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Spring water quality in the area of Rabat-Sale-Zemour-Zaer (Morocco): microbiological indicators

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Abstract: The absence of a system of continuous monitoring of the quality of spring waters together with population's ignorance of the health risk related to the use of those waters in rural areas could expose users to waterborne diseases. The present study aims to evaluate the bacteriological quality of spring waters in the region of Rabat-Salé-Zemmour-Zaer during the period 2010-2011. The bacteriological analysis focused on 51 spring waters and was conducted according to ISO method for the detection of coliforms, intestinal enterococci and *E. coli*. The results showed that 89% of the samples analyzed do not meet drinking water standards of WHO, with a prevalence of 72.5%, 62.7%, 59.8% and 38.2% respectively for total coliforms, fecal coliforms, intestinal enterococci and *E. coli*. The absence of strain enterohemorrhagic *E. coli O157: H7* in all samples was noted. The important deterioration in spring water quality in the studied area is due to many factors: the mismanagement of waste, the shallow depth of the groundwater, the permeability of the aquifer, the distance from the water spring and the source of pollution and to a lesser extent, the development of the immediate surrounding areas in the region RSZZ. The need for a monitoring system is essential for the protection of spring waters and consequently the populations.

Key Words: spring water, bacteriological quality, Region Rabat-Salé-Zemmour-Zaer, Morocco

Introduction

Spring water, originated underground, is microbiologically safe due to its natural filtration within the aquifer. Reaching the surface, it could often be used without treatment (Festy *et al.*,

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2003). Spring waters are not sterile, but they are free of pathogenic microorganisms (Leclerc and Moreau, 2002). However, due to contamination by human activities it may carry several waterborne diseases (cholera, typhoid, dysentery, enteritis, hepatitis etc.). Indeed, specific studies have revealed frequent fecal contamination of groundwater in many agricultural areas in Morocco and point out that this pollution comes from the raw wastewater reuse in agricultural regions (Lyakhloufi *et al*., 1999; Bricha *et al*., 2007; Hassoune *et al*., 2010). Other studies have shown that the pollution of groundwater comes also from leachate of a uncontrolled landfill (Smahi *et al*., 2013) and direct discharges of wastewater into groundwater and surface water (Hmama *et al*., 1993, El Addouli *et al*., 2009).

However, the impact of domestic and industrial waste, without prior treatment, on the quality of superficial waters in the region of Rabat-Salé-Zemmour-Zaer (RSZZ) has been demonstrated by several studies (Cheggour *et al*., 2005; Tahri, 2005).

However no studies were interested in the quality of groundwater waters particularly in the large rural area like the RSZZ region, where there is a large number of spring waters used by the population.

For this reason, we decided to evaluate the bacteriological quality of the spring waters of RSZZ region, in order to measure the extent of the impact of anthropogenic activities on groundwater quality and to establish a spatial typology for possible actions to protect these resources. The bacteriological quality of spring waters was assessed using the WHO guideline (2004) on drinking water by looking for organisms such as: total coliforms, fecal coliforms, intestinal enterococci and *Escherichia coli*.

Material and methods

-Study sites and sampling stations

RSZZ region is characterized by a geographical diversity of habitats (oceanic coastal, plains, semi-continental and mountains areas (Fig. 1).

This prospective study was conducted during two hydrologically different campaigns, March 2010 and June 2011. It is based on bacteriological analysis of 102 samples from 51 spring waters in the region RSZZ. Indeed, the criteria of selection for equipped and unequipped spring waters are based on the continuity of the water flow in all seasons and the frequency of use by households. Equipped spring waters are defined as the ones that can be protected from the external pollution by constructing a surrounding fence.

The coordinates of each selected water spring were identified using a GPS Explorist 400. The area of study was divided into sub-regions: the sub-region of Zaer Sehoul, and sub-region of Tifelt Oulmès (Fig. 1 and Tab. 1).

For each spring water was assigned a unique code, composed of the letter S followed by a number indicating the spring water.

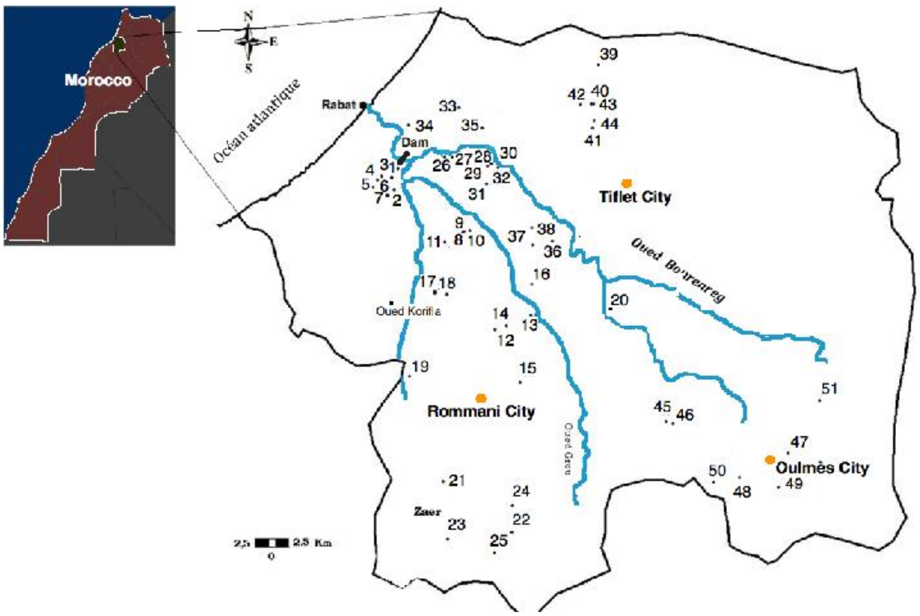

Figure 1: Geographical location of the springs.

Tab. 1: Distribution of sources analyzed by sub-region and type of development.

Sub-region	A	B	C	D
Zaer	Bouregreg	25 (From S1 to S25)	72	28
Sehoul	Bouregreg	13 (From S26 to S38)	53.8	46.2
Tifelt	Out of the watershed	6 (From S39 to S44)	100	0
Oulmès	Bouregreg	7(From S45 to S51)	85.7	14.3

A: Watershed, **B:** Number of springs analyzed, **C:** Percentage of springs equipped, **D:** Percentage of the number of unimproved springs

-Methods of analysis

Different water samples were collected in sterile 1L glass bottles and transported in a refrigerated chamber at 4 °C. They were analyzed (between 8 and 12 hours) in Laboratory of Microbiology and Environmental Health of the National Institute of Hygiene.

-Physicochemical analysis

The temperature of water springs was measured using a mercury thermometer graduated to 1/10 of a degree Celsius in situ.

pH measurements were made using a pH meter ORION Research, Ionalyser model 607 with model specific electrode ORION 91-05. The conductivity measurements (expressed in µS/cm) were made using a conductivity type YSI (model 33 SCT meter).

-Bacteriological analysis of water samples

The detection and enumeration of coliforms and intestinal enterococci bacteria in water samples were made by the methods ISO 9308-1 and ISO 7899-2 respectively.

Enumeration of total coliform (TC), fecal coliform (FC), intestinal enterococci (IE) and *E. coli* was performed by filtration of 100 ml of sample on a sterile cellulose ester membrane, with a porosity of 0.45 µm. Then the membrane is deposited on the selective culture medium. Culture of TC and FC is performed on Lactose Agar TTC (Triphenyl tetrazolium chloride 2.3.5) and Tergitol 7 agar that IE on Slanetz and Bartley and that *E. coli* on Chromagar coli. The cultures were incubated at 37 ± 1 °C for 24 hours, except for FC (thermotolerant bacteria) and *E. coli* which incubated at 44 ± 0.5 °C for 24 hours. The results were expressed as the number of colony forming units (CFU) per 100 ml of water. Identification of IE was based on the presence of a black color after culture on bile esculin azide medium (BEA) and *E. coli* the presence of a metallic luster after culture on the medium-Eosin Methylene Blue (EMB) and confirmation by the negative oxidase reaction but positive indole reaction.

Serological diagnosis was made by demonstration of serum antibodies against lipopolysaccharide (LPS) serogroup O157: H7 that has a predominance over other sero-groups: O26, O55, O91, O103, O111, O128 and O145 (Paradis, 1998; Bopp *et al*., 1999).

Results

-Physicochemical quality

Conductivity, temperature and pH are used to define the basic characteristics of water. Research contrasts these parameters between different measurement points facilitate the identification of polluted areas or different geology. Thus, the analysis of these parameters shows springs water temperatures ranging from 19 to 25 °C except spring 50 which recorded a temperature of 42 °C. The pH measured are generally in the range of standard and drinkability are close to neutral with values that range between 6.4 (S24) and 7.8 (S19, S18) except for springs S46, S22 , S50 and S23 which show pH 5.38, 5.5, 5.87 and 6.06 respectively.

However, though the temporal variability of the electrical conductivity is relatively stable, the spatial variations are very important and indicated a large diversity of lithologic facies of the region. Indeed, the measured values ranged from 91.7 (µS/cm) recorded at the source 46 and 2150 (µS/cm) recorded at the source 23. All values are in the range of potability standard required by the WHO and not exceeding 2700 (µS/cm) except source 18 which records a conductivity of 3800 (µS/cm) this may be due to the heavy pollution in emergence from the source.

-Bacteriological quality

Analysis of bacteriological quality was conducted in accordance with the required standard for drinking water by the WHO (2004). Indeed, it requires concentrations TC, FC, IE and *E. coli* less than 1 CFU /100 ml in water sources.

The compliance rate of water springs in the RSZZ region with the WHO standard on the bacteriological quality of drinking water are presented in Table 2. Thus, high levels of non-compliance were recorded for TC, FC and IE respectively 72.5% 62.7% and 60%. While *E. coli* is a non-compliance rate of 38.2% in both water sources managed as undeveloped.

-Total coliform (TC)

For the entire study area the TC ranged from a minimum of 0 CFU/100 ml to a maximum 28,000 CFU/100 ml (S18) which had a very high pollution at its emergence and the highest conductivity 3800 ms/cm. However, different regions showed significant spatial variability. Indeed, the region of Sehoul had values between 0 CFU/100ml (S33, S34, and S35) and to CFU /100ml 8000 (S36). In the Tifelt region, spring 39 had a high concentration of 4300 CFU/100 ml in 2011, other sources (S40, S41, S42, S43, S44) had a load of 0 CFU/100 ml. Similarly, Oulmès region had the lowest load of CFU /100ml TC ranging from 0 sources (S48, S49 and S50) to 100 CFU/100 ml (S47).

Tab. 2: Distribution of non-compliances with the WHO standard for contaminant studied.

Parameter	A	B	C
Total coliforms	102	74	72.5%
Fecal coliform	102	64	62.7%
E. coli	102	39	38.2%
Intestinal enterococci	102	61	60%

A: Total number of sampling point, **B:** Number of sampling points not conform, **C:** Rate of non conformity

-Fecal coliform (FC)

FC varies between a minimum of 0 CFU/100 ml to a maximum of 2000 CFU/100 ml increased in spring S21 (Oum Azza-Nkhila-Merchouch-Zhiliga region). Around Sehoul, the values of FC have values between 0 CFU/100 ml (S27, S29, S33, S34 and S35) and 1000 CFU/100 ml (S36). However, only S39 in the Tifelt region had a very high concentration (112 CFU/100 ml) in 2011. The lowest observed load was between 0 CFU/100 ml (S48, S49, S50 and S51) and 60 CFU/100 ml (S47) in the Oulmès region.

-Intestinal enterococci (IE)

Analysis of Intestinal enterococci by region showed that the Oum Azza-Nkhila-Merchouch-Zhiliga regions had the highest values at spring 6 (800 CFU/100 ml). Around the Sehoul locatity it was between 0 CFU/100 ml (S27, S29, S32, S33, S 34, S35 and S38) and 480 CFU/100 ml (S 36).

We also recorded a prevalence of *E. coli* more than 100 CFU/100 ml in 25% of springs in the region RSSZ (Tab. 3).

Tab. 3: Prevalence of *E. coli* in spring waters in the region RSZZ

Density of *E. coli* UFC/100ml	Prevalence %
Less than 100	75%
More than 100	25%

All spring waters with a higher density than 100 also have high contamination TC and CF. The improved water spring are also vulnerable to pollution by *E. coli* as unimproved.

Discussion

It appears from the results that the contamination of spring waters in the area study by TC, FC, *E. coli* and IE varies from one spring to another and from one sub-region to another. While some springs (n = 11) showed a decrease of bacterial contamination between the first and the second campaign, in others (n = 21) the increase of the contamination was found. High bacteriological pollution of equipped springs was also observed. This confirms that the equipped springs are vulnerable to pollution like unimproved spring.

Indeed, the construction of the spring, which is usually superficial, does not directly affect the quality of water flowing deep underground. Failure and lack of maintenance of the pipes very worn by time promote the formation of microbial biofilms (Heiermann and Thut, 2007) and thus could explain this contamination.

This bacterial contamination evaluated exceeds drinking water standards set by the WHO requires concentration TC, FC, IE and *E. coli* less than 1 CFU /100ml. This is certainly due to the polluted environment springs to their emergence.

Indeed, several threats with the origin of various pollutants affect the bacteriological quality of these springs; the increasing size of households, household activities (leaching near the emergence of water springs), and waste dumped directly around the emergence droppings of breeding animals. These unsanitary conditions recorded around the environment of these springs promote bacterial growth (especially micro-organisms of fecal origin) and groundwater contamination. The absence of a sewerage network and treatment system in the study area aggravates the situation by the presence of septic dug for this purpose without any protection measure outside houses.

In fact, the majority of water springs is proving a heavy fecal contamination. This observation is similar to those observed by Hassoune *et al*. (2010) and Bricha *et al*. (2007) on the impact of wastewater on groundwater, those of Nola *et al*. (1998), Djuikom *et al*. (2009) and Chippaux *et al*. (2002) on the bacteriological pollution of water springs and wells in Cameroon and Niger respectively.

Locally our study area showed a spatial contamination between sub-regions identified. In fact, the groundwater in the Zaer region and some familiar springs in the Sehoul area showed a high bacterial contamination compared to other sub-regions. This is due to frequent fetching water by the population, the use of these springs as watering livestock. However, it is worth noting that within this region some springs showed no trace of bacteriological pollution (S1, S9) despite of the presence of a polluted spring in the surrounding environment. The intervention of factors could explain this.

Indeed, the action of the water transfer through the surface layers of soil would be a real filter against microbial infiltration to groundwater and in cause with its adsorbing power of bacteria, contribute to the reduction of bacterial loads in the groundwater (Lyakhloufi *et al*., 1999). The structure and texture of the soil layers are important parameters in water protection of groundwater.

In areas of Tiflet (Zemour) and Oulmes, all springs show a very good bacteriological quality with even exploitable springs (Sidi Ali, Oulmes, Atlas) with perimeter protection around emergence. However, the open spring (S 39), semi equipped located in the sub-region Tiflet, has a very high fecal contamination probably due to the proximity of the aquifer surface and therefore the infiltration of pollution and temporarily due to construction works of the bridge in the vicinity (campaign 2011).

Pollution to the emergence of some springs allows enrichment of organic matter that makes soil favorable to the development of total coliforms which generally do not represent a direct threat to health (Edberg *et al*., 2000), but indicate the presence of organic contamination. By contrast, fecal coliforms, intestinal enterococci and *E. coli* are more valid indicators of risk (Zmirou *et al*., 1987; Edberg *et al*., 2000).

The analysis of different bacterial loads in all springs has highlighted the presence of a similarity between the intestinal enterococci and fecal coliforms in these waters.

Indeed, compared to coliforms (including *E. coli*), intestinal enterococci are more resistant to severe environmental conditions and persist longer in the water (Gleeson and Gray, 1997) such conditions are typical of groundwater are oligotrophic and where the temperature is generally cooler. Although intestinal enterococci are part of the normal flora of the human intestine, we showed an increased risk of developing gastroenteritis in their presence (Zmirou *et al*., 1987).

The proportion of springs contaminated with *E. coli* in this study (38%) is similar to capture private springs in England and Scotland (37%) (Anonymous, 1999). The presence of *E. coli* in water indicates not only recent contamination by fecal matter (Elmund *et al*., 1999), but also the possible presence of viruses (Payment and Locas, 2005). In addition, a study in Canada (Raina *et al*., 1999) was a link between the contamination by *E. coli* water and the occurrence of episodes of gastroenteritis.

Moreover, the strain *E. coli O157: H7* was detected in a large number of animal species (Beutin *et al*., 1996, Chapman *et al*., 1997, Wallace *et al*., 1997) but seems to be the main reservoir cattle without that it shows clinical signs of disease (Vernozy-Rozand *et al*., 2002). This explains the presence of *E. coli O157: H7* in all products derived from animal feces (manure and slurry), which is a potential source of contamination of soil and water, leading to outbreaks of *E. coli O157: H7*. In this study, the research of *E. coli O157: H7* in different spring analyzed has not revealed its presence. The absence of this strain can be explained by its low concentration and stress state due to low water pressure, all makes detection more difficult. In fact, the bacteria can be presented but non-cultivable (Chedad and Assobhei, 2007) even if the sources of contamination exist (slurry and manure) in the environment of the study area.

Conclusion

Monitoring and spatio-temporal analysis of several bacterial strains (The count of total coliform TC, fecal coliform FC, intestinal enterococci EI and *E. Coli*) in the different springs of the RSZZ region have shown a variation of bacterial contamination. However, the values measured and compared with international standards classified the majority of spring waters in the category of non-potable water with a percentage of 89% of non-compliance and expose the population to a health risk. The remaining 11% of the sources respond to drinking water standards of WHO.

The construction of spring waters alone is not enough to protect against a possible source of bacterial contamination. Indeed, it is important to note that improved spring waters like as unimproved spring waters are exposed to surface pollution (solid waste, animal excrement).

Our results confirm previous studies on the influence of bacterial contamination on the quality of spring waters and that is more important than the top of the water is near the surface, as land that overcome the aquifer are permeable and surface sources of pollution are important and close to the emergence of water.

However, it would be useful to monitor the possible occurrence of water-borne diseases among the rural population of the study area.

This study also demonstrated the need for the establishment of a system of surveillance and monitoring of water springs in the study area particularly those most threatened by human activities. This system should ensure the protection of the emergence of springs, maintenance of pipes furnished sources, control of bacteriological quality of the water and basic health education.

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