

# ASSESSMENT OF MICROBIAL CONTAMINATION OF GROUNDWATER IN OUALIDIA AREA, MOROCCO

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## Abstract

Analysis of the water used for drinking and agriculture is important to control the microbiological water quality. The presence of several fecal bacteria indicates water pollution and its health risk. Samples of 63 wells are analyzed with (32 wells in Douar Bakir, 23 wells Douar Ouled Lahlal, 4 wells in Dour Ouled Youssef, 1 well in Dour El Kahia and 6 sampling waters of the lagoon) by standardized methods for seven indicators. The principal component analysis is used to determine zones which have most high rate in contamination. The results show that the proportion is for viable microorganisms that are present in excess of the standard 95 % of the samples followed by total coliforms (92%), anaerobic sulfite reducing (78%), thermo tolerant coliforms (68%), E. coli (54%), enterococci (68%) and the presence of salmonella in a single well (4 CFU/51) .The principal components analysis (PCA) shows many wells which have high rate of contamination by zone. Our results indicate that the water of groundwater of Oualidia area is not suitable for drinking water in totality and agriculture uses in many wells according the Moroccan standard NM 03.7.001.

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**Keywords:** Epidemiology, waterborne diseases, Groundwater, Principal component analysis, Oualidia Morocco

## **Introduction**

Contamination of water by human activities is the primary cause of water-related diseases, therefore the lack of sanitation led to the contamination of water by feces. This contaminated water is the origin of epidemics [1]. The use of contaminated groundwater for food and cooking is one of the most disturbing and dangerous aspects for health [2].

In Oualidia, the groundwater is the principal source of water for tap water and irrigation. It moves slowly through the earth's crust, as well as pollution caused by human activities may persist for long periods ranging several years or decades for specific aquifers. The site of wells near the coast in contact with the lagoon and pressured by human activities like agriculture, tourism and aquaculture. From the other side the wells are located nearby neighborhoods is potentially threatened by infiltration of wastewater and other elements resulting from the excessive use of water fertilizer.

The humans pathogenic micro-organisms in water estimated by the coliform fecal indicator (FC) and the streptococci fecal [3,4]. The probability of enteric viruses and enteric protozoa presence in an untreated water is very high concentrated of thermo tolerant coliforms (including *E. coli*) [5, 6].

The Microbiological testing of water based primarily on the research of bacterial indicator of fecal contamination. These bacteria were not necessarily pathogenic but their presence indicates fecal contamination and epidemiological risks.

The aim of this work is to search for the microbial indicators of fecal contamination in groundwater determined by the Moroccan standard (NM 03.7.001). In sixty-three samples with (32 wells in Douar Bakir, 23 wells in Douar Ouled Lahlal, 4 wells Dour Ouled Youssef, 2 samples of lagoon water and 1 sample of the ocean in the area Oualidia.

## **Study area**

The coastal basin of Oualidia is a coastal inland basin. It is part of the Sahel Doukkala making himself part of the western Meseta of Morocco between El Jadida and Safi. It covers a surface area of approximately 250 km<sup>2</sup> and encompasses the Oualidia Lagoon.

The coastal basin Oualidia consists of two different areas morphologically and morph structural:

- An eastern sector: it forms inland, separated from the ocean by Oulja. The profile of coastal dunes imposes a characteristic morphology parallel to the coast.

- A West sector: formed of Oulja, generally consisting of a sand dune most recent consolidated at least locally fixed by vegetation. The Oualidia lagoon protected from the sea by a cord, isolated beach formed a monotonous major part by leaps resulting from dissolution of calcareous soils [7].



Figure 1 Morocco Map [8]

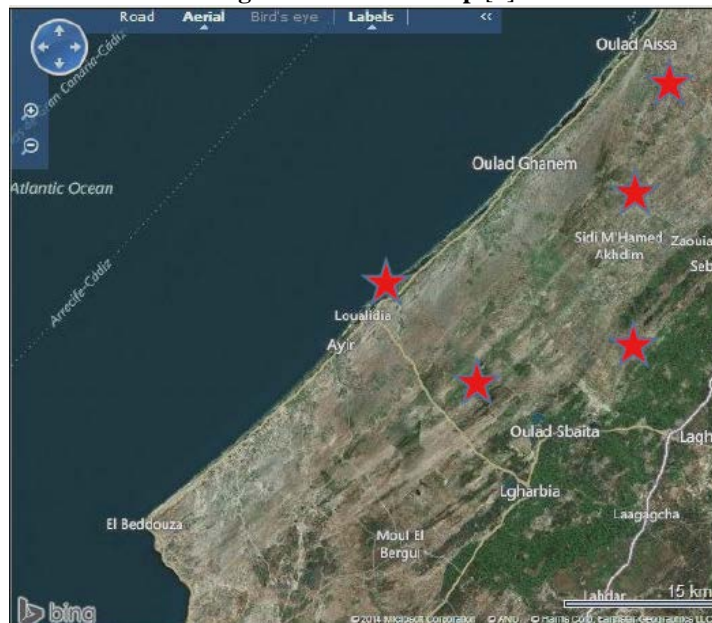


Figure 2 Sampling site ( ) in the Ouaidia area in the Douar (Bakir, Ouled Youssif, Ouled Lahlal, Elkahia) and the lagoon where water samples were collected.



## **Methods and materials**

### **Bacteriological analysis**

In this work we evaluated the degree of fecal bacteria contamination in groundwater Oualidia. There are 63 wells analyzed with (32 wells in Douar Bakir, 23 wells Douar Ouled Lahlal, 4 wells in Dour Ouled Youssef, 1 well in Dour El Kahia and 6 sampling waters of the lagoon). Enumeration of total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS) was performed according to the method of the most probable number (MPN) [9, 10]. This technique is to start a given sample in a number of tube and volume for the presence or absence of germ studied. The results are given by the tables Mac Grady for calculating MPN [11]. The samples are collected in glass bottles 500 ml previously autoclaved and then transported in a cooler at a temperature of 4 ° C to the laboratory of analysis.

The culture media used and incubation conditions : for coliforms , lauryl medium after incubation for 48 h at 37 ° C ( presumptive test) , the "positive" tubes ( lactose fermentation and gas production ) are transplanted to a confirmatory test in a selective medium containing bile salts BGBB ( lactose bile broth Brilliant Green ) incubated for 48 h at 37 ° C ( total coliforms (TC) and medium EC broth for fecal coliforms CF incubated for 24 h at 44 ° C . As for streptococci, their research is done on medium Rothe at 37 ° C for 48 h (presumptive test). From tubes Rothe positive dubbing is performed on Litsky medium at 37 ° C for 48h.

### **Typological analysis**

In order to characterize the pathological bacteria of fecal origin (CT, CF and SF) Well, we have established a bacteriological cluster by applying principal components Analysis (P.C.A) on bacteria germs indicator of fecal contamination: AM, CLF, THC, E Coli, ASR, and Enter). Recall that the statistic factorial method (P.C.A) to simplify the description of a table measuring difficult to handle, consisting of quantitative variables, from an observation plane space-time [12]. It is widely exploited in the field of biology [13]. For this analysis, we interpreted the wells that bacteriological analyzes around 63 samples. The software used for this analysis is SPSS v22 developed by IBM [14, 15, 16].

## **Results and Discussion**

### **Bacteriological analysis**

The present study was undertaken to investigate indicators of pathogenic bacteria associated with boreholes and shallow wells protected Oualidiah area. The results of the analyzes of the untreated ground water show a high prevalence of research that bacteria strongly exceeds tap water or irrigation water standards.

The depth of the wells tested is between 3 and 130 m. In theory, the depth of water is important because there is a great opportunity for contaminant attenuation when the depth of water increases through time longer and more contacts with potential displacement absorbing [17, 18]. With an average depth of 36 m the results show no relationship with depth. In ocean water near by the Douar analyzed, it is shown that, low temperatures (6 °C) favor the survival of CF in seawater [19]. Although the high salinities are lower rates of fecal coliforms in water [20].

The temperature is an essential parameter for the survival of the bacteria which is not examined in this study setting. If the temperature decreases with increasing depth, so that at low temperature, the bacterium limit its energy consumption by reducing metabolic activity, allowing it to survive much longer than at high temperatures. The correlation between the depth and the bacteria is negative except for coliforms at 36 ° C is positive but less influence as  $r = 0.05$ .

**Table 1 Max, Min, Moye. and standard deviations of bacteriological parameters analyzed**

	<b>N</b>	<b>Min</b>	<b>Max</b>	<b>Moyenne</b>	<b>Ecart type</b>
Depth (m)	55	3	130	36,19	32,79
Viable microorganisms at 22 °C (CFU/ml)	66	13	30000	8 458,00	11 162,69
Viable microorganisms at 36 °C (CFU/ml)	66	10	28000	6 531,82	9 472,65
Coliforms (CFU/100ml)	66	0	3600	256,55	481,35
Thermo tolerant coliforms (CFU/100ml)	66	0	500	24,11	66,02
E. coli (CFU/100ml)	66	0	100	13,06	25,88
Anaerobic sulfite reducing (CFU/20ml)	66	0	1200	66,29	170,38
Enterococci (CFU/100ml)	65	0	800	74,25	158,68

Aerobic microorganisms analyzed as indicators pathogenic bacteria required by the NM 03.7.001 in two different temperatures. At 22 ° C, the bacteria have a lower limit of 13 CFU / ml and up to  $3 \times 10^4$  CFU / ml. The criterion standard for water supply is 100 CFU / ml. In these results of 95.50 % about 63 wells exceeds the norm. At 36 ° C, the bacteria have a minimum of 10 CFU / ml and the largest is  $28 \times 10^3$  CFU / ml. The criterion standard for water supply is 20 CFU / ml. In these results of 95.45% about 63 wells exceeds the norm. In both temperatures 23 ° C and 36 ° C, there is the same positive percentage and the same number of contaminated wells.

Well contamination may reduce the potential benefits of water and has a high risk resulting from poor wellhead, such as poor sanitary seals the risk factors for the quality of water rather than the proximity of latrines [21]. Coliforms at 36 ° C have a minimum of 0 CFU/ml and a maximum of  $36 \times 10^2$  CFU/100ml. The criterion standard for water supply is 0 CFU / ml for coliforms at 36°C. There are 92.42 % wells exceeds Moroccan standard of water supply about 61 wells .There are 45.45 % of the wells

does not confirm the standard irrigation Moroccan NM 03.7.001 about 30 wells which the criterion is 1000 CFU/100ml . Low correlation with the depth coliforms at 36 °C therefore low temperatures prolong the survival of fecal coliforms [22, 23, 24]. Despite the unfavorable conditions for the development of bacteria, but there are several bacteria can survive and enter in VBNC states «Viable but non culturable bacteria." These bacteria take in a certain activity, but unable to multiply .This VBNC state has been demonstrated for indigenous bacteria and various enteric bacteria in the aquatic environment [25, 26].

The thermo tolerant coliforms at 44 ° C have a minimum of 0 UFC/100ml and a maximum of 500 CFU/100 ml. The criterion standard for water supply is 0 CFU / ml for coliforms at 36 ° C. There are 68.18 % of the wells exceeded the standard Moroccan water supply about 45 wells and all wells confirms the Moroccan standard irrigation NM 03.7.001 Whose standard is 1000 UFC/100ml.

The results indicate a decrease in bacterial numbers more important than during the study in the laboratory. The study showed that the microbial antagonists (protozoa) have an effect on the survival of fecal coliforms and the latter increases with depth. The comparative study of counting techniques used shows that abiotic and biotic factors have a bactericidal or bacteriostatic effect, which involves the translation of a no cultivable bacteria viable state allowing them to retain their pathogenicity [27].

There is a presence of E.Coli at 44 °C. These bacteria have a minimum of 0 UFC/100ml and a maximum 100 UFC/100 ml. The criterion standard for water supply is 0 CFU / ml for E. Coli. There are 54.55 % of the wells exceeded the standard Moroccan water about 36 wells. Samples studied contain fecal coliform type *Escherichia coli*. This shows that the water is subjected to microbiological pollution of human origin [28].

The geographical distribution of E. Coli. In the shallow ground water were related to the rate of recharge local groundwater, using groundwater as a proxy for the age of the shallow groundwater [29]. It can be carried by all in the lagoon, rivers, soil and underground water [30, 31]. The hydrological regime of the lagoon is linked to the tides, and water renewal is provided by the intake of seawater invades the entire lagoon at high tide [32].

Indeed, the nature of soil aquifer is an important factor for the filtration and in areas where the soil is cracked (granitic terrain, karst), surface water can pass off to the water table after a coarse filter that only allows not always a good clarification of the water, so a good retention of microorganisms All safety [33]. The soil of the Oualidia coastal basin is formed by consolidated Polio -Quaternary of age and sandstones, calcareous dunes. The rainfall is abundant and runoff erodes soil and deposited in rivers

and springs. During the period (1991-2005), the average monthly rainfall is 304.3 mm with a single wet season. In 1996, the station recorded 687.2 mm rain outstanding, the maximum rainfall is recorded in December (379.5 mm) [7]. Another source of contamination is the wastewater seepage from septic systems [34, 35] are the main continental inputs to the lagoon.

**Table 2 Frequencies and percentages of wells examined**

Parameter	Wells frequency	% Wells in interval	Total mean	% wells polluted according NM03.7.001 (tap water)	% wells polluted according NM03.7.001 (irrigation)
<b>Viable microorganisms at 22 °C</b>					
0-100	3	4,55			_____
100-1000	3	4,55	36,19	95,50%	
>100	60	90,91			
<b>Viable microorganisms at 36 °C</b>					
0-20	3	4,55			_____
20-1000	8	12,12	8 458	95,45%	
>1000	55	83,33			
<b>Coliforms</b>					
0	5	7,58			
0-100	30	45,45	256,55	92,42%	45,45%
100-1000	1	1,52			
>1000	30	45,45			
<b>Thermo tolerant coliforms</b>					
0	21	31,82			
0-100	44	66,67	24,11	68,18%	0
100-500	1	1,52			
<b>E. coli</b>					
0	30	45,45	13,06	54,55%	_____
0-100	36	54,55			
<b>Anaerobic sulfite reducing</b>					
0	14	21,21			
0-100	45	68,18	66,29	78,79%	_____
100-1200	7	10,61			
<b>Enterococci</b>					
0	13	19,70			
0-100	46	69,70	74,25	68,18%	_____
100-800	6	9,09			

\_\_\_\_\_ Parameter not required by the standard for the irrigation

Sulphite-reducing anaerobes at 36 °C are families Bacillaceae, gender Clostridia. These bacteria have a minimum of 0 CFU/20 ml and a maximum

1200 CFU/20 ml. The criterion standard for water supply is 0 CFU/20ml for these microorganisms. There are 78.79 % of the wells exceeded the standard Moroccan water about 52 wells. Enterococci, gender Clostridia has a minimum of 0 CFU/100ml and a maximum of 800 UFC/100 ml. The criterion standard for water supply is 0 UFC/100 ml for these microorganisms. There are 68.18 % of the wells exceeded the standard Moroccan about 52 wells.

For Salmonella, only one well contains 4 CFU/51 exceeding the supply and irrigation standard, which the criterion of 0 CFU/51 water standard. Retain a single carrier of typhoid fever can excrete up to 200 billion Salmonella typhosa per day [36]. The presence of pathogenic bacteria and several favorable environmental parameters are therefore the idea that areas of rapid recharge, egg unconfined sand that are not capped with an impermeable layer of clay [37].

### Principal component analyzes (P.C.A)

Analysis of the results shows that the majority of information is explained by the first three factorial axes (Tables 3 and 4, Figures 3). The contributions of the different parameters of the expression of the first three factorial axes C1, C2 and C3 are respectively 32,98%, 23,70% and 14,75%, for a total of 71,45% of the information explained.

The maximum of the total inertia is accumulated by the planes formed by the factorial axes  $C1 \times C2$  and  $C1 \times C3$ . Thus, the importance of biological parameters in factors C1, C2 and C3 is required. Table 4 shows the degree of contribution of seven bacteriological variables of inertia factor axes C1, C2 and C3, and plans of Figures 3 show the projection of these variables in plans  $C1 \times C2$  and  $C1 \times C3$ . It is therefore recommended to evaluate the significance of the cosine of the angle between the vector of this point and the projection plane of the variable to evaluate the quality of the projection of this variable on a given factorial axis or determine the correlation between the axis and the variable factor. Indeed, the higher the degree of correlation is close to unity (1) as the variable is related to the factorial axis. Conversely, the higher the correlation is close to 0 (zero), unless the variable is related to this axis.

**Table 3 Eigen values, contributions and percentages of inertia explained by the first three axes.**

Axis	Eigenvalues	Variance %	Cumulative %
C1	2,309279	32,989706	32,989706
C2	1,659203	23,702895	56,692601
C3	1,033026	14,757509	71,450110

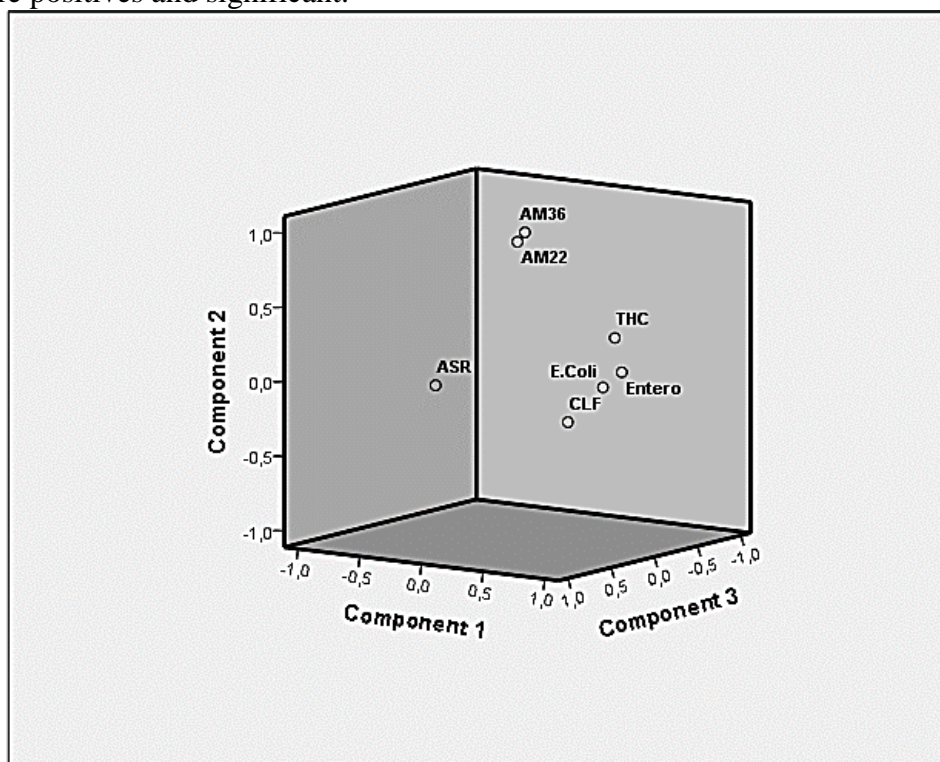


**Table 4 Degree of correlation between variables and different axes.**

Variable	Code	C1	C2	C3
Enterococci	Entero	0,830	0,097	-0,022
E. coli	E. Coli	0,807	0,019	0,164
Thermo tolerant coliforms	THC	0,679	0,293	-0,155
Coliforms	CLF	0,432	-0,271	0,035
Viable microorganisms at 36 °C	AM36	0,013	0,947	-0,067
Viable microorganisms at 22 °C	AM22	0,135	0,933	0,193
Anaerobic sulfite reducing	ASR	0,022	0,072	0,979

Analysis of the results (Tab. 4 and Fig. 3) shows that the variables that contribute most to the formation of the C1 axis are Enterococci (0,83%), E. coli(0,8%), Thermo tolerant coliforms (0,67) and Coliforms (0,43). This first component represents an increasing gradient of the negative side toward the positive side. For the C2 axis (Tab. 4 and Fig. 3) only two variables contribute significantly in its constitution, namely aerobic microorganisms at 36 °C (0,94%) and aerobic microorganisms at 22 °C (0,93%). This component represents a gradient increasing of the negative side to the positive side for all variables.

The variable which has a significant correlation with the axis C3 axis (Tab.4 and Fig. 3) is anaerobic sulfite reducing (0,979%). All correlations are positives and significant.

**Figure 3 Projection of the variables in the factorial plan C1 × C2 × C3**

The projection of "simples" (Fig. 4) on the C1XC2 plan shows a difference and high rate in zones:

- Douar Bakir : present high rates in CLF in wells(25,12,14) and in THC, Entero and E. coil in wells (23);
- Douar Ouled Lahla : present high rates in THC, E.coli and Entero in wells(47) and in AM22 and AM36 in wells (52,45);
- Oueld Youssef: present high rates in CLF in well (63) and in THC in well (60).

Moreover, the projection plane C1xC3 (FIG. 5), shows a classification of four groups:

- Douar Bakir : present high rate in ASR in well (20) ,AM22 in wells (31,60), CLF in wells (12,63,14) and E. coli in well (25);
- Douar Ouled Lahla : present high rates in E. coil and Entero in well (47) , THC in well (45);
- Douar Ouled Yossif: present high rates in CLF in well (63) and in THC in well (60).

The origin of this high bacterial contamination may be linked to the action of the infiltration of the lagoon which has the same levels of contamination or strong household activity (washing dishes, laundry, direct discharge of garbage, watering animal) that enriches the environment in organic matter and nutrients they are essential sources of nutrition for survival and multiplication of the bacteria where heavy contamination.

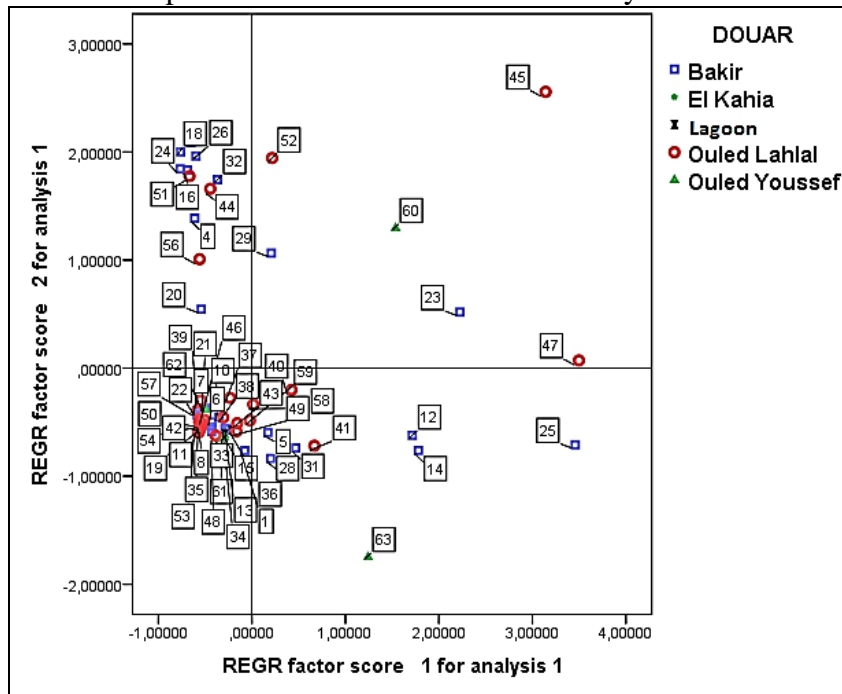


Figure 4 Projection of records in the factorial plan C1 × C2.

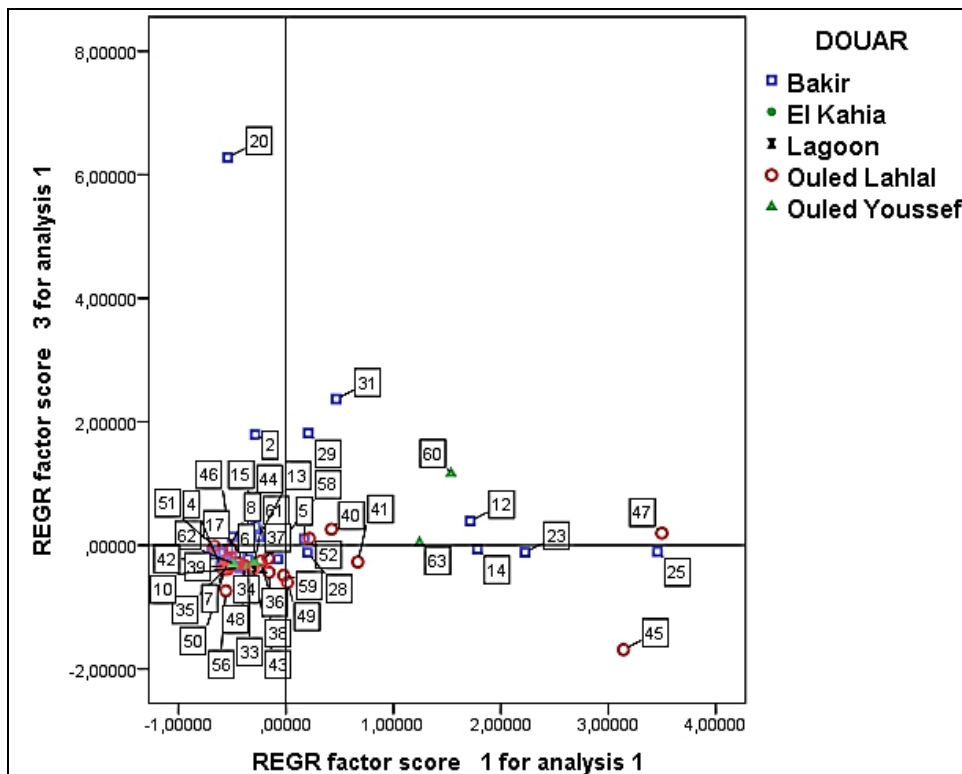


Figure 5 Projection of records in the factorial plan C1 × C3.

### Conclusion

Contamination by fecal germs and segues in groundwater has very high rate. The study found that well exceed the Moroccan standards NM 03.7.001 on power and irrigation water in the bacteriological parameters.

The anthropic action represents by household activities, discharge of liquid or solid waste at unprotected catchments, the position of latrines, water infiltration lagoon and the ocean seem to be the main sources of this bacteriological pollution.

The principal component analysis has determined many wells which have the highest contamination in three Douars: Bakir, Ouled Lahlal and Ouled Youssef.

It's recommended to do more studies on the nature of soil and rocks in the area to find out the source of the contamination, make known the utilization of these wells and survey in hygiene and good practices.

### Competing interests

All authors declare that they have no competing interest.

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