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Original Research

Temporal and Spatial Distribution of Faecal Bacteria in a Moroccan Lagoon

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Abstract

The origin and distribution of microbial contamination in Oualidia lagoon were assessed using faecal coliform and streptococci bacteria. The lagoon is used for recreation, oyster farming, and fishing. Samples were collected from 9 different sites, and physicochemical and microbiological analyses were performed monthly during 2 years (October 2003-October 2005). These samples were subjected to bacteriological analysis faecal coliform count and faecal streptococcal count. The physicochemical analysis of the water samples includes pH, temperature, and specific conductance. The bacterial genera were identified on the basis of their morphological and physiological characteristics. The present study confirmed the presence of bacterial indicators of faecal origin at various stations in every stretch of the Oualidia lagoon system. The samples collected from different sites of the lagoon showed wide variations in the counts of bacteria. The Bacterial population varied with seasonal variations in the water body. The Faecal coliform counts increase during the summer months and decrease during winter. The opposite happens for streptococci bacteria, which increase during winter and decrease during the summer, depending on rainfall. Positive correlation was established between faecal coliform and streptococci bacteria. The results of bacteriological analysis of water revealed that the situation is alarming. The Bacteriological analysis of lagoon water indicated that water was polluted by faecal contaminants to the extent that it was unsuitable for recreation and hence needed thorough impoundment.

Keywords: Oualidia Lagoon, faecal coliform, oyster management, Morocco

Introduction

Coastal lagoons are natural systems of great ecological concern because they are highly productive and teeming with biodiversity. These systems are frequently located in the interface of land, freshwater, and seawater environments. The management of these areas in view of spatial planning optimizing habitat conservation, environmental protection, resource exploitation, and economic uses is urgently needed, and user conflicts are acute and difficult to resolve.

Several studies have suggested that land-use characteristics can have an effect on coastal water deterioration. Non-point source pollution is the main cause of the impact on coastal water quality in urban areas [1]. Non-point source pollutants detrimental to water quality include heavy metals and other toxic substances [2], nutrients (particularly phosphorus) [3], and oil and gasoline runoff from roadways [4]. However, bacterially contaminated, organic pollution may strongly contribute to water quality deterioration [1, 5].

Water quality degradation from faecal contamination may result in increased health hazards to recreational users

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and shellfish consumers, and often result in the closure of shellfish harvesting areas. The standard for detection of faecal pollution in surface waters is the determination of faecal coliform bacteria density [6]. Although this standard is considered to be inadequate to assess viral contamination [7], faecal coliform (FC) bacteria have been used as indicators of contamination by humans and other warm-blooded animals from agricultural activities [8, 9]. These indicators can be found in monitoring programmes of bivalve harvesting areas world-wide [10-12]. The presence of these organisms is also used to estimate the potential health risk of other pathogenic organisms of faecal origin to the users of the waterway [13]. This research provides a comprehensive analysis from October 2003 to October 2005 of faecal coliform and streptococcus spatial distribution in the Oualidia lagoon, an oyster area of Morocco. First we will establish the geographic database distribution of faecal bacteria (FS, FC), then spatially analyze these data using ArcGIS software. Second, we will establish the correlation between environmental factors and faecal pollution of water.

Material and Methods

Study Area

Oualidia Lagoon (32º40'42"N-32º47'07"N and 8º52'30"-9º02'50"W) is located on the Atlantic Ocean (Fig. 1). This lagoon is 7 km long, averages 0.5 km in width, and exchanges water with the ocean through a major inlet about 150 m wide and 2 m deep. This basin is made up by the morphology of a depression, in a north-south direction, limited by a continental cliff and by a coastal consolidated dune ridge. Oualidia was chosen as a study area for several reasons:

1. The lagoon has multiple public uses, including recreation, oyster farming, commercial fishing, and shellfish harvesting,
2. Differing land-use patterns: the southwestern portion of the lagoon is highly developed and it includes areas with active septic tanks, while the northeastern portion is an undeveloped state with agricultural activities,
3. Relevant GIS data layers for land-use investigations in the lagoon were developed previously.

The annual average rainfall, estimated from 1977 to 1998, is about 390 mm, with a maximum in December and no rain during the dry period. The annual estimate of evaporation minus precipitation is 650 mm. Rainfall over the region accounts for only 1% of the fresh water entering the lagoon. The predominant wind directions are WSW to NW during the wet season and NNE to NE during the dry season. Sporadic violent winds (*Chergui*) occasionally blow from the ENE during the dry period and may contribute to high evaporation rates over the lagoon, as well as to extreme air temperatures that can reach 40ºC. More generally, winds blowing from the northern sector will produce southerly geotropic currents along the coast, offshore transport and coastal upwelling of nutrient-rich deep waters close to the coast. Upwelled waters with high nutrient content can be advected by flood tides into the lagoon, supporting biological production and enhancing aquaculture yields (oyster farm). The lagoon has a relevant importance for the national commercial production of bivalves. This extractive activity is practiced along the intertidal area.

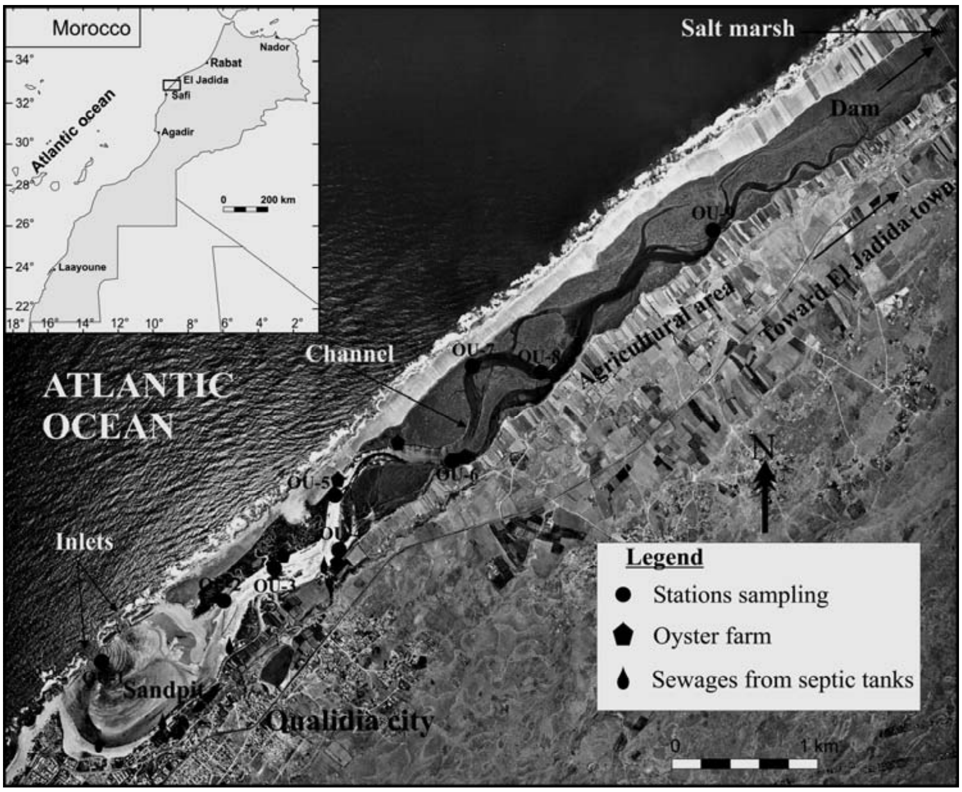

Fig. 1. Study area.

The annual production of oysters in the lagoon is estimated at 120 tons per year. Most of the bivalve production is exported to Europe.

In recent decades, population growth and development in adjacent watersheds raised new environmental concerns. Urban areas are sources of pollution, impacting the sustainability of human activities dependent on good water quality in the lagoon, such as tourism-related activities and bivalve culture [2]. Coastal aquaculture is a traditional practice in the Oualidia Lagoon. Accelerated development in the last three decades has created negative environmental impacts, such as changes in hydrologic regimes in enclosed waters due to the proliferation of aquaculture structures, and discharge of high levels of organic matter into coastal waters. Similarly, the increasing deterioration of coastal water quality resulting from the discharge of domestic, agricultural, and industrial wastes into coastal waters has affected aquaculture production and profitability [14, 15]. Furthermore, the increased frequency of red tides in the lagoon has posed serious threats to coastal aquaculture, especially to oyster cultivation. The introduction of management measures to mitigate deteriorating coastal water quality and the adverse environmental impacts of aquaculture development has now become a matter of urgency to the region. Recently, local authorities tried to control the problem of faecal pollution in the lagoon through the transference of urban effluents to the wastewater treatment plant.

Our study looked at FC levels in the surface water from 9 stations, as well as the environmental parameters in the lagoon. It also characterized land use and land cover in the adjacent sub watersheds to assess their relative contributions as sources of faecal contamination. This kind of information could be particularly useful for authorities wanting to develop monitoring strategies and to estimate risks to human health associated with the consumption of bivalves, as well as for authorities involved with land use planning.

Sampling and Analysis

Faecal indicator bacteria employed in this study were faecal coliform and faecal streptococci. To estimate the number of these bacteria, water samples were collected in ethanol-rinsed high density polyethylene bottles that were washed with ambient water before sampling to prevent ethanol-related die-off. Samples were collected monthly, at 9 georeferenced stations in duplicate, from 25 cm below the surface of water and transported to a laboratory on ice, and analyzed within 6 h of collection (Fig. 1).

A multiparametric probe (PM 2000, Belgium) designed for oceanic measurements in seawater is used. The probe was adapted to measure in situ physico-chemical parameters such as temperature (T, ºC), electrical conductivity (EC, mS/cm), and pH while all other parameters were determined in the laboratory.

Microbiological tests were carried out in order to evaluate the presence of microorganisms that are indicators of fecal pollution and are possibly associated with pathogens: faecal coliform, faecal streptococci. To perform these tests, we used the membrane filtration technique [16]. In particular, the following media, at temperature and incubation time were used: Tergitol 7 TTC agar (Biokar) incubated at 44±0.2ºC for 24 h to individual colonies of faecal coliform, and Bille Esculin Azide Agar (BEA) incubated at 37±0.5ºC for 48 h to individual colonies of faecal streptococci. Results are expressed as the number of colony forming units (CFU) 100 ml^{-1} of water for faecal streptococci and for faecal coliform. Statistical analyses were performed using STATISTICA software.

GIS Approaches

The methods implanted in the GIS for the support of mapping of environmental pollution are mostly focused on data collection and basic analyses, modelling, and predictions. The results can be displayed by the GIS in the map themes. ESRI's ArcGIS 9.2 was used to manipulate data and produce the maps. Mapping intervals were assigned using Manual classifications. EC data were mapped via satellite-generated coordinates (GPS bearings) for ease of future reference. A database was constructed matching GPS data to water data on EC gathered at 9 sampling sites in the lagoon.

Results

Environmental Parameters

The environmental parameters registered during the study period are summarized in Table 1. Rainfall ranged between 0 mm (summer 2003) and 380 mm (December 2004). Salinity ranged between 26.3 (±2.2) ppt (autumn 2004) and 33 (±0.4) ppt (summer 2005) in the lagoon. Water temperature ranged between 16.1 (±1.5)ºC (autumn 2000) and 27.6 (±1.2)ºC (summer 2004). Statistically significant relationships ($p<0.05$) were only obtained between rainfall and monthly faecal bacteria levels in water samples (Table 2).

Spatially, water temperature, salinity, pH, and conductivity levels displayed strong axial gradients, with a sharp decrease in values from the mouth to the upper reaches of the lagoon during both tidal phases (Table 1). The greatest decline in these values was observed between the last two sites. Fresh water was present at the last site, with salinity readings of less than 0.5 psu. Turbidity levels in surface waters have been found to vary due to variations in precipitation and the percentage of impervious surface in a watershed High turbidity levels in surface waters have been linked to high percentages of impervious surfaces within a watershed caused by sediment loading from runoff and erosion [17, 18].

Microbiological Characteristics

The high counts for faecal coliform at stations Ou-4 and Ou-5 during 2003-04 tests can be explained in part by their location which was within an oyster farm and pastures regularly inhabited by dairy cattle. High faecal coliform counts have been positively related to urban development, agricul-

Table 1. Monthly variation of the environmental parameters considered in this study.

Months	Rainfall (mm)	T (ºC)	Sal. (ppt)	pH
Oct-03	125	20.5	33	20.5
Nov-03	80	16.2	32.43	16.2
Dec-03	380	17.3	32.76	17.3
Jan-04	15	17.53	26.53	17.53
Feb-04	20	16.96	28.93	16.96
Mar-04	75	19.4	31.36	19.4
Apr-04	30	18.43	31.53	18.43
May-04	60	20.1	32.43	20.1
Jun-04	0	21.1	30	21.1
Jul-04	0	26	29.26	26
Aou-04	0	27.6	32.59	27.6
Sep-04	0	22.76	31.63	22.76
Oct-04	0	21.1	29.33	21.1
Nov-04	60	17.7	26.13	17.7
Dec-04	125	17.38	29.86	17.38
Jan-05	10	17.13	32.8	17.13
Feb-05	30	16.76	31.66	16.76
Mar-05	20	19.5	28.86	19.5
Apr-05	0	20.16	31.66	20.16
May-05	0	20.83	28.86	20.83
Jun-05	0	22.1	31.16	22.1
Jul-05	0	24.83	29.26	24.83
Aou-05	0	26.66	29	26.66
Sep-05	0	22.4	26.26	22.4
Oct-05	0	20.76	30.66	20.76

ture, and the amount of erodible soils [17]. High coliform counts are not only dependent on the total area of a particular land use within a watershed, but also on the amount of precipitation that the watershed received in the previous days [17].

Temporal variation in FC and FS in Oualidia Lagoon during the sampling period is shown in Fig. 2. All measurements showed a considerable variation from month to month. Most displayed a general increasing trend during the sampling period. Monthly FC levels in water ranged between 1.9×10/100 ml (June 2005) and 1.4×10^4/100 ml (August 2004) (n=40), whereas monthly FS levels varied between 3.6×10/100 ml (July 2004) and 1.1×10^4/100 ml (December 2003).

Seasonal means of FS levels in water were higher than FC in the lagoon, where seasonal means of FS levels water ranged between 5.9×10/100 ml ($\pm1.7\times10$) (spring 2004) and 4.5×10^3/100 ml ($\pm5.9\times10^3$) (autumn 2003), and of FC between 2.0×10/100 ml ($\pm1.5\times10$) (spring 2004) and 1.5×10^3/100 ml ($\pm2.0\times10^3$) (autumn 2003).

FC displayed the higher concentrations during summer, usually ranging from 1 to 65,000 CFU per 100 ml lagoon water and had lower values during winter, usually ranging from 1 to 6,000 CFU per 100 ml lagoon water. In contrast, FS displayed higher concentrations during the winter season, usually ranging from 1 to 50,000 CFU per 100 ml lagoon water. Spatially, there is a significant increase in average FC counts below the lagoon in all years (Student's t=5.171, p=0.001). Indeed, levels associated with raw sewage were recorded at all sites in the reach below the outfall. The FC/FS ratio allowed us to define the faecal contamination origin. So, values lower than 0.7 would testify to animal origin of faecal pollution, while those greater then 4 would indicate human origin.

The FC/FS ratio tends to be significantly greater than 4.0 near ocean in the summer (tourism activities). When water shows a higher faecal streptococcus count than faecal coliform, it most likely contains animal wastes, particularly livestock. Ratios tend to be less than 1.0. When ratios fall in-between values of significance, an estimate may be made according to how near the ratio is to human or animal values.

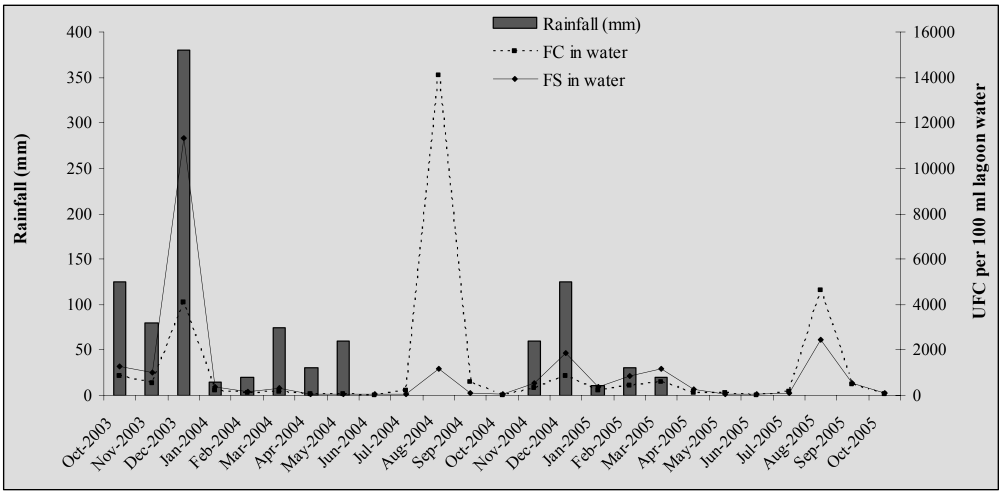

Fig. 2 Monthly variations of FC and SF levels in water (means) and rainfall (mean) in Oualidia Lagoon.

Table 2. Pearson correlation coefficients obtained in the linear regressions established between the environmental parameters and faecal bacteria levels.

Environmental parameter	Faecal bacteria	
	Faecal coliform	Faecal streptococci
Rainfall	0.55	0.74
Water temperature	0.06	-0.34
Salinity	0.17	0.17
pH	-0.35	-0.33

GIS Analysis

GIS mapping provides the first reference for the development of a geographic database on distribution of faecal contamination along the lagoon.

We have adhered to the following methodology for mapping biological pollution of the lagoon: first, the sampling points in the lagoon are georeferenced into the Morocco mapping system (Merchich projection, which is a Lambert projection). They are imported into an ArcGIS 9.2 geodatabase in a shapefile format. This shapefile has a point topology and at each point are associated fields with biological information (FC, FS, and FC/FS ratio). Second, the limits of salt marshes are manually digitalized from a georeferenced aerial photography. These limits are some polylines and placed into another shapefile into the geodatabase. Third, an interpolation is then performed upon each field of the point shapefile. We have used Inverse Distance Weighted interpolation. The choice of the interpolation fulfills two requirements:

(i) the influence of at least one upstream and one downstream point, taking into account the calculation of the interpolated point

(ii) the polyline shapefile is used like a barrier line in order to stop the interpolation process beyond the barrier.

This second requirement is necessary to avoid mistakes in the bent channel: in such a meander, the nearest point from an interpolated point into the middle of the channel measured on a straight distance lacks necessary significance because this point isn't necessarily the nearest point if we followed the distance along the bent channel. The result is a set of maps: each map emphasizes each spatialized field of the point shapefile (FS, FC, and the ratio of two variables for each date).

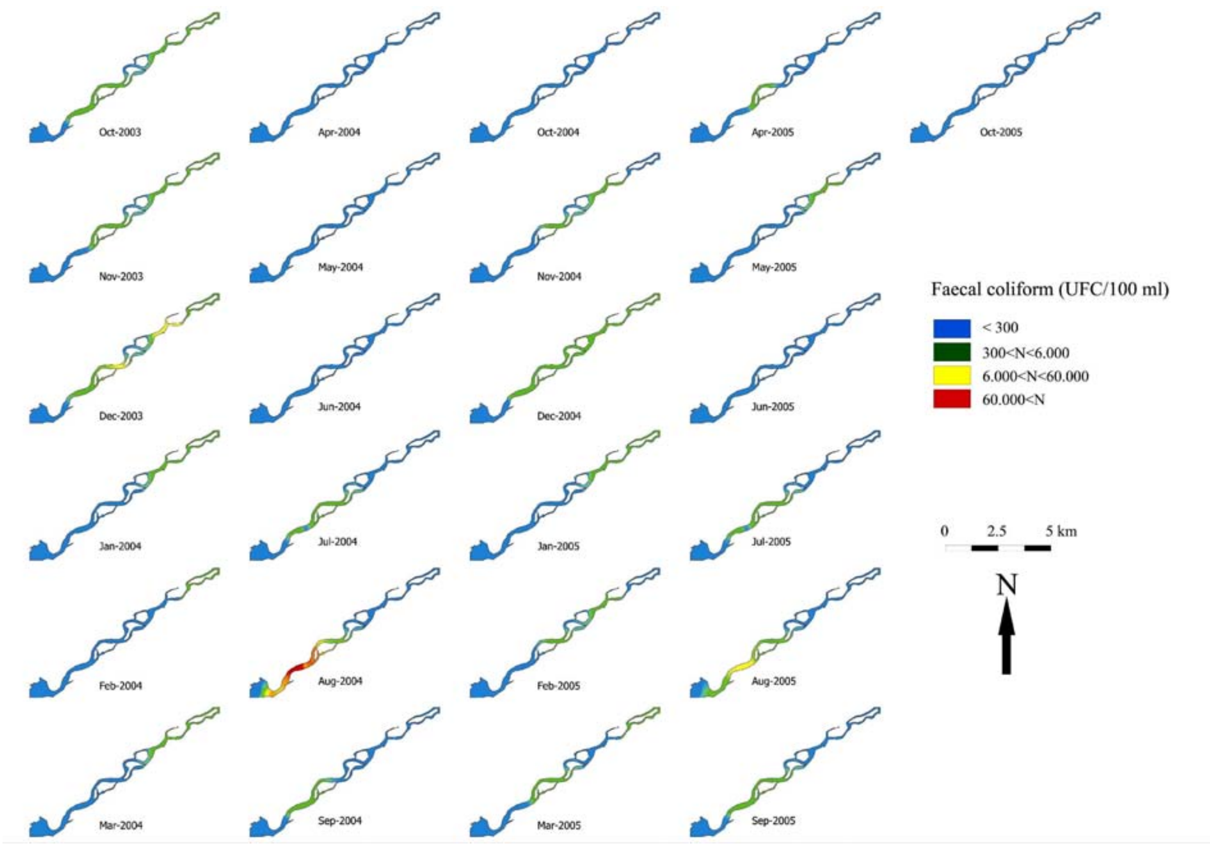

Fig. 3. Distribution of feacal coliform in lagoon water.

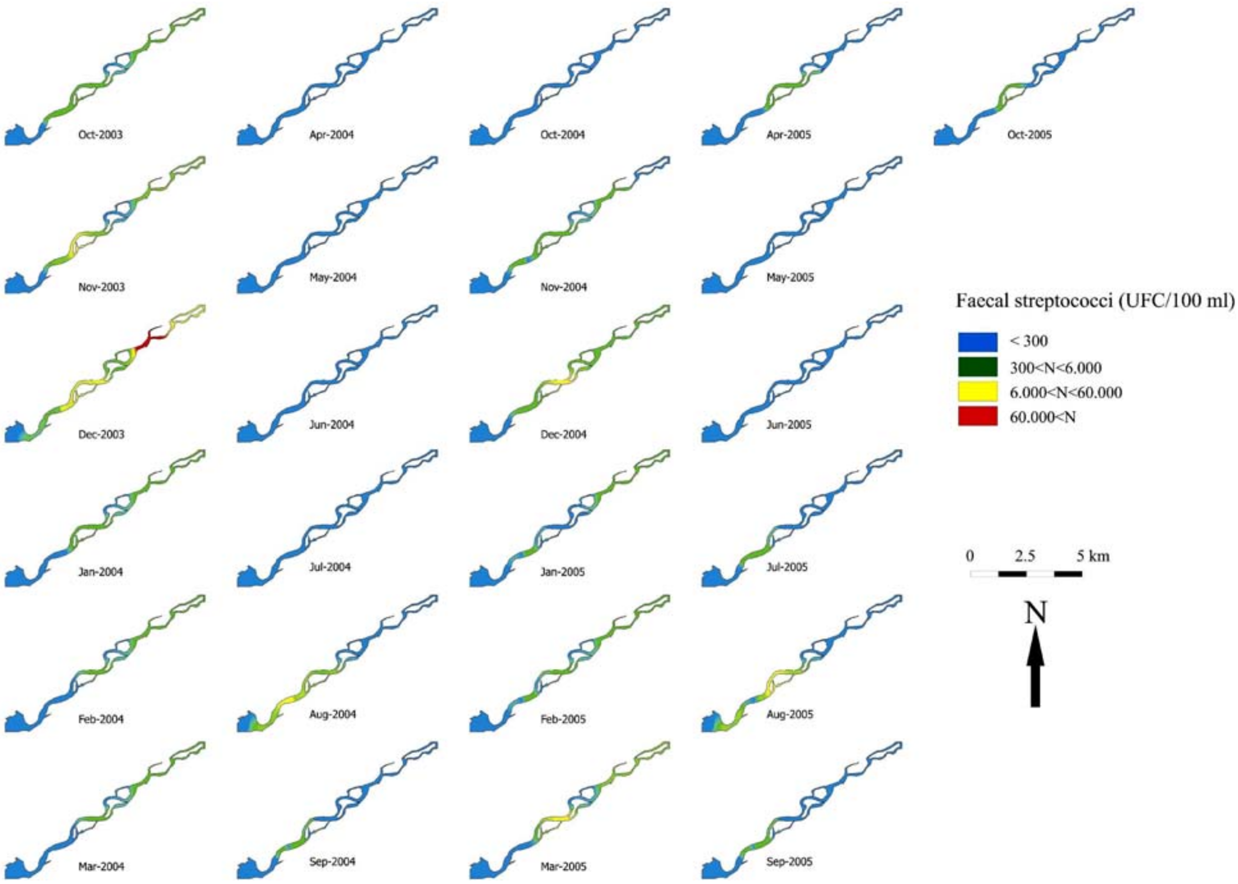

Fig. 4. Distribution of feacal streptococci in lagoon water.

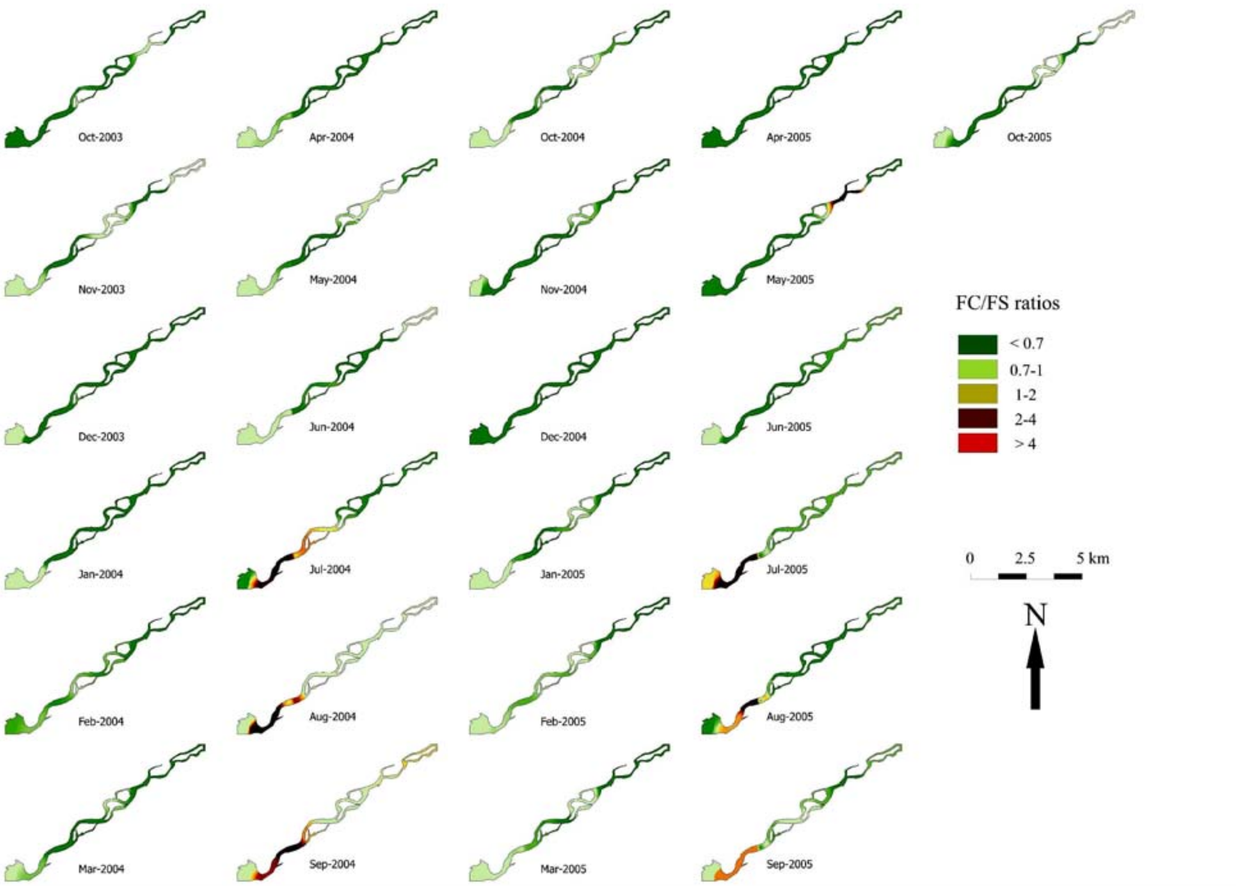

Fig. 5. FC-FS ratio distribution in lagoon water.

Maps showing the geographical distribution of FC and FS are shown in Figs. 3 and 4. Visually high faecal bacteria rates appear to be concentrated around the main human activities, including urban centres (urban sewage, tourism activities), oyster farms, and agricultural areas. The Temporal distribution shows that faecal contamination of animal origin yearround may be taken as strong evidence that pollution derives predominantly or entirely from livestock or poultry waste. If the ratio is greater than or equal to 4, it may be taken as strong evidence that pollution derives from human waste in the summer season, where human actions (tourism activities) are marked well near the ocean. These results confirm the animal origin of the faecal contamination bound to the fertilizer flow by stream waters and\or to pasture activities, and human, related to the anthropic action due to an intensification of tourism activities downstream of the lagoon during the summer (Fig. 5).

Discussion

Many studies address the combined effects of human activities and environmental factors on the levels of microbiological contamination found in coastal areas used for the commercial production of bivalves [13, 14, 19, 20].

In urban watersheds, a strong correlation exists between the percent of impervious surfaces in the watershed and means of faecal coliform levels in surface waters. [13] also found a similarly strong correlation between population densities and mean faecal coliform levels in surface waters. Faecal coliforms are consistently higher during both base flow and high flow periods in urban watersheds compared to other land use [21].

The number of domestic animals in an urban watershed may be another cause of elevated faecal coliform levels. Mallin et al. [22] and Kistemann et al. [23] found that high levels of faecal coliforms and *E. coli* in urban watersheds were the result of animal sources rather than human. They also found that faecal coliform counts from septic systems in surface waters of an urban watershed were negligible in comparison to other sources of faecal coliforms.

The seasonal differences in the disappearance rates of faecal coliform in this study may be due to seasonal variations in temperature and/or intensities of rain. Faecal coliform levels in surface waters often peak after a major rain event [13, 24]. Significant and positive correlations between the microbiological characteristics and monthly rainfall were reported in this study (R^2 for FS=0.74 and R^2 for FC=0.55). We observed that in the case of rainfall, the bacteria loads of running water suddenly increase. Thereafter, they decrease or disappear from water with time and can concentrate in sediments at high densities.

The highest FC/FS ratio was obtained in the rainy season and was negligible in winter, which may be because the relative frequency of FS from animal sources increases in rainfall ($R^2 = 0.74$). When deposited on the floor of animal rearing facilities or spread onto pastures of adjacent watersheds, microbial pathogens in animal waste can be washed and conveyed into creeks, especially during rainy periods [13, 24]. Therefore, the positive significant correlations can be found between land use associated with livestock production and anomalous concentrations of pathogenic bacteria during high-flow periods in water courses [25]. Under such circumstances the assimilatory capacity of the local landscape may become compromised and the microbiological contamination of a lagoon is likely to occur.

In our study, faecal coliform are used as indicators of enteric pathogenic organisms in lagoon environment, although recent studies indicate that they are not reliable predictors of the presence of pathogenic viruses [26]. They are regularly monitored to ensure that water bodies meet established sanitary standards for water use in recreational activities. Information on faecal coliform levels is also required for classifying shellfish growing waters as approved, conditionally approved, or restricted, in order to protect humans from consuming contaminated shellfish.

Conclusion

Our study is believed to contribute to the knowledge of the FC and FS levels in surface water, and variations of environmental parameters in Oualidia Lagoon (Moroccan coast). In addition, it also characterizes land use and land cover characteristics in adjacent sub-watersheds as well as assesses their relative contributions as sources of faecal contamination to lagoon water.

The results of this study show a periodic occurrence of faecal bacteria levels higher than the end-product standard, and a positive relationship with rainfall in water. Both occurrences indicate that the deterioration of the microbiological quality of water derives from the cumulative impact of the reservoir of faecal contamination created in adjacent urban and suburban areas in the Oualidia sub-watershed, entering into the lagoon during stormwater runoff.

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