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Evaluation of Bacteriological Parameters of Water Quality in The Bouregreg Estuary Along the Moroccan Atlantic Coast

M. El Amraoui [1,2], M. Tarbaoui [3,*], J. Benba [4], B. El Amraoui [1,5], T. Bamhaoud [1], N. Lazrak [2], M. Monkade [6]

Abstract- **On the Morrocan Atlantic coast, the Bouregreg estuary was submitted to many anthropic effects. After the dam Sidi Mohammed ben Abdellah was built in 1974, the flow became almost null. The waters of the estuary are meant for recreational activities, fishing, but at the same time they receive water collecting domestic and industrial wastewater. The waste in old landfills was not treated and leaked into the river water. The matter gets worse with the action of tides which make it difficult to escape the pollution load (physicochemical, bacteriological, heavy metals, etc.). The main objective of this study is to monitor the bacteriological quality of the surface waters from Bouregreg estuary (Moroccan Atlantic coast). The bacteriological parameters were determined upstream (S1, S2 and S3) and downstream (S4, S5 and S6) of the Bouregreg estuary. The parameters studied are fecal coliform (FC), fecal streptococci (FS), Salmonella, Staphylococcus aureus and Pseudomonas aeruginosa. The evaluation of the bacterial contamination of the studied water samples shows that the rate of contamination by fecal coliforms is very high in station S3 compared to other stations. This is explained by the emplacement of this station which is located next to a main rejection of Oued Akrach. The bacterial loads are maximal in this station: 7 10^3 CF/100ml for fecal coliforms and 2 10^3 SF/100ml for fecal streptococci. The ratio values (CF/SF) for the different stations show that the fecal pollution observed at the studied estuary is of mixed origin with human dominance. The search for pathogenic bacteria namely Salmonella, Staphylococcus aureus and Pseudomonas aeruginosa, was negative for all the studied samples.**

Index Terms— **Atlantic coast; Bacteriological parameters; Bouregreg estuary; Morocco; Pollution; Water quality.**

Mohammed El Amraoui, [1]Laboratoire Contrôle Qualité en Bioindustrie et Molécules Bioactives, Faculté des Sciences, Université Chouaib Doukkali, BP 20, 24000 El Jadida, Maroc, [2]Laboratoire du Maghreb, BP 2863 Rabat, Maroc
Meriem Tarbaoui, [3]Laboratoire Biomolécules et Synthèse Organique, Faculté des Sciences Ben M'sik, Université Hassan II-Casablanca, B.P 7955 Casablanca, Maroc
Jamila Benba, [4]laboratoires de Biotechnologie Marine et de l'Environnement, Faculté des Sciences, Université Chouaib Doukkali, BP 20, 24000 El Jadida, Maroc
Belkassem El Amraoui, [1]Laboratoire Contrôle Qualité en Bioindustrie et Molécules Bioactives, Faculté des Sciences, Université Chouaib Doukkali, BP 20, 24000 El Jadida, Maroc, [5]Physicochimie des Milieux Naturels et Molécules Bioactives (PMNMBA), Faculté Polydisciplinaire de Taroudant, Université Ibn Zohr, BP 271, 83000 Taroudant, Maroc,
Toufiq Bamhaoud, [1]Laboratoire Contrôle Qualité en Bioindustrie et Molécules Bioactives, Faculté des Sciences, Université Chouaib Doukkali, BP 20, 24000 El Jadida, Maroc, [2]Laboratoire du Maghreb, BP 2863 Rabat, Maroc
Nicole Lazrak, [2]Laboratoire du Maghreb, BP 2863 Rabat, Maroc
Mohammed Monkade, [6]Département de Physique, Faculté des Sciences, Université Chouaib Doukkali, BP 20, 24000 El Jadida, Maroc .

I. Introduction

An estuary is the place of transition between salt water and fresh water. The historical importance of these geographical areas is due to the existing shops and to the shipping of products. On the Moroccan Atlantic coast, the Bouregreg estuary was submitted to many anthropic effects. After the dam Sidi Mohammed Ben Abdellah was built in 1974, the flow became almost null. The estuary is bordered by two cities, Rabat and Sale in which the process of urbanization and industrialization has continually increased, affecting the water quality. The waters of the estuary are meant for recreational activities, fishing, but at the same time they receive water collecting domestic and industrial wastewater. The waste in old landfills was not treated and leaked into the river water. The matter gets worse with the action of tides which make it difficult to escape the pollution load (physicochemical, bacteriological, heavy metals, etc.). Knowing that the fishing sites and shellfish gathering sites are close to discharge points, we can imagine how serious the consequences could be. Biological monitoring is essential in assessing the quality of the estuary water. In Moroccan estuaries, several authors have shown such contamination [1] -[2]- [3]- [4] due to domestic and industrial activities along the Moroccan coast. The objective of this study is to evaluate the bacteriological quality of the water of the Bouregreg estuary and to predict their impact on the global state of the environment and the state of the marine ecosystems in particular.

II. Materials and Methods

A. *Presentation of the study area (Bouregreg estuary)*

The Bouregreg estuary is located on the Atlantic between the two cities Rabat and Sale 34° N and 6° 50 'west. It has a length of 23 km, limited by the dam of Sidi Mohammed Ben Abdellah and an average width of 150 m. It is generally facing south-east and north-west, except in the area ranging from kilometer point (kp) 13.5 to the confluence of Oued Akrech where it is oriented sud-ouest/north – East. Upstream and up the river Akrach (18 km), the estuary has an appearance of deep valley surrounded by highlands; Downstream of kilometer point (kp) 14.5 (Both islands), the collection disappears and the estuary through an alluvial plain in which he describes many meanders. This terrace, called Oulja disappears, about 4 km from the ocean where marshes are observed. Near its mouth are two sandy beaches of Rabat and

Sale which constitute the terminal portion of the estuary (Fig. 1).

B. Sampling

The water samples were taken using a Van Dorn bottle 1 liter in PVC. Sampling was conducted at two scales: a spatial and temporal scale during the 2013-2014 hydrological cycles and a vertical scale from the surface to the bottom every 2 m.

C. Choice of stations

The bacteriological parameters were determined upstream and downstream of the Bouregreg estuary. All samplings were performed in subsurface waters and along the river. The samples were taken from the downstream sites (S4, S5 and S6) between the Moulay Al Hassan bridge and the Al Fida bridge and upstream sites (S1, S2 and S3) near the dam Sidi Mohamed Ben Abdellah (Approximately 5 km) (Fig. 2). Upstream sites are characterized by stagnant waters where maritime influence is very weak. By against downstream sites are located at the mouth of the ocean, this is a crossing area between Rabat and Sale. This area receives polluted effluents mainly from the city of Sale which are discharged directly into the ocean without treatment, and from the work of the development of the Bouregreg Valley Project.

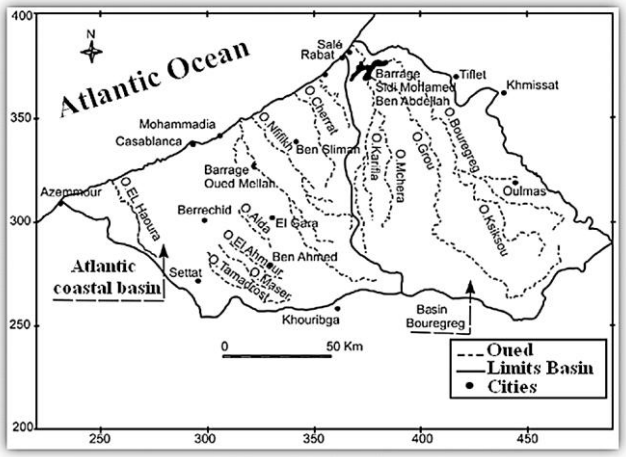

Fig. 1. Location of the study area (Becking, 1998)

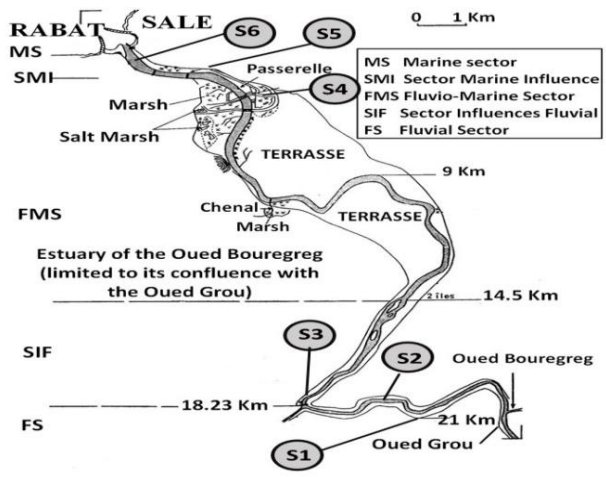

Fig. 2. Location of sampling points upstream and downstream of the Bouregreg Estuary

D. Analytical Methods

Bacteriological parameters

The bacteriological analysis of the various samples of water consists of an enumeration of the indicator germs of fecal contamination to know fecal coliforms (FC) and fecal streptococci (FS), and search for pathogenic contamination to know pathogens germs (*salmonella, staphylococcus aureus* and *pseudomonas aeruginosa*).

Enumeration of bacteria

The search for these organisms consists in taking 1 ml of the sample to be analyzed previously homogenized and introducing it into a tube containing 9 ml of sterile physiological water (NaCl 9 ‰). This gives the dilution 10^{-1} from which the dilutions 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} are prepared. The appropriate culture media are then inoculated with the dilutions obtained.

Fecal coliforms

Fecal coliforms (FC) or thermotolerant coliforms are a sub-group of total coliforms able to ferment lactose at a temperature of 44.5°C. The most important species of this bacterial group is *Escherichia coli* (*E. coli*) and to a lesser extent some species of the genera Citrobacter, Enterobacter and Klebsiella [5]-[6]. The spatio-temporal evolution of the abundances of FC was assessed by counting the colonies on the yellow-orange Tergitol agar and triphenyl tetrazolium chloride (TTC - Tergitol 7). The inoculated petri dishes were incubated at 44.5 °C for 24 hours [7].

Fecal streptococcus

Fecal streptococcus are spherical, in pairs or chains, Gram positive, catalase-negative, facultative anaerobic, which hydrolyze esculin in the presence of bile [8]. This test is characteristic of bacteria in group D Lancefield. Under the general description of fecal streptococci and according to WHO, fecal streptococcus are largely of human origin. However, some bacteria of this group were also collected from feces of animals such as Streptococcus bovis, S. equinus, S. gallolyticus and S. alactolyticus [9]- [11], or even meet on plants. They are still considered indicators of fecal pollution, and their main interest lies in the fact that they are resistant to desiccation and persist longer in water [12]. They thus provides additional information on pollution. The presence of fecal streptococcus was evaluated by counting black colonies with black halo on the agar medium in the bile, esculin and sodium azide, after incubation at 37 ° C for 48 h [7]. It is important to mention that the value of the report fecal coliform / fecal streptococci is used as an informative element of the first order. Indeed, when it is greater than 4, the pollution is of human origin. When this ratio is below 0.7, the pollution is of animal origin [13]. However, this ratio is not recommended unless the contamination is really recent.

Salmonella

Salmonella is a pathogenic bacterium that usually causes gastrointestinal disturbances. The variants of Salmonella are very numerous (2575 listed) but all have 90% or more of their genetic in common. The most common variants of Salmonella are: Typhimurium, Enteritidis, and Typhi. The search for Salmonella lasts 4 days and is much more qualitative than quantitative; to get it we need 5 liters of water.

- The pre-enrichment

On the first day, 5 liters of water to be analyzed is filtered and the filtration membranes are placed in a flask of buffered peptone water and incubated at 37 °C for 24 hours to revive the stressed bacteria.

- The enrichment

On the second day, 1 ml of the buffered peptone water is placed in a Selenite and GN broth and then incubated at 44 °C for 24 hours.

- Isolation

On the third day, the isolation is carried out from selenite and GN on Hektoen medium.
Salmonella give black colonies with a greenish halo on Hektoen.

- Enumeration of germs

For salmonella we can only use the method of presence or absence, we cannot make an enumeration since we have made enrichment.

Staphylococcus aureus

Staphylococcus aureus is a gram-positive coccal bacterium that is a member of the Firmicutes, and is frequently found in the nose, respiratory tract, and on the skin. It is often positive for catalase and nitrate reduction and is a facultative aerobe that can grow without the need for oxygen. [14]
Although S. aureus is not always pathogenic, it is a common cause of skin infections such as abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies.

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a bacterium found frequently in some water systems and in the environment. Its origin may be human and possibly fecal. Its presence in groundwater can result in contamination by surface water. It is found frequently in wastewater with concentrations up to 106/100 ml. It causes infections of the ears, eyes, burns and urinary tract as well as enteritis.

Identification by ATB EXPRESSION

To confirm the identification of the germs found in water samples, the identification by ATB EXPRESSION was used. It is a semi-automated biochemical identification of which the identification galleries containing the dehydrated substrates are inoculated with a standardized bacterial suspension and then incubated for 4 or 24 hours at a given temperature.
After incubation, the galleries are read by the ATB identification system and then the data are interpreted.

III. Results and Discussions

A. *Evaluation of the degree of fecal contamination in the different sampling points*

The results of the bacteriological analysis of water from six studied stations reveal the presence of the germs of fecal contamination (Table I). The fecal coliform load average (FC) varies between 2.6 10^3 and 7 10^3 (CFU / 100 mL). In terms of bacterial load, the station S3 is more concentrated with fecal staphylococci than other stations. The fecal staphylococci load average (FS) varies between 0.9 10^3 and 2 10^3 (CFU / 100 mL). In terms of bacterial load, the stations S3 and S4 are slightly more concentrated with fecal staphylococci than other stations. The result of the enumeration of the germs of fecal contamination is in good agreement with the bibliographical data about the state of bacterial contamination of urban effluents [15]- [17], but it far exceeds the standard set by the World Health Organization 1000 CFU / 100 mL [18]. The ratio values (FC/FS) for the different studied stations show that the fecal pollution observed at the studied estuary is of mixed origin with human dominance (Table II).

Table I. The average values of bacteriological parameters of water samples in the six studied stations

Parameters / Stations	Fecal coliform (CFU /100 mL)	Fecal staphylococci (CFU /100 mL)
Station S1	3 10^3	1 10^3
Station S2	2.6 10^3	0.9 10^3
Station S3	7 10^3	2 10^3
Station S4	3.7 10^3	2 10^3
Station S5	4 10^3	1.1 10^3
Station S6	3.2 10^3	1.2 10^3

Table II. Origin of contamination in the six studied stations

Sampling stations	Report (FC/FS)	Origin of contamination
Station S1	3	Mixed with human dominance
Station S2	2.88	Mixed with human dominance
Station S3	3.5	Mixed with human dominance
Station S4	1.85	Uncertain
Station S5	3.63	Mixed with human dominance
Station S6	2.6	Mixed with human dominance

B. *Evaluation of the degree of contamination of the different sampling points by pathogenic germs*

After having used all the biochemical tests necessary for the identification of Salmonella, none was found to be salmonella for all the water samples studied. These results are in agreement with some anterior studies [19]. The search of staphylococcus aureus and pseudomonas aeruginosa was also negative for all the water samples studied. Several causes can be at the origin of the absence of the pathogenic germs of which the most important are the high elevated values of the pH and the concentrations of oxygen dissolved in the water which are not very favorable to the survival of these germs [19]-[21].

C. *Environmental risks of bacteriological parameters*

Classical indicator bacteria have proved to be useful in studies describing the environmental distribution of waste water discharges [22]-[29]. Pathogen contamination of aquatic ecosystems is known to occur from a range of sources including municipal waste water effluents, agricultural wastes, and wildlife [30]. The World Health Organization (WHO) has stated that infectious diseases are the world's single largest source of human mortality [31]. Many of these infectious diseases are waterborne and have tremendous adverse impacts in developing countries. Fecal Coliforms are

bacteria found in the digestive tract of warm-blooded animals. The level of coliforms and streptococci in the environment is an indicator of other contaminants. Shellfish can become contaminated through filtering fecal coliforms from the water during feeding, which can make humans sick if the shellfish are eaten. The studied bacteria are excellent indicators to confirm the quality and the level of biological contamination of waters of Bouregreg estuary. The results of the bacteriological analysis showed high levels of fecal coliforms in water samples collected at the station S3 in comparison with the other stations. This is explained by the emplacement of this station which is located next to a main rejection of Oued Akrach and by the conditions and the elements necessary for the survival of these bacteria in the studied waters. Indeed, the waters of this station are of mixed origin with human dominance, In addition to that this station is located in upstream sites which are characterized by stagnant waters where maritime influence is very weak.

IV. Conclusion

The study area (Bouregreg estuary) is an area of natural (continental and marine) and anthropogenic (liquid waste and / or solid industrial and domestic). From the economic and social plan, the collected data from this research will allow better management of the protection of the coastal marine environment, to the benefit of the development of various riverine activities, notably fishing, tourism, swimming and water sports. The need for the installation of sewage treatment plants in the study area is of major and immediate importance in order to improve the quality of fluvial waters on the one hand and to contribute to the improvement of the state of the coastal marine environment on the other hand.

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