ESI Preprints Not Peer-reviewed

Assessment of the Shellfish Production Areas' Quality: The Oualidia and Sidi Moussa Lagoons Case

B. Khbaya
Institut National de Recherche Halieutique, Oualidia, Morocco
F-Z. Bouthir
Institut National de Recherche Halieutique, Bd Sidi Abderrahmane, Casablanca, Morocco
R. Cherkaoui
Laboratoire de microbiologie, centre spécialisé en pathologie des animaux aquatiques / INRH. Cap Malabata, Tanger, Morocco
E. M. Kabiri
Institut National de Recherche Halieutique, Oualidia, Morocco

Doi: 10.19044/esipreprint.12.2023.p837

Approved: 22 December 2023
Posted: 25 December 2023

Cite As:
Khbaya B., Bouthir F-Z., Cherkaoui R. & Kabiri E.M. (2023). *Assessment of the Shellfish Production Areas' Quality: The Oualidia and Sidi Moussa Lagoons Case.* ESI Preprints. https://doi.org/10.19044/esipreprint.12.2023.p837

Abstract

Based on European regulation 91/492/EC, Morocco, very early, established legislation with conditions for producing and marketing live bivalve molluscs. In applying this legislation, the National Institute of Fisheries Research (INRH) has set up a system for sanitary monitoring of the marine environment, through which several harvesting areas have been classified while others are in progress. In January 2020, the Oualidia and Sidi Moussa lagoons were categorized respectively in classes B and C with respectively 52.77% of the results, which were between 230 and 4600 MPN E. coli / 100 g of flesh and intravalvular liquid (FIL) and 11.11% of results that fell between 4600 and 46000 MPN E. coli / 100 g FIL. Sidi Moussa lagoon has been classified as a clean area category C since 2006. As a result, the oyster farming activity has been suspended in this area. This incident is a warning sign of the significant weakness of these ecosystems in addressing multiple social and economic challenges. On another side, INRH has sufficient data and tools to progress towards a better optimization of the

marine environment sanitary monitoring program management.

Keywords: Shellfish, oyster farming, Sanitary monitoring, Oualidia and Sidi Moussa lagoons, Morocco

1. Introduction

It is well known that shellfish contamination occurs because bivalve molluscs are filter-feeding animals that selectively filter and accumulate small particles of phytoplankton, zooplankton, viruses, bacteria, and inorganic matter from the environment (Carella et al., 2010; Leoni et al., 2017). This highlights the role of shellfish as a vehicle for several hazards that could result in products that are unsuitable to guarantee the safety of consumers, particularly if live bivalve molluscs are consumed raw or undercooked (Iwamoto et al., 2010; Rubini et al., 2018 and Sferlazzo et al., 2018). The adoption of regulations that specify acceptable levels of bacterial enteric pathogens in shellfish-growing water (United States National Shellfish Sanitation Program) or shellfish tissues (European regulation 91/492/EC, Moroccan regulation[1]) has significantly decreased the impact of bacteria as causes of shellfish-associated disease outbreaks (Butt et al., 2004). In these two later regulations, shellfish harvesting areas are classified on the basis of the microbiological (*Escherichia coli*) and chemical (mercury, cadmium and lead) levels, as category A, B, and C. Bivalves in category A, which contain less than 230 MPN MPN *E. coli* /100 g FIL for at least 80% of the samples without the level of contamination exceeding 700 MPN *E. coli* / 100 g FIL for the remaining 20%, can be marketed without any preventive treatment. Bivalves collected from category B areas (at least 90% of samples are < 4600 MPN *E. coli* / 100 g FIL and the remaining 10% < 46000 MPN *E. coli* / 100 g FIL), can only be destined for human consumption after a depuration process to meet category A requirements or cooked by an approved method. Bivalves found in category C areas (< 46000 MPN *E. coli* /100 g *FIL*), must be subjected to relaying for a period of at least 2 months in category A area or cooked by an approved method (Council Directive 91/492/EEC, 1991 and Moroccan regulation[1]). Chemical contamination must remain at the levels required for category A. The RSSMM network[2] was created in 1996 by INRH with the aim of carrying out sanitary monitoring of areas classified A, B and C under the conditions provided for by national food regulations. This network is implemented by six laboratories (LSSMM) housed in 6 regional centers of the INRH based at

[1] Arrêté du ministère de l'agriculture, de la pêche maritime, du développement rural et des eaux et forêts n° 1950-17 du 14 kaada 1438 (7 août 2017) relatif au classement sanitaire des zones maritimes de production conchylicole. BO-6696-19 kaada 1439., (2018), 1529-1535.
[3] Réseau de Suivi de la Salubrité du Milieu Marin.

Dakhla, Laayoune, Agadir, Oualidia, Tangier and Nador. In 2021, the RSSMM conducted sanitary monitoring at 68 representative sampling points in 27 harvesting areas classified and professionally operated.

In 2006, a new Moroccan regulation established the concept of "sanitary classes A, B, C, and D" based on the level of chemical and microbiological contamination. Consequently, the Oualidia lagoon has been classified into category B, with 19% of the microbiological results superior to 230 MPN *E. coli* / 100g FIL without contamination exceeding 4600 MPN *E. coli* / 100g FIL. At the same time, the Sidi Moussa lagoon was classified in category C with around 31% of the results which ranged between 4600 and 46000 MPN *E. coli* / 100g FIL (Arrêté ministériel, 2006[3] and Khbaya et al., 2011). As a result, oyster farming has been prohibited in this lagoon. The sanitary monitoring of this lagoon has since been carried out on the *Ruditapes decussatus* species, which constitutes the main part of the natural deposit of this lagoon. In comparison, the Oualidia lagoon has maintained their breeding of *C. gigas* species provided that the product be purified in the purification facilities annexed to the harvesting area.

In 2008, INRH conducted a comparative study of the microbiological contamination level in the oyster farmed (*C. gigas*) and in two burrowing species present in the natural deposit, *R. decussatus* and *Ensis ensis*. The results of this study show: *i.* the homogeneous nature of the *E. coli* contamination levels in the lagoon and, *ii.* the differences in the contamination levels between burrowing bivalves molluscs group and species *C. gigas* (Cherkaoui et al., 2010). Failures in the lagoon ecosystem management were highlighted, which prompted the public authorities to set up an integrated development project in April 2010. The public administration has carried out several works in Oualidia and its lagoon to improve the quality of aquaculture water, such as constructing a liquid sewerage network.

The purpose of this study is to assess the quality of the harvesting areas known as the Oualidia and Sidi Moussa lagoons based on microbiological and chemical monitoring results for the three calendar years 2017 - 2019. This assessment is based on computer processing following the regulatory food safety criteria. This report thus enables the necessary information to revise, if required, the monitoring strategy and / or classifications of harvesting areas.

[4] Arrêté du ministère de l'agriculture et de la pêche maritime relatif au classement des zones de ramassage des mollusques bivalves vivants., (2006).

2. Material and methods

a) Characteristics of the study area

Sidi Moussa - Oualidia complex lagoon is located on the Atlantic coast of Morocco, about 35 km south of the city of El Jadida. The complex is a Site of Biological and Ecological Interest, well-known as a stopover site for migratory coastal birds, and a wetland of international interest classified as RAMSAR since 2005. The complex has two lagoons (Oualidia and Sidi Moussa) separated by an area of 40 km, distributed by four marshes, and salt marshes separated from the sea by a dune ridge. Oyster farming activity is significant in the Oualidia lagoon. At the same time, the two lagoons are the subject of potent activity of collecting shells such *R. decussatus*, *Cerastoderma edule* and *E. ensis*. The human activities in and around the lagoons (agriculture and tourism) exert multiple pressures on these ecosystems and result in changes in the environment and aquaculture water quality.

b) Sampling and Analysis

Enumeration of β-glucuronidase positive *E. coli* in shellfish.

Table 1. Characteristics of sampling points

Area names and code	Areas limits	Sampling point codes	Geographical coordinates of sampling points	Class	Exploited seashell
Oualidia lagoon (10-03)	32°45’26 N / 09°00’52 W 32°44’57 N / 09°01’31 W	100302 100303	32°44'47.1"N 9°01'25.0"W 32°45'13.9"N 9°00'44.3"W	B	*C. gigas*
Sidi Moussa lagoon (10-01)	32°59’86 N- 08°43’72 W 32°58’53 N- 08°45’54 W	100102 100103	32°58'48.6"N 8°44'38.6"W 32°58'59.2"N 8°44'33.1"W	C	*V. decussatus*

INRH officers conducted sampling according to the collecting points listed in table n°1. The samples were shipped refrigerated to the laboratories of marine microbiology of SSMM / INRH at Oualidia and were analyzed within 24h of harvesting. Depending on the size of the *C. gigas* or *R. decussatus*, 15 to 30 bivalves of each sample were randomly selected for microbiological analysis. Samples were processed for microbiological detection, enumeration, and graduated dilutions according to the ISO 6887-3 method (NM ISO 6887-3, 2017).

All the bivalve samples were examined using the five-tube Most Probable Number (MPN) method in accordance with the EU reference method ISO 16649–3 (NM ISO 16649-3, 2015). In briefly, 75-100g of flesh and intervalvular liquid were added to 2 parts of tryptone-Sel (Biokar diagnostics, France) and homogenized using a blender for 2 min. Ten ml of the liquid part of the 1+2 suspension were added to a flask containing 90 ml of tryptone-Sel (Biokar diagnostics, France) resulting in a final 1+9 dilution. Aliquots of 10 ml of the initial suspension (1+2) were transferred to each of five tubes of double strength Mineral Modified Glutamate Medium (MMGB) (Biokar diagnostics, France). Aliquots of 1.0 ml of the 1+9 dilution were transferred to each of five tubes of single strength MMGB. Further dilutions were prepared in the same way. All the double and single-strength MMGB were incubated aerobically at 37±1°C for 24±2h. Subcultures from positive MMGB tubes that changed color from purple to yellow, were plated on chromogenic Tryptone Bile Glucuronide Agar (TBX) plates (Biokar diagnostics, France) incubated aerobically at 44±1°C for 21±3h. At the end of incubation, the number of positive tubes of double or single-strength MMGB tubes were counted in order to estimate the level of *E. coli*/100g using the MPN table, generated with the MPN calculator referenced in NM ISO 7218 (NM ISO 7218, 2014). Counting *E. coli* in bivalve molluscs using the MPN technique is limited to the detection of less than 18 E. coli / 100g FIL.

Heavy metal analysis

After sampling, the shellfishes *C. gigas* and *R. decussatus* were kept in seawater collected at the sampling location for about 24 hours to remove the digestive contents so as not to interfere with the content of trace metals in the shellfish tissue. Soft tissue was removed and mixed for good homogeneity of samples.

All precautions are taken to avoid contamination during the steps of preparation and measurements. Ultrapure water and acid of high purity cleaned by sub boiling distillation are used. The digestion procedure is carried out according to a method for the pressure digestion of foodstuffs NM EN 13805 (2015). Determinations of Cd and Pb are performed by inductively coupled plasma mass spectrometry (ICP-MS) for direct analysis of liquid samples (iCAP-Q series mass spectrometry Thermo Fisher Scientific), according to NM EN 15763 (2012).

The total mercury contents are determined directly on fresh sample aliquots by DMA according to the methods of the U.S., Environmental Protection Agency (2007), EPA 7473 and the User manual (MILESTONE DMA 80). The samples' expected values of trace metals are expressed in mg/kg of fresh weight (F.W.).

Precision was estimated through replicated samples (every 10th sample was a replica) and was better than 10% in all cases. In addition, a quality control program was performed to ensure both precision and accuracy of sample results. It includes treatment and analysis of Standard Reference Material (the SRM was Nist 2976) and blanks with the samples.

c) Assessment of the quality of an area

The estimation of the sanitary class was determined for each harvest area based on microbiological and chemical monitoring results. Harvest areas which have exceeded the maximum content of at least one of the chemical contaminants or which have a microbiological quality less good than the regulatory criteria of a class C are considered "of very poor quality"[1]. Nevertheless, the classes A, B, or C estimation is determined according to the distribution of the frequencies of the microbiological monitoring results under the legislation limits.

d) Annual update of sampling frequencies for microbiological monitoring

A statistical procedure determined the stability of microbiological contamination levels. This procedure calculates the geometric mean of the E. coli concentrations obtained over the last three calendar years. For the geometric mean calculation, the value of results below the detection limit is set at 10 (Meteigner, 2017; Piquet, 2018).

This geometric mean is compared to predefined limits (Table 2). The sampling frequency being able to be applied (Bimonthly or Monthly) is thus determined as a function of the geometric mean (XG).

e) Trend in the microbiological quality of an area

Quality	A	AB	B	BC	C
X_G		13	40	200	750
Frequency	Bimenthly	Monthly	Bimenthly	Monthly	Bimenthly

Table 2 : Sampling frequency determination according to the quality of the area and the geometric mean (XG) of the results.

Trend analysis of microbiological parameters considers ten years of acquired data and is based on the nonparametric Mann Kendall test (Meteigner, 2017; Piquet, 2018). The purpose of the Mann-Kendall (MK) test (Gilbert, 1987) is to statistically assess if there is a monotonic upward or downward trend of the variable of interest over time. A monotonic upward (downward) trend means that the variable consistently increases (decreases) through time, but the trend may or may not be linear.

3. Results and discussion

A total of 72 microbiological and 18 chemical element analysis results were obtained during the period 2017-2019 for both Oualidia and Sidi Moussa lagoons, and no alerts were reported concerning these two criteria.

Table 3 shows the chemical contamination levels obtained for the two lagoons during 2017-2019. The maximum levels reached for mercury and lead during this period remained below the food regulatory limits. Indeed, these contents remained at least 13 and 7 times lower than the limit set for mercury, respectively, for Oualidia and Sidi Moussa lagoons. Lead maximum content was at least 9 and 3 times inferior to the limit set of 1.5 mg/kg F.W. for Oualidia and Sidi Moussa lagoons, respectively. As a result, the maximum cadmium levels obtained during the chemical monitoring were 1.4 mg/kg F.W. and 0.85 mg/kg of F.W. for Oualidia and Sidi Moussa lagoons, respectively.

These results show a precise increase in levels of cadmium compared to lead and mercury but lower than legislation limits for class A harvesting areas. However, we observed that these levels showed the same order of magnitude as those of previous years, indicating a possible classification of the two lagoons in categories A, B, or C according to the microbiological contamination levels. Indeed, due to significant physiological differences between the species monitored *C. gigas* (filtering species) and *R. decussatus* (burrowing species), the Oualidia lagoon shows higher levels of Cd contamination than the Sidi Moussa lagoon. The reason for these high concentrations could also be due to anthropogenic sources (phosphate industry) and natural sources (upwelling activities) (Chafik et al., 2001; Gaudry et al. 2007). It is well known that the metal bioaccumulation process in bivalves can be influenced by biotic and abiotic factors and the structural and functional properties of the biological barriers that separate living organisms from their environment (Bouthir et al., 2004; Maanan, 2008; Mejdoub et al., 2018).

During the 2017-2019 period, microbiological monitoring results obtained in two lagoons are shown in Figure 1, while the frequency distribution of results according to different legislation limits is summarized in Table 4. For the Oualidia lagoon, 52.77% of microbiological monitoring results were above the legislation limit for class A harvesting areas (230 MPN *E. coli* / 100 g FIL). 19.44% of these results were above limit of 700 MPN *E. coli* / 100 g FIL limit but without exceeding the legislation limit for category B which is 4600 MPN *E. coli* / 100 g FIL. These results are compatible with a sanitary classification of Oualidia lagoon in class B. For the Sidi Moussa lagoon, 83.33% of results are above the legislation limit of 230 MPN *E. coli* / 100 g FIL.

Table 3. Heavy metals data from Oualidia and Sidi Moussa lagoons during surveyed periods 2017- 2019

	Number of data		**Metal heavy content***		**Legislation limits**	**Units**
	Oualidia lagoon	**Sidi moussa lagoon**	**Oualidia lagoon**	**Sidi moussa lagoon**		
Mercury (Hg)	6 (100%)	6 (100%)	0.027 ± 0.0078 (0.014 - 0,038)	0.063 ± 0.0077 (0.052 - 0.070)	≤ 0.5	
Lead (Pb)	6 (100%)	6 (100%)	0.139 ± 0.051 (0.10 - 0,16)	0.176 ± 0.136 (0.088 - 0.45)	≤ 1.5	*mg/kg of Fresh Weight (FW)*
Cadmium (Cd)	6 (100%)	6 (100%)	0.803 ± 0.368 (0.402 - 1.44)	0.707 ± 0.098 (0.61 - 0.85)	≤ 2 for mussels & oyster ≤ 1 Other shellfish	

****Values are in "mean ± standard deviation". (Min – Max) of the results obtained during the monitoring period.**

Among these results, 11.11% are between regulatory thresholds of 4600 E. coli / 100 g FIL and 46000 MPN E. coli / 100 g FIL. These results are compatible with a sanitary classification in category C. Therefore, this assessment of the sanitary quality of two lagoons, Oualidia and Sidi Moussa, is consistent with the administrative type published in January 2020[4]. However, taken separately, the results of the microbiological monitoring of the Sidi Moussa lagoon show that the frequency of values above the legislation limit for class B (4600 MPN *E. coli* / 100 g FIL) is only 8.33% for the years 2018 and 2019 (Fig. 1). These results suggest that the lagoon of Sidi Moussa may be categorized in class B which is not in accordance with the administrative classification. Therefore, the preventive measure taken can be explained by:

- the fact that one of the three sampling points of this lagoon, which is also the most susceptible to contamination, is no longer used due to depletion of the shellfish stock and,

[4] Arrêté du ministère de l'agriculture, de la pêche maritime, du développement rural et des eaux et forêts relatif classement des zones de ramassage des mollusques bivalves vivants., (2020).

- the lack of effective management of domestic waste. The levels of chemical contamination in the marine environment are known to change slowly and evenly.

Consequently, a single sampling point per harvesting area with a six-monthly monitoring frequency is generally sufficient for monitoring this parameter[1]. National legislation always assumes a monthly frequency for microbiological sampling. However, the determination of the sampling frequency is generally based on a statistical approach of the distribution of results acquired during the last three years. The statistical approach allows arriving at a reading grid, allowing, according to the geometric mean (XG) of results obtained, over the year, for each sampling point to identify microbiological monitoring frequency for the area (Meteigner, 2017; Piquet, 2018). The frequency is therefore adapted to the classification and the risk of episodic deterioration (alert) of the sanitary quality of the classified area.

Figure 1 : ***E. coli*** **enumeration in shellfish from the Oualidia and Sidi Moussa lagoons (period 2017-2019). (*only the maximum contamination value over the period is indicated*).**

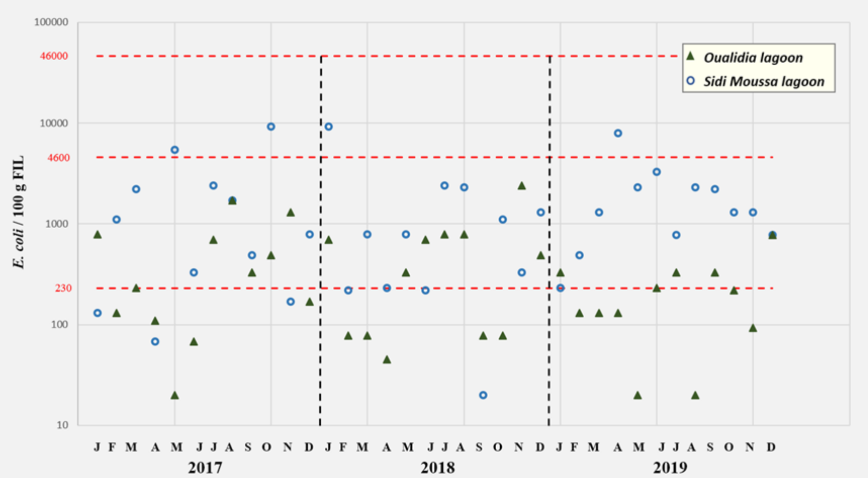

Table 4 : Frequencies distribution of the microbiological contamination results according to the different legislation limits.

Name of area	Number of data	< 230	[230-700]	] 700-4600]	] 4600-46000]	> 46000	Number of alerts	Maximum value	Estimated quality	Geometric mean X_G*	Frequency
Oualidia lagoon	**72** (100%)	**34** (47,22%)	**24** (33,33%)	**14** (19,44%)	**0**	**0**	**0**	**2400**	**B**	**67,3** **95,1**	**Bimonthly**
Sidi Moussa lagoon	**72** (100%)	**12** (16,66%)	**12** (16,66%)	**40** (55,55%)	**8** (11,11%)	**0**	**0**	**9200**	**C**	**800,4** **936,1**	**Bimonthly**

*Geometric mean of the two sampling points of the area based on microbiological monitoring results obtained during the year 2019.

Environmental and socio-economic importance make considered both lagoons as sensitive harvesting areas by public authorities. Consequently, a fortnightly frequency (every 15 days) was applied by INRH to microbiological monitoring until December 2011, even though national food regulation set a monthly frequency (Cherkaoui et al., 2010). As part of the monitoring strategy revision, we updated frequencies based on results geometric mean obtained from 2009 to 2011, assigning a monthly frequency to microbiological monitoring of these lagoons (khbaya, 2011). Categorized B, Oualidia lagoon has a geometric result means obtained in 2019 for two sampling points of 67.3 and 95.1 with no exceeding of legislation limit of 4600 MPN *E. coli* / 100 g FIL during the last three years. Therefore, a bimonthly frequency could be applied during 2020 to monitor this harvesting area. This is also the case of the Sidi Moussa lagoon, which is classified as C and has a geometric means of 800.3 and 936.1 for two sampling points. So, the frequency that has been applied during 2020 to this area is bimonthly. Using this statistical approach to microbiological monitoring in other areas will lead to applying a bimonthly frequency to at least 2 by 4 other shellfish harvesting areas monitored in the LSSMM-Oualidia (results not shown). Indeed, areas called Cap Beddouza and Oum Tyour – Chouika, classified A, show a geometric means of 12.0 and 12.6, respectively, with no alert recorded during the last three years. Applying this statistical approach could contribute to good optimization of the monitoring program management by the INRH.

The evolution of microbiological quality of a classified area for a given shellfish group is based on trend analysis. This analysis takes into account the data acquired over ten years, and it is based on the non-parametric test of Mann Kendall (Meteigner, 2017; Piquet, 2018). Monitoring data obtained from the various points sampling in the area over the last ten years are aggregated before treatment. This choice makes it possible to avoid the influence of a brief modification due, for example, to particular climatic circumstances. Trend analysis of sanitary monitoring results for the two lagoons by the Mann Kendall test does not make it possible to detect a significant trend for the two harvesting areas (results not shown). This can signify the development in each of these two areas of comparable levels of microbiological contamination from year to year.

Since 2006, sanitary monitoring has always been stopped for the Sidi Moussa lagoon. However, over the last three years, the Oualidia lagoon continues to benefit from sanitary state B, which allows it to preserve the oyster farming activity. This is evidenced by the fact that less than 20% of the results are between 700 and 4600 MPN E. coli / 100 g FIL and that the maximum values hardly exceed 2400 MPN E. coli / 100 g FIL.

Conclusion

Based on national legislation, the main objective of the regular sanitary monitoring of the marine environment is to ensure safety for shellfish consumers. The chemical and microbiological monitoring results confirm the sanitary class B for the Oualidia lagoon, which therefore remains suitable for oyster farming. On the contrary, this activity continues to be prohibited in the Sidi Moussa lagoon without water quality improvement. This shows the significant vulnerability of these ecosystems, despite their environmental, social, and economic importance.

Acknowledgements

The authors would like to thank A. Bessa, and R. Chfiri for technical assistance. We thank B. Moutaki, O. Adil, A. El Garni, Z. Boumenkhar, and A. Ayyoubi for their contribution to the sampling.

Conflict of Interest: The authors reported no conflict of interest.

Data Availability: All of the data are included in the content of the paper.

Funding Statement: The authors did not obtain any funding for this research.

References:

1. Bouthir, F. Z., Chafik, A., Souabi, S., Benbrahim, S. (2004). Qualité physico-chimique des eaux côtières du littoral de la Wilaya du grand Casablanca (océan Atlantique marocain) utilisation de la moule (*Mytilus galloprovincialis*) comme indicateur de la contamination métallique. Journal of Marine Life, No. 14(1–2), 59–70.
2. Butt, A. A., Aldridge, K.E., Sanders, C. V. (2004). Infections related to the infection of seafood. Part I. Viral and bacterial infections. The Lancet Infectious Diseases, No. 4, 201-212.
3. Carella, F., Aceto, S., Marrone, R., Maiolino, P., De Vico, G. (2010). *Marteilia refringens* infection in cultured and natural beds of mussels (*Mytilus galloprovincialis*) along the Campania coast (Tirrenian sea, South of Italy). Bulletin of the European Association of Fish Pathologists, No. 30, 189.
4. Chafik, A., Cheggour, M., Cossa, D., Benbrahim, S., Sifeddine, M. (2001). Quality of Moroccan Atlantic coastal water monitoring and mussel watching, Aquat. Living Ressources, No. 14, 239-249.

5. Cherkaoui, R., Azhari, H., Moutaki, B. (2010). Etude comparative du niveau de contamination microbiologique des espèces *C. gigas*, *Venerupis decussata* et *Ensis ensis* au niveau de la lagune d'Oualidia. Rapport INRH, 32 pages.
6. Council Directive 91/492/EEC. (1991). Health conditions for the production and the placing on the market of fishery products.
7. EPA. US. (2007). Environmental Protection Agency, EPA 7473, Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry.
8. Gaudry, A,, Zeroual, S., Gaie-Levrel, F., Moskura, M., Boujrhal, F. Z., El Moursli, R. C., Guessous, A., Mouradi, A., Givernaud, T., Delmas, R. (2007). Heavy metals pollution of the Atlantic marine environment by the Moroccan phosphate industry, as observed through their bioaccumulation in *Ulva lactuca*. Water, Air, & Soil Pollution, No. 178, 267–285.
9. Gilbert, R.O. (1987). Statistical Methods for Environmental Pollution Monitoring, Wiley, NY.
10. Iwamoto, M., Ayers, T., Mahon, B. E., Swerdlow, D. L. (2010). Epidemiology of seafood-associated infections in the United States. Clinical Microbiology Reviews, No. 23, 399-411.
11. Khbaya, B. (2011). Mise à jours de la fréquence d'échantillonnage pour le suivi microbiologique des lagunes d'Oualidia et de Sidi Moussa. Rapport INRH., 15 pages.
12. Leoni, F., Chierichetti, S., Santarelli, S., Talevi, G., Masini, L., Bartolini, C., Rocchegiani, E., Naceur Haouet, M., Ottaviani, D. (2017). Occurrence of *Arcobacter spp*. and correlation with the bacterial indicator of faecal contamination Escherichia coli in bivalve molluscs from the Central Adriatic, Italy. International Journal of Food Microbiology, No. 245, 6-12.
13. Maanan, M. (2008). Heavy metal concentrations in marine molluscs from the Moroccan coastal region. Environmental Pollution, No. 153, 176 -183.
14. Mejdoub, Z., Zaid, Y.; Hmimid, F.; Kabine, K. (2018). Assessment of metals bioaccumulation and bioavailability in mussels *Mytilus galloprovincialis* exposed to outfalls pollution in coastal areas of Casablanca. Journal of Trace Elements in Medicine and Biology, No. 48, 30–37.
15. Meteigner, C. (2017). Evaluation de la qualité des zones de récolte conchylicole. Département des LANDE. RST/LER/AR/LER/17. 006.

16. NM EN 13805. (2015). Produits alimentaires : Dosage des éléments traces Digestion sous pression.
17. NM EN 15763. (2012). Dosage des éléments traces - Dosage de l'arsenic, du cadmium, du mercure et du plomb par spectrométrie d'émission avec plasma induit par haute fréquence et spectromètre de masse (ICP-MS) après digestion sous pression.,
18. NM ISO 16649-3. (2015). Microbiologie de la chaîne alimentaire - Méthode horizontale pour le dénombrement des *E. coli* bêta-glucuronidase positive - Partie 3 : Recherche et technique du nombre le plus probable utilisant le bromo-5-chloro-4-indolyl-3 ß-D-glucuronate.,
19. NM ISO 6887-3. (2017). Microbiologie de la chaîne alimentaire - Préparation des échantillons, de la suspension mère et des dilutions décimales en vue de l'examen microbiologique - Partie 1 : Règles générales pour la préparation de la suspension mère et des dilutions décimales.
20. NM ISO 7218. (2014). Microbiologie des aliments - Exigences générales et recommandations.,
21. Piquet, J-C. (2018). Procédure nationale de la surveillance sanitaire microbiologique des zones de récolte de coquillages. REMI – IFREMER.,
22. Rubini, S., Galletti, G., D'Incau, M., Govoni, G., Boschetti, L., Berardelli, C., Barbieri, S., Merialdi, G., Formaglio, A., Guidi, E., Bergamini, M., Piva, S., Serraino, A., Giacometti, S. (2018). Occurrence of *Salmonella enterica subsp. enterica* in bivalve molluscs and associations with *Escherichia coli* in molluscs and faecal coliforms in seawater. Food Control, No. 84, 429-35.
23. Sferlazzo, G., Meloni, D., Lamon, S., Marceddu, M., Mureddu, M., Consolati, S. G., Pisanu, M., Virgilio, S. (2018). Evaluation of short purification cycles in naturally contaminated Mediterranean mussels (*Mytilus galloprovincialis*) harvested in Sardinia (Italy). Food Microbiology, No.74, 86-91.