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RESEARCH OF BACTERIAL INDICATORS OF FAECAL CONTAMINATION IN WATER OF THE HASSAN II DAM (PROVINCE OF MIDELT, MOROCCO)

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ABSTRACT

To satisfy its water needs increasingly growing, Morocco has an important infrastructure of dams. However, demographic and economic expansion of Morocco could lead to a deterioration of the water quality of these artificial lakes. The Moulouya watershed is among the most important watersheds in Morocco, it contains to the present five major dams, among them the Hassan II dam, which became operational in 2005. This dam is one of the largest Moroccan reservoirs with a maximum storage capacity of 400 million cubic meters. However, increased human activities upstream of the dam associated with the phenomenon of erosion could lead to pollution of this water mass. The aim of our study was to assess the water quality of the reservoir through the search of bacterial indicators of fecal contamination, via a monthly followed, during the study period from September 2011 until August 2012.
Detection of fecal coliforms (CF) and fecal streptococci (SF) in the waters of the dam was a presumption of its contamination by the feces. With the exception of bottom waters that were loaded with spores of sulphite-reducing anaerobes (ASR), the waters of the other stations in the water column did not contain these spores. However, water levels in total coliforms (CT), CF and SF were qualified water quality of the dam as good to excellent, this according to the Moroccan standards of quality of surface waters.

Keywords: Hassan II dam, water, faecal pollution, Morocco

INTRODUCTION

Morocco's water resources are both limited and under pressure accompanied by an increasing deterioration of their quality (MATEE, 2001). This situation could be exacerbated by climate change announced for Morocco; the consequences could have adverse effects on the potential water resources, both in terms of quantity and quality (Alibou, 2002). The unpredictable weather and the irregularity of water intakes and allochthones materials combined with stagnant nature of lentic ecosystems are all factors responsible for the fragility of these bodies water to pollution (Chahlaoui 1990).

Located to the east of the country, Moulouya basin is characterized by a semi-arid to arid climate and limited water resources constitute the limiting factor of its socio-economic development (Melhaoui and Boudot, 2009). The basin contains to the present five major dams, among them the Hassan II dam, which became operational in 2005. After the partial siltation of the Mohammed V dam (Belguenani et al., 2002; Snoussi et al., 2002), the Hassan II dam currently ranks, viewpoint capacity, as the largest dam in the Moulouya basin with a maximum storage capacity of about 400 million cubic meters (Mm^3). It is a wealth of varied economic potentialities. This is an important reserve of water for the production of drinking water and irrigation. The dam also contributes to the protection of downstream areas against flooding, and it participates in the alleviation of the siltation of Mohammed V dam, located downstream (DHA, 2008). This lake is an attractive tourist environment for practitioners of sport fishing, emanating from the surrounding regions, and a refuge for migratory birds.

Increasing human activities developed upstream of the dam (Rahoui 2003; Lamri and Belghiti, 2011), associated with the phenomenon of erosion (Ahamrouni, 1996), could negatively affect the ecosystem and could lead to water pollution threatening, thus, its bacteriological quality. To our knowledge, this site is a virgin land that has never been subject to bacteriological studies, and this is what makes the originality of this work, which was spread over the period from September 2011 until August 2012. The aim was to assess the water quality of the reservoir through the search of bacterial indicators of fecal contamination.

MATERIALS AND METHODS

Presentation of the study areas

The Hassan II dam, which morphometric and hydrological characteristics are reported in Table 1, is situated in Midelt plain (South-East Region Meknes-Tafilalt in the center of Morocco) (Figure 1).

Table 1: Morphometric and hydrological characteristics of Hassan II dam (DAH, 2008)

Hydrological and general characteristics of the watershed and reservoir		Natural catchment basin area: 3300 km^2 Average annual intake: 220 Million m^3 (Mm^3).
Characteristics of the reservoir		Normal reservoir level: 1370 NGM (Level General of Morocco); Normal reservoir area: 12.7 km^2; Normal reservoir volume: 400 Mm^3; Mean of annual volume regularized 100 Mm^3.
Characteristics of the works	**Dam**	Type: weight of Betton Roller Compacted (BCR); Maximum height on Foundation: 115m; Crest length: 600 m; Crest width: 7 m; Land foundation: Granite; Level crest: 1375 NGM; Volume of the dam body: 600 000 m^3.
	Draining of bottom	Level of the threshold: 1298 NGM.
	Drinking water intakes	Type: concreted tower with four levels for taking water: 1323, 1333, 1343 and 1353 NGM; Maximum flow: 1m^3/s.
	Agricultural intake	level of taking: 1315 NGM

The dam is located in the high Moulouya characterized by a cold arid climate with mountainous tendency (El Hashimi et al., 2005). The rainfall regime is extremely variable and erratic. Stormy rainfall brings products eroded from upstream. Sometimes, the region receives snowfall.

The soils of the study area is distributed between soil on granite, soil on arkosic Triassic forms, and soils on Jurassic carbonate formations (Amrani et al., 2006). The scattered vegetation is represented mainly by tufts of alfa (*Stipa tenacissima L.*) and wormwood (*Artemisia herba alba*), which undergo the action of a constant and prolonged overgrazing (Rhanem, 2009). The implantation region of the dam is a pastoral activity area. Sheep farming is dominant.

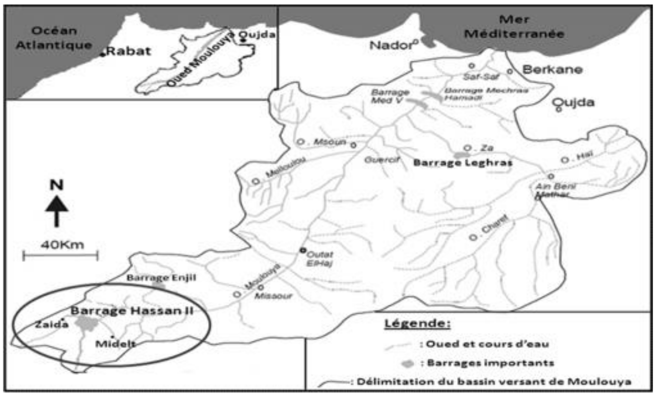


Figure 1: Geographical situation of the Hassan II dam in the Moulouya basin (Dakki et al, 2003.) (With modifications)

The catchment of the dam (Figure 2) is drained by two main tributaries: Moulouya and Ansegmir who receive, throughout their upper streams, domestic discharges of agglomerations: Boumia, Zaida and Ait Ayach (Rahoui 2003; Lamri and Belghyti , 2011). They also receive waste from agriculture developed in the valleys of the two wadis. In addition, the Moulouya drains water from the abandoned mining district of Zaida.

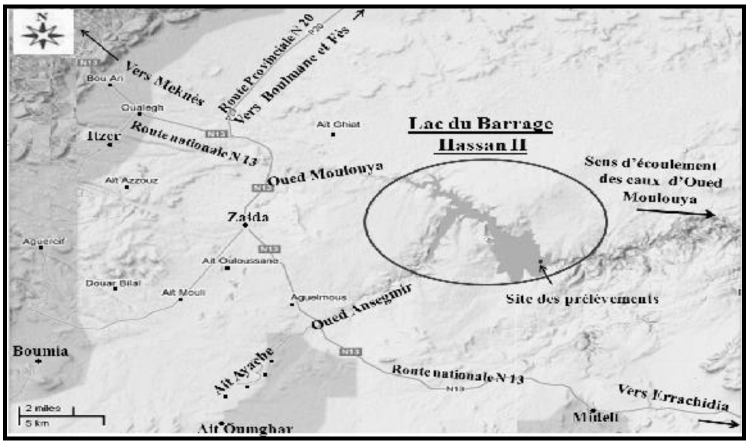


Figure 2: Localization of the sampling site in lake of the Hassan II reservoir (Source map: http://earth.google.fr, with modifications).

Choice of stations and sampling frequency

In limnology, the deepest point is, as standard, place of sampling a lake (Barbe, 2003). To determine the sampling point at the reservoir (Figure 2), and due to the absence of a bathymetric profile, we used dam's morphometric data

(Table 1) and dam's pictures just before it was filled. The sampling campaigns were held once a month during one year, so, 12 campaigns.
The selected stations are: Surface (S), 10m (P1), 20m (P2), 30m (P3), 50m (P5), 60m (P6) and the bottom (F). We note the absence of stations P5 in August and P6 in June, July and August; this was due to changes at level of lake of the dam following releases water whose the dam has been.
The choice of stations's depths to explore was the result of monitoring of the water temperature and dissolved oxygen, meter by meter, at the rate of twice a month for three consecutive months during the period of thermal stratification of the lake. Indeed, the temperature is considered as a determining factor that strongly influences the chemical and biological kinetics in lakes (Alaoui et al, 2000.). Dissolved oxygen is considered as the parameter most fundamental of the water quality (CCME, 1999).

Takings and analytical methods

The samples were taken according to standardized techniques: using a Van Dorn-shaped bottle (volume: five liters) for physico-chemical analysis, and sterile glass bottles (volume: 500 milliliters) for bacteriological analysis. The samples were stored at 4°C in cold boxes, and they were transported to the regional laboratory of epidemiological diagnosis and environmental hygiene of Meknes, in which we have made bacteriological analysis, according to the following standards: revivable microorganisms at 37°C (GT) [NM ISO 6222 (2007)], total coliforms (CT) and fecal coliform (CF) [NM ISO 9308-1 (2007)], fecal streptococci (SF) [NM ISO 7899-2 (2007)], spores of sulphite-reducing anaerobes (ASR) [NM ISO 6461-2 (2007)].
The other parameters were measured *in situ*, the temperature that was taken using an electro-thermometer. A measuring probe (HANNA Hi 8519N) available to assess the hydrogen potential (pH), and another probe (CONSORT K912) for measuring the electrical conductivity. Dissolved oxygen was also assayed on site, according to the Winkler method modified by Alsterberg (Rodier et al., 2009).

Data analysis

The comparison of means of physico-chemical parameters, at risk of error of 5%, was measured by t-test using SPSS software (version17.0). To determine the relationship between the parameters studied and distribution of stations, all measured variables were submitted to principal component analysis (PCA) used the Statistica software (version 9).

RESULTS

Descriptive analysis

Temperature

The temperature of the lake ranged from a low of 8.1°C at the bottom and a maximum of 24.3°C at the surface (Figure 3A). The means of temperatures showed a decreasing gradient from the surface to P6 and a slight temperature inversion occurred in the lake bottom. However, the comparison of means showed that, with an error probability of 5%, the temperature of the surface compared to the others stations, and the P1 compared to the others stations were significantly different ($p<0.05$). The temperature in P2 did not differ significantly with that in S, P1 and P3. The means of temperatures in P3, P5, P6 and F stations, compared them showed no significant difference ($p>0.05$).

Dissolved Oxygen

Throughout the entire water column, it is in the bottom of the lake where we find the lowest value of dissolved oxygen (4.97 mg / l). For others levels of the water column, the dissolved oxygen concentrations fluctuated between a minimum of 5.25 mg/l in P6, and a maximum of 12.66 mg/l in P3 (Figure 3B). However, the lake at all stations of the water column, had never come to an anoxic stage. It was very rarely that is lower oxygen levels below 5 mg/l. The evolution of the means dissolved oxygen (Figure 3B) showed an increasing gradient from S to P2, then a decreasing gradient from P2 to F. However, the comparison of means showed that, with a risk of error of 5%, we observed, a significant increase ($p<0.05$) from S to P1. The means of dissolved oxygen in bottom waters compared to those of water in S, P1, P2, P3 and P5 were significantly different ($p<0.05$). The means concentrations of dissolved oxygen in stations P1, P2 and P3, compared between them and means in P3, P5 and P6, compared between them, showed no significant difference ($p>0.05$).

Electrical conductivity

The maximum (534 µS/cm) and minimum (440 µS/cm) values of conductivity were recorded in P5 and surface respectively (Figure 3C). The evolution of the means of electrical conductivity of the water showed an increasing gradient from S to P3, then a reduction is signaled in P5, and then an increase occurred to the bottom.

However, comparison of means showed that, with a probability of error of 5%, the means of conductivity of the water at surface revealed a significant difference between it and those in P2, P3, P5 and F ($p<0.05$). The mean electrical conductivity of the water in P1 differed significantly only with P3 and P6. The means of electrical conductivity was not evolved significantly from P2 to F ($p>0.05$).

pH

The dam waters were slightly alkaline, values ranged generally between 7.06 and 7.53 (Figure 3D). However, no significant difference was revealed by comparing the means of pH of the water in different stations ($p>0.05$).

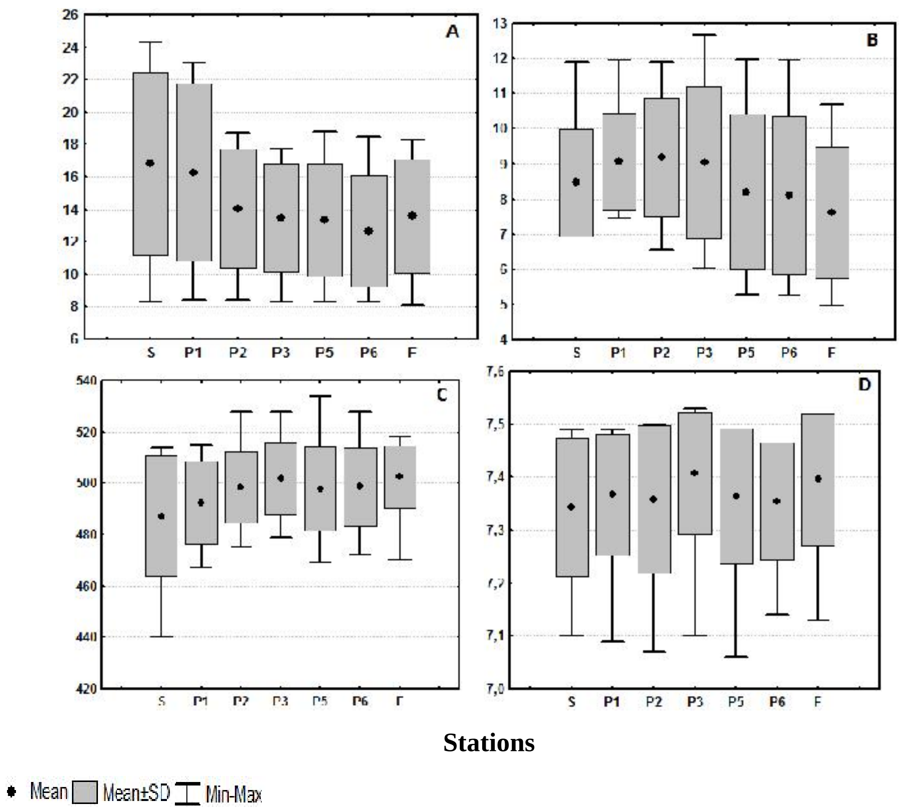


Figure 3: The evolution of values of: means ± standard deviations (Mean ± SD), maximum (Max) and minimum (Min) of the various physico-chemical parameters sought in the dam waters Hassan II during the study period: **A** (Temperature (°C)), **B** (dissolved oxygen (mg/l)), **C** (conductivity (µS/cm)), **D** (pH).

Bacteriology

The summary results of bacteriological analyzes are reported in Table 2. In terms of viable microorganisms at 37°C (GT), the results showed that the highest content was observed at the bottom of the lake with a mean of 109±47 colony forming units per milliliter (CFU/ml). For total coliforms (CT), the mean ranged from 12±21 CFU/100 ml at F to 22±28 CFU/100 ml at P5. However, the maximum value of 80 CFU/100 ml was observed in P5.
At the different stations of the water column, the number of fecal coliforms (CF), ranged from a minimum of 00 CFU/100 ml and a maximum of 4 CFU/100 ml in P2. The means contents of fecal streptococci (SF) ranged from 6±6.8 CFU/100 ml, as low mean in P6, and 24±39 CFU /100 ml, as high mean found in P5, however, a maximum concentration of 130 CFU/100 ml is reported in P5.
Apart from the bottom waters which showed content of spores of sulfite-reducing anaerobes (ASR), which ranged from a minimum of 6 CFU/100 ml and a maximum of 88 CFU/100 ml, the waters of others stations of column water were empty.

Table 2: Means (Men), standard deviations (SD), maxima (Max), and minima (Min) of germs sought in different stations of Hassan II dam during the study period.

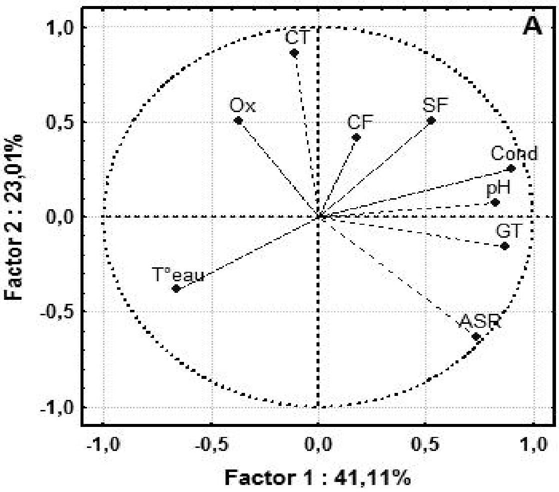
Stations		GT (UFC/ml)	CT (UFC/100ml)	CF (UFC/100ml)	SF (UFC/100ml)	ASR (UFC/100ml)
S	Men ± SD	72±57	15±20	0,67±1,07	9±16,4	00
	Max	170	52	03	46	00
	Min	03	00	00	00	00
P1	Men ± SD	75±48	13±15	0,08±0,29	15±23,6	00
	Max	143	40	01	64	00
	Min	06	00	00	00	00
P2	Men ± SD	95±62	17±19	0,75±1,54	19±27,1	00
	Max	210	43	04	90	00
	Min	18	00	00	00	00
P3	Men ± SD	92±57	17±18	0,75±1,06	19±29,7	00
	Max	188	48	03	108	00
	Min	08	00	00	00	00
P5	Men ± SD	74±47	22±28	0,36±0,5	24±39	00
	Max	144	80	01	130	00
	Min	13	00	00	01	00
P6	Men ± SD	72±44	17±23	0,22±0,67	6±6,8	00
	Max	161	70	02	20	00
	Min	24	00	00	00	00
F	Men ± SD	109±47	12±21	0,42±0,9	18±15,9	40±28
	Max	160	60	03	54	88
	Min	32	00	00	01	06

Explanatory Analysis

This analysis realized on an array of data from seven individuals (stations) and nine variables: water temperature (T°eau), dissolved oxygen (Ox), pH (pH), conductivity (Cond), GT, CT, CF, SF and ASR. The F1-F2 factorial plane allowed us to explain 64.12% of the total variability. The projection of the variables on the factorial plane F1-F2 (Figure 4A) showed that:
Electrical conductivity, pH, GT, ASR and SF were positively correlated with F1, unlike the temperature of water which was negatively associated with this axis. So this axis opposes most mineralized waters, characterized by the high values of pH, loaded in GT, SF and ASR, against waters with high temperatures. CT, CF and dissolved oxygen were positively correlated with axis F2, the positive pole of this axis characterized the oxygenated water with an important load in CT and CF.

The juxtaposition of the two F1-F2 factorial planes of the variables (Figure 4A) and stations (Figure 4B) showed that:

- Stations S and P1 were characterized by high temperatures and low values of pH.
- The important contamination in CT and CF was a characteristic of stations: P2, P3 and P5.
- The station F had the distinct characteristics of other stations in the reservoir: water with low temperatures, more mineralized, with high values of pH and rich in GT, SF and ASR.



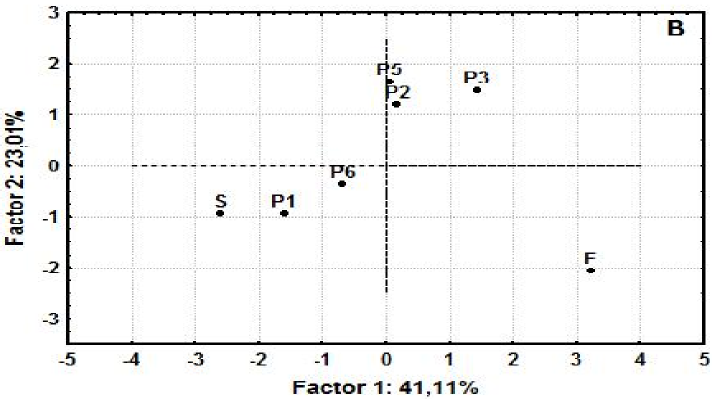


Figure 4: Principal Component Analysis of physico-chemical and bacteriological parameters: **(A)** Map of physico-chemical and bacteriological parameters: water temperature (T°eau), dissolved oxygen (Ox), pH (pH), conductivity (Cond), GT, SF, CT, CF and ASR.
(B) Map of stations.

DISCUSSION

The study of the thermal regime of the lake of Hassan II dam classified it in the category of hot monomictic lakes (Chahboune et al., 2013), however, the change in the means of temperatures gave a cool character to water of dam. That was an indicator of good health of the lake.

The good oxygenation of the water of Hassan II dam could be related to the different activities jointly undertaken by the water managers which include: frequent releases of the bottom waters and the seeding reservoir by silver carp (Chahboune et al., 2013), a species known by its algivore power (Foutlane et al., 1997).

Waters of Hassan II dam exhibited moderately accentuated mineralization (Rodier et al., 2009). Oxic conditions in bottom waters were presumably minimize fluctuations in the conductivity of the water. Indeed, anoxic conditions could cause a release of elements contained in sediments, these elements could then help to increase the quantity of salts and minerals dissolved in water (Tremblay et al., 2001).

Despite the granitic nature of foundation soil of dam, capable to act on the water by making it acid tendency (Nisbet et al., 1970), the pH of the water from the Hassan II dam remained slightly alkaline. This could be explained, probably, by the carbonate nature of the geological outcrops traversed by the waters of the tributaries feeding the dam (Igmoullan et al., 2001). According to the classification given by Nisbet and Verneaux (1970), the pH values were classified waters of the dam in the fourth class that corresponded to the waters close to neutrality, favorable to the development of living organisms and it is a class from limestone regions, favorable for fish farming.

Bacteriological contamination of water from the dam differed from one station to another. Detection of fecal coliforms and fecal streptococci in the dam waters presumed fecal contamination. This could be due to anthropogenic activities due to direct discharge of raw wastewater produced in common Zaida and Boumia in the Moulouya river (Rahoui 2003; Lamri and Belghiti, 2011), sanitations nonconforming or absent in the waterfront communities of the two rivers feeding the dam, as well as agricultural activities involving the land application or storage of manure on agricultural plots developed throughout the valleys of Moulouya and Ansegmir wadis in upstream of the dam, but it could also be linked to watering herds, mainly sheep, by the nomads of the region in the lake of the dam, as it could be natural, due to wild fauna, represented mainly by migratory birds that nesting in the dam.

The maximum concentration of CF 4UFC/100 ml appears to be low. This low content may not reflect the reality, due to the probable existence of germs viable but non-culturable. Bacteria surviving in this state are not detectable by classical cultural techniques used in this work. Indeed, many publications showed after their introduction in natural aquatic, fecal bacteria lose their ability quickly to grow in specific culture media used to count them but it retained much longer certain metabolic activities (Colwell et al. 1985; Grimes and Colwell, 1986; Roszak and Colwell 1987; Oliver, 2005). The presence of fecal coliforms, even in small numbers, should suspect the presence of all kinds of pathogens of intestinal origin, including the resistant forms which to fear are: viruses, cysts, spores and oocysts (Payment and Hartemann 1998).

In relation to the content of CF and SF, our results were not consistent with the observations made by Chahlaoui (1996) in Lake of Ait lamrabtiya dam (Morocco) and Hmaidi and Hmaidi (2009) in the Lakhal dam (Algeria), who found a low number of SF compared to the CF. Thus, the waters of different stations of Hassan II dam was characterized by a low concentration of fecal coliform compared to fecal streptococci. This result could be explained by variability in the length of survival of these germs in the aqueous environment, which is high for coliforms and very long for fecal streptococci (Leclercq, 2001).

Spores of ASR and CT are often considered indicative of faecal pollution, but it should nevertheless be noted that some of them can, on occasion, come from other habitats (Leclercq, 2001). Spores form of ASR is much more resistant than vegetative forms of fecal coliforms and fecal streptococci, and allowed to detect old or intermittent faecal pollution (Rodier 2009). Spores of ASR were found only in the bottom waters, this result was reasonable because the spores were from germs to strict anaerobic respiration. The results of analyzes of dissolved oxygen have revealed that the dam waters had never reached an anoxic stage, that did appear detection of ASR spores as illogical. However, repeated releases of dam's waters could favor the resuspension of spores

trapped in the bottom sediments of the lake, where anaerobic conditions are likely to be predominant.
The comparison of our results with those provided by the national grid defining the quality of surface waters (Official Bulletin N°. 5062 of 5 December 2002) supported the conclusion that the water quality is good to excellent, as: quality water in different stations of the water column was excellent if we consider only the contents of CF, and she was as good at just interpreting the concentration of SF. However, if we consider only content of CT, the water quality was excellent in P1, P2 and P3, and it was good in S, P5, P6 and F.
Staffing of dam by several levels of drinking water intakes provides to the manager's dam several alternatives to choose the appropriate level of water destined for the production of drinking water.

CONCLUSION

This research work is a first contribution to the search of bacterial indicators of fecal contamination in raw waters of Hassan II dam before they undergo the action of the treatment process. It follows that, these waters were characterized by a low load of bacteria indicatives of fecal contamination. The detection of CF and SF was a presumption of contamination of dam water by the feces whose origin could be anthropogenic or natural or a combination of both. The bottom waters were particularly loaded in spores of ASR. However the water contents in total coliforms, fecal coliforms and fecal streptococci were qualified water quality of the dam as good to excellent, this according to the Moroccan standards of quality of surface waters. However, the presence of fecal coliforms and fecal streptococci, must be feared the potential presence of pathogenic micro-organisms of all kinds.
Ignorance of self-purification power of Moulouya river, incites us to recommend the installation of treatment stations of wastewater in common Zaida and Boumia, which stood upstream of the dam, before they are discharged directly into the Moulouya river.

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