Bacterial Examination of Wells of the Maâmora Tablecloth: A community based survey in Kenitra, Morocco

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Abstract—*Pollution of water resources is a global problem. Unfortunately, groundwater is more sensitive to contamination by industrial waste, agriculture, household waste and wastewater that lead to physicochemical (pesticides, heavy metals, nitrates) and biological (viruses, bacteria, parasites) pollution. Bacterial contaminations of water are responsible for the emergence of many serious epidemic diseases (Cholera, Typhoid, Tuberculosis etc). The purpose of this study is to find out the impact of farms intensification and untreated sewage discharges on the microbiological quality of Maâmora water wells. Initially, bacteriological analysis done to identify not only pathogenic faecal germs but also indicator germs which have the same origin and which indicate that the water has been in contact with feces. In a second step, these identified germs were counted. Germs included in this study were Escherichia coli and Streptococci. Analysis were done about total & faecal coliforms and faecal Streptococci from the raw water of 16 boreholes on the Maamora aquifer, Kenitra. Microbiological analysis of the waters of these studied boreholes were compared with WHO standards of potability i.e. Zero bacteria/100mL for Faecal Coliforms, Total Coliforms and Faecal Streptococci. This study reveal that there was more (33% to 42%) contamination in studied wells with total germs who attained 1 to 13 bacteria/1mL. These results calls for an improvement of the control water quality of the Maâmora and develop vigilance tools for decision-makers to managers.*

Keywords— Pollution of water, Bacterial contamination, Maâmora Tablecloth, Morocco.

I. INTRODUCTION

Many microorganisms, viruses, bacteria and protozoa, even fungi and algae are present in the water.[1] The anaerobic conditions generally encountered in groundwater. Bacteria, viruses, and other pathogens found in groundwater come from septic tanks, landfills, sewage spreaders, livestock, fermented materials, cemeteries, and surface water discharges.[2-4] These pollutions can also be due to leaks of pipes and sewers or the infiltration of surface waters.

The great majority of these harmful microorganisms, likely to generate formidable human infections. Drinking water must not contain pathogens, which could lead to biological contamination and cause an epidemic.[5-6] For this purpose, the National Office for Drinking Water (ONEP) and the Autonomous Board of Water and Electricity distribution (RAK) are responsible for the control of the bacteriological quality of water & intended for the production of drinking water for human consumption.[7]

The microbiological monitoring of drinking water concerns the following parameters: thermo-tolerant coliforms (*Escherichia coli*, *Enterobacter cloacae*, Salmonella, *Yersinia enterocolitica* etc), faecal streptococci (*Streptococcus bovis, S. equinus, S. gallolyticus, S. alactolyticus* and *Enterococcus faecalis, E. faecium*) and aerobic bacteria revivable at 22 °C and at 37 °C.[8-9].

The bacteriological analysis makes it possible to identify and to count the germs indicating the presence of pathogenic fecal germs. They indicate that the water has been in contact with feces. In this study *Escherichia coli,* and *Fecal Streptococii* were taken into account.

II. METHODOLOGY

A community based observational study was conducted by examination of 32 water samples of 16 wells selected from the set of wells of Maamora plateau of Moracco.

The Gharb plain corresponds to a large basin located in the downstream part of the Sebou basin. 80% of which is at an altitude of less than 20 m. It covers an area of about 4000 km^2 and is limited to the North and East by the rif wrinkles, to the West by the Atlantic Ocean, to the South by the region of Zemmour. The Maâmora plateau, which is part of the entire Sebou basin, with an area of 1890 km^2 is bounded in the south by the Meseta massif, in the north by the Gharb basin. The topography of the region is a succession of hills and valleys elongated parallel to the shore, the altitude is relatively low and does not exceed 250 m.

The Maamora tablecloth (Figure 1) can be subdivided into two slices. A slice with depths between 0 and 40 m which represents the vast majority of the water table. It corresponds to areas very easily accessible to exploitation. These areas are located mainly in the center, east and north of Maâmora at the level of the loading line of the water table. It is the same in the coastal zone, in the interdunal spaces and finally in the valleys of the river. These areas are interesting because they allow easy exploitation of the aquifer & they are nonetheless vulnerable areas. Current farms and fields are in fact largely in these areas. By way of example, mention may be made of the Taicha zone. The other section, where the depth of the aquifer is greater than 40 m, is generally in the southwestern part of the Maâmora where there is a protuberance with depths up to 100 m.

2.1 Method for sampling

Ensure that the container used once clogged should not open to ensure total protection against any contamination. For the purpose 500 ml, preferably borosilicate, glass bottles with emery clogging are used. Before use, these vials have been thoroughly washed and rinsed as there should be no trace of any detergent or antiseptic. They are then dried and then corked with cotton. It is recommended to affix a label allowing the identification of the sample to be entered later. The emery plug, intended for closure after sampling, is washed, rinsed, dried and then wrapped separately in a piece of filter paper. Add 1 ml of N/50 solution of sodium hyposulphite per liter of vial volume to the vials for the collection of water treated with chlorine or its derivatives. It is advantageous, for the convenience of subsequent manipulations, to use flat caps. The packaged cap and vial are then wrapped in filter paper and sterilized or autoclaved at 120 °C for 15 minutes.

2.2 Sampling of well pumps

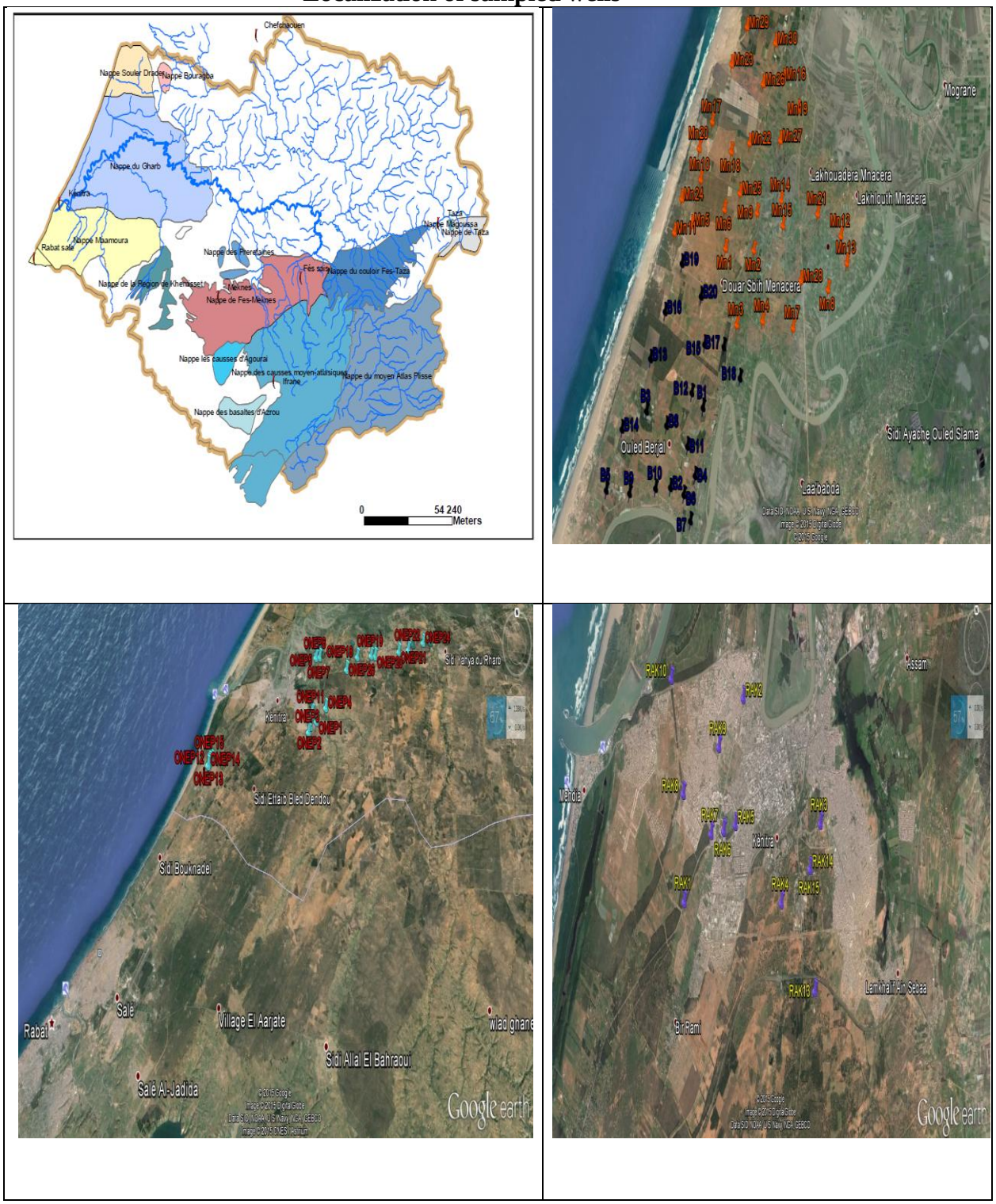
Before proceeding with the actual sampling, following steps should be carried out to ensure sterility:-

1. Wash your hands and forearms very thoroughly, rinse with alcohol, allow to dry;
2. Flame the pump for at least 1 minute using, for example, a portable butane gas torch;

3. Open the pump and let the water run for 3 to 5 minutes before taking the sample.

Once the sample has been collected, write on the label the indications necessary to identify the sample. Replace the bottle in its paper envelope, preferably protecting the cap and the neck. On an attached sheet, write down all the information you need to interpret the analysis. Introduce everything in a cooler.[10]

Figure 1
Localization of sampled wells



2.3 Transport and conservation in laboratory

The initial germ content of the water may be modified in the bottle after sampling also, so for this reason any analysis must be carried out as quickly as possible. Evolution is quite difficult to predict and depends on many factors: temperature, bacterial competition of species present, chemical composition of water.

Specimens for bacteriological analysis may be stored at a temperature between 0 and 4 °C for a maximum of 8 hours for treated water and 18 hours for raw water.[11]

2.4 Method for enumeration of bacteria

Concentration by filtration on membranes is the concentration technique most used in the microbiological laboratory. Most generally, filtration is carried out on cellulose ester membranes with a porosity of 0.22 μm or 0.45 μm, which can retain the bacteria.[12-13]

2.5 Coliform Count

Filter membrane (MF) method consists of filtering a given volume of the sample onto a membrane of 0.45 μm porosity which is deposited on a selective medium before incubation. The filter membrane method comprises two tests: the presumptive test and the confirmatory test (Moroccan Standard 03-7-003).[14]

2.6 Presumptive test

Dry in an oven at 37 °C the surface of medium Tertigitol-7, the Petri dish open, the middle down.

Seeding:

- The sterile filtration funnel is placed on the empty vial.
- The sterile filter membrane is placed aseptically on the porous disk at the base of the funnel.
- The assembly is tight and connected to a vacuum pump, a water pump or a simple hand pump.
- The sample is shaken vigorously 25 times and then 100 ml of the sample is filtered.
- The membrane is deposited on the tertigitol-7 medium with a flambé gripper captivating by its extreme edge, the surface that was in contact with the water upwards.
- No air bubbles should exist between the membrane and the culture medium.

Incubation: The Petri dishes are incubated in the inverted position:

- At 37 °C + 1 °C for 48 hours.
- At 44 °C + 0.5 °C for 48 hours.

2.7 Reading the results

In general, yellow or yellow-orange colonies with yellow halo are presumed coliforms. Some faecal Streptococci that develop into yellow yellow-halo colonies are easy to differentiate from those of coliforms because of their extreme small size.

2.8 Confirmatory test

The coliforms colonies presumed on Tergitol-7 medium are confirmed by the absence of oxidase and possibly by the fermentation of lactose in bright green medium at 37 °C and gram negative staining.

Isolation: Bacterial colonies are often the result of developing aggregates of cells of different species or genus. The study of the characteristics of its colonies can be significant only if one carries out the test on pure cultures obtained after isolation, if necessary on medium EMB it is the case for the study of the oxidase and the coloration of gram.

Incubation:

- At 37 °C + 1 °C for 48 hours.
- At 44 °C + 0.5 °C for 48 hours.

Reading and results: The bright green bile lactose broth tubes with bacterial growth, and a gaseous release of at least 1/10 of the volume of the gas bell are considered positive and taken into account for enumeration of coliforms.

2.9 Presumptive test for Streptococci count

Dry in an oven at 37 °C the middle surface Slanetz and Bartley, the Petri dish open, the middle down.[15]

Seeding:

- The sterile filtration funnel is placed on the empty vial.
- The sterile filter membrane is placed aseptically on the porous disk at the base of the funnel.
- The assembly is tight and connected to a vacuum pump, a water pump or a simple hand pump.
- The sample is shaken vigorously 25 times and then 100 ml of the sample is filtered.
- The membrane is deposited on the middle Slanetz and Bartley with a flambé gripper by captivating by its extreme edge, the surface which was in contact with the water upwards.
- No air bubbles should exist between the membrane and the culture medium.

Incubation: The Petri dishes are incubated in the inverted position: At 37 ° C + 1 °C for 48 hours.

Reading the results: Count all the red, purple or pink colonies, visible on the box resulting from the TTC reduction by faecal Streptococci

2.10 Confirmatory test for Streptococci:

Seeding: The suspected faecal Streptococci colonies are confirmed in Litsky's medium, transplanted with a sterile loop to up to 5 characteristic colonies, each in a tube containing Litsky medium.

Incubation: At 37 °C + 1 °C for 48 hours.

Reading and results: The appearance of a microbial disorder confirms the presence of faecal Streptococci; the culture with time, agglomerates at the bottom of the tube fixing the dye and forming a purple pellet of identical meaning.

2.11 Optional *Aerobic and Anaerobic Germs (Total Germs):*

The water is inoculated by incorporation in a strictly defined and nonselective medium. The method used is called incorporation, it consists of inoculating two series of Petri dishes (the volume of the sample to be analyzed is poured before the flow of the culture medium).

Seeding:

- 2 sets of 2 Petri dishes with a diameter of 100 mm per sample are prepared.
- Inoculated with 1ml of the sample to be analyzed in each box.
- The agar must be poured within 5 minutes after the preparation of the water to be analyzed in the Petri dishes.
- The contents of the boxes are gently stirred by a circular motion and a rocking motion to ensure a homogeneous mixture.
- Cool on a perfectly horizontal and cold surface.

Incubation: The reading is made after 48 hours of incubation at 37 °C or after 72 hours of incubation at 22 °C. This method, however, causes a thermal shock to the microorganisms at the time of incorporation of the agar undercooled (at 45 °C).

Reading and results: The reading is done on the boxes containing:

- 30 colonies at least if possible.
- 300 colonies at most.

Expression of results:

- Enumeration at 37 °C after 48 hours of the number of colonies / mL
- Enumeration at 22 °C after 72 hours of the number of colonies / mL

$$\text{UFC/100mL} = \frac{A}{B} x100$$

CFU: Unit Forming a Colony

A: total number of coliform colonies, counted on all membranes.

B: the sum of the quantities of filtered water expressed in mL

III. RESULTS

Total 32 borehole well water sample were examined in this study. It was found that out of 32 water sample 3 (9.38%) were completely safe other wise 29 (89.62%) sample were having any type of bacterial contamination. (Figure 2)

Out of these 32 water sampled, 12 were processed further to identified bacteria at 37^0C and 22^0C. It was found that on 37^0C and 22^0C, 58% and 67% respectively of well water sample was found to have contamination. (Figure 3 & 4).

Figure 2

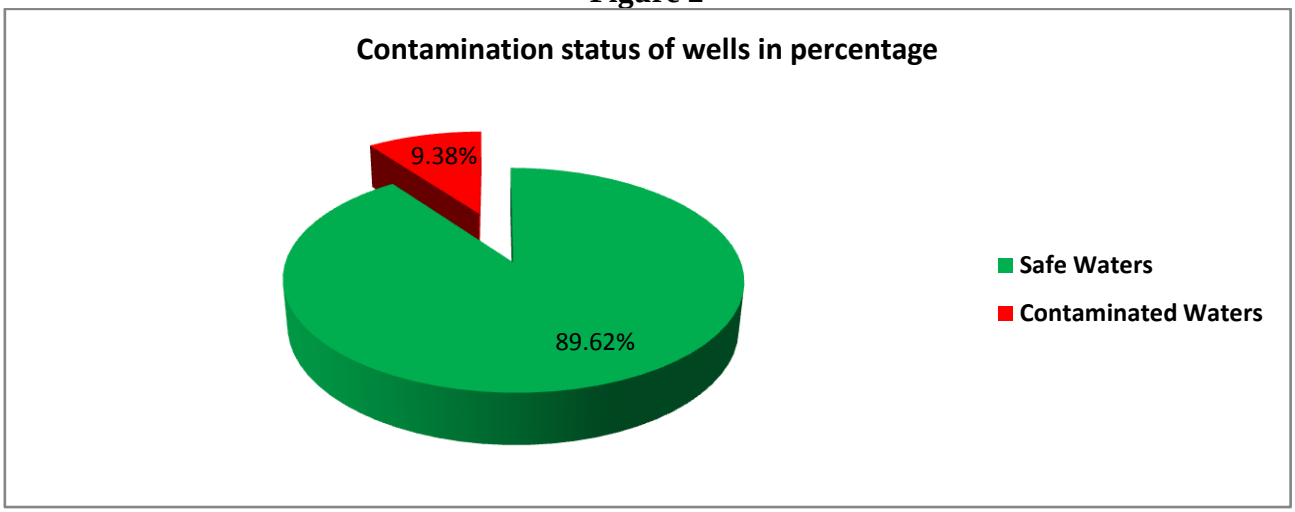


Figure 3

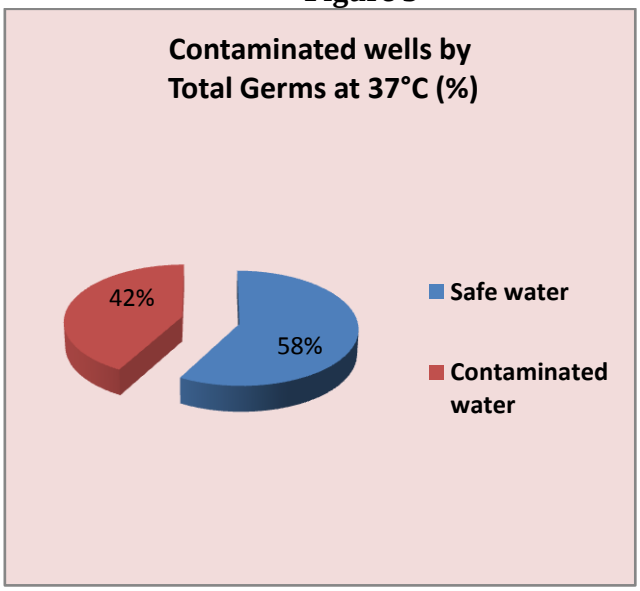


Figure 4

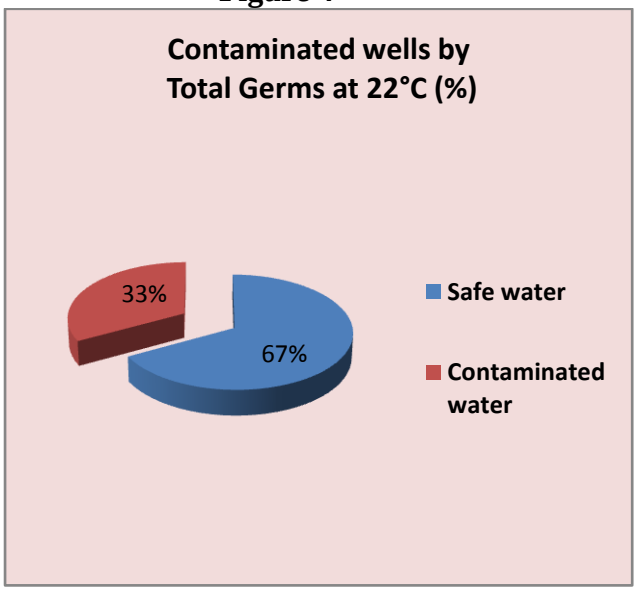


Table 1
Bacteriological examination of RAK drilling (Year 2011)

S. No.	Type of Bacteria	Contaminated Wells		Uncontaminated Wells	
		No.	%	No.	%
1	Total Germs at 22°C (N=12)	8	66.67	4	33.33
2	Total Germs at 37°C (N=12)	7	58.33	5	41.67
3	Total Coliforms (N=32)	0	0	32	100
4	Feacal Coliforms (N=32)	0	0	32	100
5	Faecal Streptococci at 37°C (N=32)	0	0	32	100

On further examination it was found that there is a complete absence of faecal Coliforms & streptococci in studies sample of ONEP borehole water in year 2010. But in year 2011, although Coliforms & streptococci were not present but other germs were there at 22 °C and 37 °C . There were 1 to 13 germs / mL were found in 8 of 12 (42%) wells and 7 of 12 (33%) in the ARK (Table 2 & 3).

Table 2
Bacteriological examination of RAK drilling (Year 2011)

S. No.	Type of Bacteria	Unit	F_3	F_4	F_{13}	F_{14}	F_{15}	F_{16}
1	**Total Coliforms**	UFC/100ml	0	0	0	0	0	0
2	**Faecal Coliforms**	UFC/100ml	0	0	0	0	0	0
3	**Total Germs at 22°C**	UFC/1ml	4	1	13	3	0	1
4	**Total Germs at 37°C**	UFC/1ml	1	4	9	0	0	6
5	**Faecal Streptococci at 37°C**	UFC/100ml	0	0	0	0	0	0

Table 3
Bacteriological examination of RAK drilling (Year 2013)

S. No.	Type of Bacteria	Unit	F_3	F_4	F_{13}	F_{14}	F_{15}	F_{16}
1	**Total Coliforms**	UFC/100ml	0	0	0	0	0	0
2	**Faecal Coliforms**	UFC/100ml	0	0	0	0	0	0
3	**Total Germs at 22°C**	UFC/1ml	1	2	0	0	0	3
4	**Total Germs at 37°C**	UFC/1ml	2	0	1	1	0	0
5	**Faecal Streptococci at 37°C**	UFC/100ml	0	0	0	0	0	0

IV. DISCUSSION

The evaluation of the bacteriological quality of the water from the boreholes made on the Maâmora aquifer is based on the search for control bacteria for faecal contaminations (sanitary effect) and bacteria indicative of the deterioration of the quality of the water at the sources. The study is based on the examination of 32 analyzes on 16 wells selected from the set of wells that have been monitored for bacteriological.

In this study, there was a absence of total coliforms from the water from the boreholes carried out by ONEP and RAK. This result is in line with Moroccan and WHO standards[14] (0 bacteria/100ml) for the quality of human drinking water. Likewise total coliform, there was a complete absence of faecal Streptococci from the water from the boreholes. This result is in line with Moroccan and WHO standards (0 bacteria / 100mL) for the quality of human food water. (Table 4 & 5)

Regarding the results of analyzes of the total germs at 22 °C and 37 °C we find that there is compliance with Moroccan standards (<20 germs / 1 mL or <100 germs / 1mL) because the maximum value of 20 germs / mL (or 100germs / mL) was not found in any of the 32 analyzes. But values of 1 to 13 germs / mL were found in 42% and 33% of samples in the ARK. In general, the counts obtained do not exceed the Moroccan standards of the quality of the human food water (100 germs / 1mL) and a treatment with chlorine is essential to make the water conform. In conclusion, the results of bacteriological analyzes comply with Moroccan standards of the quality of human food water, so it is deduced that the water of the boreholes studied has a very good bacteriological quality. (Table 4 & 5)

Table 4
Moroccan Standard for Bacteriological parameters with sanitary effect

S. No.	Type of Bacteria	VMA : Maximal Admitted Value
1	Total Germs at 22°C	100 bacteria/1 mL
2	Total Germs at 37°C	20 bacteria/1 mL
3	Total Coliforms	0 bacteria/100 mL
4	Feacal Coliforms	0 bacteria/100 mL
5	Faecal Streptococci at 37°C	0 bacteria/100 mL

Table 5
Bacteriological examination of RAK drilling (Year 2011)

S. No.	Type of Bacteria	Norms	Percentage of wells Contaminated
1	Total Germs at 22°C	100 bacteria/1 mL	66.67
2	Total Germs at 37°C	20 bacteria/1 mL	58.33
3	Total Coliforms	0 bacteria/100 mL	0
4	Feacal Coliforms	0 bacteria/100 mL	0
5	Faecal Streptococci at 37°C	0 bacteria/100 mL	0

V. CONCLUSION

According to the WHO and others authors, about 30000 people a day, about 10 million a year, die because of inadequate or poor water supply and deplorable hygiene conditions.[16-19] A bacteriological examination can only be validly interpreted if it is carried out on a sample taken correctly, in a vial present during the sampling. The sample should be stored in a cooler from 0 °C to 4 °C. The analysis must be carried out as soon as possible after sampling within a period not exceeding 24 hours, otherwise the sample must be fixed at the time of sampling by adding exactly 2 ml of a mercuric solution ($HgCl_2$) at 20g / L one litter of sample. TC and FC, FS are counted using the filter membrane method. The result of the bacteriological analyzes reveals the contamination of the raw water resulting from the Drilling carried out by the RAK by two types of bacteria: Total sprouts at 37 °C and Total germs at 22 °C. While the results found in treated water showed a complete abatement of the bacterial population at the level of ONEP boreholes. The present study revealed a high risk of contamination of unprotected wells up to 67%, contamination of water from open wells in contact with wind and other bacterial sources. There is an urgent need to ensure good closure and well sealing of wells to prevent the release of waste or the fall of animals or contamination by raw sewage from septic tanks. The present study should be supplemented by the search for other pathogenic bacteria[20-22] such as Staphylococci, Salmonellae, Vibrio and Chlostridium.

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CONFLICT OF INTEREST

None declared till now.

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