Assessment of bacterial contamination of *Mytilus galloprovincialis*, *Cardium edule* and water quality in Bouregreg estuary (Morocco, Atlantic Ocean)

Youssef Salama^{1,4} · Othmane Salama² · Mohammed El Amraoui³ · Mohammed Chennaoui⁴

Received: 18 August 2020 / Accepted: 21 December 2020 / Published online: 11 January 2021

© The Author(s) 2021 OPEN

Abstract

The Moroccan marine environment is threatened by several types of pollution. Indeed, the littoral zone knew an urban, industrial and agricultural development without precedent; moreover, coastal waters have served as discharges for almost all activities linked to this development. The aim of this study is to determine the biological, physicochemical and bacteriological quality of waters and bivalve shells in the Bouregreg estuary. Sampling was carried out at four stations (S1, S2, S3 and S4) located downstream to upstream of the estuary. Thus, we analysed the degree of contamination by total coliform, faecal coliform and faecal streptococci in the water and in the flesh of *Mytilus galloprovincialis* and *Cardium edule* and determined the physicochemical parameters of seawater samples, which included temperature, dissolved oxygen, electrical conductivity, pH, total hardness, biological oxygen demand for five days (BOD5), complete alkalimetric title, NO_2^- , NO_3^- , NH_4^+ , Fe^{2+} , K^+ , SO_4^{2-} , HCO_3^- , Cl^- , Na^+ , Mg^{2+} and Ca^{2+} . The S1, S2 and S3 stations were shown to have more or less homogeneous physicochemical properties. The evolution of faecal bacteriological contamination of water and bivalves has shown that faecal pollution of the environment varies according to the stations and according to the seasons. The degree of contamination is high in autumn and winter and comes from wastewater and runoff discharges which, by leaching agricultural land, bring a large number of microorganisms to the environment. This influences the quality of the aquatic fauna, in particular, the infestation of lamellibranch molluscs such as in *Mytilus galloprovincialis* and *Cardium edule*. We have noted that the contamination of mussels by these types of microorganisms follows, more or less, that of the biotope. It should also be noted that in summer, the faecal contamination of the environment increases with the arrival of summer visitors.

Keywords Shellfish · Mussels · Water quality · Faecal bacteria · Bouregreg estuary · Morocco

1 Introduction

Pollution of the marine ecosystem is one of the major problems that harm the environment. The factors responsible for them are continuing to increase and become

unbalanced, especially by human action (oil spills, chemical pollution, industrial and agricultural waste, etc.).

For the preservation of the marine environment, several national and international organizations were interested in the study of this pollution. The Mediterranean is one of the most endangered seas in the world, whence the creation of

✉ Youssef Salama, salama.youssef@gmail.com | ¹Department of Biology, Polydisciplinary Faculty of Khouribga, University Sultan Moulay Slimane, Khouribga, Morocco. ²Laboratory of RMI, Faculty of Sciences and Technologies, Hassan 1st University, Settat, Morocco. ³Quality Control in Bio-Industry and Bioactive Molecules Laboratory, Faculty of Science, University Chouaib Doukkali, El Jadida, Morocco. ⁴Laboratory of Life Science and Earth (SVT), Regional Centers for the Professions of Education and Training (CRMEF), El Jadida, Morocco.

the Programme for the Assessment and Control of Marine Pollution in the Mediterranean (MED POL). For the same objective, is part of our study which is interested in the analysis of the water quality of the Bouregreg estuary because of its importance in terms of tourism and its potential in aquaculture. Several authors are interested in this activity [1–5].

The Bouregreg estuary, the site of our study, is located between Rabat as capital and Sale as the third most populous city. Neglected in recent decades, the Bouregreg estuary is home to many human activities. The ecosystem is affected by complex waste discharges from various sources, a situation that is further complicated by the action of the tides in the estuary, which makes the natural elimination of pollutant loads resulting from human activities difficult. The estuary constitutes a transition zone between the aquatic/continental middle and presents a great physical and environmental variability leading to a very high biological diversity. However, the estuary is also a meeting place for various activities: urbanization, industrialization, leisure, etc. However, these activities are most often harmful to the surrounding environment, both aesthetically and ecologically [6, 7]. It is therefore very useful to assess and monitor the degree of pollution of the estuary. Note also that the estuary, which constitutes the interface between the river and the ocean, is an ecosystem particularly vulnerable to pollution. It is under a double continental and oceanic influence [8] and forms a breeding area for many marine aquatic species (fish, crustaceans, molluscs, etc.).

According to [9–11], the poorly planned urban development along the rivers (Case of the Bouregreg estuary) constitutes a serious threat to the quality of the water which transits by these streams to the sea and the seawater itself. He adds that urban and industrial effluents are often discharged without prior treatment and directly into the receiving environment. As a result, high pollutant loads and wastes accumulated are directly transported in the Coastal Marine Environment.

Thus, by this work, we contribute to the study of the Bouregreg estuary by monitoring the degree of physico-chemical pollution of the waters and the quality of the bacteriological contamination of two lamellibranch molluscs of significant economic and ecological interest (*Mytilus galloprovincialis* and *Cardium edule*) and find out the causes of this contamination which makes the environment conducive to the proliferation of different microorganisms (bacteria of faecal origin, anthropogenic, etc.).

2 Materials and methods

2.1 Study area and sampling location

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 Mohamed Ben Abdellah and an average width of 150 m. The Bouregreg Basin (Morocco) has a surface area of around 10,000 km². The altitude varies from sea level to 1627 m, with 50% of the surface area falling within an altitude range of 500–1000 m. The Bouregreg River has a fairly low overall slope of around 0.6% and rarely exceeds 1.0% throughout its course. From a geological point of view, the basin is mainly shaped of hills and plateaux of the Central and Coastal Meseta and a marshy alluvial plain with recent deposits of grey silt and sludge. It is therefore subdivided into two distinct sub-basins: the north-eastern part which corresponds to the actual Bouregreg Basin (around 3830 km² of the surface area) and the south-western part, corresponding to the basin of its main tributary (Oued Grou) which itself has two tributaries (Oued Korifla and Oued Akreuch) and a surface area of 5670 km² (Fig. 1) [12].

The mean annual rainfall varies from around 400 mm in the coastal region to 760 mm in the high mountains, with a big seasonal contrast from the dry season (May–September) when it receives 8% of annual rainfall to the rainy season (October–April) when it gets 92% of it.

The seawater and the mussels were sampled at four stations (S1, S2, S3 and S4) on the Bouregreg estuary of the region of Sale. The stations were chosen by taking into account a number of parameters (access facility, the abundance of mussels and the consumption of these molluscs by the waterside population) (Fig. 2).

- S1: Mouth of Bouregreg, site closest to the two beaches of Rabat and Sale.
- S2: Hassan II Bridge, located at 2.2 km from the ocean.
- S3: located at 3.7 km from the ocean, near an urban outlet and the tailings of the artisanal pottery complex of the city of Sale, whose daily peak flow is 40 L/s.
- S4: located at 7.5 km from the ocean, downstream from the largest discharge of wastewater with a daily peak flow of over 100 L/s.

The sample was carried out on an annual cycle (2019–2020) at a rate of five per season for four stations (S1, S2, S3 and S4).

Fig. 1 Location of the study area

2.2 Seawater samples

Samples were taken monthly of each site from December 2018 to January 2020; 500-mL polyethylene bottles were used to collect water samples in the morning and the afternoon.

The water samples were taken using a Van Dorn bottle 1 L in PVC. Sampling was conducted at two scales: a spatial and temporal scale during the 2019–2020 hydrological cycles and a vertical scale from the surface to the bottom every 1.3 m [13].

2.3 Mussel samples

Two stations were chosen representing the different molluscs' habitats under different conditions in Bouregreg estuary. The molluscs individuals were sampled during the period from December 2018 to January 2020.

At each station, the bivalves were sampled manually at low tide level from the intertidal estuarine mudflats of two different study sites (S1 and S3) as shown in Fig. 2. All the studied species feed by filter-feeding (or suspension-feeding) and live more or less in shallow water and were transported to the laboratory in controlled temperature conditions below 10 °C.

About 30 individuals of tow molluscs species were collected monthly from the Bouregreg estuary; *Mytilus gallo-provincialis* (Lamarck 1819); *Cardium edule* (Linnaeus 1758).

The collected mollusk species were identified according to Bosch and Bosch (1982) [14] and Sharabati (1984) [15].

2.4 Preparation of tissue suspensions

They were transferred to the laboratory in dry plastic bags and examined within 2–3 h. Barnacles were removed from the shell with a blunt scalpel, and the shell was cleansed with a small scrubbing brush and then rinsed with cold running water. The shells were dried with absorbent paper, and each mussel was opened using the procedure described by Thomas and Jones (1971) [16] except that the shell water was discarded.

Aliquots of 25 g of each sample were aseptically weighed into sterile plastic bags and homogenized with 225 ml of 0.1% (w/v) peptone water. Decimal dilutions from dilution 10^{-1} were prepared in tubes containing 9.0 ml of 0.1% peptone water. All samples were homogenized in Colworth Stomacher Circulator. The suspension was allowed to stand for 2–5 min and the supernatant used for bacteriological examination.

For examination of the digestive tract and contents, the intact stomach and intestine were dissected aseptically from each opened mussel and the volume of digestive tract derived from five mussels was measured in a sterile measuring cylinder. Four volumes of sterile distilled water were added; the mixture was homogenized and used for bacteriological examination as described above for the total tissue.

Fig. 2 Location of study sites in the estuary of the Bouregreg

2.5 Microbiological analysis

The microorganisms sought in this study were adopted as indicators of faecal pollution to ensure the sanitary quality of water and meat products, coliforms and faecal streptococci, and pathogenic bacteria, with water-borne transmission (*Salmonella* and *Cholera vibron*). Following suggested procedure from Bacteriological Analytical Manual (BAM) [17], triplicates were maintained to obtain mean value and average bacterial densities values were expressed as CFU/ml for seawater samples and CFU/g for mussels samples.

2.5.1 Faecal coliform (FC) and faecal streptococci (FS)

The total viable bacterial populations were enumerated in seawater samples by spread plate method using nutrient agar medium with 2.5% NaCl supplementation. The appropriate volume of seawater was serially diluted with sterile saline, and 0.1 ml samples were spread on nutrient agar medium. Coliforms from each location were detected and identified by membrane filter technique as described by APHA [18]. Experiments were performed in triplicates.

For mussels, coliforms are counted on lactose deoxycholate agar in a double layer. One millilitre of the suspension prepared is placed in a sterile Petri dishes, and 13 ml of agar melted and brought to 47 °C is added. The whole is homogenized by rotating the Petri dishes and then solidified at room temperature. The second layer is then poured. Incubation is at 37 °C for the enumeration of total coliforms and at 44 °C for 24 h for the enumeration

of faecal coliforms. Coliforms form dark-red- to purple-coloured colonies larger than 0.5 mm in diameter.

2.5.2 *Salmonella* and *shigella*

For water, the filtration membrane is seeded directly into sodium selenite broth and incubated at 37 °C for 24 h.

For mussels, pre-enrichment is performed in peptone water for 4 h at 37 °C, and enrichment in sodium selenite broth at 37 °C for 24 h. Isolations from selenite broth are carried out on SS (*Salmonella*–*Shigella*) agar. Colonies will appear colourless and/or black-centred which are identified according to biochemical (API automatic systems) and antigenic characteristics.

2.5.3 *Vibrionaceae*

For water, the filtration membrane is seeded in hypersaline alkaline peptone water and incubated at 37 °C for 18 h.

For mussels, 1 ml of the suspension is inoculated in hypersaline alkaline peptone water (enrichment) for 18 h at 37 °C. The isolations are carried out on TCBS agar (Thio-sulfate Citrate Bile Saccharose). Incubation takes place at 37 °C for 24 h. Yellow colonies are identified after fresh examination, and Gram stain was performed.

2.6 Physicochemical analysis

The physicochemical parameters (Table 1) were analysed using the techniques developed by APHA [19] and Rodier [20]. Methods for measurement of physicochemical

Table 1 Water quality parameters and measurement methods

Parameters	Abbreviations	Units	Measuring equipment and method analysis
Temperature	T °C	°C	Mercury thermometer
Potential hydrogen	pH	–	pH-meter INOLAB pH7110
Electrical conductivity	EC	μS/cm	Conductimeter INOLAB cond720
Dissolved oxygen	DO	mg/L	Iodometric method according to WINKLER
Biological oxygen demand in 5 days	BOD ₅	mg/L	Respirometry BOD
Complete alkalimetric title	TAC	°F	Titrimetry
Chlorides	Cl [–]	mg/L	Volumetric dosage with mercuric nitrate
Sulphates	SO ₄ ^{2–}	mg/L	Nephelometric method with barium sulphate
Nitrates	NO ₃ [–]	mg/L	Sulphanilamide method after reduction on a column
Nitrites	NO ₂ [–]	mg/L	Sulphanilamide method
Ammonium	NH ₄ ⁺	mg/L	Indophenol blue method
Sodium ion	Na ⁺	mg/L	Flame photometry
Potassium ion	K ⁺	mg/L	Volumetric titration methods
Ferrous ion	Fe ²⁺	mg/L	Volumetric titration methods
Magnesium ion	Mg ²⁺	mg/L	Volumetric titration methods
Calcium ion	Ca ²⁺	mg/L	Volumetric titration methods
Bicarbonate	HCO ₃ [–]	mg/L	Volumetric titration methods

variables of water, which included temperature, dissolved oxygen, electrical conductivity, pH, total hardness, biological oxygen demand during five days (BOD_5), complete alkalimetric title, NO_2^- , NO_3^- , NH_4^+ , Fe^{2+} , K^+ , SO_4^{2-} , HCO_3^- , Cl^- , Na^+ , Mg^{2+} , Ca^{2+} , are regrouped in Table 1.

2.7 Statistical analysis

2.7.1 The Data

The spatiotemporal variability of physicochemical concentrations was investigated by the correspondence factor analysis (CFA) performed on a data matrix consisting of 16 variables (water temperature, pH, NO_2^- , NO_3^- , NH_4^+ , Fe^{2+} , K^+ , SO_4^{2-} , HCO_3^- , Cl^- , Na^+ , Mg^{2+} , Ca^{2+} , TAC (Complete Alkalimetric Title), TH (Total hardness) and BOD_5) by adding the seasonal contents of streptococci faecal and faecal coliforms as two supplementary variables.

2.7.2 Correspondence factor analysis (CFA) method

We chose to use CFA for several reasons: it was specifically designed to compare profiles or patterns; it is a multidimensional method that achieves appropriate data reduction, filters out noise and objectifies correlations among variables; it is a method that provides graphic outputs (e.g. maps) that are easier to grasp than series of numbers (Benzdéri [21] and Blasius and Greenacre [22]; Doré et al. [23], and references cited therein). Its principles are described in an appendix to Doré et al. [23] in several textbooks (Greenacre [24]; Jambu [25] and Krzanowski [26]).

3 Results

3.1 Microbiological contamination in seawater samples

The results of bacteriological analysis of seawater samples at the four stations are shown in Figs. 3 and 4.

In the four stations, the rates of coliforms and faecal streptococci are gradually increasing in summer and autumn and then reach 5–6 times rate in winter. The faecal concentration rate is high in station S4 which is located near the upstream the Douar Doum discharges. The maximum bacterial loads observed during the summer and present the following results (Figs. 3 and 4): for station S1, FC: $6 \times 10^3/100$ mL, FS: $1.8 \times 10^3/100$ mL; for station S2, FC: $7.9 \times 10^3/100$ mL, FS: $6.69 \times 10^3/100$ mL; for station S3, FC: $8 \times 10^3/100$ mL, FS: $2 \times 10^3/100$ mL; and for station S4, FC: $3.34 \times 10^4/100$ mL, FS: $2.55 \times 10^4/100$ mL. The coliforms and faecal streptococci values are high since the analyses were carried out at low tide.

Indeed, the station S3 received directly discharges of domestic wastewater [6]. The research of *Salmonella* and *Vibrio cholerae* was negative during the study period on all the water and mussel samples.

The FC/FS ratios were calculated to determine the origin of faecal pollution (Table 2). The mean values were considered with respect to season in order to synthesize the information contained in the set of variables considered at each sampling point.

Fig. 3 Faecal coliforms and faecal streptococci concentrations in seawater samples

Fig. 4 Average values of FC/FS ratio in the seawater of Bouregreg

Table 2 FC/FS ratios and origin of faecal pollution [27]

(FC/FS) = R	Origin of faecal pollution
$R > 4$	Exclusively human
$2 < R < 4$	Mixed with human prevalence
$1 < R < 2$	Dubious Origin
$0,7 < R < 1$	Mixed with animal prevalence
$R < 0,7$	Mainly or entirely of animal origin

The results presented in Fig. 4 show that faecal pollution has a mixed origin with human prevalence at the S1 and S3 and dubious origin at the S2 and S4.

3.2 Microbiological contamination in mussel samples

The results show that the average bacterial load varied during the warm summer–autumn seasons: for *Mytilus galloprovincialis* between FC: 5.24×10^4 CFU/g and FS: 1.61×10^4 CFU/g for the station S1 and FC: 6×10^4 CFU/g and 1.95×10^4 CFU/g for S3, while those of *Cardium edule* are FC: 4.4×10^4 CFU/g and FS: 2.2×10^4 CFU/g for S1 and FC: 5.2×10^4 CFU/g and FS: 2.8×10^4 CFU/g for S3. Generally, from station S1 to station S3 (Fig. 5), there is an increasing trend in the levels of microorganisms in the

water and flesh of the two mussels. Station S3 is more contaminated than station S1.

3.3 Data analysis

The cumulative variance for the two axes F1 and F2 is 51%, a value sufficient for the interpretation of the foreground factorial axes $F1 \times F2$ to largely explain the phenomenon studied.

On the other hand, the analysis of the eigenvalues of the inertia rates of the various abiotic descriptors and the correlation of these descriptors to the factor axes provide an ecological explanation for these axes and subsequently determine the hydrological functioning of the ecosystem studied.

3.3.1 Interpretation of axes F1, F2 and F3

3.3.1.1 Axis F1 The results (Table 3 and Figs. 6 and 7) show that the variables which contribute remarkably to the constitution of this axis are NH_4^+ , NO_3^- and SO_4^{2-} which are parameters largely assessing the rate of nitrogenous matter, in other words, the degree of eutrophication of the medium. There is only one increasing gradient from the negative side to the positive side of the F1 axis for NH_4^+ , NO_3^- and SO_4^{2-} .

Fig. 5 Bacteriological concentration of *Mytilus galloprovincialis* and *Cardium edule* in stations S1 and S3

Table 3 The eigenvalues, inertia and cumulative variance of the three factorial axes F1, F2 and F3

	Eigenvalues	Rate inertia	Cumulative variance
F1	0.28	28	28
F2	0.23	23	51
F3	0.15	15	66

3.3.1.2 Axis F2 Four active variables contribute to the constitution of this axis, namely TAC (Complete Alkalimetric Title), T, Na⁺ and Cl⁻. In addition to temperature (T) and alkalinity, this axis therefore represents the variation in the salinity of the medium. Similarly, two gradients are also represented by the axis F2:

- An increasing gradient from the negative side of F2 to its positive side for TAC.
- An inverse gradient for the other variables mentioned (T, Na⁺ and Cl⁻).

3.3.1.3 Axis F3 It is an axis made up (Fig. 7) by the main contribution of three physicochemical variables: K⁺, Ca²⁺ and BOD₅ representing an increasing gradient from the negative side to that positive of the same axis for the first two parameters and decreasing for the last. Furthermore, high concentrations of FS and FC are noted in the quadrant limited by the positive portions of the axis and the axis F1–F2 (Fig. 6); these are also noted in the scale bounded by the positive parts of the F1 axis and the F3 axis (Fig. 7). Consequently, the ecological conditions which seem to favour the multiplication of these micro-

Fig. 6 Projections of the physicochemical and bacteriological variables in the F1–F2 plane

Fig. 7 Projections of the physicochemical and bacteriological variables in the F1–F3 plane

organisms are characterized by a richness in NH_4^+ , NO_3^- , SO_4^{2-} and high TAC. These conditions are best achieved in station S4. Thus, it was this station that showed the highest contamination in FS and FC.

Faecal coliforms are abundant there most of the year, while at stations S1, S2 and S3 these microorganisms are more numerous in summer autumn than in winter spring. We know that station S4 is the most exposed to wastewater. Consequently, it is these types of water that participated in sowing these microorganisms and favouring their multiplication.

The F1x3F3 plan (Fig. 7) shows in addition high values of K^+ and Ca^{2+} which can favour the development of these microorganisms indicative of the hygienic quality (Coliforms and faecal Streptococci) of the waters.

4 Discussion

The gradual increase in the rate of contamination by coliforms and streptococci (flesh and water) in the four stations is probably due to the drought period which prevailed in Morocco during the period from 2018 to 2019. In addition, the hydrodynamics of Bouregreg is influenced by the presence of the Sidi Mohamed Ben Abdellah dam, which deprives the estuary of a good amount of fresh water except during times of dam releases, which are generally done in November [10, 28].

Only the tide perpetually and cyclically determines the volume of water entering or leaving the estuary; hence, the variations in bacterial loads are noted in the different stations. Thus, the reduction in freshwater supplies after the constriction of the dam in 1974 and the multiplication of wastewater discharges contributed to the establishment of conditions favourable to the installation and the multiplication of coliforms and streptococci. The domestic pollution constituting most of the

effluents makes the waters of this estuary unsuitable for swimming [29, 30].

These results agree with the studies of Elkaim [31] and Ezzaouak [6] which all agree on the seriousness of the bacterial pollution of the waters of the Bouregreg estuary if we take into account the most permissive WHO quality standards [13]: 2000 faecal coliforms per 100 ml and 2000 faecal streptococci per 100 ml, as well as the [32]. This high degree of bacterial pollution is well illustrated during the analyses of samples of individuals of the two species of molluscs in which the bacterial loads are very high (Fig. 5).

Due to lack of resources, the samples of *Mytilus galloprovincialis* and *Cardium edule* (Table 3) were taken at only two stations S1 and S3. At these two stations, the sample of *Cardium edule* is less contaminated than those of *Mytilus galloprovincialis*.

This last result is not in agreement with that of Nicolas et al. and Frehi et al. [33, 34] who reported that the mussel is the most contaminated species which presents a major risk to public health. They explained this phenomenon by the time factor which has an impact on the toxicity of shellfish and made it possible to note the existence of two periods of risk: a winter and another summer with the importance of the first because the winter rainfall leads to increased contaminant transport in runoff and increases faecal contamination. These periods follow the time of appearance in the environment of toxic effluence of dinoflagellates capable of producing paralysis.

S1 is less contaminated than S3; this can be explained by its proximity to the mouth, which is weakly influenced by the discharge of wastewater and which is influenced by the tides. Likewise, the seasonal variation in faecal bacteria shows their predominance in estivo-autumnal (Fig. 3).

For their feeding, these molluscs filter from 20 to 120 L of water, favouring a massive accumulation of interval concentrations 2–6 times higher than that of

the environment [31]. This phenomenon explains the results obtained and the difference in the degrees of contamination of the flesh of *Mytilus galloprovincialis* and *Cardium edule*. (Table 3). Signalling that, by filtering large quantities of water to feed (from 100 to 650 L/hr/kg of living animals), marine bivalves concentrate microorganisms and toxic substances from the environment or of faecal origin. Their ingestion therefore exposes consumers to a risk of toxic infection [35, 36]. According to Holmes and Earampamoorthy [37, 38], molluscs filter and concentrate particles, toxins which can cause human pathologies due to their ingestion.

These consumed shellfish, often raw, contain biotoxins, viruses and bacteria which are badly eliminated. This results in many foodborne illnesses due to the ingestion of this type of food.

Thus, Cherkaoui and others [39–41] found that the presence of pathogenic microorganisms of faecal origin in surface water poses important health problems when these waters are used for the production of drinking water, for nautical activity (case of Bouregreg) or for irrigation. As indicated by Nassri [42], it is necessary to carry out the disinfection of wastewater in sewage treatment plants in order to reduce the level of contamination of surface water by pathogenic microorganisms such as faecal coliforms, total coliforms and faecal streptococci.

In the work of Tahri [7], the first station reveals very high bacterial contamination, compared to the WHO guidelines [13] for bathing water. The contents of faecal coliforms and faecal streptococci remain largely higher, and the waters of this station can be classified as highly polluted water. Remember that this is an area close to the two beaches of Rabat and Sale, which is very active throughout the day, especially during the summer. For stations S3 and S4 pollution becomes very critical.

In 2015, Ferdaous [43] found that the concentration of coliforms varied between 1.1×10^4 FC/100 ml and 2.4×10^7 FC/100 ml. The fecal coliforms showed high concentrations at the S4 station in the study area because the S4 station is near the urban wastewater discharges from Douar Doum and Takaddoum and which receives the discharges from the Akrech slum. With regard to faecal streptococci, Bounouira and Cheggour [44, 45] find values which oscillate between $2, 3 \times 10^2$ FC/100 ml and $2, 4 \times 10^6$ FC/100 ml. The highest concentrations 10^5 and 10^6 were recorded at stations S3 and S4 which receive large quantities of wastewater. The latter station is located in the most upstream part of the estuary. These waters are only exceptionally renewed, which also explains the high contents of streptococci and thermotolerant coliforms.

Comparison with Tunisian standards relating to discharges into the maritime public domain (2,000 coliforms, 1,000 faecal streptococci) shows that the waters of the

Bouregreg estuary are very laden with microorganisms of faecal contamination. This pollution is due to uncontrolled discharges of urban wastewater without treatment in the estuary. This contamination of waters with wastewater poses a great risk to public health, and the more intense the contamination, the greater the risk, despite the state of health of the people exposed to the contamination. Therefore, enteric diseases and the other pathologies mentioned are related to the intensity of contamination of the water.

However, it can be said that the deterioration in water quality is mainly due to pollution of domestic (agglomeration without treatment stations) and industrial origin. As for the Bouregreg, recurrent floods resulting from the combination of heavy precipitation on relatively impermeable soil and an anarchic occupation of the river bed place the fight against floods at the forefront of concerns [45]. It should also be noted that the sediments made up of very fine particles contain a large number of bacteria because they offer a large colonization surface which is large in relation to the mass. Thus, 90.5% of faecal coliforms can be fixed in small particles (0.45–5 μ m).

According to Radenac [46], these microorganisms can be found in sediments such as *Escherichia coli* (*E. coli*), faecal streptococci, *Vibrio cholerae*, etc. The fate of the microbial load in water, as with other pollutants, is a function of the dilution, dispersion and sedimentation of fine particles.

Regarding the origin of bacterial contamination of the environment, the application of the FC/FS report allows conclusions to be drawn about the origin of the source of this pollution. Thus, this ratio varies depending on the stations and the distance from the sea. In half of the cases, there is pollution mainly of human origin, with a ratio ($R > 4$ and $2 < R < 4$); the other half is of uncertain origin with a ratio of $1 < R < 2$.

The first report ($R > 4$ et $2 < R < 4$) confirms the contribution of summer visitors to marine pollution. This same result was reported by El Amraoui and El Harim [40, 47]. The second report ($1 < R < 2$) can be explained by several facts such as the leaching of agricultural land by runoff which carries microorganisms in the effluents then in the sea or the proximity of the industrial zone of Youssoufia and Douar Doum at the level of station S4. In addition, the sewage of wastewater flows into the estuary at the station S4.

Runoff water causes significant bacterial contamination of the aquatic environment. Elattar et al. [48], for example, reported that the FC/FS ratio calculated at the level of the waters of the Oualidia lagoon in Morocco shows that the contamination is generally of animal origin. This phenomenon can be explained by the considerable quantities of manure used to fertilize pasture fields. They also noted a

correlation between faecal contamination and periods of heavy rainfall.

Regarding the comparison of the FC concentration in surface water and certain Moroccan rivers, the analysis of the water quality parameters of Bouregreg is moderately degraded. With this level of bacterial contamination, it can be concluded that the molluscs of Bouregreg Rivers are unsuitable for direct consumption. However, it is possible to improve the edibility of these molluscs, living in areas of up to 5000 faecal coliforms/100 ml, by causing a purge by stabling in clean water. In the same concept, Chafik et al. [49] confirmed this same phenomenon by analysing the quality of the waters of the Atlantic coast. Molluscs removed from an environment containing more than 5000 faecal coliforms/100 ml, as is the case with Bouregreg River, should be considered inedible, even after cleaning in a basin. This significant faecal contamination can be a presumption of the existence of other pathogenic microorganisms with water transmission such as *Yersinia*, *Campylobacter* and especially *Salmonella* and *Vibrio cholerae* whose research in our work has proved negative. This can be due either to their presence in small numbers in the waters, to competitiveness with other microorganisms, to the poor environment in nutrients or to the dilation effect caused by the tide.

Compared to water contamination, the mussels concerned by this study have higher bacterial loads than the water at the four stations where the concentration of CF in the water is, respectively, lower than that of the flesh. The significant bacteriological concentration noted in the mussels studied is in agreement with the results reported by Plusquellec et al. [50] according to which shellfish are good indicators of microbial pollution of coastal waters. In addition, Passerat et al. [51] reported that faecal coliforms are one of the microorganisms of faecal origin because they are common indicators of microbiological contamination of water.

El Sharkawi and Mabrouk [52, 53] found highly significant correlations between water contamination and that of shellfish for both coliforms and streptococci. However, faecal streptococci currently constitute a more significant contamination test than faecal coliforms and a better indicator of water safety [54]. Thus, the sanitary condition of the shellfish samples is to be feared especially since the presence of bacterial indicators can indicate that of pathogenic microorganisms [55, 56]. *Salmonella* and vibrios were not detected in any sample. Similar results were found by Bouchriti and Hassou [57, 58] by studying the bacterial pollution of the Oualidia lagoon and by Ezzaouk [6] by studying the estuary of the Bouregreg River. Mabrouk [53] isolated only two salmonella in the Nador lagoon. Bahou [59] did not detect any salmonella or *Vibrio parahaemolyticus* in the coastal seas of northern Morocco.

El Amraoui [40] showed that on the bacteriological level, total coliforms, faecal coliforms, faecal streptococci were found systematically in all the samples analysed in the marine environment. With regard to pathogenic microorganisms, no vibrio was isolated and only seven out of 16 samples (44%) contain *Salmonella*. According to Rodrigues and HOFER [60], this absence can be explained by the inability of mussels (*Mytilus galloprovincialis* and *Cardium edule*) to retain these microorganisms as concentrated particles.

Likewise, El Alaoui [61] proclaims that the presence of bacteriological, physico-chemical, organic and metallic pollution explains that anthropic action exerts considerable pressure on the waters of the Bouregreg estuary. According to Berraho [1], the Citadines landfills installed near the estuary and the releases of the dam, Sidi Mohamed Ben Abdellah, generate a significant contribution of pollution during rainy periods, a significant supply of water, anoxic, turbid and charged with organic matter and in suspended particles. Note also Cherkaoui [39] confirms that in addition, we must put an end to activities harmful to the environment and the balance of the ecosystem (such as illegal and intensive exploitation of quarries, dumping of garbage and of rubble), the Bouregreg Agency seized for this purpose the Prefecture of Sale and the Ministry of Habous and Islamic Affairs, owners of the land, so that they take the necessary measures against the polluters and offenders.

For this, it was decided to separate the wastewater pipes from the rainwater pipes. If the second, natural, will pour into the river, the first, very polluting, will be transported to the coast through pumping stations. Two emissaries will conduct these discharges to the seabed, 3 km from the coast. Furthermore, monitoring the various physicochemical and bacteriological parameters considered [Correspondence Factorial Analysis (CFA)] enabled us to determine the level and trends of contamination in the Bouregreg estuary, therefore giving an initial assessment of the impact of rejects from the cities of Rabat-Sale and confirming the previous results.

We deduce that actually faecal indicators are not supposed to multiply in the environment. Their presence in high densities in association with the parameters mentioned is probably reflecting the wastewater contribution to biotic and abiotic characteristics of the water. These conditions are less represented in station S1, hence the low load of this station in FS and FC. In the same sense, various authors [62–67] reported that in coastal waters, eutrophication is known to be one of the conditions favouring pathogenic microorganisms in the aquatic environment. Our study also shows that, with regard to the ecological parameters studied, the ecological behaviour of coliforms and faecal streptococci is roughly the same.

But coliforms are more abundant than streptococci. This dangerous situation contributes to the degradation of the physicochemical quality of this environment and could have harmful effects on all the components of this system. It is therefore important that the competent authorities become aware of the reality of the problem of pollution of the Bouregreg estuary by urban wastewater in order to purify these discharges before being discharged into the estuary. Finally, as pointed out by Cherkaoui [9], the installation of observatories for monitoring the quality of surface water must be generalized and must not only concern threatened rivers.

5 Conclusion

During the study period, the physicochemical parameters showed that the temperature of the seawater follows a seasonal evolution and varies depending on an increasing gradient or descending from downstream to upstream. BOD₅ values correlate well with dissolved oxygen values and indicate contamination of the medium of organic and mineral matter.

The bacteriological study of seawater and bivalves has enabled us to observe that the faecal pollution of the environment is variable both in space and in time. It is a medium rich in nitrogenous matter and relatively eutrophic. The winter period has shown a large number of microorganisms which are probably due to leaching water near the study stations. During the summer period, the Bouregreg site showed significant faecal contamination. Consequently, that of molluscs tends to increase again during this period.

The origin of the pollution, as determined by the FC/FS ratio, is human in half of the samples. The other half show for pollution of uncertain origin.

According to these results, the Bouregreg estuary has been affected by pollution. The discharge of domestic wastewater into the estuary causes faecal contamination to be very high. This influences the quality of aquatic fauna, in particular lamellibranch molluscs such as in *Mytilus galloprovincialis* and *Cardium edule*. These contaminated molluscs can carry water-borne pathogens; hence, there is the need to establish a monitoring system for this type of pollution. In addition, urban pollution due to industrial discharges results in a risk of aggravating the ecological imbalance of the environment. The current state of bacteriological pollution therefore requires awareness to save this fragile ecosystem.

The downstream sector of the Bouregreg wadi is polluted to such an extent that its ecological balance is involved in addition to endangering the health of neighbouring populations. Pollution, especially of organic and

biological origin, is attributable to domestic and industrial wastewater discharges.

In addition, the microbiological quality of the beaches does not meet the permissible standards for bathing water and the situation is even more serious on the sale side than in the city of Rabat.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

1. Berraho A (2006) Évaluation du milieu marin. INRH–Casablanca, Morocco
2. DdRV IFREMER (1999) Aquaculture Maroc. Appui à l'étude des potentialités. Cahiers Options Méditerranéennes 43:87–107
3. Food, Organization A (2008) FAO Yearbook: FAO Annuaire. Fishery and Aquaculture Statistics [Elektronische Ressource]: Capture Production, Aquaculture Production, Commodities. FAO
4. Boudinar B (2007) Diagnostic du secteur de la pêche maritime au Maroc. Ministerio de Medio Ambiente y Medio Rural y Marino/OIT
5. Orbi A, Berraho A (1999) Potentialités de l'aquaculture marocaine. Cahiers Options Méditerranéennes 43:77–81
6. Ezzaouaq M (1991) Hydrodynamic, physico-chemical and bacteriological characterization of surface water of the estuary of the Bouregreg (Morocco) subjected to discharges from the cities of Rabat-Sale. Université Mohammed V. de Rabat, Morocco
7. Tahiri L, Bennasser L, Idrissi L, Fekhaoui M, Abidi AE, Mouradi A (2005) Contamination métallique de *Mytilus galloprovincialis* et des sédiments au niveau de l'estuaire de Bouregreg (Maroc). Water Quality Research Journal 40(1):111–119
8. Benabdellouahad S (2006) Structure, dynamique et typologies physico-chimiques et phytoplanctoniques de l'estuaire du Bou Regreg (Côte Atlantique Marocaine)
9. Cherkaoui E, Bayed A, Hily C (2003) Organisation spatiale des peuplements macrozoobenthiques subtidiaux d'un estuaire de la côte atlantique marocaine: l'estuaire du Bou Regreg. Cah Biol Mar 44(4):339–352
10. Laouina A, Coelho C, Ritsema C, Chaker M, Nafaa R, Fenjiro I, Antari M, Ferreira A, Van Dijck S (2004) Dynamique de l'eau et gestion des terres dans le contexte du changement global,

- dans le bassin du Bouregreg (Maroc). Science et changements planétaires/Sécheresse 15(1):65–77
11. Bounouira H (2007) Etude des qualités chimiques et géochimiques du bassin versant de Bouregreg. Université Ibn Tofail de Kénitra, Morocco
 12. Becking GC (1998) The effect of essentiality on risk assessment. Biol Trace Elem Res 66(1–3):423–438
 13. WOH (2003) Guidelines for safe recreational water environments: coastal and fresh waters, vol.1. World Health Organization
 14. Bosch K (1997) Elementare Stichprobentheorie (Beschreibende Statistik). In: Elementare Einführung in die angewandte Statistik. Springer, pp. 1–34
 15. Sharabati D, Sharabati IA (1984) Red Sea Shells. KPI
 16. Thomas K, Jones AM (1971) Comparison of methods of estimating the number of *Escherichia coli* in edible mussels and the relationship between the presence of salmonellae and *E. coli*. J Appl Bacteriol 34(4):717–725
 17. Food US, Microbiology DADo (1976) FDA Bacteriological analytical manual for foods. Association of Official Analytical Chemists
 18. (APHA) APHA, (1995) American water works association and water environment federal, 1995: standard methods for the examination of water and wastewater, 19th edn. APHA, Washington DC
 19. APHA (2018) Standard methods for the examination of water and sewage. Creative Media Partners, LLC
 20. Rodier J, Legube B, Merlet N (2009) Analysis of Water. Dunod, Paris
 21. Benzécri J (1973) L'Analyse des Données, tome 1: La Taxinomie, tome 2: L'Analyse des correspondances. Dunod, Paris
 22. Blasius J, Greenacre M (1998) Visualization of categorical data. Academic Press, Cambridge
 23. Doré JC, Ojasoo T, Okubo Y, Durand T, Dudognon G, Miquel JF (1996) Correspondence factor analysis of the publication patterns of 48 countries over the period 1981–1992. J Am Soc Inform Sci 47(8):588–602
 24. Greenacre MJ (1984) Theory and applications of correspondence analysis. Academic Press, Cambridge
 25. Jambu M (1991) Exploratory and multivariate data analysis. Elsevier, Amsterdam
 26. Krzanowski W (2000) Principles of multivariate analysis: a user's perspective (oxford statistical science series (21)). Oxford University Press, Oxford
 27. Borrego A, Romero P (1982) Study of the microbiological pollution of a Malaga littoral area II. Relationship between fecal coliforms and fecal streptococci, VIème journée d'étude, Pollutions, Cannes, France, pp. 561–569
 28. Haddout S, Priya K, Ljubenkov I (2020) The calculation of estuarine flushing times in convergent estuaries using freshwater fraction method. Int J River Basin Manag. <https://doi.org/10.1080/15715124.2020.1719121>
 29. El Alaoui I, Benbouzid M, El Hajjaji S, Benmessaoud F, Bellahcen TO (2019) Potential impact of the latest developments in the Bouregreg Estuary. In: International Conference on Advanced Intelligent Systems for Sustainable Development. Springer, pp. 520–535
 30. Essediya C (2006) Structure et organisation des peuplements macrozoobenthiques de l'estuaire du Bou Regreg après la construction du barrage. Université Mohammed V. de Rabat, Morocco
 31. Elkaim B (1974) Contributions à l'étude écologique d'un estuaire atlantique marocain: l'estuaire du Bou Regreg. Université de Bordeaux I, France
 32. Chafik A, Cheggour M, Cossa D (2001) Quality of Moroccan Atlantic coastal waters: water monitoring and mussel watching. Aquat Living Resour 14(4):239–249
 33. Nicolas M, Belin C, Favre P, Rudloff L (2017) Surveillance des phycotoxines dans les coquillages. Bulletin Epidémiologique, santé animale et alimentation n° 77/numéro spécial-surveillance sanitaire des aliments 77: 23–27
 34. Frehi H, Couté A, Mascarell G, Perrette-Gallet C, Ayada M, Kara MH (2007) Dinoflagellés toxiques et/ou responsables de blooms dans la baie d'Annaba (Algérie). CR Biol 330(8):615–628. <https://doi.org/10.1016/j.crv.2007.05.002>
 35. Beaumais O, Appéré G (2010) Recreational shellfish harvesting and health risks: a pseudo-panel approach combining revealed and stated preference data with correction for on-site sampling. Ecol Econ 69(12):2315–2322
 36. Flegel T, Fegan DF (2002) Strategies for preventing the spread of fish and shellfish diseases. Fish Sci 68(1):776–788
 37. Holmes M, Teo SM (2002) Toxic marine dinoflagellates in Singapore waters that cause seafood poisonings. Clin Exp Pharmacol Physiol 29(9):829–836
 38. Earampamoorthy S, Koff RS (1975) Health hazards of bivalve-mollusk ingestion. Ann Intern Med 83(1):107–110
 39. Cherkaoui E, Nounah A, Khamar M (2013) The impact of pollution on the Bouregreg estuary (Morocco, Atlantic Ocean): the molluscs as an indicator of metal contamination. J Environ Sci Eng B 2(7B):432
 40. El Amraoui M, Tarbaoui M, Benba J, El Amraoui B, Bamhaoud T, Lazrak N, Monkade M. Evaluation of bacteriological parameters of water quality in the Bouregreg estuary along the Moroccan Atlantic Coast. World J Innov Res 1(1):20–23
 41. Kabbour A, Mouhir L, Laaouan M, El Hafidi M, Saafadi L (2020) Effectiveness of vertical system planted with *Renealmia Alpinia* used in sub-humid climate to treat urban wastewater: a case study in the pilot site Bouregreg-ONEE RABAT. In: E3S Web of Conferences. EDP Sciences, pp. 02001
 42. Nassri I, Bellaouchou A, El Abidi A, Senouci S, Assobhei O, Fekhaoui M (2013) Spring water quality in the area of Rabat-Sale-Zemour-Zaer (Morocco): microbiological indicators. Int J of Aquatic Sci 4(2):59–68
 43. Ferdaous L, Mohammed B, Nouzha B, Hamid L, Abrerrahim L (2015) Etude de la qualité physicochimique et de la contamination métallique des eaux de surface du bassin versant de Beht (Maroc). Eur Sci J 11(11):132–147
 44. Cheggour M, Chafik A, Fisher N, Benbrahim S (2005) Metal concentrations in sediments and clams in four Moroccan estuaries. Mar Environ Res 59(2):119–137
 45. Bounouira H (2007) Study of chemical and geochemical qualities of Bouregreg river basin. Université Ibn Tofail de Kénitra, Morocco
 46. Radenac G, Miramand P, Tardy J (1997) Search for impact of a dredged material disposal site on growth and metal contamination of *Mytilus edulis* (L.) in charente-maritime (France). Mar Pollut Bull 34(9):721–729. [https://doi.org/10.1016/S0025-326X\(97\)00011-8](https://doi.org/10.1016/S0025-326X(97)00011-8)
 47. El Harim A, Cherkaoui E, Khamar M, Nounah A (2019) The impact of the depollution project on the quality of the estuarine ecosystem of Bouregreg (Morocco Atlantic Coast). Int J Conserv Sci 10(4):763–772
 48. El Attar J, Assobhei O (2001) Étude de la pollution fécale d'une zone ostréicole du Maroc (lagune de Oualidia). Marine Life (Marseille) 11(1–2):39–47
 49. Chafik A, Cheggour M, Rharbi N, Bouhallaoui A (1996) Evaluation de la salubrité de la lagune de Oualidia: étude de la contamination métallique de l'huître creuse *Crassostrea gigas*. Travaux et Documents INRH 93:26
 50. Plusquellec A, Beucher M, Legal Y (1984) Bivalves: indicateurs de pollution microbienne des eaux littorales. IFREMER, Actes de colloques 3:541–548

51. Passerat J, Ouattara NK, Mouchel J-M, Rocher V, Servais P (2011) Impact of an intense combined sewer overflow event on the microbiological water quality of the Seine River. *Water Res* 45(2):893–903
52. El-Sharkawi F, El-Derea H, Akel M (1982) The effect of marine pollution on the hygienic quality of shellfish caught in Alexandria beaches. *Bull High Inst Public Health Alex* 12:47–57
53. Mabrouk A (1988) Etude bactériologique des huîtres de la lagune de Nador. Université Mohammed V. de Rabat, Morocco
54. Bouhayene S, Djebbar A (2014) Evaluation of the microbiological quality of the seawater of the main beaches of Skikda (East-Algerian). *Ann Biol Res* 5(12):34–40
55. Brisou JF, Denis FA (1978) Hygiène de l'environnement maritime. Masson
56. Robertson W, Tobin R (1983) The relationship between three potential pathogens and pollution indicator organisms in Nova Scotian coastal waters. *Can J Microbiol* 29(10):1261–1269
57. Bouchriti N, El Marrakchi A, Fahim A (1992) The microbiological contamination of an oyster growing area in Morocco: the Oualidia lagoon. *Hydroécologie Appliquée* 4(2):189–202
58. Hassou N, Maanan M, Hennani M, Zourarah B, Assobhei O (2014) Spatial and temporal variation of faecal pollution indicators (*Escherichia coli* and faecal streptococci) and physico-chemical parameters at the Oualidia lagoon and its watershed (Morocco). *Int J Curr Microbiol Appl Sci* 3:675–694
59. Bahhou J (1991) Contribution à l'étude bactériologique des eaux marines littorales du nord du Maroc. Université Mohammed V. de Rabat, Morocco
60. dos Prazeres RD, Hofer E (1986) *Vibrio* species from the water-oyster ecosystem of Sepetiba Bay in Rio de Janeiro State. Brazil *Revista de Microbiologia* 17(4):332–338
61. El Alaoui I, Benbouzid M, Hajjaji SE, Benmessaoud F, Bellahcen TO (2020) Potential impact of the latest developments in the Bouregreg estuary. In: Ezziyyani M (ed) *Advanced intelligent systems for sustainable development (AI2SD'2019)*. Springer International Publishing, Cham, pp 520–535
62. Verdelhos T, Neto J, Marques J, Pardal M (2005) The effect of eutrophication abatement on the bivalve *Scrobicularia plana*. *Estuar Coast Shelf Sci* 63(1–2):261–268
63. Schlacher TA, Liddell B, Gaston TF, Schlacher-Hoenlinger M (2005) Fish track wastewater pollution to estuaries. *Oecologia* 144(4):570–584
64. Preece EP, Hardy FJ, Moore BC, Bryan M (2017) A review of microcystin detections in estuarine and marine waters: environmental implications and human health risk. *Harmful Algae* 61:31–45
65. Whittall D, Bricker S, Ferreira J, Nobre AM, Simas T, Silva M (2007) Assessment of eutrophication in estuaries: pressure–state–response and nitrogen source apportionment. *Environ Manage* 40(4):678–690
66. Elliot EL, Colwell RR (1985) Indicator organisms for estuarine and marine waters. *FEMS Microbiol Rev* 1(2):61–79
67. Oualid C, Abdellah M, Abdelhakim EOA (2018) Impact of urban activities on water quality. *Int J Appl Eng Res* 13(11):9442–9449

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.