RESEARCH ARTICLE

CHARACTERIZATION OF BENTHIC MACROFAUNA AND WATER MICROBIOLOGY IN THE OURIKA REGION

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Manuscript Info	*Abstract*
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The current study was carried out at the level of the Ourika valley which is located 35 km from Marrakech and which is one of the most famous tourist sites in Morocco, in order to monitor the pollution status of the area and its impact on public health.Sampling was carried out seasonally at seven areas of the basin for the biological study (substrate samples) and at four areas for the microbiological study (water samples). The choice of areas is linked to accessibility and socio-economic activities near each area.The region is characterized by a fairly significant taxonomic richness (28 taxa identified, grouped into 6 major Orders: Ephemeroptera, Diptera, Plecoptera, Trichoptera, Coleoptera and Odonata), with less diversification in 2017 with the disappearance of five families (Hydrophylidae, Chaoboridae, Psychodidae, Ephydridae and Ephemerellidae), which could be due to the poor water quality recorded in certain areas according to the Normalized Global Biological Index, Biotic Index of New Caledonia and BioSedimentary Index.Regarding the bacteriological quality of groundwater intended for domestic consumption, the latter was found to contaminate with various faecal germs, with rates exceeding the standards with a maximum of 25x103 CFU / 100 mL for coliforms, 700 CFU / 100 mL for streptococci and 14 * 103 CFU / 20 mL for clostridiums.Following its results, good monitoring and management of resources is more than necessary to preserve biodiversity and the health of the local population.

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Introduction:-
The present work on the Ourika sub-basin, one of the ten sub-basins of the Haouz-Mejjate basin, fits into the framework of the Convention for the Integrated Management of Water Resources (IWRM).This project, launched by the Tensift Hydraulic Basin Agency, aims to present the state of water resources in the sub-basin and deduce the dysfunctions in order to put in place the conditions for the protection and safeguard of these resources for sustainable development.The Ourika Valley, located 35 km from Marrakech is considered to be one of Morocco's most famous tourist sites, known mainly for its agricultural, tourist and livestock activities.

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Following the famous flood which affected the watersheds of the High Atlas of Marrakech on August 17, 1995, attention was particularly drawn to this region with the need to study flood phenomena with a view to forecasting and protection. Since then, several studies have followed one another.

A biological study based on benthic macro-invertebrates and a microbiological approach to the region's wells would be relevant in order to understand, complete and establish an inventory of the waters of this region.

The assessment of the quality of an aquatic environment using biological methods is based on the application of a general principle according to which a given environment corresponds to a particular biocenosis **(Compagnat et al, 2000)**. Benthic macro-invertebrates which have the capacity to integrate these variations in the environment are then often used as potential indicators of the faunistic response to these disturbances **(Archaimbault, 2004)**.

Bacteria are ubiquitous in nature, they are found in all environments; air, soil, water and even on other living things. In humans they are then said to be commensal. They can be part of the cutaneous, digestive, oral, genital flora. The objective of bacteriological analysis is to find species that are likely to be pathogenic, or what is often easier, those that accompany them and which are in greater numbers present in the intestines of mammals **(Rodier et al. 2009)**.

Material and Methods:-

In order to follow the spatio-temporal evolution of benthic populations, 8 substrate samples are taken per station, along the Ouad Ourika at the level of the zones (O1, O2…O7),once per season during the years 2016-2017**(Fig 1)**.Quantitative samples were taken using a "Surber" net (0.3 mm mesh), in different biotopes. In the field, the collected macro-invertebrates are fixed immediately with 10% formalin. Once transmitted to the laboratory, the identification of the fauna is done under a binocular magnifying glass up to the family, and for some to the genus according to the identification guide **(Tachet et al, 2010)**.As macro-invertebrates are not determined at the same level, we therefore speak of a "taxon" **(Huet, 2014)**.

Regarding the microbiological study, the water samples were taken from the zones (S1, S2, S3 and S4) **(Figure 1)**, once per season during 2016 and 2017. Specialized bottles previously rinsed and sterilized by autoclaving at 120 ° C for 20 minutes have been dedicated for this use. The methods used for the various bacteria count tests are those described by **(Rodier et al, 2009)**.
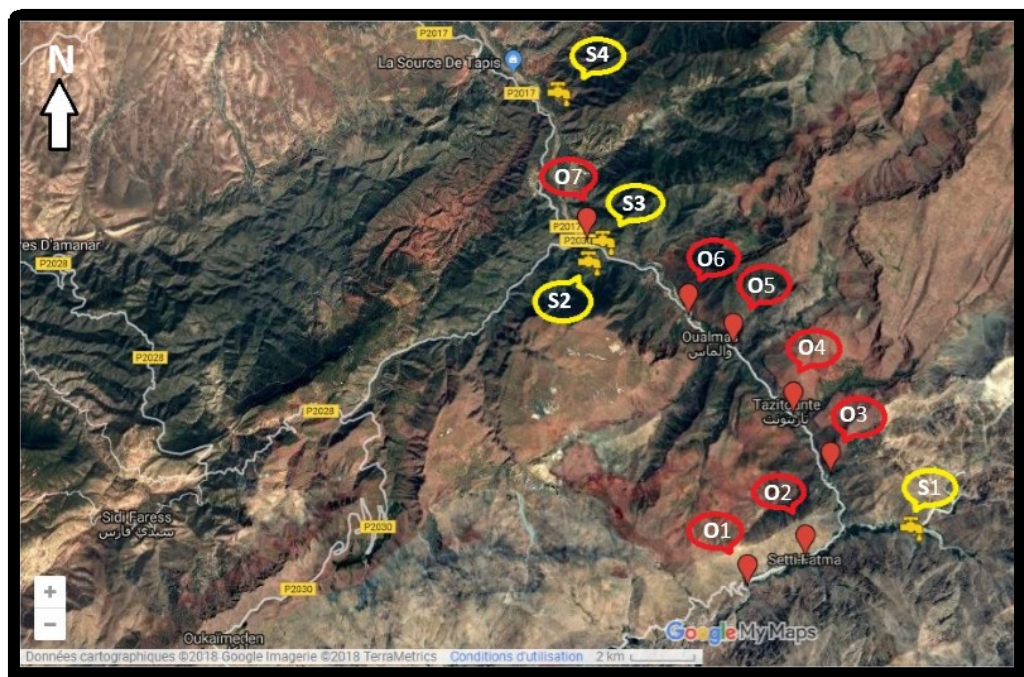

Figure 1:- Sampling points for well and surface water.

Results:-
The sorting and identification of benthic species allows the calculation of a set of biological indices. Indeed, the latter are used to assess the overall health of aquatic ecosystems, given that these living organisms integrate, in time and space, all the characteristics of their habitat.

Taxonomic richness:
It represents the number of taxa present in a sample. This wealth reflects the health of the community. High taxonomic richness is usually indicative of the good health of a river **(Moisan; Pelletier, 2013)**.

All the taxa identified during the two years 2016 and 2017 are represented in the following graphs:
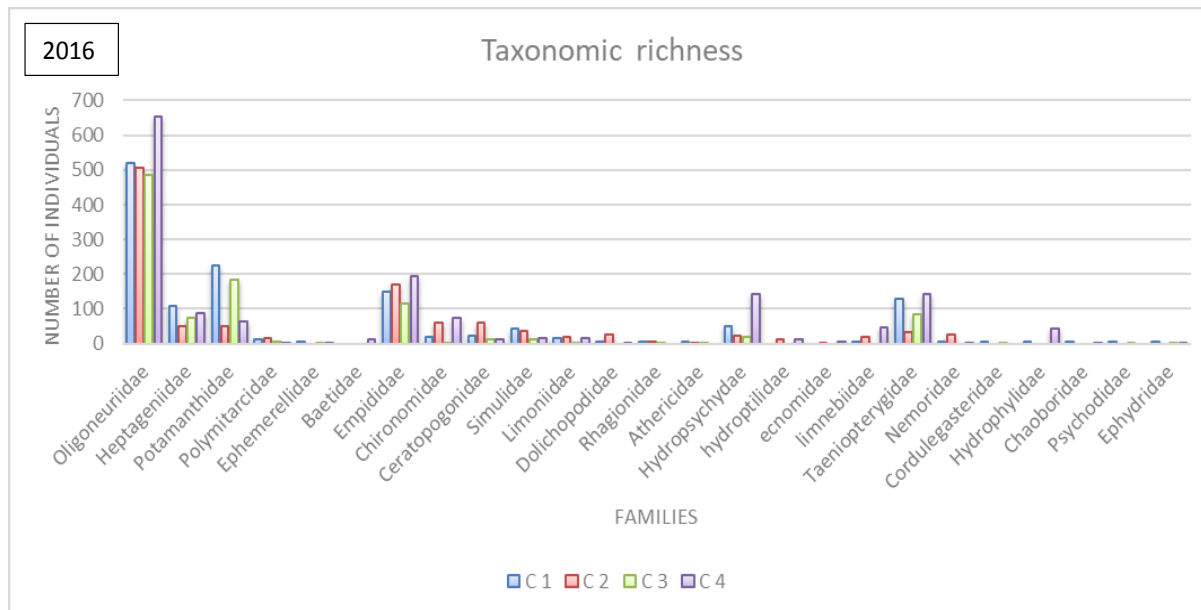

Figure 2:- Graphic representation of all the taxa identified during 2016.
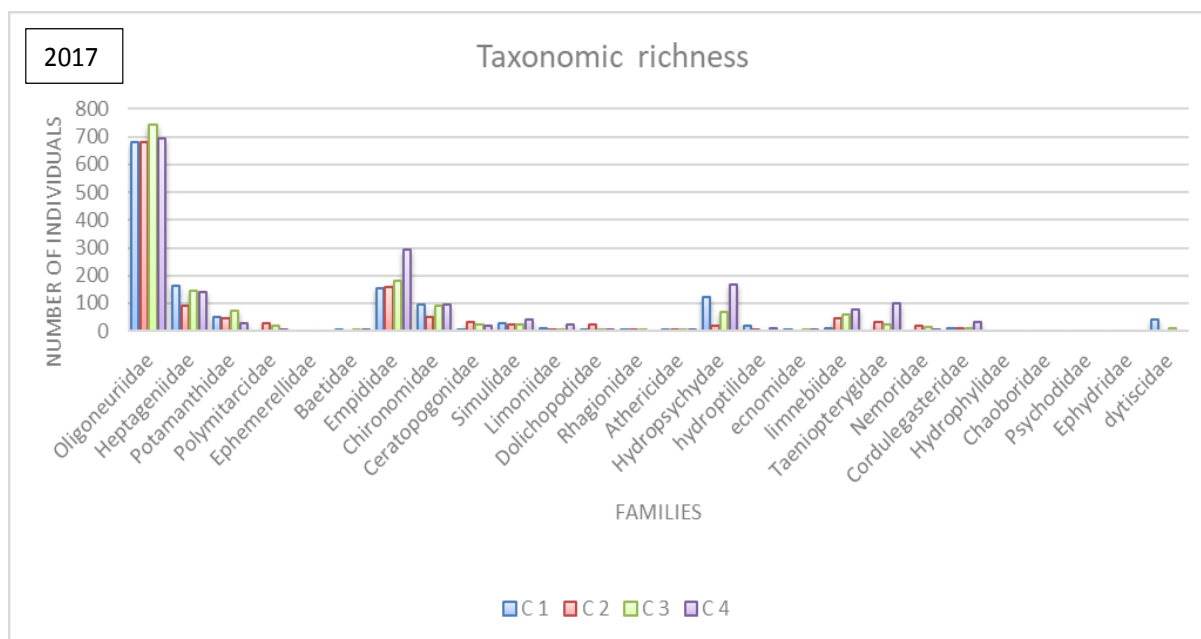

Figure 3:- Graphic representation of all the taxa identified during the year 2017.

The year 2017 was slightly less diverse than the year 2016 with the disappearance of five families (Hydrophylidae, Chaoboridae, Psychodidae, Ephydridae and Ephemerellidae), however it saw a greater number of individuals across all the taxa (5902 individuals in 2017 compared to 5,032 individuals in 2016).

Normalized Global Biological Index (IBGN):
Based on the benthic macro-invertebrates, the standardized global biological index allows a score from 0 to 20 to be calculated depending on the indicator faunal group and faunistic diversity.Which gives us an idea of the general quality of a river **(Rodier et al, 2009)**.
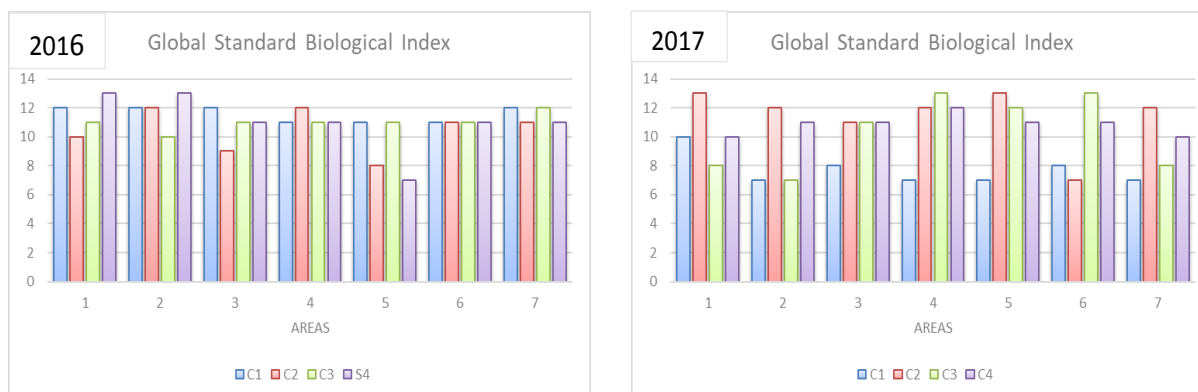

Figure 4:- Results of the normalized global biological index, during the four seasons of the years 2016 and 2017.

In 2016, the Ourika region is generally characterized by average water quality during the four seasons and at the level of the majority of the zones, with the exception of the O5 zone which knew a degradation in Winter and in Summer in revealing poor quality water. As well as the O1 and O2 zones which showed good quality water in summer.

In 2017, the Ourika region recorded a deterioration in water quality during the Autumn from average to poor in all areas. During Winter and Spring the water quality improved on average in most areas except for O6 in Winter and O2 in Spring. However, all areas showed average water quality during the summer.

Biotic Index of New Caledonia (IBNC):
The IBNC Index is based on a list of 66 indicator taxa. Each taxon has a score between 1 and 10, depending on their sensitivity to 8 indicators (chlorides, sulphates, sodium, potassium, ammonium, phosphates, MES, BOD5). The interest of this index is to detect organic pollution generated by domestic effluents, livestock, etc **(Mary, 2015)** **(Huet, 2014)**.
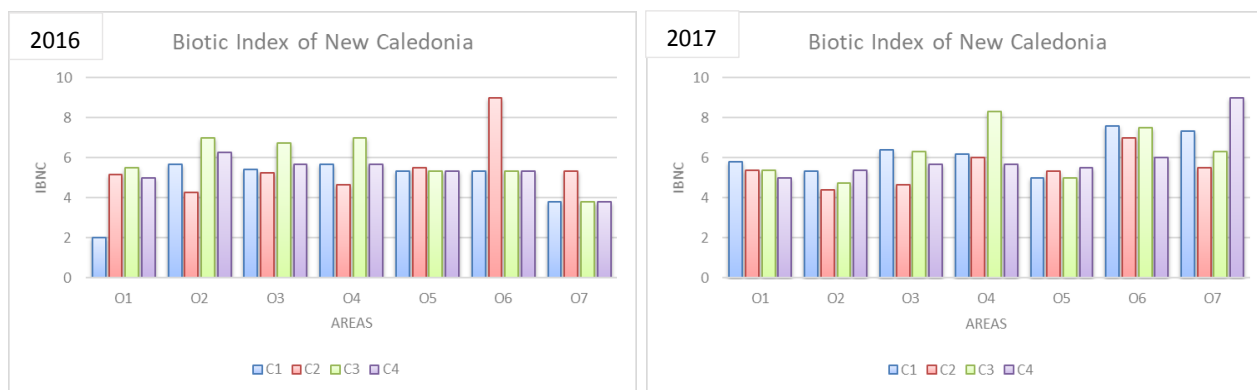

Figure 5:- Results of the Biotic Index of New Caledonia, during the four seasons of 2016 and 2017

For the year 2016, apart from the zone O1 in Autumn, O2 in Winter and O7 in Autumn, Spring and Summer which present a poor quality, the year in general is characterized by a fair to good quality which varies slightly during the seasons.

Likewise for the year 2017, only certain areas such as O2 in Winter and Spring and O3 in Winter which presented a mediocre quality, the rest of the areas generally present a good to very good quality, varying a little during the seasons but which remain within the standards.

BioSedimentary Index (IBS):
This index, varying from 1 to 10, is based on a list of 56 pollution indicator taxa. The interest of this index is to detect sediment-type pollution, in particular pollution with fine particles from lateritic soils. This index was developed in order to highlight the degradation of the quality of the watercourse linked to the transport of suspended matter such as sands, silts and clays**(Mary, 2015) (Huet, 2014)**.
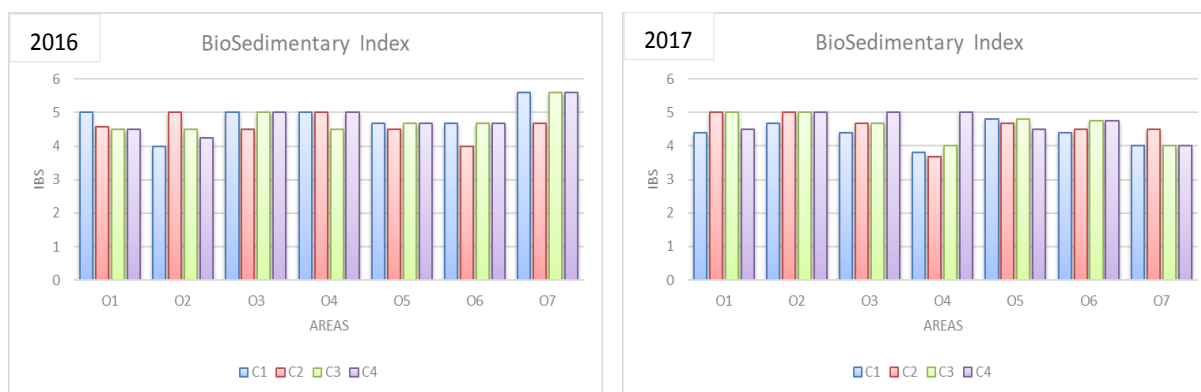

Figure 6:-Results of the BioSedimentary Index, during the four seasons of 2016 and 2017

For the year 2016, the O7 zone is the only one which presents a good quality recorded during the three seasons Autumn,Spring and Summer. The other areas have poor to fair ecological quality over all seasons

While in 2017, the ecological quality of the watercourse is characterized as being poor to fair in all areas and throughout the year, or even poor as in the case of areas O3 and O7.

Hilsenhoff biotic index (HBI, FBI ou FBIv):
A community strongly dominated by a few taxa may indicate the presence of stress. Variables or indices based on the degree of tolerance to organic pollution are frequently used (% of EPT,% of EPT without Hydropsychidae,% of tolerant organisms, etc.) **(Hilsenhoff, 1987) (Hilsenhoff, 1988) (Bode et al, 1996) (Bode et al, 2002)**.

The Hilsenhoff Index (HBI) takes into account the tolerance ratings of each of the organisms in the community. Odds adapted to the family (level 2) **(Moisan; Pelletier, 2013)**.
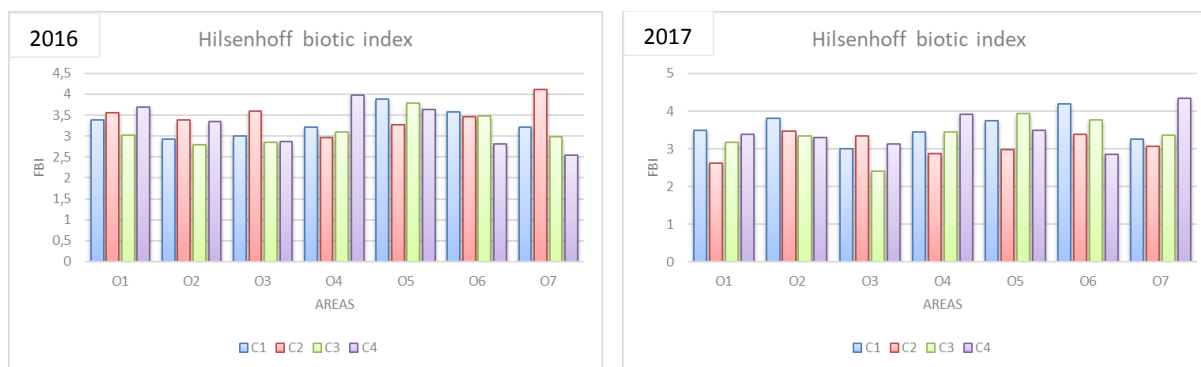

Figure 7:- Results of the Hilsenhoff biotic index, during the four seasons of 2016 and 2017.

The Hilsenhoff Biotic Index based on the Family Tolerance Score indicates excellent to very good quality over all study areas and over the two years, with slight variation from season to season.

Even if this index indicates the presence of a very low organic pollution, all the taxa identified, their distribution as well as the abundance of certain taxa compared to others clearly indicates the presence of a stress in the zone of 'study.

Enumeration of total germs:
Facultative aerobic and aero-anaerobic microorganisms grow in a defined, non-selective nutrient agar medium incubated at 37 ° C for 24 hours. They appear as colonies of different sizes and shapes **(Manceur; Djaballah, 2016)**.

The count of total germs which is expressed in CFU / mL "colony forming unit" is often used as an indicator of pollution. Our results show the presence of germs in all areas, ranging from 20 CFU / mL recorded in zone S1 during winter, up to 45,000 CFU / mL in zone S3 in autumn.
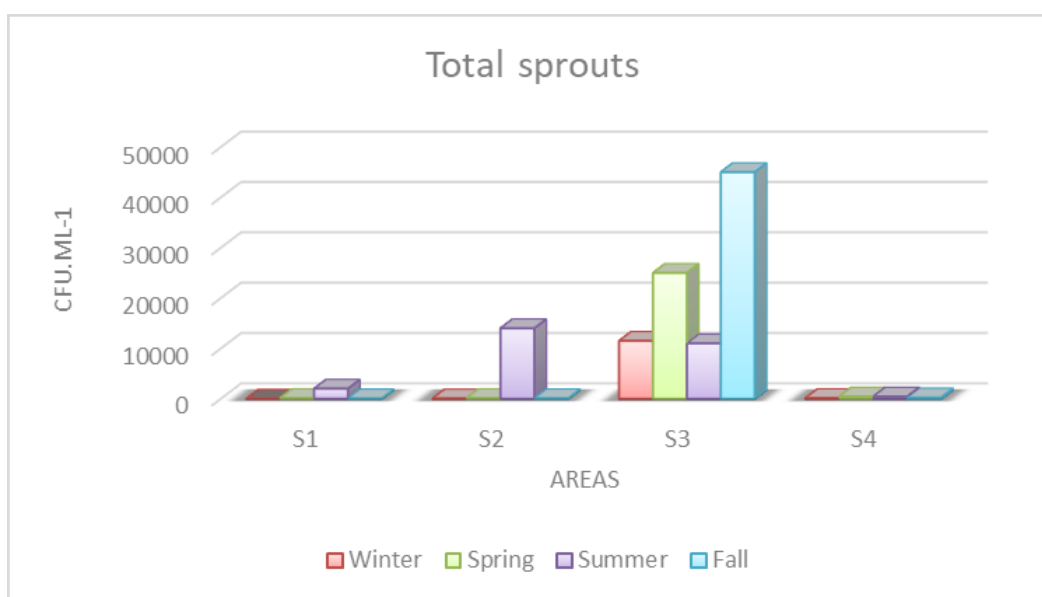

Figure 8:-Variation of Total Germs in Spring Waters

Enumeration of coliforms:
The coliform count was carried out on a Macconkey agar culture medium (selective medium for the isolation of enterobacteria).

The reading is generally done after 24 hours at 37 ° C under aerobic conditions, giving rise to:
1. _Red (pink) colonies: acidification of the medium, lactose strain +.
2. _Colorless colonies: absence of acidification of the medium, lactose strain -.

Our results show values between 0 CFU / 100 mL in the rainy period and 25x103 CFU / 100 mL in the dry period. These contents greatly exceed **(Normes marocaines, 2006)**(0 CFU / 100 mL). This contamination is generally due to the infiltration of surface water in wells, or by domestic waste.
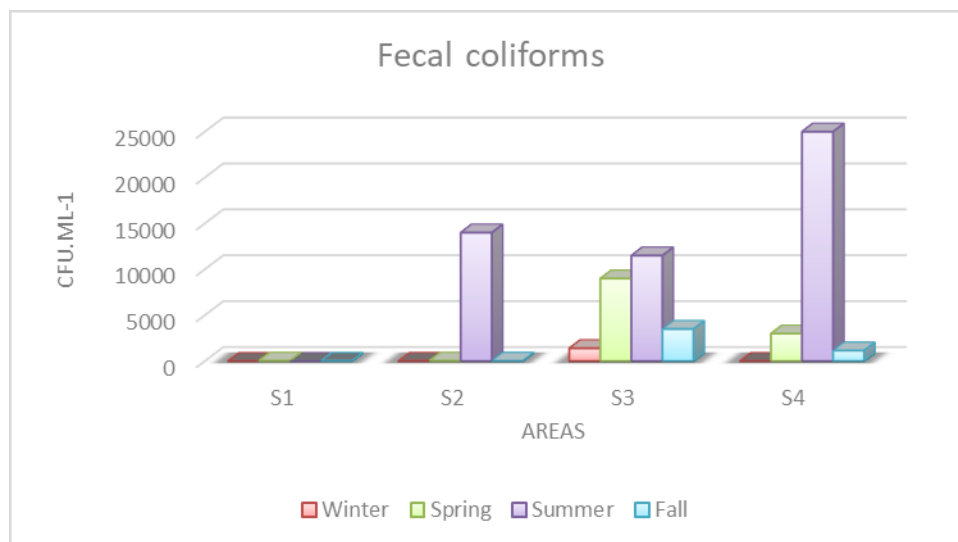
Figure 9:- Variation of faecal coliforms in spring waters.

Enumeration of streptococci:
Presumption test on Rothe medium:
Preparation of a series of tubes containing Rothe's selective medium at the rate of three tubes per dilution.Consider as "positive" the tubes in which cloudiness occurs throughout the liquid mass.

Confirmatory test on Litsky medium:
Each Rothe tube found positive will be subcultured to a tube containing Litsky's medium. Incubation takes place at 37 ° C for 24 hours. Tubes showing both a microbial cloudiness and a whitish or purple pellet at the bottom of the tube are considered "positive".

The count is carried out according to the prescriptions of the MAC GRADY table, taking into account the positive Litsky tubes **(Manceur; Djaballah, 2016) (Rodier et al, 2009)**
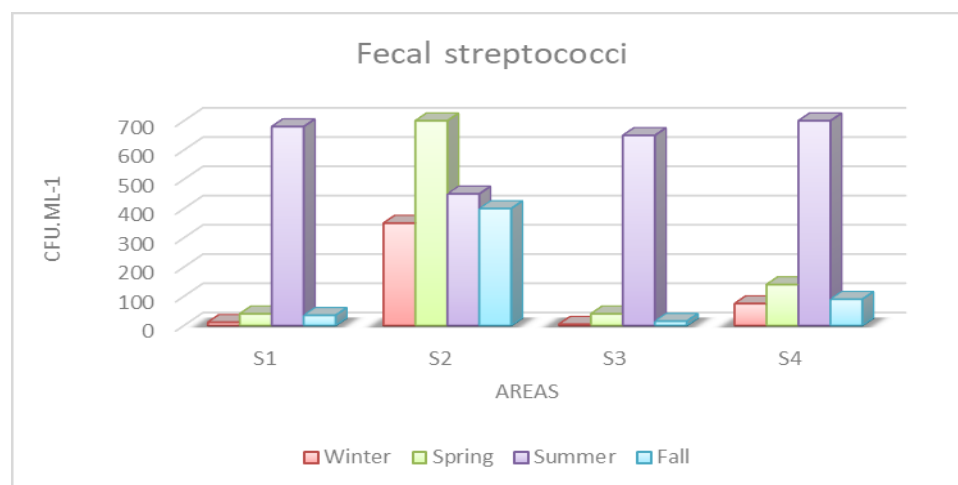

Figure 10:- Variation of faecal streptococci in spring waters.

No germ must be present in 100ml of water intended for consumption **(Ayad; kahoul, 2016)**. Our study revealed values of up to 700 CFU / 100 mL, which qualifies this water as non-potable.

The presence of such a large number of faecal streptococci in the water results in contamination with faeces **(Ayad; kahoul, 2016).**

Enumeration of sulfite-reducing clostridiums:
Clostridium sulfito-reducing are often considered as witnesses of faecal pollution. The spore form, much more resistant than the vegetative forms of faecal coliforms and faecal streptococci, would thus make it possible to detect old or intermittent faecal pollution **(Manceur; Djaballah, 2016) (Rodier et al, 2009).**
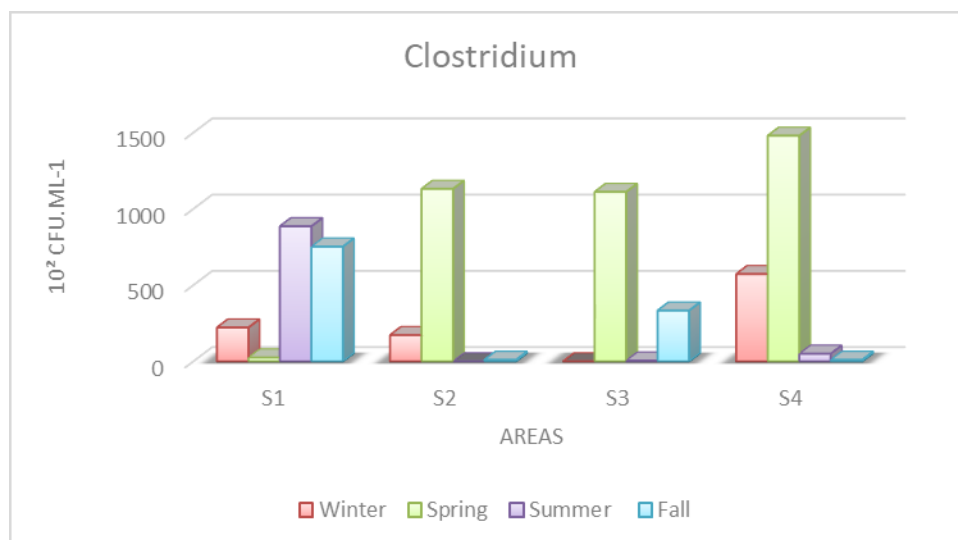

Figure 11:- Variation of sulfite-reducing Clostridium in spring waters.

The sulfite-reducing anaerobic bacteria are present in most areas except S3 in Winter and S2 in Summer, with values between 166 CFU / 20 mL and 14 * 103 CFU / 20 mL.According to**(Normes marocaines, 2006)**, no bacteria should be present in 100 mL of the sample. Suddenly our values far exceed the standards, and qualify our water as non-potable.

Discussion:
All of the biological indices used, defined average water quality with suboptimal habitat capable of supporting aquatic life, hence the presence of fairly significant biological diversity despite the dominance of certain species **(Karrouch ; Chahlaoui, 2009)**.

The region is characterized by a fairly significant taxonomic wealth (28 taxa identified, grouped into 6 major Orders: Ephemeroptera, Diptera, Plecoptera, Trichoptera, Coleoptera and Odonata), with the dominance of certain families, namely `` Taeniopterygidae '' belonging to the group 9 of indicator taxa composed essentially of the most polluo-sensitive families, followed by the families " Heptageniidae " and " Potamantidae " which belong to group 5 moderately resistant to pollution and " Hydropsychydae " belonging to group 3.

The structure and composition of the benthic community has clearly changed over the years, this is what was noticed when comparing our results with those of the study of **(Zuedzang Abessolo, 2014)**carried out at Ouad Ourika. The stations targeted in his study were '' OR1, OR2 and OR3, respectively equivalent to zones '' O2, O5 and O7 '' in our study.

For station OR1, there was a clear domination of the Simulidae, Baetidae and Caenidae, which represent nearly 92% of families. In our study, the O2 zone was mainly characterized by the dominance of the Oligoneudirae, Taeniopterygidae and Empididae families, with the disappearance of Caenidae.
For station OR2 and OR3, the region experienced the disappearance of Baetidae, Caenidae, Chironomidae, Hydropsychidae, Heptagenidae and Simulidae and the appearance of new families: Ephydridae, Psychodidae, Empididae, Polymitarcidae, Ceratopogonidae and Heptageniidae

The changes in the structure and composition of the benthic community between 2014 and 2016/2017 could clearly be interpreted as an improvement in the ecological quality of the water in the region, but it should not be forgotten

that the region is constantly changing. and change according to the needs of the population and visitors who play a very important role in the economy of the region.

According to **(Oubraim, 2002)**, temporal variations in density and taxonomic richness are closely related to the seasons. The spring and summer periods offer favorable conditions for the installation of a rich fauna in the different stations, and these variations can be explained by the life cycle of the species, by the variations in flow, the nature of the substrate as well as by the presence of organic matter.This is also the case in our study for some families who experienced an increase during the summer. The rapid flow, observed during the winter, generates floods which cause a drift phenomenon at the origin of the decrease in the number and the richness of the benthic fauna **(Oubraim, 2002)**.

Thanks to the microbiological study, carried out through research and the enumeration of the various germs of fecal origin, namely fecal coliforms, fecal streptococci and sulfite-reducing Clostridium, we have succeeded in determining the bacteriological quality of the water. from the region of Ourika, precisely groundwater intended for domestic consumption.The latter was found to be contaminated by various faecal germs, with rates exceeding Moroccan standards for drinking water and which are (0 CFU / 100 ml)**(Normes marocaine, 2006)**, which could constitute a risk to public health.

This contamination could be of geological and anthropogenic origin, in particular from the infiltration of wastewater and the use of chemical fertilizers in agriculture. Other studies have revealed that groundwater pollution is linked to the presence of septic tanks, the lack of treatment, the lack of a sanitation network and the non-compliance with public hygiene conditions**(Ayad, 2016)**.Also**(Otchoumou, 2017)**states that animal husbandry is one of the biggest sources of groundwater pollution through animal droppings, (an activity that is also present in our study area).

Our results join those of other authors such as: **(Kelome et al, 2012)**, whose study revealed the existence of a large contamination by germs of fecal origin, mainly coming from the bad conditions mentioned above.

The highest concentrations of bacteria of faecal origin were recorded during the summer when faecal coliforms reached a value of 25x103 CFU / 100 mL, and Streptococci a value of 700 CFU / 100 mL, which is also the case study carried out by**(Ben Moussa et al, 2012) (Boudraa et al, 2011)**.

According to **(Guessoum et al 2014)**,The presence of sulfite-reducing anaerobic spores with the absence of Coliform bacteria in natural water may reflect old fecal contamination. However, in our study the two families are present in the water of the region, especially in Summer.This further aggravates the situation.

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