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Bioaccumulation and health risk assessment of bacterial pollutants to bivalve species in Ras-Kabdana-Saïdia coasts (Eastern Morocco).

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ABSTRACT

This study was carried out to assess the level of fecal pollution from seawater, sediment, and bivalves (*Chamelea gallina*) of the Ras Kabdana-Saidia coastline, monthly during 2018. The seasonal variations were affected significantly (P<0.001) by the concentrations of fecal coliforms (FC) and fecal streptococci (FS) in seawater, sediment, and mollusks. The highest bacterial population in mollusks was recorded in autumn/winter and the lowest values were founded in summer. The physicochemical conditions are known to govern the bioavailability of fecal indicator bacteria in the marine environment and their bioaccumulation by marine biota. Monitoring of bacteriological parameters revealed relatively high bacterial loads, especially in bivalves. Mollusks are filter feeders that can capture bacteria, and retain them for long periods. The FC (1.32 log CFU. 100 g^{-1}) and FS (1.93 log CFU. 100 g^{-1}) levels of *Chamelea gallina* from the studied area met the regulation limits set by various international organizations. The results of the study show that molluscs produced in this area are obviously safe for raw consumption based on their bacterial quality.

INTRODUCTION

The Moroccan Mediterranean Coast is the seat of a great fishing wealth, in 2019 the production is around 18 947 tons, of which 577 tons are shellfish (**Office National des péches, 2019**). This wealth is in fact a remarkable pole of economic and social development. Moreover, the growing urbanization of the coast, linked to both tourist and industrial activity, is leading to a very significant increase in polluting discharges into the marine environment (**Azizi *et al.*, 2020, 2021; Hicham *et al.*, 2021**). Bacterial pollution is one of these discharges and leads, among other things, to the contamination of seafood, where the consumption can cause health risks and present a real danger to humans (**Campos and Cachola, 2007**; **Moumen *et al.*, 2008**). Due to their high filtration capacity, bivalve mollusks can have an impact on the levels of planktonic micro-

organisms (**Darakas *et al.*, 2011**). The relationship between microorganisms and benthic filter feeders may be functionally important for aquatic ecosystems (**Loredana *et al.*, 2005**). In addition, as a result of their filter feeding, bivalve mollusks accumulate and concentrate many of the pollutants found in seawater, particularly those that are particulates or associated with particles (**Azizi *et al.*, 2018**). They also accumulate other pollutants such as fecal bacteria (**Kambire *et al.*, 2012**). This ability to accumulate material facilitates the detection and measurement of pollutants that may be in the water at very low concentrations (**Azizi *et al.* 2020**). The fact that bivalve mollusks can accumulate bacteria and viruses and that they are harvested for human consumption has led to their being considered as bio-indicators of water quality in terms of hygiene and public health (**China *et al.*, 2003**). Therefore, this study would investigate the effect of seasonal variations on the concentration level and distribution of the bacterial load (flora) in bivalves, sediments and seawater from Ras Kabdana-Saidia coasts; and to study if bivalve concentrations are within the permissible limits for human consumption. To compare the levels of bacterial loads in bivalves and its environment, the bacterial concentrations were measured in the sediments, and seawater from the same sampling sites. The physico-chemical parameters were also determined in the seawater.

MATERIALS AND METHODS

1. Description of the study area

The Ras Kabdana - Saïdia area: Located in the extreme northeast of Morocco between latitudes 35°03’ N and 35°08’ N and between longitudes 02°19’ NW and 02°26’ W, it is limited from the mouth of the Kiss river and Ras Kebdana harbor. The coastal zone of Ras Kabdana - Saidia extends by 20 km of entirely sandy beach, includes a Site of Biological and Ecological Interest (SBEI) at the mouth of the Moulouya (**Mouzouri and Irzi, 2011**). The SBEI represents an undeniable heritage value because it is the largest Mediterranean estuarine complex in the Maghreb and comes second in terms of importance in North Africa. The site of biological interest and Moulouya is the least anthropized estuarine system in Morocco (**Bouabdallah and Larune, 2009**). The area is located in a semi-arid bioclimatic atmosphere with a temperate winter, the dry period of which begins in May and ends in October (**Bouabdallah and Larune, 2009**; **Ben Chekroun *et al.*, 2013**). In addition, the rainfall regime is characterized by an interannual irregularity with remarkable proportions depending on the year. The average annual is around 329 mm in 2018. As for the average annual temperature, they vary between 5.2 and 18.7 °C in winter and 18.5 and 31 °C in summer. The prevailing wind is from WSW from November to May and from ENE from May to October (**Sbai and Lasgaa, 2012**).

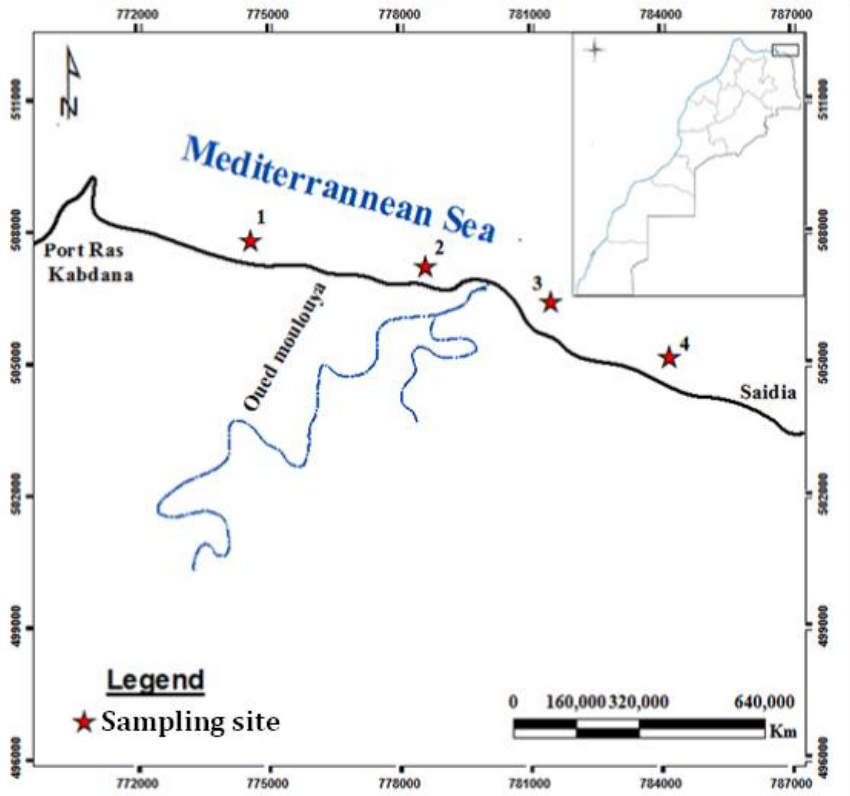

Figure 1. Geographic location of the study area

2. Sampling Strategy

Waters, sediment and mollusks (*Chamelea gallina*) were collected monthly in 2018, from four sampling sites (1, 2, 3, and 4) (Figure 1). The water samples were collected from a depth half meter from the water surface, in sterile flasks, the sediment samples were collected by using core sampler, while the mollusks were harvested using a boat equipped with a dredge. Samples are placed in sterile bags and transported in a cooler with cold accumulator as quickly as possible to the laboratory for analysis (**APHA, 1995**; **Molina-Alcaide *et al.*, 2009**; **Ngadi *et al.*, 2021**).

3. Physicochemical parameters

pH, temperature (°C), salinity (psu) and dissolved oxygen (mg/l) was measured *in situ* using a multi-parameter probe (type WTW), turbidity (NTU) was determined by a turbidimeter. The determination of suspended matter (SM) was carried out by membrane filtration and drying in accordance with standard AFNOR T90-105. The concentrations of chlorophyll *a* were determined by colorimetric methods as we described in previous works (**Ben chekroun *et al.*, 2013**; **Azizi *et al.*, 2018**; **Ait Hmeid *et al.*, 2021a**).

4. Enumeration of fecal bacteria

Enumerations of fecal coliforms (FC), fecal streptococci (FS), for the water samples were carried out using the filtration technique on grid membranes (cellulose nitrate), which are then deposited on the surface of the agar.

For mollusks, prior to analysis, the individuals are brushed then scales aseptically, a quantity of 25 g of their contents (pulpit and intravalvaire liquid) are subjected to

grinding in physiological water at 3% NaCl by a stomacher type crusher (**Lee *et al.*, 2008**). For the sediment, 10 g of test sample is suspended in a sterile flask containing 3% NaCl physiological water. Homogenate is analyzed in the raw or dilute state (**Boukef *et al. 2008***; **Ait Hmeid *et al.*, 2021b**).

The different types of counts were performed according to the agar box counting technique, based on the use of selective media: Eosine with Methylene Blue (EMB, Bio-Rad) for fecal coliforms and Slanetz and Brathely (SB, Bio-rad) for fecal streptococci. The experiments were repeated three times to get mean and averages bacterial density values were represented as log CFU. 100 mL^{-1} for seawater samples and log CFU. 100 g^{-1} for sediments and mollusks samples.

5. Determination of the origin of fecal contamination in the seawater

According to the criteria defined by **Borrego and Romero (1982)**, contamination is of animal origin if the ratio (R) fecal coliforms (FC)/fecal streptococci (FS) is less than 0.7 and of human origin if this ratio is greater than 4. The origin of the contamination is of mixed and predominantly animal origin if R is between 0.7 and 2. The origin of the contamination is mixed and predominantly human if R is between 2 and 4.

6. Statistical Analysis

The effects of time on bacteria concentrations in seawater, sediment, mollusks and the chemical and physical characteristics of seawater were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test. ANOVA data with a $P < 0.05$ was classified as statistically significant. Pearson correlation coefficients were determined using SPSS. The regression lines were used to visualize relationship between the load of mollusk bacteria and the contamination of the other two compartments (water and sediment).

RESULTS AND DISCUSSION

Table 1 show the average values of the physicochemical parameters in seawater from the coastal areas of Ras Kabdana-Saïdia. All the abiotic parameters measured show a clear seasonal variation ($P < 0.001$), which are very typical to the mediterranean marine environment. The average seawater temperature is 21 °C, this average fluctuated between 17.97 °C in autumn and 27.95 °C in summer. The observed dissolved oxygen values ranged between 7.41 and 8.17 mg/l. The minimum dissolved oxygen was observed in winter and the maximum was registered in spring. The salinity concentration varied between 35.09 and 36.87 psu, registering lowest value in autumn and highest content in summer. These physico-chemical parameters (temperature, dissolved oxygen and salinity) have been influenced by the intensity of solar radiation, evaporation of Mediterranean seawater and freshwater influx coming from the Moulouya River (**Balakrishnan *et al.*, 2017; Azizi *et al.*, 2020**). The turbidity measured in the Ras Kabdana - Saidia area remains below 7.43 NTU, the value is recorded in winters. These results were lower than those previously reported by **Rosa *et al.* (2001)** in the Gulf of Gaeta (Tyrrhenian Sea). The increased turbidity content during winter can be attributed to less salinity recorded during this period and to the turbulent nature of seawater during this period which triggers vertical mixing of water column (**Balakrishnan *et al.*, 2017**). The

suspended matter fluctuates between 27.75 mg/l (in spring) and 42.75 mg/l (in winter), these results were higher than those reported by **Kambire *et al.* (2012)** in the Aby lagoon of Ivory Coast. However, this increase corresponds to a resuspension of the sediments due to periods of strong winds (**Koffi-Nevry *et al.*, 2008; Azizi *et al.*, 2020**). The concentration of chlorophyll *a* varied from 0.4 to 2.42 µg/l during the study period with maximum values recorded during the autumn season, the same results were found by **Boukef *et al.* (2008)** in the Bizerte lagoon of Tunisie. Average pH value was 8.15, and was higher in winter than other seasons, similar results have been reported by **Bennani *et al.* (2012)** in the Moroccan Mediterranean Sea. The observed high pH content might be due to the influence of freshwater influx coming from the Moulouya River and the photosynthetic activity of phytoplanktons (**Subramanian and Mahadevan, 1999**).

Physicochemical parameters revealed significant variation according to seasons (**Azizi *et al.*, 2018, 2020, 2021; Ngadi *et al.*, 2021**). These conditions are known to govern the bioavailability of contaminants (fecal indicator bacteria) in the marine environment and their bioaccumulation by marine biota (**Darakas *et al.*, 2011; Bennani *et al.*, 2012; Skander *et al.*, 2017**).

Table 1. Seasonal changes in the abiotic parameters of seawater from Ras Kabdana - Saidia area during sampling periods

Season	Spring	Summer	Autumn	Winter	P-value
T (°C)	$21.34^{c} \pm 0.3$	$27.95^{d} \pm 0.4$	$17.97^{a} \pm 0.6$	$18.75^{b} \pm 0.5$	***
pH	$7.9^{b} \pm 0.3$	$7.6^{a} \pm 0.3$	$8.5^{c} \pm 0.4$	$8.6^{c} \pm 0.5$	***
Sal (psu)	$36.25^{b} \pm 0.8$	$36.87^{b} \pm 0.5$	$35.09^{a} \pm 0.6$	$35.29^{a} \pm 0.8$	***
O_2 (mg/l)	$8.17^{c} \pm 0.2$	$7.71^{b} \pm 0.1$	$8.1^{c} \pm 0.2$	$7.41^{a} \pm 0.1$	***
Turb (NTU)	$2.44^{a} \pm 0.81$	$2.59^{a} \pm 0.815$	$6.71^{b} \pm 1.54$	$7.43^{b} \pm 2.17$	***
SM (mg/l)	$27.75^{a} \pm 8.25$	$35.5^{b} \pm 1.5$	$33^{ab} \pm 1.5$	$42.75^{c} \pm 3.25$	***
Chl *a* (µg/l)	$1.51^{bc} \pm 0.4$	$0.4^{a} \pm 0.13$	$2.42^{c} \pm 1.48$	$0.89^{ab} \pm 0.29$	***

T: temperature; Sal: salinity; O_2: dissolved oxygen; Turb: turbidity; SM: suspended matter; Chl *a*: Chlorophyll *a*. Values in the same row marked with different letters (a, b, c and d) indicate significant differences (Tukey's test, $P < 0.05$), among seasons. ***: $P < 0.001$.

Seasonal variations in the bacterial load in seawater, sediment and mollusks (*Chamelea gallina*) in the Ras Kabdana - Saidia area is illustrated in Table 2. The results have shown that in seawater, the highest fecal coliform loads were observed mainly during the winter period ($P < 0.001$), it reaches the value of 1.18 log UFC. 100 mL^{-1}, and the lowest values are recorded during the summer season of 0.84 log UFC. 100 mL^{-1}. The most abundant fecal streptococci are in the fall period with a value of 1.58 log UFC. 100 mL^{-1}. At the sediment level, the average abundance of FC and FS were significantly different between seasons ($P < 0.001$). The highest loads are recorded during the fall season for the two groups of bacteria. For mollusks, a significant difference between seasons ($P < 0.001$) for bacterial loads were also observed, and the average load ranged from 1.09 to 1.45 log CFU.100 g^{-1} for fecal coliforms and from 1.66 to 2.09 log CFU.100 g^{-1} for fecal streptococci, for summer and autumn, respectively for both microorganisms. The average load of fecal pollution indicators (fecal coliforms and fecal

streptococci) slight but significative increase during the wet seasons (autumn/winter) for all the compartments studied (seawater, mollusk and sediment). Higher values recorded during the wet periods are often associated with increased inputs of fecal bacteria from urban wastewater and agricultural discharges and their accumulation in the water due to increased rain (**Bouchriti *et al.*, 1992; Molina-Alcaide *et al.*, 2009; Ngadi *et al.*, 2021**). In the present study, the high concentrations of fecal bacteria during the wet periods could be explained by significant agricultural, domestic and industrial discharges that Moulouya River drains (the first longest river in northern Morocco) from its catchment basin of about 74000 Km^2 (i.e. 10% of Moroccan territory) during the rainy season. Besides, strong presence of fecal indicators bacteria may be attributed to untreated wastewater effluents from a large part of Saïdia and Ras-Kebdana cities that discharged directly into the sea via the youngest ramified Wadis of the Moulouya River.

In addition to anthropogenic activities, the microorganisms were also influenced by the environmental parameters. In our study, the climatic changes were very probably responsible for the observed differences in fecal contamination levels during the seasons. Indeed, our studies demonstrated that the relative low salinity observed in winter period could explain the abundance of fecal loads during this season, these results are in agreement with the observation of **Skander *et al.* (2017)** who revealed that in seawater with low salinity there is a greater survival of the bacteria load contaminants. Besides the salinity, temperature is also a crucial variable for the survival of the microorganisms in the marine environment (**Šolić *et al.*, 2010**). In the coastal area of Ras Kabdana–Saïdia, the high levels of bacterial fecal indicators in *Chamelia gallina* in the winter period is due to the low temperature. According to **Bernard (1989)**, the highest uptake of coliforms bacteria by four marine bivalve molluscs are recorded at 17°C. These investigations confirmed the observed results in our study, the seawater of Ras Kabdana–Saïdia are characterized by median temperatures (18.36 °C) during the wet periods. Another probable reason for the high contents of fecal contaminations in *Chamelia gallina* in this period is likely the physiological status of the bivalve. Authors (**Jozić *et al.*, 2012; Skander *et al.*, 2017; Hmeid *et al.*, 2021**) indicated that the biological status of the mussels is mainly regulated by the abiotic factors. During the rainy periods, the moderate temperature and/or the low salinity observed in the Ras Kebdana-Saîdia area promote the filtration rates in the bivalves and the animal can retain more fecal bacteria than other seasons.

In our study, the *Chamelia gallina* bivalves show higher contamination in FS than in FC. In a previous work, **Zegmout *et al.* (2011)** indicated similar results, studied the bacterial loads from differents bivalves of the Moroccan Mediterranean Sea. **Skander *et al.* (2017)** demonstrated that different indicators of fecal pollution are not bioaccumulate in the same way, molluscs accumulate FS much more effectively than FC. **Skander *et al.* (2017)** reported that FC could be easily digested or more quickly excreted by the molluscs. Taking into account the conditions prevailing in the digestive tract of the

bivalve, **Noble *et al.* (2004)** indicated that fecal streptococci show a high resistance to pH, salinity and bile in the digestive system of the mussels which allows them to survive a long time to these conditions.

Table 2. Seasonal variations in the cultivable bacterial load of seawater (log CFU. 100 mL^{-1}), sediment (log CFU. 100 g^{-1}), and mollusks (log CFU. 100 g^{-1}) in the Ras Kabdana - Saidia area

		Spring	Summer	Autumn	Winter	*P*-value
Seawater	**FC**	$1.0^{b} \pm 0.08$	$0.84^{a} \pm 0.08$	$1.17^{c} \pm 0.15$	$1.18^{c} \pm 0.04$	***
	FS	$1.4^{b} \pm 0.05$	$1.12^{a} \pm 0.1$	$1.58^{c} \pm 0.01$	$1.54^{c} \pm 0.01$	***
Sediment	**FC**	$1.12^{b} \pm 0.08$	$0.93^{a} \pm 0.08$	$1.29^{c} \pm 0.15$	$1.28^{c} \pm 0.05$	***
	FS	$1.52^{b} \pm 0.05$	$1.24^{a} \pm 0.1$	$1.7^{c} \pm 0.01$	$1.65^{c} \pm 0.01$	***
Mollusks	**FC**	$1.29^{b} \pm 0.09$	$1.09^{a} \pm 0.07$	$1.45^{c} \pm 0.15$	$1.45^{c} \pm 0.04$	***
	FS	$1.94^{b} \pm 0.05$	$1.66^{a} \pm 0.11$	$2.09^{c} \pm 0.01$	$2.04^{c} \pm 0.01$	***

FC: fecal coliforms; FS: fecal streptococci. Values in the same row marked with different letters (a, b and c) indicate significant differences (Tukey's test, $P < 0.05$), among seasons; ***: $P < 0.001$.

A significant difference for bacterial concentrations ($P < 0.001$) was observed between different compartments (seawater, sediment and mollusks) (Table 3). The highest values for bacterial loads were observed in mollusks (1.32 and 1.93 log CFU. $100g^{-1}$, for FC and FS, respectively), and the lowest contents were observed in the seawater (1.04 and 1.41, log CFU. 100 mL^{-1}, for FC and FS, respectively) for both bacteria. Ours results reveal that the densities of fecal pollution indicators were more concentrated in the bivalve samples compared to the other compartments (sediment and seawater), which is consistent with the results found by **Mok *et al.* (2016)** which showed that shellfish are hosts of a large bacterial population. According to **Marino *et al.* (2005)**, mollusks can capture bacteria, particularly coliforms in water, more quickly and retain them for long periods. Bivalves are known for their ability to accumulate contaminants as bacterial loads in their tissues (**Malham *et al.*, 2014**). They are filter-feeding organisms with a high capacity to accumulate bioavailable pollutants in water at higher levels than those in the environment (**Loredana *et al.*, 2005; Azizi *et al.*, 2018, 2020**). Therefore, they can be used as sentinel organisms to monitor pollution in coastal waters (**Mok *et al.*, 2016; Azizi *et al.*, 2018, 2021; Jeamsripong *et al.*, 2018; Bozcal and Dagdeviren, 2020**). Whereas, fecal bacteria serve as bio-indicators of seafood product contamination (**Martinez and Oliveira, 2010**).

Table 3. The means of bacterial concentrations in seawater (log CFU. 100 mL^{-1}), sediment (log CFU. 100 g^{-1}) and mollusks (log CFU. 100 g^{-1}) from Ras Kabdana-Saidia during the sampling period of 2018

	Seawater	Sediment	Mollusks	P-value
FC	$1.04^{a} \pm 0.183$	$1.15^{b} \pm 0.184$	$1.32^{c} \pm 0.183$	***
FS	$1.41^{a} \pm 0.196$	$1.52^{b} \pm 0.195$	$1.93^{c} \pm 0.185$	***

FC: fecal coliforms; FS: fecal streptococci. Values in the same row marked with different letters (a, b and c) indicate significant differences (Tukey's test, $P < 0.05$), among seasons. ***: $P < 0.001$.

In order to check whether there is a relationship between the bacterial load in the shellfish and the contamination of the other two compartments (seawater, sediment), we established regression lines for each pair of values (Figure 2). The results obtained show that the relationship is positive and that the contamination of the bivalve is highly dependent on the level of water contamination. The same is true for the sediment, on the other hand. The contamination of the mollusk from water is greater than that from sediment: the correlation coefficients for the pair of value bivalves/water are 0.966 and 0.996, respectively for the FC and FS of the mollusk. While for the bivalve/sediment pairs are of the order of 0.946 for FS and 0.965 for FC. Studies by **Martinez-manzanares *et al.* (1991)** have shown that when the water temperature and salinity conditions are adequate, the bivalves increase their filtration capacity and consequently they can retain many more microorganisms. **Jozić *et al.* (2012)** reported that enterobacteriaceae and FS are not purified at the same rate and that FS removal is slower. FS are characterized by their high resistance to a large number of hostile factors (salinity, pH) and may be considered to belong to a class of bacteria capable of long survival in the digestive tract of bivalves (**Raji *et al.*, 2015**).

Table 4 shows the origin of fecal contamination in the study area. The four stations studied were dominated by mixed, predominantly animal pollution ($0.7 < FC/FS < 2$).

Fecal coliforms are generally more abundant in human feces than fecal streptococci. Fecal streptococci are thought to be more important in number than fecal coliforms in animals (**Martín-García *et al.*, 2006**; **Koffi-Nevry *et al.*, 2008**), hence the importance of the ratio of fecal coliforms to fecal streptococci.

The concentration of fecal streptococci was higher than that of fecal coliforms in waters as well as in sediments and mollusks. Similar results were reported by **Bouchriti *et al.* (1992)** studied the microbiological contamination of the Oualidia lagoon (Morocco) as an oyster growing area and, by **Skander *et al.* (2017)** investigated the bacteriological quality of the bivalve in the North East Algerian coast. According to **Leclerc *et al.* (1981)**, fecal coliforms are abundant in the feces and are the predominant environmental indicators. In this work, the value of the fecal coliform/fecal streptococci ratio mostly indicates mixed pollution with animal predominance, this is related to inputs from discharges from agricultural land in the catchment area. The application of this ratio to the level of the Aby lagoon, Ivory Coast, has shown human pollution caused by inputs of excreta from sanitary facilities located on the lagoon, untreated sewage and the excreta of some domestic and wild animals walking along the lagoon's edge (**Kambire *et al.*, 2012**).

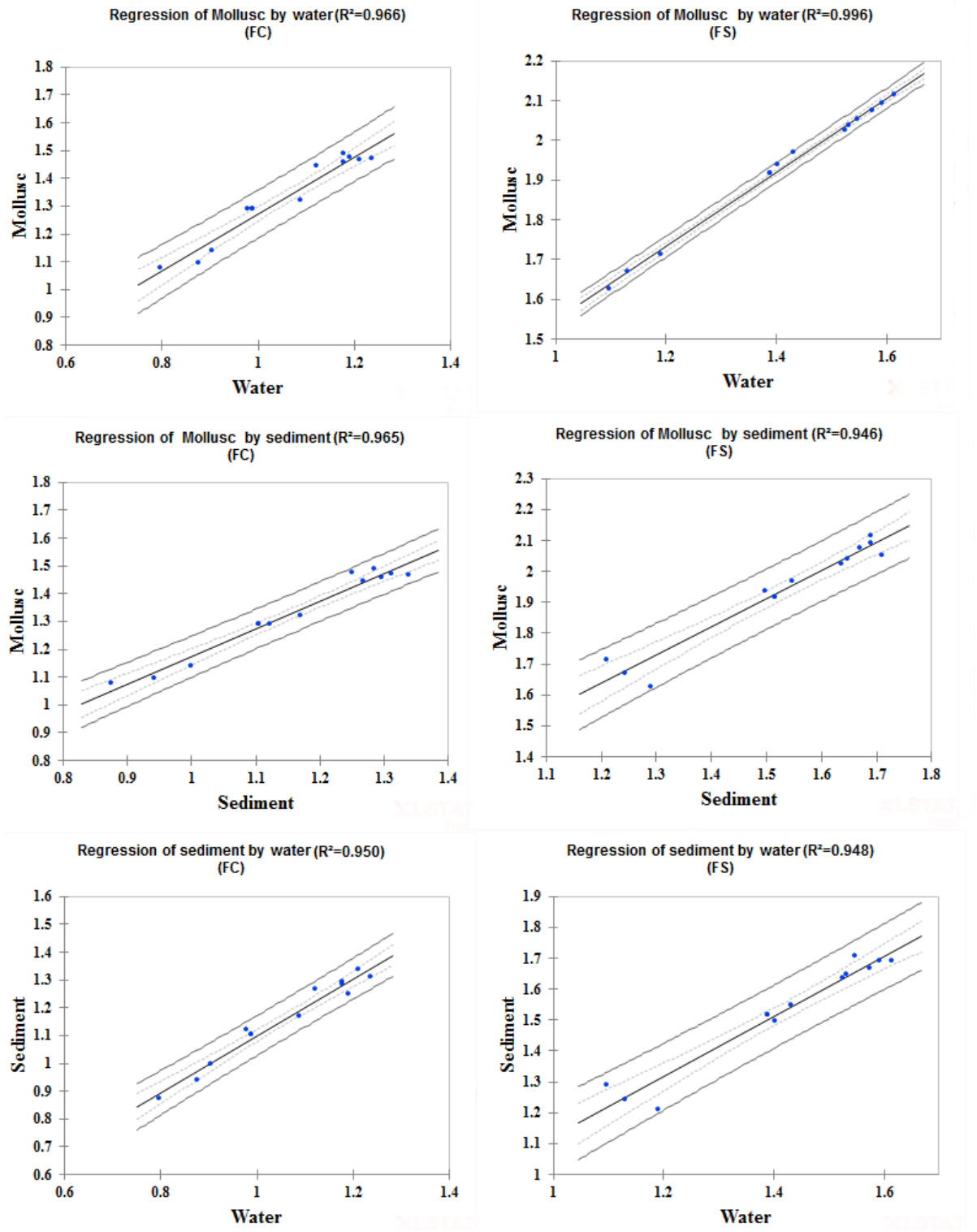

Figure 2. Correlation between bivalve contamination and sediment and seawater contamination at the small grassland level

Table 4. Determination of the source of fecal pollution based on the FC/FS ratio

Stations	FC/FS	Origin
1	0.77	mixed (animal - dominated)
2	0.83	mixed (animal - dominated)
3	0.86	mixed (animal - dominated)
4	0.75	mixed (animal - dominated)

FC: fecal coliforms; FS: fecal streptococci.

The results obtained from the Pearson's correlation coefficients (Table 5), show at the mean FC concentration in the Ras Kabdana - Saidia area was significantly correlated during the four survey seasons at pH ($r = 0.981$ and 0.980, $p < 0.05$ and $r = 0.991$, $p < 0.01$ for the seawater, sediment and mollusks, respectively). FC level in water was inversely correlated with temperature ($r = -0.965$, $p < 0.05$), salinity ($r = -0.998$, $p < 0.01$) and FC levels in mollusks and sediment were inversely correlated with temperature ($r = -0.966$ and $r = -0.968$, $p < 0.05$) and salinity ($r = -0.997$ and $r = -0.996$, $p < 0.01$). Likewise for the mean FS concentrations, which were positively correlated with a pH ($r = 0.955$ and $r = 0.953$, $p < 0.05$ for the water and sediment respectively) but negatively correlated with temperature ($r = -0.982$, $p < 0.05$, and $r = -0.982$, and $r = -0.983$, $p < 0.01$ for the water, mollusk and sediment respectively), and salinity ($r = -0.988$, $r = -0.977$ and $r = -0.998$, $p < 0.05$, for the water, mollusk and sediment, respectively). According to studies by **Mill *et al.* (2006)**, temperature and salinity are believed to influence the increase of these fecal pollutants in marine waters. **Jozić *et al.*, (2012)** revealed a significant correlation between fecal indicator bacteria contents in seawater and molluscs. Authors (**Rajesh *et al.*, 2001; Jozić *et al.*, 2012**) indicated that the retention of bacteria loads by bivalves were highly controlled by the salinity and temperature of the seawater. The simultaneous action of both physicochemical parameters could be highly significant than when each factor acts alone (**Jozić *et al.*, 2012**), which is our case (Table 5). In addition, the mean concentrations of FS, were significantly positively correlated for all three compartments to those of FC. These results are consistent with the findings of **Bennani *et al.* (2012)**.

Table 5. Pearson's correlation coefficients between bacterial loads and environmental parameters in the Ras Kabdana - Saidia area

		Temperature	pH	Salinity	FC
Water	**FC**	-0.965*	0.981*	-0.998 **	1
	FS	-0.982*	0.955*	-0.988*	0.949**
Mollusk	**FC**	-0.966*	0.991**	-0.997**	1
	FS	-0.982**	0.942	-0.977*	0.987*
Sediment	**FC**	-0.968*	0.980*	-0.996**	1
	FS	-0.983**	0.953*	-0.998*	0.994**

* Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)

The mean fecal pollution indicator concentrations in the bivalve studied here are compared with values from the literature in Table 6. The acceptable levels of fecal pollution indicators in mollusks for human consumption are presented in Table 7.

For the mean levels of fecal coliform, the observed values are lower than those observed for Izmir coastal area, Turkey (**Kacar, 2011**), Adriatic Sea, Italy (**Vernocchi *et al.*, 2007**), Northern Ionian Sea, Italy (**Loredana *et al.*, 2005**), Karnataka Coast, India (**Geetha and Krishnamoorthy, 2010**) and Bizerte lagoon, Tunisia (**Boukef *et al.*, 2008**). Fecal coliform levels were lower than the World Health Organization (**WHO, 1996**), the Food and Agriculture Organization (**FAO, 1993**), the European Community (**EC, 1998**), the United Nations Environment Program (**UNEP, 1996**), and the French Food Safety Agency (**FFSA, 1999**) permissible limits which are 300 CFU.100 g^{-1}.

Fecal streptococci concentrations found in mollusks from Ras Kabdana-Saïdia were lower than those found in the literature (**Loredana *et al.*, 2005; Boukef *et al.*, 2008**). Although there is no information about maximum fecal streptococci levels in bivalves in World Health Organization (**WHO, 1996**), Food and Agriculture Organization (**FAO, 1993**), and United Nations Environment Program (**UNEP, 1996**). Results of the present study are below the maximum levels reported by the European Community (**EC, 1998**) and French Food Safety Agency (**FFSA, 1999**) for mollusks,which are 2500 CFU.100g^{-1}.

Table 6. Levels of bacterial loads in bivalves obtained in several monitoring studies (log CFU. 100 g^{-1})

	FC	**FS**	**References**
Ras Kabdana-Saidia, Morocco	1.32	1.93	**Present study**
Adriatic Sea, Italy	3.31		**Vernocchi *et al.*, (2007)**
Izmir coastal area, Turkey	5.03		**Kacar, (2011)**
Northern Ionian Sea, Italy	1.4	3.05	**Loredana *et al.*, (2005)**
Karnataka coast, India	3. 1		**Geetha and Krishnamoorthy, (2010)**
Lagoon Bizerte, Tunisie	3.56	2.7	**Boukef *et al.*, (2008)**

Table 7. Permissible limits (log CFU. 100 g^{-1}) for bacterial loads in mollusks recommended by various organizations

Authorities	**FC**	**FS**
WHO, (1996)	300	
FAO, (1998)	300	
EC, (1998)	300	2500
UNEP, (1996)	300	
FFSA, (1999)	300	2500

WHO: World Health Organization; FAO: Food and Agriculture Organization; EC: European Community; UNEP: United Nations Environment Program; FFSA: French Food Safety Agency.

CONCLUSION

The present study provides primary information on the distribution of concentrations of fecal pollution indicators in seawater, sediment and mollusks (*Chamelea gallina*) from the Ras Kabdana-Saidia (East Moroccan Mediterranean Coast). Mollusks showed a seasonal variation of bacterial load with higher values during the fall/winter seasons and lower values during the summer period. The temporal variation of mollusk bacterial levels were attributed to the changes in the environmental parameters (temperature, pH and salinity). Bacterial concentrations were higher in soft tissues of mollusks than in seawater and sediment. Because of their tendency to concentrate fecal pollution-

indicative bacteria from the environment, *Chamelea gallina* has been found to be useful as bioindicator organisms. The average levels of fecal pollution indicators found in tissues of *Chamelea gallina* from coastal Ras Kabdana-Saidia were generally in agreement with the literature, and did not exceed the established quality standards for mollusks, as recommended by various organizations. Therefore, it can be concluded that the *Chamelea gallina* produced in the area do not present health risk based on fecal contamination, and are apparently safe for raw consumption.

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