Assessment of Microbiological Contamination of Marine Ecosystems : Can we Continue to Limit Ourselves to the Analysis of Fecal Parameters for the Assessment of the Coastal Ecosystems Quality?

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Abstract

This study reports the relationship between the level of faecal contamination and the prevalence of *salmonella* in the coastal and shellfish areas of the Agadir coast (south of Morocco), 801 samples of mussel, sea water, and marine sediment collected for48 months from six site along the Moroccan Atlantic coast between Essaouira and Sidi Ifni.Two methods were used for this study:(AFNOR NF EN ISO 6579 V08-013) to detect the presence of *salmonella* spp, and the most probable number method (ISO/TS 16649-3 standard) to detect *E. coli* the overall prevalence of *salmonella* spp.is 7.1%. The number of *E.Coli* was between 18/100g and 28.10^3/100g of flesh and Liquid intervals .This study indicates the potential health risk associated with the presence of these bacteria and their relationship in the assessment of microbiological contamination of the marine environment in this populated region of southern Morocco where shellfish production and maritime tourism are important to the local and national economy.

I. INTRODUCTION

Salmonella and *E.coli* are enteric bacteria entering coastal waters from remote sources by rivers and precipitation [2-10]. These non-indigenous bacteria undergo significant stress in the marine environment and their survival in this environment will depend on their adaptations to the abiotic and biotic constraints of this new support. Therefore, these bacteria pose a potential risk to consumers by ingesting undercooked bivalves or crustaceans [4]. Several cases of gastroenteritis due to the consumption of seafood have been recorded in Morocco [3] and their etiology is often not known. They could be caused by enteric pathogens or toxin.

In Morocco, few studies [3-8-11] have been done on the occurrence of the pathogenic bacteria in the seafood in order to better estimate the prevalence of these pathogens in the marine environment. Such studies are necessary to carry out the analysis of risk associated with these pathogens to reduce the manifestation of collective food poisoning infection.

The first aim of this study was to investigate the occurrence of Salmonella along the Moroccan Atlantic coast between Agadir and Essaouirra.

The second aim was to determine the existence of any correlation between the occurrence of *salmonella* pp. And *Escherichia coli* (indicator bacteria) and better contribute to the improvement of the coastal and marshy monitoring programs of the Moroccan coast of Agadir which is of great importance in the fisheries and tourism sectors in this country.

II. METHODS AND MATERIAL

2.1. Study Area

Our study area is located on the coast of Agadir from the Essaouira city region northwards to the Sidi Ifni region to the south 120 Km (fig.1) it is characterized

by an alternation of rocky cliffs and of beaches showing wide foreshore. Along this strip there are large shellfish deposits, mainly mussels. Three shellfish-growing areas are classified at the level of this region, Tamri-Capghir (30°42'687''N-09°51'730''W), Douira- Sidi Rbat (30°12'198''N-09°38'152''W), and Sidi Boulfdail (29°40'970''N-09°58'691''W). Despite its tourist attractions, the coast of Agadir suffers from the pollution risks. It is directly affected by discharges from urban agglomerations (industrial zone of Anza), as well as that comes from harbor activity. The annual rainfall in Agadir is 250 mm. The rainy period lasts a few months, from late October to March. The sunshine is more than 300 days a year, interrupted by fog and dew. Temperatures are strongly influenced by the trade winds present throughout the year, and vary little between winter and summer. Average temperatures range from 14°- 16°C in January to 19°- 25°C in July. However, the region sometimes experiences rising Saharan air which can exceptionally increase temperatures above 40°C.The Agadir coast is permanently influenced by the currents coming from the Canary Islands which go from southwest to north, there are also the offshore currents which cause the rise of cold waters (upwelling), which are especially important in summer (Orbi et al.1991).Samples were taken from six sites spanning more than 122 kilometers along the coast of Agadir: Tamri and Capghir in the northern zone, Anza in the central region, Tifnit, Douira and Sidi Rabat in the southern areas (Fig 1).
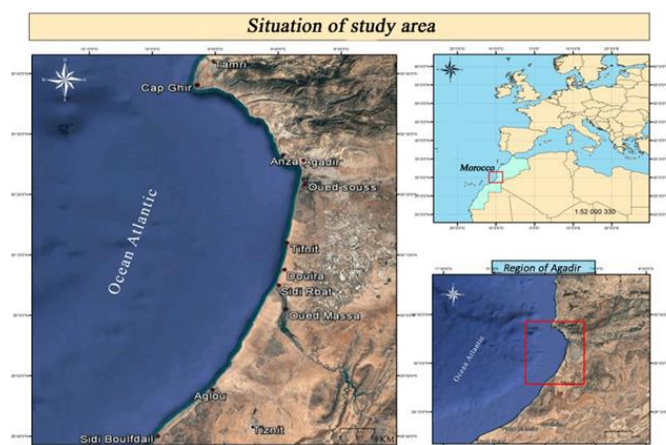
Figure 1 : situation of study area

2.2. Physico-chemical parameters

Temperature, salinity and pH were measured along the sampling period. The sea water temperature is measured by the laboratory calibrated thermometer, pH was measured by the pH meter (WTWpH522), and Salinity was detected by a salinometer (WTWLF18, Measuring Cells Tetracon 325).

2.3. Environment parameters

The environmental parameters analysed in this study are: Environmental temperature, wind, precipitation, solar radiation, salinity and water temperature. The minimum, maximum and average air temperatures were recorded daily. Wind direction was measured in each of the four quadrants (northwest, northeast, southwest and south-east). The wind speed (wind flow velocity) was expressed in kilometres per hour. Precipitation was measured in millimetres of precipitation per day, and solar radiation was measured in watts per square meter (W / m2.). All climatic data were provided by the Climate Department of the Regional Directorate of National Meteorology Agadir-Inzegane station (9°34'58.5''W, 30° 25'10.18''N).

2.4. Sampling program

The samples are collected monthly at six sites along the Agadir coast (fig.1). each sampling consists of commercial-sized mussels, those samples were collected in each of the different stations and placed in plastic bags , Sea water and sediment were collected in sterile glass jars; The samples are then stored in coolers which its temperature maintained between 2°C and 15°C by cold accumulators and transported to the laboratory. On arrival at the laboratory, samples are stored at a temperature of 6°C +/- 2°C. The time limit between sampling and analysis should not exceed 24 hours. These stations where samples were collected have been chosen according to their accessibility, depending on the tourist, agricultural activity, and function of their proximity to streams, and waste water. The presence of *E.coli* and salmonella spp. was studied in seawater (277), marine sediments (241), and mussels (283).

2.5. Enumeration of *Escherichia.Coli*

The most probable number (MPN) of *E. Coli* in samples was determined using the most probable number (MPN) method for mussels and sediment, with a series of five tubes out of three. According to ISO / TS 16649-3: 75 to 100g of flesh and interval fluid were diluted in double quantity with tryptone diluent (Biokar Diagnostics) and the mixture was homogenized using a Blendor (120 revolution per second) for 60 to 120 sec, the resulting

stock solution was diluted to 1/3, after 15 min of rest, 30 ml of the same solution are mixed with 70 ml of diluted solution to give a dilution of 1:10

- The first series of five tubes of 10 ml of doubly concentrated glutamate broth are inoculated with dilution to 1/10.
- The second series of the simply concentrated glutamate broth is seeded with 1 ml of the same dilution.
- The 3 series of simply concentrated broth glutamate is inoculated with 1 ml of the diluted 1: 100 solution.

After homogenization, all the tubes were incubated at 37°C for 24 hours. Escherichia coli confirmation was carried out by Subculture on Tryptone-Bile-Glucuronat (TBX) (Oxoid, Wesel, Germany) of positive tubes (tubes showing acid production by a yellow color) after incubation at 44°C for 24 h, The number of boxes growing blue or greenish blue colonies is considered a positive result, this number determines the MPN or the most likely number of E. coli.

2.6. Isolation and Biochemical Identification of *Salmonella*

These bacteria were isolated according to ISO Standard 6579 (2002). 225 ml of buffered peptone water is added to 25 g of homogenized mold and incubated at 37°C. for 20 h. After the pre-enrichment step, 0.1 ml of the solution was transferred in to 10 ml of Rappaport Vassiliadis Broth (ScharlauChemie, Spain) and the mixture was incubated at 41.5°C for 24 h as well as 1 ml was added to 10 ml of Muller Kauffman au tétrathionate-novobiocine (MKTTn) (Scharlau Chemie, Spain) and incubated at 37°C for 24 h. The enriched suspensions were then placed on Rambach Agar (Merck) and Xylose Lysine deoxycholate Agar (Scharlau Chemie, Spain), and incubated at 37°C for 24 h. Suspicious colonies were purified with Tryptone sulphite neomycin agar (TSN) (Scharlau Chemie, Spain) also they were confirmed biochemically with API E system (bioMérieux, Marcy-France). For the sea water samples 100 ml of them were filtered through a 0.45 μm sterile filter membrane (Millipore Corporation, Bedford, MA),

and the filter was then placed in 225 ml of buffered peptone water (Merck Darmstadt, Germany).

2.7. Statistical analysis

The relationship between the presence of *salmonella* spp., E. coli and the environmental parameters included in the study was studied by simple logistic regression analysis, these statistical analyzes were performed with SPSSversion14.0.1 (SPSSInc) and the level of significance was Set at $P < 0.05$.

III. RESULTS AND DISCUSSION

3.1. Occurrence of E.coli:

Based on the results of the *E. coli* enumeration obtained by the NPP method for all the sites, we note that the Colimetry shows a greater contamination at the site of Anza compared to the other sites in the 3 compartments (85% in sediment, 63% in seawater and 75% in the mold) this would be explained By the nature of the site,which is unhealthy because of its proximity to the urban area polluted by domestic and industrial discharges that flow directly into the marine environment, unlike other sites of Tamri, Douira, Sidi Rbat and Tfinit which are located in safe area (figure. 2-3-4).
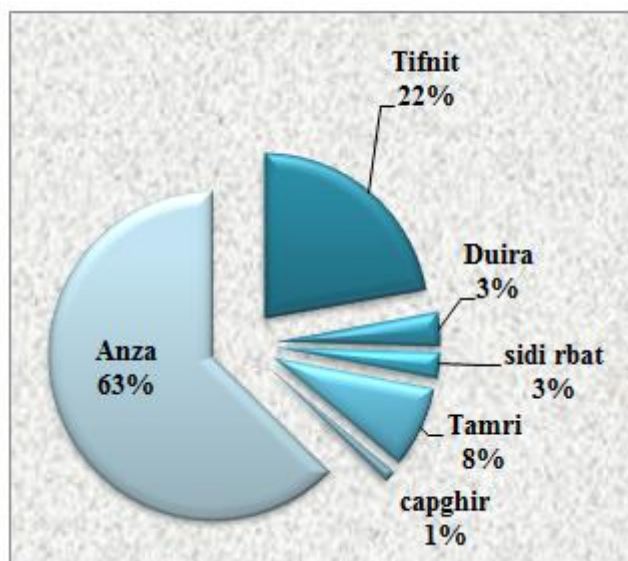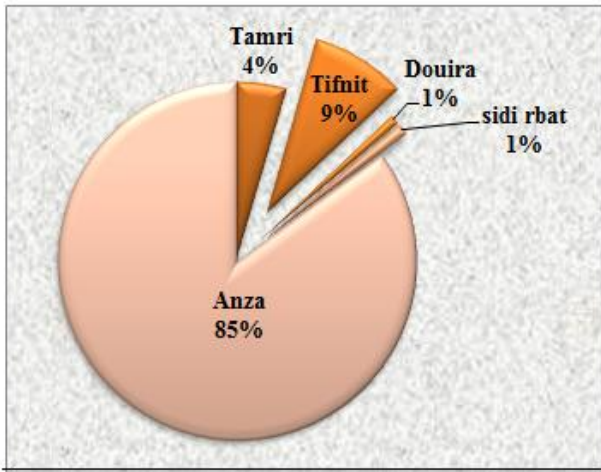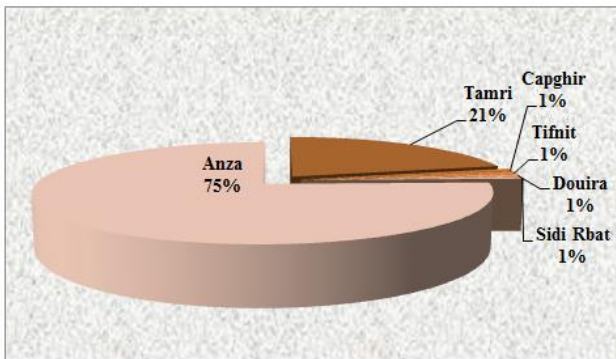

Figure 2: Distribution of *E.coli* in seawater at the various site

Figure 3: Distribution of E.coli in sediment at the various site

Figure 4: Distribution of E.coli in Mussels at the various sites.

3.2. Occurrence of *Salmonella:*

Salmonella showed an absence at the site of Capghir (ranked A) on 74 samples unlike the other sites; Tifnit (ranked A) showed 13.8% incidence of Salmonella on 144 samples, Anza (ranked D) showed 11% salmonella in 138, Tamri (ranked A) showed a relatively low incidence of 5.7% on 157 samples, Sidi Rbat (ranked A) 5.5% out of 144 samples and Douira (ranked A) 3.5% out of 144 samples.

Figure 7: Proportion of salmonella distribution in study site

Fifty-seven samples (7.1%) were contaminated with Salmonella spp of 801samples analyzed. The highest prevalence of Salmonella bacteria was detected in mussels, with 28 positive samples occurring in the 283 analyzed (10%), followed by sediments, with 19 positive samples (7.9%) and seawater with 10 positive samples (3.6%).
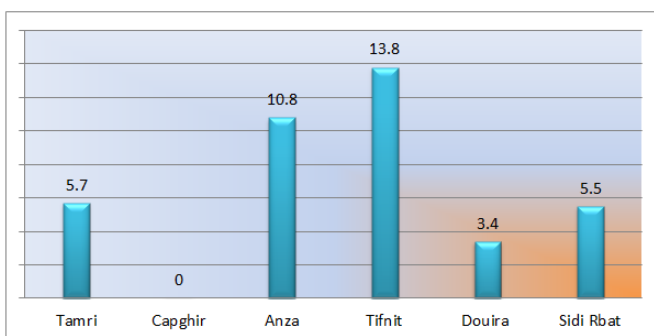
Table1: Distribution of *salmonella* in the compartments studied

compartment	Number of samples	No. of strains (% *Salmonella* positive)
mussel	283	28(9.9)
Sediment	241	19(7.9)
Sea water	277	10(3.6)
Total	801	57 (7.1)

3.3 *Salmonella* distribution according to colimetry

In order to discern a possible correlation between colimetry and the incidence of salmonella spp contamination, we calculated the percentage of strains of these pathogenic bacteria in each compartment analyzed according to the sanitary distribution of the shellfish aquaculturesites (Table2). This percentage is determined by the number of *E.coli* per 100g of flesh and interval fluid. Twenty two strains of salmonella, or 38.6%, are detected in *E.coli* contaminated mussels with a level between 0 and 230 *E coli* per 100g of flesh and interval fluid, which interval is determined for a health classification in A.Still in the same compartment five salmonella strains, or 8.8%, are detected in mussels with a contamination rate between 230 and 4600 *E.coli* per 100g of flesh and liquid intervals 1.8% Remains the percentage of salmonella strains detected in mussels contaminated with 4600 and 46000 *E.coli* per 100 g of flesh and interval fluid. For marine sediment and sea water the same ascertainment had been made, that means the percentage of salmonella is greater for low values of *E.coli* (Table 2).

Table 2: Percentage of *salmonella* isolated from each compartment analyzed according to the health distribution of *E.coli*

Number of *E. coli*/100g flesh and liquid intervals	Number of strains *salmonelle*/Compartment analyzed (%)			Total (%)
	Mussel	**Sédiment**	**Sea water**	
0- 230	22 (38.6)	15 (26.3)	2 (3.5)	39 (68.4)
230-4600	5 (8.8)	2 (3.5)	7 (12.2)	14 (24.5)
4600-46000	1 (1.8)	2 (3.5)	1 (1.8)	4 (7.01)
>46000	0 (0)	0 (0)	0 (0)	0 (0)
Total	28	19	10	57

The study of the correlation coefficients between the variables (Table3) shows that sea water temperature does not have a significant association with *Salmonella and E.coli*, The salinity of sea water is negatively associated with *E. coli*, environmental temperature and pH did not show any significant association with the two germs, logistic regression analysis identified the presence of rain during the first day and the fourth Day before sampling as a dominant factor in the presence of *Salmonella* in the Agadir region, which is also valid for *E.coli* but with less importance, insolation is negatively associated with the presence of *Salmonelle.*

Table 3: Correlation matrix obtained from the analysis of bacteriological components and environmental parametrs

parameters	*S.spp.*	*E.coli*
Sea water temperature	-0.455	-0.634
salinity	0.567	-0.039
Environnement temperature 2	-0.006	0.086
Environement temperature 3	-0.327	0.721
PH	-0.154	-0.698
precepetation_1	0.000	0.029
precepetation_4	0.000	0.033
Insolation_1	-0.010	-0.897
Insolation_4	-0.004	0.890
S. spp.	1	0.2336
E.coli	0.2336	1

Discussion

The main objective of this study is to understand the relationship between fecal contamination and the presence of salmonella on the Agadir coast in order to better contribute to the improvement of the monitoring programs of the coastal and shellfish areas of the Moroccan coast. The results obtained reflect a low incidence of salmonella in the coastal environment of the Agadir region, with an overall value of 7.11% as well as the total absence of salmonella at the Capghir site which has a rocky structure with a near absence of sediment. Low levels of incidence of salmonella in the marine environment have been observed in other regions of some countries that have oceanographic conditions and temperate seawater temperatures similar to the coastal region of Agadir namely the Galicia region (NW Spain), the United States, the United Kingdom and

Mexico, with impacts of 2.4%. 7.4%. 8% and 4.8% [1-10-12] respectively.

According to the tendency observed in the region of Agadir, the arrival of salmonella in the marine environment has been mainly governed by the presence of rainfall which transports the contamination from the source of origin to the sea by water streams, Contamination reaches the sea, the presence of salmonella is mainly affected by atmospheric conditions, these interpretations are supported by the statistical analysis, which has identified the presence of rainfall as a factor favoring the direct salmonella in coastal areas. [2-5- 6]. According to the results of this study, salmonella was detected, mainly when rainfall occurs consecutively in the days prior to sampling, which is also valid for *E.coli* but with less importance, it Is not consistent with the work of Kleinheinz et al. [7]. Who found that the concentration of *E.coli* decreased with precipitation.

Twenty two strains of *salmonella*, or 38.6%, are detected in E. coli-contaminated mussels with a level between 0 and 230 *E.coli* per 100g of flesh and interval fluid, which interval is determined for a health classification in A.While 1.8% of salmonella are detected in mussels contaminated with 4600 and 46000 *E.coli* per 100 g of flesh and interval fluid for a health classification D. This shows that the high levels of *E.coli* do not necessarily suspect the presence of salmonella and vice versa. These results are consistent with those already observed by Martinez-Manzanares et al. (1991) and Martinez-Urtaza et al. (2003), which show that fecal coliforms do not correlate with the presence of Salmonella [9-10].The absence of correlation between *E. coli a*nd *salmonella* contamination make us asking about the assessment of fecal contamination as an indicator. The effectiveness of assessing the health hazard due to the possible presence of pathogenic microbes and of estimating the health of the shellfish areas by shellfish colimetry has been proved but under special conditions [9].

IV. CONCLUSION

The absence of correlation between the number of fecal coliforms and the presence of salmonella in each of the compartments analyzed make us asking about the effectiveness of colimetry as a biological parameter for assessing the potential danger due to salmonella in shellfish, This absence of correlation between *E.coli* and salmonella recommends contributing to the improvement of coastal and shellfish monitoring programs and the development of risk management systems by regularly detecting salmonella and particularly for Shellfish aquaculture sites, classified as "A", during rainy periods for good monitoring of consumer health and the marine ecosystem Through this article, we hope that our country will also participate in the European epidemiological surveillance network for salmonellosis through the establishment of a National Reference Center for *salmonella* as the developed countries (France, United States..), which will have as objective; Epidemiological surveillance and prevention based on the strengthening of the control of the the water quality distributed, the treatment of waste water, the strengthening of the control of shellfish harvesting areas.

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