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Full Length Research Paper

Comparison of *ESCHERICHIA COLI* levels in shellfish from Mediterranean coast, Morocco

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Monitoring of *ESCHERICHIA COLI* levels in commercial bivalve shellfish is intended to protect consumer health from foodborne diseases. This study aims to identify if one species can be used as indicator for microbiological contamination of other species present. During 2012, 168 samples of shellfish (clams, cockles and mussels) were analysed for *E. COLI* by MPN technique. 62.5% of samples complied with regulatory threshold ≤230 MPN and geometric mean were higher in cockles than in clams or mussels in each site. Statistical significance was observed between sites. Seasonally, the highest levels were recorded in wet weather (winter and spring) due to runoff of waters from rainfall and lowest levels were recorded in dry weather (summer) when high temperatures show bactericidal effect. Our findings show cockles could be as sentinel specie for burrowing shellfish, but for non burrowing (mussels), monitoring should be done on this specie.

Keywords: Monitoring, *Escherichia coli*, Shellfish, MPN

INTRODUCTION

Bivalve molluscs shellfish are foodstuffs of economic interest and consumed for their richness of proteins and vitamins. Due to their filter feeding from the surrounding waters, bivalve can concentrate contaminants, including microorganisms that can cause several infectious diseases to Humans (Brands et al., 2005); (Robertson, 2007). As the consumption of shellfish raw or lightly cooked constitutes a potential risk to public health (Fleming et al., 2006); (Murchie et al., 2005); (WHO and FAO, 2012), their hygiene-sanitary control is extremely important and legislated (Oliveira et al., 2011). *E. coli* is commonly associated with warm-blooded animals and is therefore a reliable indicator of contamination of human and animal origins (Savichtcheva and Okabe, 2006). In Morocco, exploitation of molluscs shellfish is governed by ministerial circular n° 1508/2012 which is derived in principle from European requirements EC n° 854/2004 (Anonymous, 2004). Production shellfish areas are classified in categories depending on levels of Most Probable Number (MPN) *Escherichia coli* (*E. coli*) in 100 g of flesh. This classification determines the level of post- harvest treatment required before shellfish can be sold for human consumption. Category A: (≤230 MPN) shellfish can be placed on market without further treatment. Category B:

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(>230 and ≤4.600 MPN) shellfish have to be treated either by re-laying in category A waters or depuration in approved plant before placing on the market. Heat treatment is permitted. Category C: (> 4600 and ≤46.000 MPN) shellfish have to be relayed in cleaner waters for a minimum of 2 months before they be consumed directly. Heat treatment is permitted. Category D: (>46.000 MPN) shellfish are prohibited to harvest.

There are lagoons and coastal areas which shellfish activities are present. Among them, northwest Mediterranean coast is recognized among other by its production of bivalve mollusc. Two species are dominant in production: tuberculate cockle [*Acanthocardia tuberculata (Linnaeus, 1758*)] and smooth clam [*Callista chione (Linnaeus, 1758)]* which exist naturally in seabed. They are filter feeding burrowing, macrobenthic communities and share the same substratum. Their fishing is achieved by dredging at a depth up to 30 meters aboard artisanal fishing boats.

Furthermore, in recent years, there is aquaculture activity in this region consisting on mussels rearing in platforms. There are plans for the expansion of production with the focus on mussel culture (*Mytilus* spp). But one problem is requested: which specie will be representative of others in surveillance from the same area. Until now, monitoring of production shellfish areas is carried out on all species commercially interesting. In order to reduce surveillance costs, this study intends to answer if one species will be considered in the same area, as a sentinel and indicator for all commercial species?

MATERIEL AND METHODS

Study area

This study was carried out in northwest Mediterranean coast of Morocco between latitudes (35°50' N; 5°20' W) and (35°27' N; 05°05'W) covering a coast of approximately 80 km (Figure 1). Fishing, tourism and agriculture are the most practiced activities in this region. Population densities are remarkable in the north where urban cities near the coast are present (Fnideq, M'Diq and Martil). While, low densities of population were observed in the southeast with agriculture activities dominant in Oued Laou town. Six sites are chosen for determining shellfish quality levels by *E. coli* in this area. From the north to the south, we found S1, S2, S3, S4, S5 and S6. It is clear to precise that in sites (S1 and S3) three shellfish species (cockles, clams and mussels) were sampled together. By against, mussels rearing is limited to these sites localised in north of this region, for the rest sites, cockles and clams were sampled together. These sites were assumed to represent different degree of anthropogenic influence and land runoff. They are located at varying distances from outlets of rivers.

Sample collection and conservation

Frequency sampling of bivalve shellfish was monthly from January 2012 to December 2012 and 168 samples were collected. Among them 24 samples were represented by mussels collected from S1 and S3, while 144 samples were represented by clams and cockles harvested together from all sites. S1 and S2 are part of unclassified area. While S3, S4, S5 and S6 are part of class B area. For burrowing shellfish (cockle and clam) once caught, sorted by specie and put in a plastic bag. For non burrowing shellfish (mussels), they were extracted from the net and put in a plastic bag per sample. All samples were packed in a cool box with ice packs in manner to reach a temperature of less 8°C and more than 1°C and shipped to laboratory quickly. They were stored in a fridge at 3°C and no more 24 hours should elapse between sampling and the starting of the test.

All samples were collected by station staff of National Institute Research Fisheries (INRH) at M'Diq.

Microbiological analyses

Parameter monitoring in live shellfish was *E. coli* in 100 grams of flesh and intravalvular liquid (FIL). The enumeration method is five-tube, three-dilution MPN technique, ISO 16649-3 (ISO, 2004).

So for (cockle and clam) 10 pieces at least per sample were rinsed and shucked aseptically. Homogenisation was done in a blender bowl at high speed during 1 minute. Approximately 100 g of the FIL was taken. After, was added double weight of diluent Tryptone Water (TW). The mix was blended for 1 minute. After 15 minutes of decantation, 30 ml of the homogenate were added to 70 ml of diluent and homogenised thoroughly. This is the master 10^{-1} dilution. To make dilution to 10^{-2}, add 1 ml of 10^{-1} to 9 ml of diluent. For expected samples to be heavily polluted, further decimal dilutions were added.

Other step consists of inoculation of tubes of minerals modified glutamate broth medium, incubation of tubes at 37°C for 24 h and subculture to tryptone bile glucuronide agar with incubation at 44°C for 20-24 h for determination the MPN index from the number of positive tubes.

For mussels, at least 15 pieces were taken for the process. During shucking, byssal threads were cutting with a sterile pair of scissors and removed away. Then it is similar for the others steps until enumeration of *E. coli*.

Statistics

Bacterial numbers in samples are usually assumed to follow a log-normal distribution because they reflect exponential growth. MPN values were therefore logged to ensure a more symmetrical distribution of the data (Helsel and Hirsch, 1992). In order to see statistical

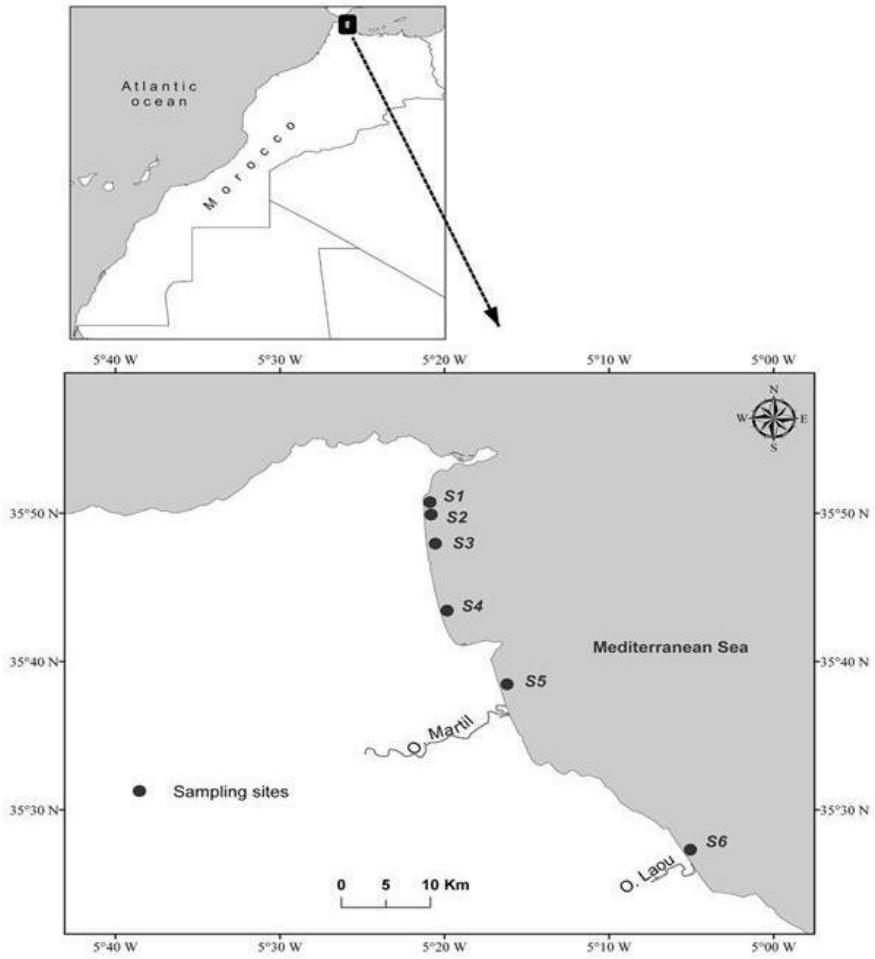

Figure 1. Map showing sampling sites in northwest coast of Morocco

significance between species studied and locations (sites), two-way ANOVA was running. Seasonal variations of *E. coli* levels were analysed by amalgamating MPN by season considering winter (January-March), spring (April-June), summer (July-September) and fall (October-December). One-way analysis (ANOVA) was used between seasons followed by Tukey HSD. Also, for sites (S1 and S3) where 3 species were present, we check significance difference between them and sites by two-way ANOVA. Statistical tests were computed using STATISTICA software version 5, 97 editions.

RESULTS

In this study, the major samples analysed in all sites consisting of clams and cockles. But also, mussels (24 samples) were compared with others in S1 and S3. Bacterial loads of *E. coli* were expressed by MPN *E. coli*/100 g FIL.

Out of 168 shellfish samples analysed, 62.5% complied with regulatory threshold ≤230 MPN whose 73.6%; 70.8% and 52.7% complied with threshold respectively for clams; mussels and cockles.

In S1, *E. coli* levels in cockles were greater and up to threshold 230 MPN almost the year except in September and October. Concentrations reached maximum value of 16.000 MPN twice in January and February 2012 (Figure 2A). For clams, during January to May, *E. coli* levels were higher than threshold 230 and reached 9.200 MPN. But from June to November, levels were less than 230 MPN, but in December levels increased to 490 MPN (Figure 2B). Concerning mussel samples collected from S1, it's observed lower contamination comparatively with the burrowing shellfish. *E. coli* levels varied from 20 to 790 MPN as peak recorded in fall (Figure 2E). Except in winter, there was alternatively contamination of mussels that exceeded threshold.

In S2, *E. coli* levels either in cockles and clams were higher than 230 MPN during the first semester. They

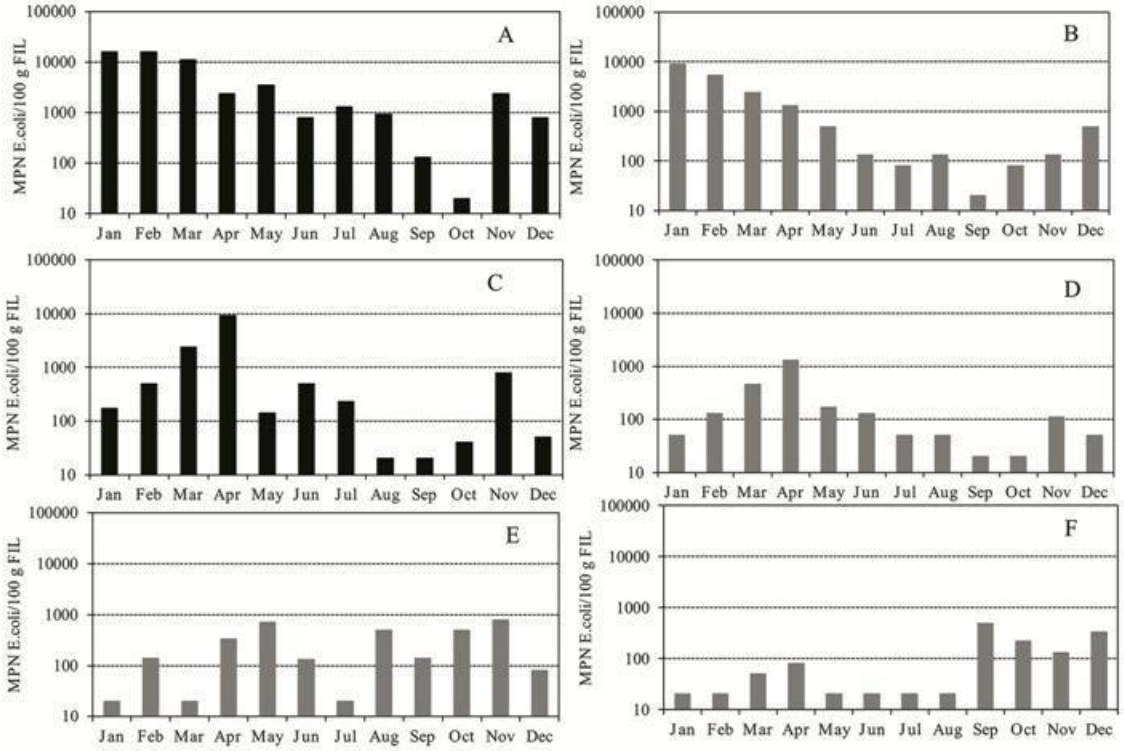

Figure 2. *E. coli* concentrations in cockles A and C, in clams B and D and in mussels E and F from S1and S3 sites respectively

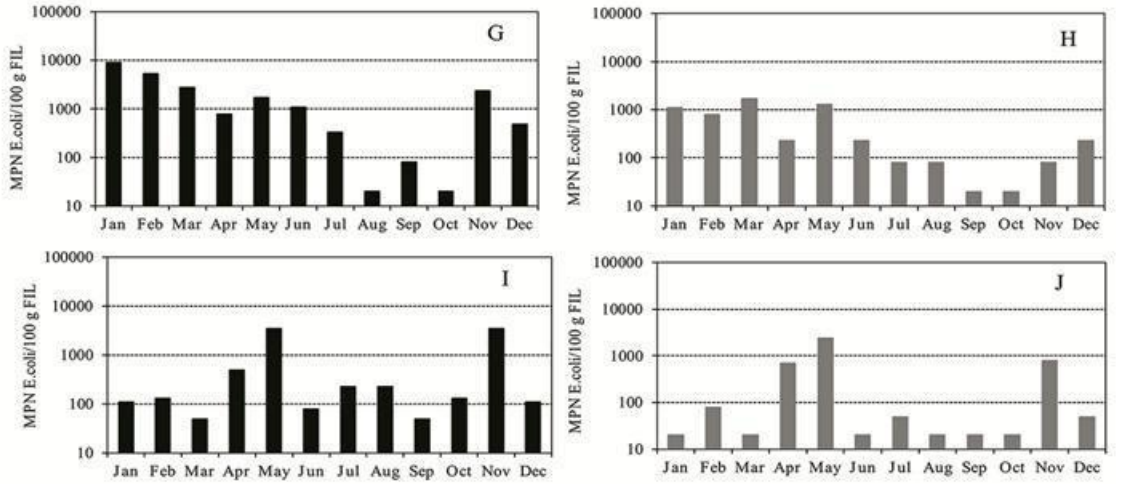

Figure 3. *E. coli* concentrations in cockles G and I, in clams H and J from S2 and S4 sites respectively

reached respectively 9.200 and 1.700 MPN for cockles and clams (Figure 3G and 3H). For summer period, levels were less than 230 MPN except on July in cockles (330 MPN). In fall, levels were higher specially in cockles than in clams with a maximum of 2.400 MPN.

In S3, levels were higher during winter and spring periods in cockles with a maximum value of 9.200 MPN. In summer, levels were decreased below threshold nevertheless in November they increased to 790 MPN but decreased after (Figure 2C). For clams, solely in spring, there were contamination reaching twice 1.300 MPN. However in other seasons, lower concentrations of *E. coli* were recorded below 230 MPN (Figure 2D). Regarding mussels, it's observed lower contamination during the year

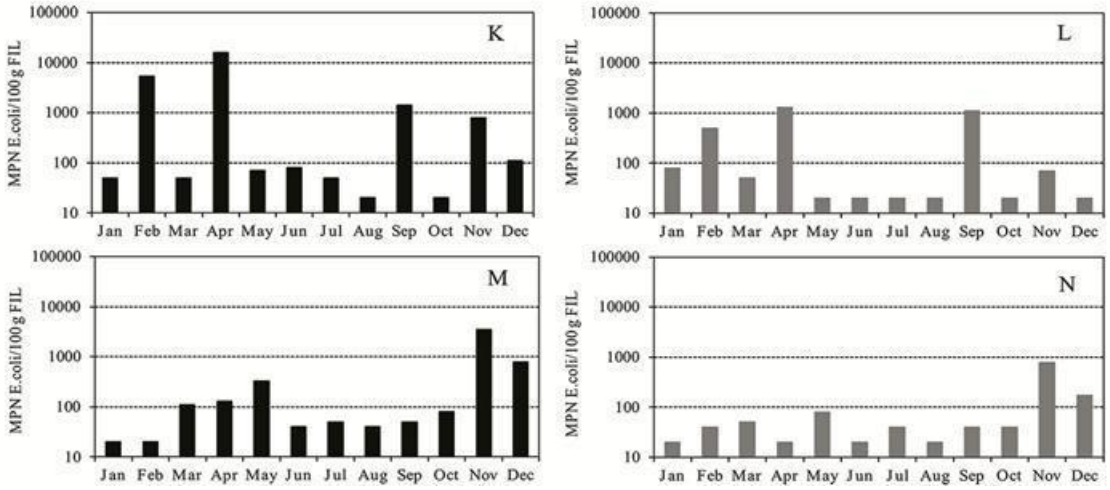

Figure 4. *E. coli* concentrations in cockles K and M, in clams L and N from S5 and S6 sites respectively

under 230 MPN, except for the last 4 months, where a light contamination was observed with a peak of 490 MPN (Figure 2F).

In S4, during spring and November, it's observed higher contamination either for cockles and clams, respectively with a maximum value of 3.500 and 2.400 MPN (Figure 3I and 3J). In other months, a light contamination was observed which varied from 20 to 230 MPN in both shellfish.

In S5, during winter and spring, it's observed variable contamination of *E. coli* that reached on April 16.000 MPN and 1.300 MPN respectively in cockles and clams (Figure 4K and 4L). In summer light contamination under threshold were recorded in both shellfish except in September where contamination upper than 230 MPN in both shellfish were observed (1.400 MPN in cockles and 1.100 MPN in clams).

In S6, it's observed a light contamination less than 230 MPN almost months of year, except in November and December which it recorded respectively a peak of 3.500 MPN in cockles and 790 MPN in clams (Fig. 4M and 4N). This site was considered the lowest polluted site in this study.

Descriptive statistics (minimum, maximum, median, geometric mean and log10 SD) were calculated for species in all sites and were given below in table 1. It noted that almost concentrations of *E. coli* in shellfish have a minimum value of 20 MPN, but maximum value varied from 490 to 16.000 MPN depending on species and sites. It's noted also a big monthly variation per specie and per site which is translated by a high log10 standard deviation compared with log10 geometric mean.

Generally, geometric mean levels of *E. coli* were higher in in clams and cockles from S1 than the others. A decreasing gradient of geometric mean levels of *E. coli* was observed from S1 towards S6 in these shellfish together. Mean levels of *E. coli* were greater in cockles than in clams in each site (Table 1).

It's remarked a variation of *E. coli* levels monthly inside a site and between species. Statistical analyses by two-way ANOVA showed statistical significance ($F=5.34^*$ at $p<0.05$) between species (cockles and clams) in bioaccumulation of faecal contamination. Moreover, statistical significance were obtained among sites ($F=11.04^*$ at $p<0.05$). Post hoc Tukey pair-wise multiple comparisons test show S1 differ significantly from others, but no statistical significance between S1 and S2.

When it's examined significance between 3 species (clams, cockles and mussels) collected from S1 and S3, it found statistical significance between species ($F=13.31^*$ at $p<0.05$) and sites ($F=7.91^*$ at $p<0.05$). Statistical data by Tukey's test show significance between cockles and mussels, but no significance between mussels and clams. Statistical analyses one way ANOVA on effect of season onto 3 shellfish showed statistical significance between seasons ($F=4.82^*$ at $p<0.05$). Comparisons by Tukey's test show significance between summer and (winter and spring) seasons.

Data pairs of *E. coli* levels between species enabled us to calculate ratio of concentration between the following species: cockles were about 2.8 fold higher than clams and about 6 fold higher than mussels. While clams were about 2 fold higher than mussels.

Table 1: Descriptive statistic for species per sampling site (**MPN *E. coli* 100 g CIL**)

Site	Status of area	Species	Min	Max	Median	log10 Geometric Mean	log10 SD
		Clam	20	9.200	310	2.58	3.45
S1	unclassified	Cockle	20	16.000	1.850	3.17	3.78
		Mussel	20	790	140	2.15	2.44
S2	unclassified	Clam	20	1.700	230	2.31	2.77
		Cockle	20	9.200	945	2.78	3.44
S3	Class B	Clam	20	1.300	80	1.97	2.56
		Cockle	20	9.200	200	2.36	3.42
		Mussel	20	490	35	1.76	2.18
S4	Class B	Clam	20	2.400	35	1.85	2.85
		Cockle	50	3.500	130	2.34	3.12
S5	Class B	Clam	20	1.300	35	1.84	2.66
		Cockle	20	16.000	75	2.29	3.67
S6	Class B	Clam	20	790	40	1.70	2.34
		Cockle	20	3.500	65	2.02	3.00

DISCUSSION

Comparisons of geometric mean levels of *E. coli* between cockles and clams samples harvested from same site show statistical significance and higher values for ever sites in favour of cockles. In previous study by (Boutaib et al., 2011), it showed significance difference between these 2 species and geometric means of *E. coli* were higher in cockles than in clams. These findings obtained in 2008 were in accordance with results reached in 2012. It should be noted that relevant information is particularly lacking concerning microbiological comparisons between smooth clam and tuberculate cockle. This difference in concentrations and release of faecal contaminants seems possible that it is up to dynamic movement of cockle via its foot on the surface of sediment and shell is often opens in comparison with clams. Moreover, when shucking shell of cockles, there was more silt and sediment with whole meat than in clams. This is contributed to more contamination by faecal bacteria and more loads of *E. coli*. It is likely there is high capacity of filtration of water by cockles more than clams, which explains higher contamination in cockles than in clams. High loads of *E. coli* were recorded in all shellfish during wet seasons than in dry season. The seasonal variation shows higher values of contamination in bivalves during winter and spring, due to runoff of waters from rainfall, leading to an increase of transport of contaminants towards coast. While in dry weather (summer) highest temperatures, solar radiation, salinity seems to show bactericidal effect on microbial contamination. Contamination due to rainfall most often results from urban wastewater discharges or from nonpoint pollution sources in the watershed (Papastergiou et al., 2009); (Chu et al., 2011); (Conn et al., 2012). In other study conducted by (Almeida and Soares, 2012) onto microbiological monitoring of bivalves from the Ria Formosa lagoon of Portugal, they showed seasonal variation when bivalves had highest *E. coli* levels in winter and fall due to the runoff of waters from rainfall relatively to those in spring and summer.

Increased levels of *E. coli* in bivalves from all monitoring points under high river flow conditions suggest that storm water runoff is contributing to significant proportion of *E. coli* accumulated by bivalves (Campos et al., 2013). In his study onto impact of rainfall on the hygienic quality of mussels, (Tryland et al., 2014) highlighted the need to consider rainfall as an important factor in water contamination in urban areas due to overflow. Also, contamination difference between cockles and clams originated from same site is higher in wet seasons like winter and spring than in dry season (summer). It also found *E. coli* levels in cockles were higher than those recorded in mussels. It seemed that shellfish burrowing concentrate bacterial contamination in guts than shellfish no burrowing. The accumulation of *E. coli* and other enteric bacteria in bivalves is a dynamic process. It is related to

filtration rate, to bacterial content of the ambient water and to the filtration efficiency of gills (Jozic et al., 2012). Our findings showed cockles concentrate more than clams and mussels. In their study, (Amouroux and Soudant, 2011) demonstrated that cockle (*Cerastoderma spp*) may be considered a sentinel species for burrowing (*Tapes* spp) and non burrowing species studied (*Crassostrea gigas*, *Mytilus* spp). However, in other study leaded by (Younger and Reese, 2013), their findings showed no significance difference found between *Cerastoderma edule* (cockles), *Tapes philippinarum* and *Mytilus* spp. These different results in classifying shellfish in order to have one specie representative for all commercial species from the same area may due to several factors like: hinterland geographical nature of production area, rain volume received in the catchment, size of shellfish, presence or absence of wastewaters discharge near the production area, waters temperature and salinity, etc.

Spatially, our findings showed decreasing gradient in contamination between sites in both shellfish studied from north to south. S1 showed the highest levels of *E. coli* for each species for many reasons. This site is localised in north of this region in front of Fnideq city which have high populated density (more than 52.000 people) over a limited surface area. Furthermore, wastewaters outfall discharges on the coast of this site and this volume increase when it was raining following runoff. Uncontrolled sewage disposal or performed without previous appropriated treatment, small river outlets or diffuse land runoff of contaminants derived from agricultural activities and septic tank leakages may also produce sporadic contamination (Hernroth et al., 2002).

S2 is the nearest site from the first. It is influenced by currents deriving from the north. Also it received storm water during wet period via temporary water canal. Furthermore, near this site, outcrops rocks on which birds settles and reject their faecal waste on water surface. This seems to explain high levels of *E. coli* in shellfish harvested from this site mainly in wet period. S3 is positioned about 200 m from the outlet of oued Negro. Hinterland of this site is formed by natural lands, but in wet period, rainwater was very loaded by mud. This influenced loads of *E. coli* in shellfish during wet period. S4 is localised about 300 m from the outlet of oued Smir. The latter crosses lagoon Smir which likely, rainwater was relatively diluted before reaching the coast. So, concentrations of *E. coli* are moderately high in shellfish from this site.

S5 is situated in north of river Martil. During wet seasons levels of *E. coli* were higher in both shellfish due likely to storm water drained by the river which crosses an agricultural plain.

S6 is localised about 1 km at the west from the outlet of oued Laou, which drained land agriculture. Based on mean levels of *E. coli* in shellfish originated from this site, it seemed that these results were the lowest in this region. These values were relatively higher in wet seasons and specifically in cockles than in clams.

CONCLUSIONS

Our results showed significant difference in geometric mean of *E. coli* between cockles and clams and mussels on the other side harvested from the same site. There was decreasing gradient in contamination levels between sites from the northwest and others towards the southeast. Runoff rainfall events increased contamination levels in both shellfish but rain volume received decreased from the northwest towards the southeast of study area. That explains the decrease of contamination from north to the south region. For all sites, precipitation seems to be the agent that most contributed to the increase of contamination, causing transport of contaminants in the runoff waters.

It's concluded cockles can be sentinel representative for clams in this region for sanitary quality, because they are dredging together. Furthermore, fishermen harvest both shellfish because it is not economically profitable to sort only one specie. But for mussels, it will be constraint of exploitation if it considers cockles a sentinel representative for mussels because levels of *E. coli* in mussels were several times complied with threshold 230 MPN than cockles. So it is recommended in this study cockles may be solely a representative for burrowing shellfish but mussels should continue to be monitoring apart.

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REFERENCES

Almeida C, Soares F (2012). Microbiological monitoring of bivalves from the Ria Formosa Lagoon (south coast of Portugal): A 20years of sanitary survey. Mar. Pollut. Bull. 64, 252–262. doi:10.1016/j.marpolbul.2011.11.025

Amouroux I, Soudant D (2011). Comparison of microbiological contamination level between different species of shellfish, in: ICMSS 2011-International Conference of Molluscan Shellfish Safety, 12-17 June, 2011, Charlottetown, Prince Edward Island, Canada.

Anonymous (2004). Regulation (EC) No. 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption. J Eur Union L22683 Bruss.

Boutaib R, Marhraoui M, Abdellah MKO, Bouchrif B (2011). Comparative Study on Faecal Contamination and Occurrence of Salmonella spp. and Vibrio parahaemolyticus in Two Species of Shellfish in Morocco. Open Environ. Sci. 5, 30 – 37.

Brands DA, Inman AE, Gerba CP, Mare CJ, Billington SJ, Saif LA, Levine JF, Joens LA (2005). Prevalence of Salmonella spp. in Oysters in the United States. Appl. Environ. Microbiol. 71, 893–897. doi:10.1128/AEM.71.2.893-897.2005

Campos CJA, Acornley R, Morgan OC, Kershaw S (2013). Trends in the levels of Escherichia coli in commercially harvested bivalve shellfish from England and Wales, 1999–2008. Mar. Pollut. Bull. 67, 223–227. doi:10.1016/j.marpolbul.2012.11.030

Chu HJ, Pan TY, Liou JJ (2011). Extreme Precipitation Estimation with Typhoon Morakot Using Frequency and Spatial Analysis. Terr. Atmospheric Ocean. Sci. 22, 549. doi:10.3319/TAO.2011.05.10.02(TM)

Conn KE, Habteselassie MY, Denene BA, Noble RT (2012). Microbial water quality before and after the repair of a failing onsite wastewater treatment system adjacent to coastal waters. J. Appl. Microbiol. 112, 214–224. doi:10.1111/j.1365-2672.2011.05183.x

Fleming LE, Broad K, Clement A, Dewailly E, Elmir S, Knap A, Pomponi SA, Smith S, Solo Gabriele H, Walsh P (2006). Oceans and human health: Emerging public health risks in the marine environment. Mar. Pollut. Bull. 53, 545–560. doi:10.1016/j.marpolbul.2006.08.012

Helsel DR, Hirsch RM (1992). Statistical methods in water resources. Elsevier.

Hernroth, B.E., Conden-Hansson, A.-C., Rehnstam-Holm, A.-S., Girones, R., Allard, A.K., 2002. Environmental Factors Influencing Human Viral Pathogens and Their Potential Indicator Organisms in the Blue Mussel, Mytilus edulis: the First Scandinavian Report. Appl. Environ. Microbiol. 68, 4523–4533. doi:10.1128/AEM.68.9.4523-4533.2002

ISO, (2004). ISO/TS 16649-3. Microbiology of Food and Animal Feeding Stuffs – Horizontal method for the enumeration of b-glucuronidase-positive Escherichia coli – Part 3: Most probable number technique using 5-bromo-4-chloro-3-indolyl b-D- glucuronide. Int. Organ. Stand. Genève Suisse.

Jozic S, Šolic M, Krstulovic N (2012). The accumulation of indicator bacteria Escherichia coli in mussels (Mytilus galloprovincialis) and oysters (Ostrea edulis) under experimental conditions. Acta Adriat. 53, 353–360.

Murchie LW, Cruz-Romero M, Kerry JP, Linton M, Patterson MF, Smiddy M, Kelly AL (2005). High pressure processing of shellfish: A review of microbiological and other quality aspects. Innov. Food Sci. Emerg. Technol. 6, 257–270. doi:10.1016/j.ifset.2005.04.001

Oliveira J, Cunha A, Castilho F, Romalde JL, Pereira MJ (2011). Microbial contamination and purification of bivalve shellfish: Crucial aspects in monitoring and future perspectives – A mini-review. Food Control 22, 805–816. doi:10.1016/j.foodcont.2010.11.032

Papastergiou P, Mouchtouri V, Karanika M, Kostara E, Kolokythopoulou F, Mpitsolas N, Papaioannou A, Hadjichristodoulou C (2009). Analysis of seawater microbiological quality data in Greece from 1997 to 2006: association of risk factors with bacterial indicators. J. Water Health 07, 514. doi:10.2166/wh.2009.135

Robertson LJ (2007). The potential for marine bivalve shellfish to act as transmission vehicles for outbreaks of protozoan infections in humans: A review. Int. J. Food Microbiol. 120, 201–216. doi:10.1016/j.ijfoodmicro.2007.07.058

Savichtcheva O, Okabe S (2006). Alternative indicators of fecal pollution: Relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. Water Res. 40, 2463–2476. doi:10.1016/j.watres.2006.04.040

Tryland I, Myrmel M, Østensvik Ø, Wennberg AC, Robertson LJ (2014). Impact of rainfall on the hygienic quality of blue mussels and water in urban areas in the Inner Oslofjord, Norway. Mar. Pollut. Bull. 85, 42–49. doi:10.1016/j.marpolbul.2014.06.028

World Health Organization, Food and Agriculture Organization of the United Nations, (2012). Prevention and reduction of food and feed contamination. World Health Organization; Food and Agriculture Organization of the United Nations, [Geneva]; Rome.

Younger AD, Reese RA (2013). Comparison of *Escherichia coli* Levels Between Bivalve Mollusc Species across Harvesting Sites in England and Wales. J. Shellfish Res. 32, 527–532. doi:10.2983/035.032.0232