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Original Research Article

An evaluation of environmental factors affecting the survival of *Escherichia coli* in coastal area, Oualidia lagoon

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A B S T R A C T

Enteric Bacteria survival in aquatic environment is impacted to a great extent by the ability of bacterial cells to survive in adverse environmental conditions. We evaluate the impact of the environmental factors, such as a temperature, salinity, pH and turbidity waters of coastal Moroccan area; lagoon Oualidia on survival *Escherichia coli*. The six diffusion chamber inoculated by a suspended bacteria were implanted into surface and depth of three investigated sites different geolocation. After exposure time in lagoon condition, all experiment samples were evaluated in terms of physicochemical and survival bacteria parameters. The viable cells was enumerated by three different methods; conventional techniques using a slide culture (selective media EMB and now selective medium TSA) and direct microscopic counts (TB 0,4%). The results revolted an important decrease, in Escherichia coli counts according time, in response to changed environmental conditions. Significantly deference ($p<0,05$) indicate that the direct microscopy counter is more efficient than the traditional method of manual counting of bacterial colonies on petri dish. That is may be explained by a presence of cell loses cultivability but remains viable and potentially able to regrow (VBNC). Environment factors have a negative impact of higher salinity ($R=-0.97$) and pH ($R=-0,98$) against a positive impact of higher turbidity ($R=0.93$) on survival Escherichia coli into lagoon systems. No impact unregistered of the temperature effect ($p>0,05$).

Keywords

Survival *Escherichia coli*, Oualidia lagoon, Diffusion chamber, environmental impact, VBNC

Introduction

Sewage water discharged without any treatment and uncontrolled human activities are the most serious threats that might destroy coastal environment especially lagoons. They have a negative impact on this environment and make it less attractive for recreation as well as for aquaculture, which is sensitive to the presence of fecal bacteria and pathogenic microorganisms. The fate of this allochthonous microorganism and bacteria reach coastal marine environment depends on various processes. Indeed, when Enteric bacteria exposed to the marine environment condition simultaneously encounter a variety of an abiotic challenges including; sunlight irradiation, salinity, temperature, pH and nutrient availability, which can result in bacterial stress or viral degradation (Troussellier *et al.*, 1998; Rozen and Belkin 2001, Byappanahalli and Fujioka, 2004), and biotic effects, including competition with other microorganisms and grazing (Barcina *et al.,* 1991; Rozen and Belkin, 2001, Feng *et al.,* 2010, Korajkic *et al.*, 2013, Wanjugi and Harwood, 2013, 2014), leading either to their disappearance or to an alteration in their physiological state. Consequently, considerable interest has been shown in the survival of enteric bacteria in natural environment and drinking waters (McFeters and Stuart, 1972; Davenport *et al.,* 1976; Xu *et al.,* 1982; Elliot and Colwell, 1985; Grimes *et al.,* 1986; Rhodes and Kator, 1990; Gonzales *et al.*, 1995).

Historically, the faecal indicator Bacteria (total and faecal coliforms, enterococci and E. coli), have been much used to diagnostics water quality, basing on a selective culturing methods. However, since more the 30 years, direct enumeration technique has shown that the traditional count methods do not give a good estimates of the number of enteric bacteria present in water analyzed. Due to the environmental factors, several bacteria which are readily cultivable may enter a state in which they retain viability but fail to grow in conventional strikes culture. The viable but non culturable (VBNC) state concept was introduced to describe cells that remain metabolically active but are unable to divide in or on nutritional media that normally support their growth (Oliver, 2000). The primary concern of this research is to examine the effects of the environmental condition specially salinity, pH, temperature and turbidity on E.coli survival in Oualidia lagoon. A Second objective was to evaluate the use of direct enumeration in coastal area and we will compared with indirect, bacterial enumeration methods.

Materials and methods

Study area

Located on the Atlantic coast of Morocco between El Jadida and Safi, the Oualidia lagoon is approximately 7 km long and 0.5 km wide. It exchanges water with the ocean with major inlets (Fig.1). The fresh water supply is provided by some resurgence distributed along the principal channel. The poor water quality of the lagoon is directly associated with sewage discharge through underground seepage waters and/or uncontrolled touristic activities. To identify the factors affecting the survival bacteria especially E.coli along the Oualidia lagoon, three experiments sites characterized by different hydrodynamical and environmental condition were investigated (Fig.2). First site was the lower zone influenced by the sea. Second site was the intermediary zone of the lagoon under both marine and continental influences. Third site was the upper zone characterized by a continental impact (presence of nitrogen element, suspend and organic matter) (Rharbi et al, 2001). The series of three experiments described in this paper were carried out in the lagoon Oualidia for three season of the year 2012 (winter, spring and summer) and were followed up in situ.

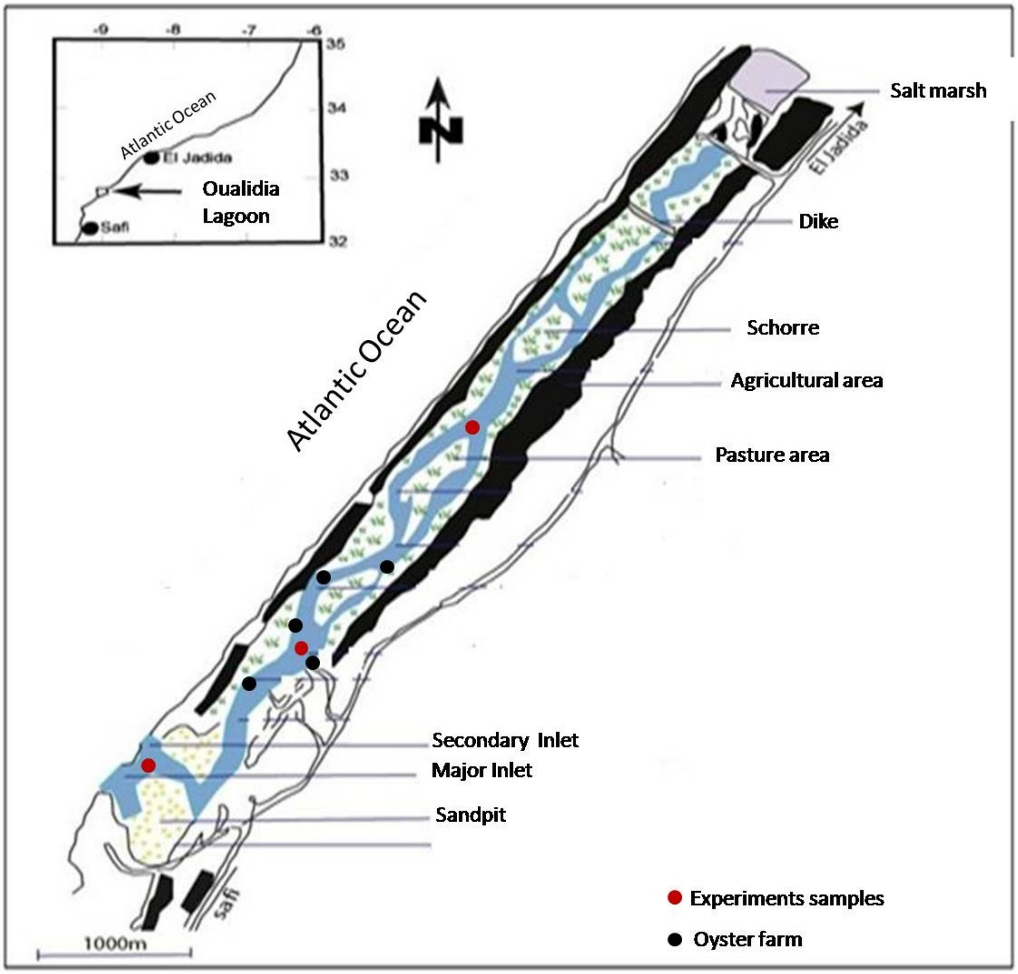

Figure.1 Map of Oualidia lagoon

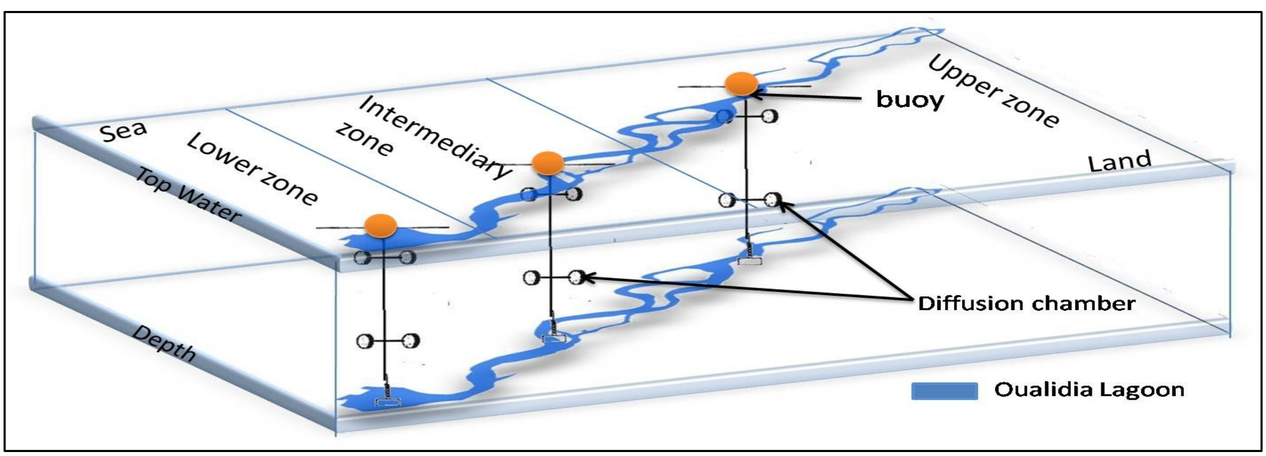

Figure.2 Experimental dispositive

Inoculum preparation and Diffusion chamber test

E.coli spaces isolated from the local wastewater were used in this study. Inoculate of the test microorganism were prepared by growing isolates E.coli in 250 ml sterile nutritive bouillon (NB) for 24H at 37°C. After incubation, the cells were harvested by centrifugation at 3000 rpm for 15 min and washed twice with sterile isotonic saline. After the final wash the cells were suspended in 10mL sterile ringer solution at a concentration of 4.108 CFU per ml. 1 ml from this suspension was inoculated into diffusion chamber with a sterile syringe. For survival studies, Plexiglas diffusion chambers (250 ml of capacity) were used with a membrane filter (0.47 µm).

The bacterial suspensions (1 ml) were placed into sterile chambers (sterilized by Ethanol 95% 24 hours before being used) just before placing them at the studies sites. Six chambers of E.coli survival test were implanted into lagoon Oualidia by descriptive experiments points. That were been exposed in the environment conditions for various lengths of time. For all experiments samples, we evaluate the physicochemical parameter of water lagoon, such as temperature, pH, salinity, and turbidity (Table 2).

Enumeration methods

The bacteria enumeration was done after the exposed times (4H, 8H……….48H), in the lagoon using three compared techniques. Two microcolony methods employing a slide culture; i) selective media; Eosin-methylene blue (EMB) and ii) now selective nutritive media; trypto caseine soya agar (TSA). Colony forming units (CFU) were determined by cultivation at 37 °C for 24 Hours on EMB for selective test and TSA for now-selective method. Results are expressed as the mean numbers of CFU mL-1. The direct microscopic counting methods using a trypan blue (0.4%). Blue stained cells were counted non-viable and now stained cell as viable.

Statistical analysis

Data were presented and analyzed using Matlab software 6.1. Bacterial numbers are expressed as mean Log UFC per ml. The environmental effect on survival bacteria and the methods comparisons were analyzed using the student test. Differences were considered statistically significant when $p<0.05$.

Results and discussion

Kinetic of *Escherichia coli* in Oualidia lagoon

The kinetic curves of E.coli population implanted into lagoon Oualidia by three experiments sites, in three different seasons, are presented in Figures 5, 6 and 7.

In response to a changing environmental conditions; for a rich environment (sewage water) to an impoverished environment (lagoon), the bacterial concentration ($N_0=4.10^8$ CFU/ml) declined rapidly under time. Several aspect of the environmental conditions effects were consider, including water temperature, salinity, turbidity, solar radiations…..etc. This result is in arguments with several researches demonstrating the negative impacts of natural environmental conditions on allochtonnus bacteria (Chraibi, 1996, Chedad and Assobhei 2007, Chandran *et al.*, 2010).

Fig.3 Shape of the survival curves of *Escherichia coli* in lower site (Inlet) of the Oualidia Lagoon

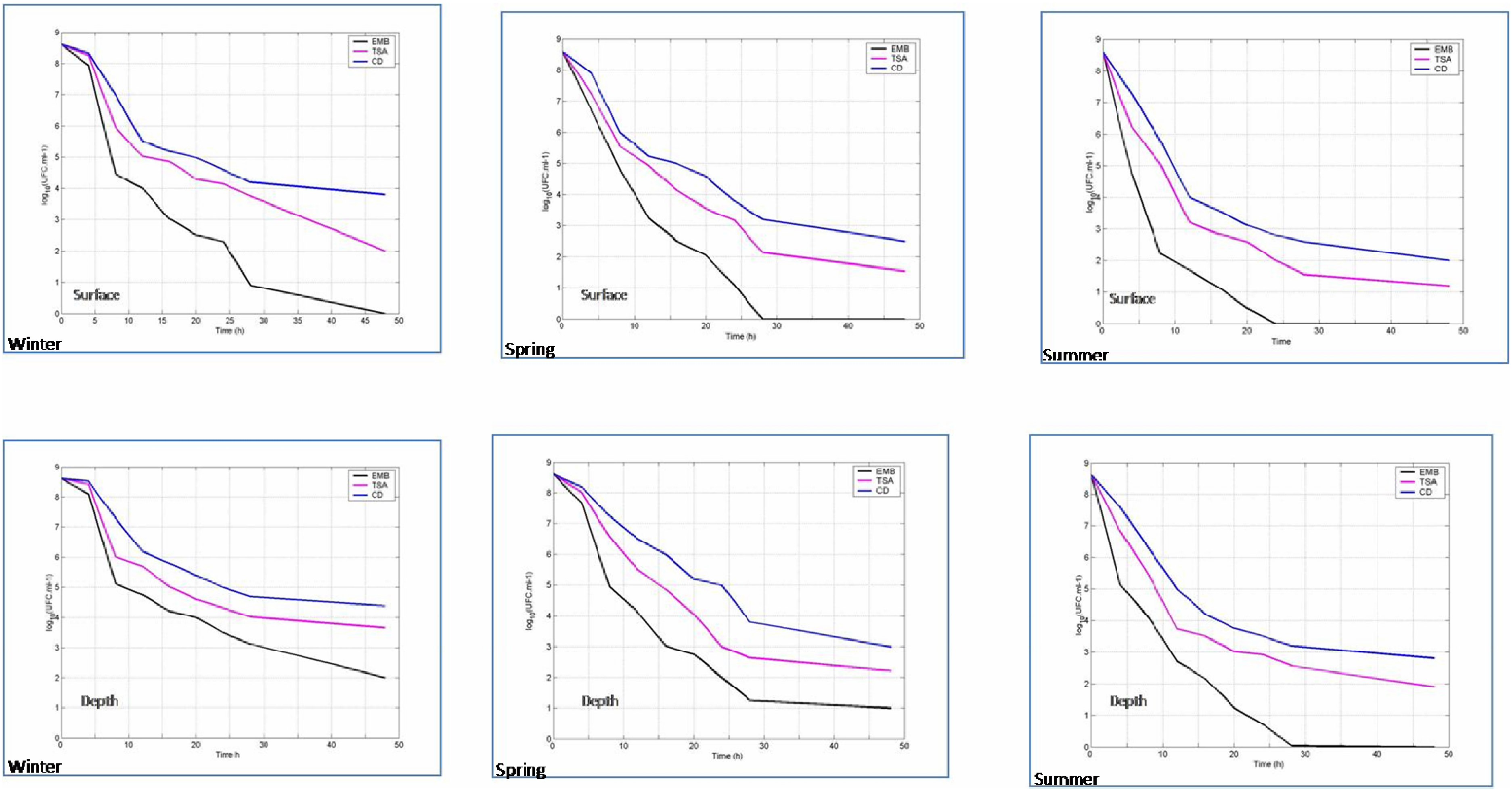

Fig.4 Shape of the survival curves of *Escherichia coli* in intermediary zone (Ostrea) of the Oualidia Lagoon

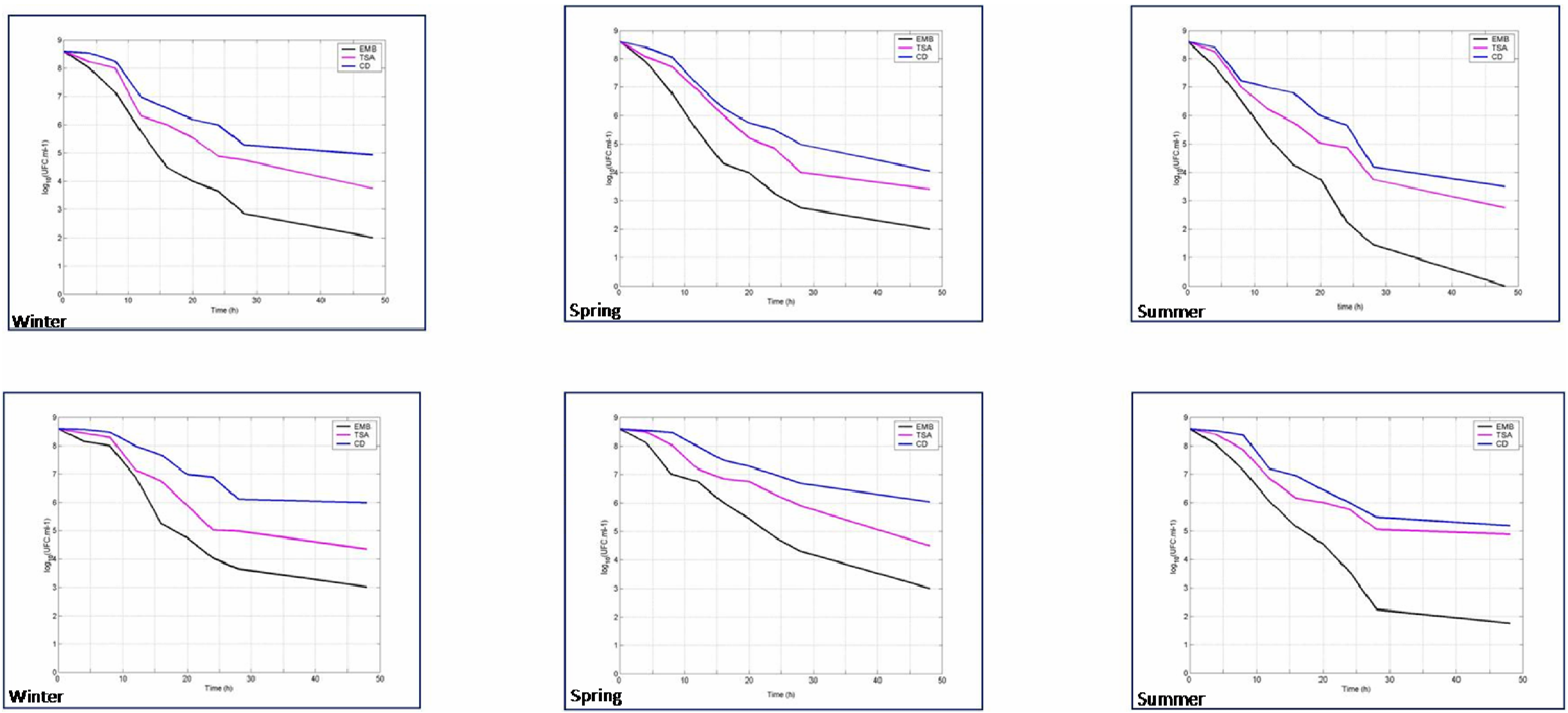

Fig.5 Shape of the survival curves of *Escherichia coli* in upper site (agricultural area) of the Oualidia Lagoon

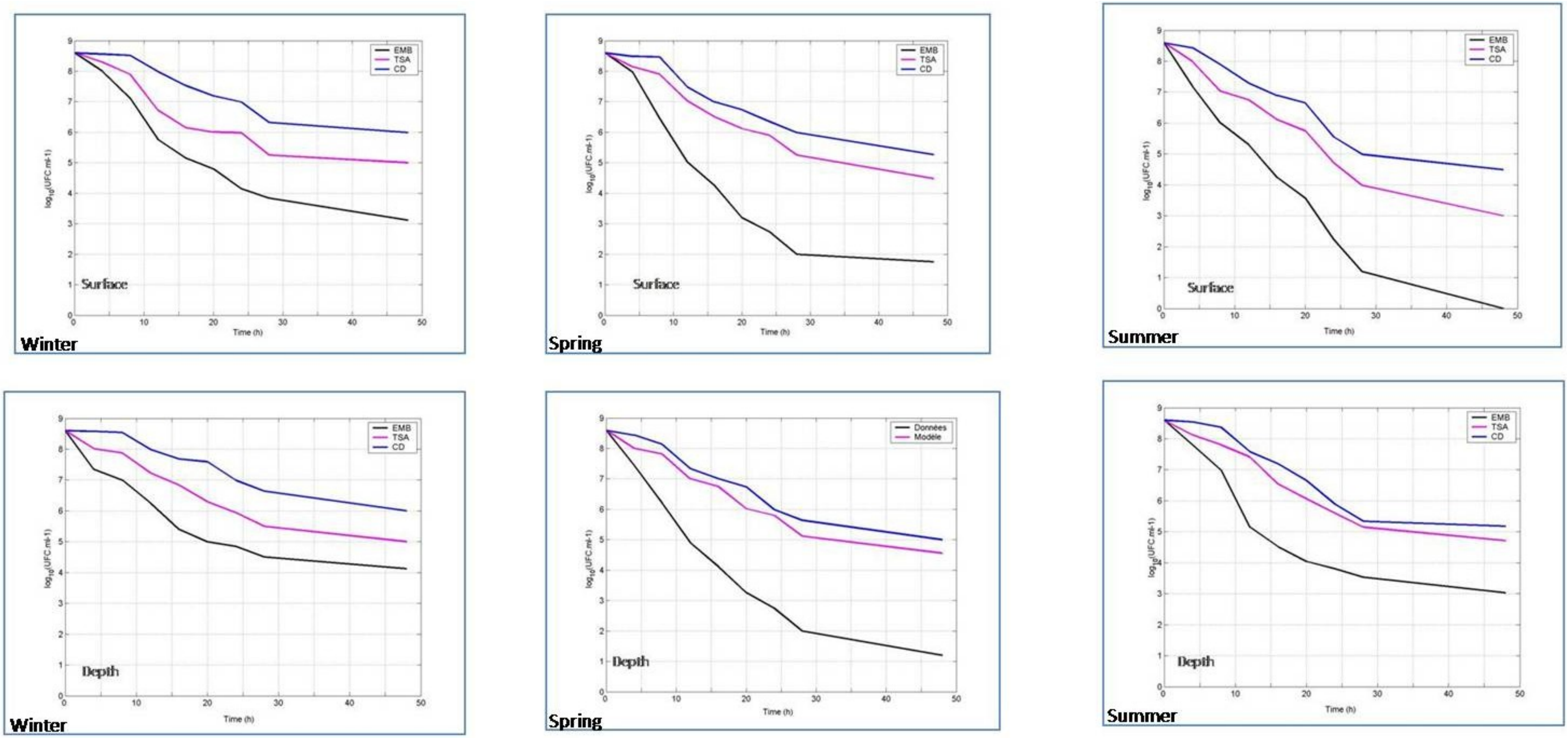

Table.1 Physicochemical parameter of water lagoon measured at all experiments samples

	Period		pH	Salinité	Turbidité	Température
Inlet	**Winter**	**Surface water**	7,95	29,3	0,55	15,3
		Depth	8,22	32	2,5	
	Spring	**Surface water**	8,1	31,46	0,15	16,4
		Depth	8,2	31	1,05	
	Summer	**Surface water**	8,34	32,49	0,04	22,3
		Depth	8,4	32,8	0,09	
Oyster farm	**Winter**	**Surface water**	7,46	24,5	3,41	16,6
		Depth	7,59	24,94	9	
	Spring	**Surface water**	7,76	28	2,34	17,9
		Depth	7,8	28,8	3,6	
	Summer	**Surface water**	7,89	29	1,67	25
		Depth	7,92	29,8	3	
Agricultural Area	**Winter**	**Surface water**	7,32	23,56	19	17
		Depth	7,5	23,89	22	
	Spring	**Surface water**	7,48	27	6,5	19,7
		Depth	7,6	27,2	9,8	
	Summer	**Surface water**	7,71	27,3	11,58	26,6
		Depth	7,9	29	14	

Table.2 Correlation coefficient R between survival *Escherichia coli* and physicochemical parameters ($p<0,05$)

	Parameter Survival *E. Coli*	**pH**	**Salinity**	**Turbidity**
Parameter Survival *E. Coli*	**1**			
pH	**-0,98**	**1**		
Salinity	**-0.97**	**0,95**	**1**	
Turbidity	**0,93**	**-0,76**	**-0,74**	**1**

Generaly, for survival study in naturel systems, diffusion culture may be a more useful tool for establishing factors-survival relationships (Chraibi, 1996; Chedad and Assobhei, 2007; Chandran *et al.,* 2010 and Hennani *et al.,* 2014). In membrane diffusion, inoculate bacteria under investigation come in contact with continuous exchanges of solute. At this time, the membrane filters prevent the entry of autochthonous bacteria and pathogens into experiment culture.

The incident of unfavorably environmental conditions increase from winter to summer, from upstream to downstream and from surface water to depth. Significant difference ($p<005$) showed an impact of season and space on survival bacteria. Well, the bactericidal action of lagoon water was most pronounced during the summer and less important in the winter. According to the study results of Vandonsel *et al.* (1967) and Majdoub *et al.* (2003), solar radiation plays an important role in the inactivation of bacteria in water environments. Indeed, the best elimination of microorganisms occurred on the water surface and that reduction of microorganisms decreased with increasing depth of the aquatic environment. Thus, the inactivation of microorganisms can be expected only at the surface. Others parts, downstream lagoon characterized by low filtered water turbidity can be correlated with low bacterial counts. Turbidity reduces solar radiation intensity and protects microorganisms from being irradiated (being either under floating solids) (Pfammatte and Wegelin, 1993). This has been observed in the study of Bell and Albright (1981) and Albright (1983) realized on the Elorn estuary, showed that about 60% of the bacterial biomass is associated with suspended particle in turbid environments, thus protecting them from adverse environmental conditions.

Total cell counts as shown by direct enumeration staining generally were significantly higher ($p<0.01$) than the conventional methods using a culture media EMB and TSA counts. In summer period, after exposing time (48 hours), to unfavorable conditions, any survival cells were observed in surface inlet water (site1), by selective media. While, 100 cells were counted by TSA nutritive media against 100000 living cells identified by direct microscopic counts. A similar result has been observed by Chedad and Assobhei 2007, Chraibi, 1996.

Nonetheless, one of the most intriguing findings on stressed bacteria in natural environments was the so-called viable but non culturable (VBNC) state (Xu *et al.*, 1982). The VBNC state is defined as a state of dormancy triggered by environmental harsh conditions, such as nutrient starvation (Cook and Bolster, 2007), temperature (Besnard *et al.,* 2002), osmotic stress (Asakura *et al.*, 2008), oxygen availability (Kana *et al.,* 2008), heavy metals (Ghezzi and Steck, 1999), exposure to white light (Gourmelon *et al.*, 1994) and chlorination of wastewater (Oliver, 2005; Oliver *et al.*, 2005). In such a state, bacteria lose the ability to grow on solid media.

Environmental impact on survival *Escherichia coli*

The environmental results of such experiments samples are presented in Table 1. Our experiments indicate that the number of living cells of E. coli decreased rapidly after exposed to the environmental factors of the lagoon. Indeed, analysis data showed that the survival of Escherichia coli in lagoon Oualidia was influenced by environmental factors ($p<0.05$), such a negative impact of salinity (R= - 0,97) and pH (R= -0,98), against a positive impact of turbidity (R= 0,93). This confirmed some research suggested that the higher temperature and pH defavorate a survival of entero-bacteria in sea water **(**Carlucci and Pramer, 1960; Ayres, 1977; Anderson *et al.*, 1979; Solic and Krstulovic, 1992; Chedad and Assobhei, 2007).

While, the fine materials suspended in aquatic systems may reduce the capacity for waters to naturally degrade contaminants including Enteric-bacteria in natural environment (Dunlop *et al.,* 2005).

As for the temperature factors, our results showed a no-significant effect on survival of bacteria implanted into lagoon ($p>0.05$). However, numerous researches have shown the contradictory results concerning the temperature impact on survival of bacteria in natural environment. Some shown that culturable bacteria were not particularly sensitive to a higher temperature as 60°C (Stroes-Gascoyne and Hamou, 2010).On the contrary, other research suggest that low temperatures favorites destruction of bacterial cells (Chedad and Assobhei, 2007, Sakyi and Asare, 2012). That is important to introduce the effect of temperature factors on survival bacteria in future research.

In conclusion, our experiments showed that exposure to an intense environmental aquatic condition were the major factors responsible for the disappearance of Escherichia coli in the studied ecosystem. Generally we have demonstrated by this study, a negative impact of higher pH and salinity against a positive impact of the higher turbidity on survival bacteria in the coastal lagoon Oualidia. However, any impact of the temperature factor on survival of Escherichia coli has been detected. We recommended introducing effect of this parameter on survival bacteria in future research. Clearly, more environmental models are necessary in future study (current study) to explicate the survival of enteric bacteria into coastal area especially lagoon and the effect correlation between all environmental factors on bacterial survives.
In comparison with colony counts, the direct microscopic methods resulted in higher bacterial counts after exposure time in natural condition of lagoon for all experiments samples. Slides were clear and dead and viable cells easy to distinguish. Adverse environmental conditions induce many situations of bacteria, where a cell loses cultivability but remains viable and potentially able to regrow. So some bacteria can not be detected by cultivation, but they still present a public health danger. While the importance to introduce the new advanced counting techniques in the supervision and quality control of aquatic environments.

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