congo who found that there was a clear contamination of undeveloped wells compared to developed wells which, moreover, did not present any cases of contamination [18]. Our result (71.42% of wells sterilized regularly but contaminated) could be justified by the excessively long time interval between the last sterilization of these wells and the samples taken.

Furthermore, we found that of the 20 water points sampled, 14 (70%) were located less than 15 meters from the latrines while the remaining 6 or 30% were built more than 15 meters away, which led us to believe that latrines would potentially be the main sources of contamination of the wells. This result corroborates with the studies conducted by several authors who revealed to us that the wells examined were heavily contaminated by bacteria. According to them, the contamination of all the wells could be due to inadequate construction of the wells, garbage dumping sites and various human activities around the wells. [19-21]. The water from these wells is generally not drinkable unless treatment is carried out. [22]

At the end of the analysis of the 20 water samples obtained, we were able to note that all the 04 boreholes examined were compliant with the total flora as well as with the TC and FC but of the 07 wells fitted out and regularly sterilized, 06 were non-compliant with the total flora while 05 were compliant with both the total flora and the TC and FC. Concerning the 9 wells fitted out and not disinfected, they were non-compliant with both the total flora and the TC and FC. The main germs found were Gram-negative bacilli of the Enterobacteriaceae family, the genera *Salmonella* (*Salmonella arizonae*, *Salmonella enterica* serotype), *Proteus* (*Proteus cholerais* or *Proteus mirabilis*) and *Escherichia* (*E. coli*). This information reveals a high contamination of the wells by fecal coliforms potentially dangerous for humans. These results corroborate with those obtained by Moussima *et al*. in their study conducted in Yaounde which revealed to us that Contrary to the recommendations of the Cameroonian standard, the indicators of fecal pollution and bacteria of the genus *Salmonella* were isolated in the water analyzed [5].

The presence of these coliforms in the water indicates contamination that could strongly be of fecal origin, which is probably due to the presence of latrines less than 15 meters from the developed wells; the failure to disinfect wells by the population also proves to be a probable source of contamination of the developed wells. Similar results were obtained in Yaoundé in the locality of Don Bosco in a study conducted by Fokou *et al* in which they justified the contamination of the various boreholes analyzed by the presence of latrines in 80% of cases near a borehole and the failure to disinfect the boreholes in 70% of cases. [6]

5. Conclusion

In the Messassi district of Yaoundé, 43.56% of households consume and use groundwater for their various needs. This groundwater, collected from boreholes and wells located less than 15m from latrines (70%), is generally contaminated with enterobacteria, in this case of the genus Salmonella (50%), Escherichia (30%) and Proteus (20%). The health hazards presenting a microbiological risk following consumption of groundwater in this district are significant and require intensification of the basic health protection measures recommended by national standard guidelines.

Abbreviations

E. coli	Escherichia Coli
EMB	Eosine Methylene Blue
TGEA	Tryptone Glucose Extract Agar
UFC	Unité Formant Colonie

Author Contributions

Lucien Honore Etame Sone: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing

Cédric Gueguim: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing

Yannick Zombo Otoulou: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology

Anne Christine Ndzana Abomo: Conceptualization, Formal Analysis, Investigation

Damase Serge Assiene Oyong: Conceptualization, Formal Analysis, Investigation

Marie Julie Manga Sipewa: Conceptualization, Formal Analysis, Investigation

Laurent Akono: Conceptualization, Formal Analysis, Investigation

Marius Noubi Feuzeu: Conceptualization, Formal Analysis, Investigation

Corneille Lawo Banga: Project administration, Validation

Jean Lagarde Betti: Project administration, Validation

Richard Ghogomu Tanwi: Project administration, Validation

Conflicts of Interest

The authors declare no conflicts of interest.

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Research Fields

Lucien Honore Etame Sone: Biochemistry, Imuunology, Microbiology

Cédric Gueguim: Microbiology, Biochemistry, Biotechnologie, Immunologie

Yannick Zombo Otoulou: Microbiology, pharmacology

Anne Christine Ndzana Abomo: Biochemistry, Imuunology, Microbiology

Damase Serge Assiene Oyong: Microbiology, pharmacology