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Spatio-Temporal Variation in the Microbiological and Physicochemical Quality of the Oualidia Lagoon, Morocco

Yousra Moujahed [1,2a], Abdelhamid El-Hissoufi [1b], Houda Ennassiri [2c], Siham Bazine [2d], Samah Ait Benichou [1e]✉

[1]*Health, Environment and Biotechnology Laboratory, Department of Biology, Faculty of Sciences Ain Chock, University Hassan II Casablanca (https://ror.org/001q4kn48), Casablanca 20650, Morocco*

[2]*Microbiology Department, Charle Nicole Laboratory, Casablanca 20360, Morocco*

[a] ***ORCID***: *0009-0003-5351-1357*; ***Email:*** *yousra.moujahed3@gmail.com*

[b] ***ORCID***: *0009-0000-9596-2584*; ***Email***: *abdoelhi1998@gmail.com*

[c] ***ORCID***: *****-****-****-*****; ***Email***: *houdaennassiri@gmail.com*

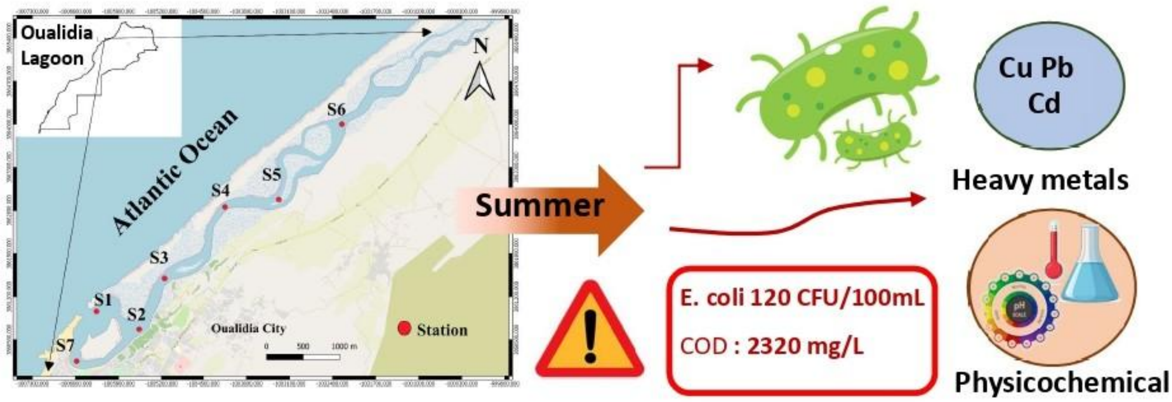
[d] ***ORCID***: *****-****-****-*****; ***Email***: *sihambazine@gmail.com*

[e] ***ORCID***: *0000-0003-4423-2893*; ***Email***: *aitbenichous@gmail.com*

✉ **Corresponding author:** Samah Ait Benichou; ***Email***: *aitbenichous@gmail.com*

Graphical abstract

This study shows how seasonal variation influences the microbiological and physico-chemical quality of the Oualidia Lagoon. Sampling revealed higher bacterial loads, greater chemical oxygen demand, and elevated heavy metal concentrations during summer. These findings point to increased anthropogenic pressures on the Lagoon ecosystem, with potential risks to environmental and human health.



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Abstract: The expansion of human activities around the Oualidia Lagoon exposes this biotope to negative environmental pressures. The aim of this study is to assess these impacts by monitoring the physico-chemicall and bacteriological quality of the Lagoon. Three water and mussel sampling campaigns were conducted between winter and spring 2023 and were used for microbiological (Escherichia coli, intestinal enterococci, Salmonella and Vibrion), physico-chemical (pH, temperature, salinity, electrical conductivity, nitrates, sulphates, chlorides, orthophosphates, chemical oxygen demand) and selected heavy metal (Fe, Cu, Cd, Pb, Cr) analyses. The results obtained show spatio-temporal variations in the different parameters analysed. The microbiological quality of the Lagoon water deteriorated in the summer season, with the bacterial load reaching a maximum of 120 CFU/100 mL for Escherichia coli. The physico-chemical analyses showed a marked increase in chemical oxygen demand, particularly in the summer months (2,320 mg L^{-1}) and the electrical conductivity and chloride reaching a maximum around 50,000 µS cm-1 and 28,400 mg L^{-1} respectively.

Keywords: *Oualidia Lagoon, Microbial contamination, Anthropogenic pollution.*

Introduction

Ecosystem deterioration, and in particular the deterioration of coastal ecosystems, results from increasing human activities. At present, the preservation and protection of coastal zones is one of the major environmental concerns given the role they play in socio-economic sectors [1]. Among the coastal ecosystems identified are Lagoons, also known as transitional waters. These ecosystems are highly dynamic and physically complex, occupying about 13% of the world's coastal zones. They are defined as ecotones between freshwater, marine and terrestrial ecosystems. They are bodies of water connected to the sea by one or more inlets, with limited freshwater influence [2]. They are often confined, with little renewal, partly because of their morphology, and their water quality is not guaranteed [3]. These ecosystems are naturally vulnerable and their ecological balance can change rapidly under the influence of natural or anthropogenic factors [4]. Interest in these ecosystems is growing, and they are now considered essential elements of national heritage in many countries.

Lagoons are known for their high sensitivity to anthropogenic influences, mainly because of limited water exchange with the sea [5]. For this reason, the conservation of Lagoon ecosystems and the management of their uses with a view to sustainable development are essential. This is not only to preserve biodiversity, but also to maintain the goods and services provided to human society [3]. The Oualidia Lagoon is one of Morocco's best-known Lagoons. It is famous for its rich shellfish resources and high level of summer tourism. Nevertheless, the Lagoon is subject to heavy pollution from wastewater and runoff from neighbouring agricultural areas [6]. These activities lead to a deterioration in water quality and contribute to its vulnerability. They can also cause contamination by numerous pathogens, posing a threat to human health and the marine ecosystem [4]. These activities have a negative impact on the environment, making it less attractive for recreation and aquaculture [6,7]. The Lagoon has been the subject of several studies (currents, sedimentology, hydrodynamic functioning, etc.). Yet, there has not been sufficient work focusing on its physicochemical and microbiological quality. Although several studies have concerned the environmental status of the Oualidia Lagoon, the originality of the present work lies in conducting comprehensive physico-chemical and microbiological monitoring in 2023. Given the Lagoon’s ecological, economic, and social significance as a RAMSAR site and a Site of Biological and Ecological Interest, it requires regular monitoring to ensure its protection. This study fills a critical gap, as no similar assessments have been performed since 2014 [1]. By providing recent data, it offers valuable insights to support surveillance and guide future conservation and sustainable management efforts. For this reason, this study aims to assess the spatio-temporal variation of the Lagoon’s water quality. This will help to understand the extent to which the environmental quality of the Oualidia Lagoon has been affected by economic and social growth in this region.

Materials and Methods

Study Area

The Oualidia Lagoon (longitude 9°02'50" W, latitude 32°44'42" N) is located on Morocco's Atlantic coast, 180 km south of Casablanca. It extends parallel to the coast over a distance of approximately 7.5 km and 0.5 km in width, with a total area of 3.5 km² [4]. It communicates with the Atlantic Ocean through two passes: one main and permanent, the other secondary, active only at spring tide in open sea conditions [8]. Intertidal zones cover about 53% (1.6 km²) of the entire Lagoon [2]. The Oualidia Lagoon is one of Morocco's principal Atlantic Lagoons. It is regarded as one of the most important given its ecological interest and its considerable economic and social importance [9]. It is also classified as a RAMSAR site [3], and since 1996 has been designated in Morocco's master plan for protected areas as a site of biological and ecological interest (SIBE) [10]. The Oualidia Lagoon provides valuable socio-economic benefits and ecosystem services to the local population. Nonetheless, irrational use of this ecological space has led to various types of degradation [7]. Within the Lagoon, there are various anthropogenic activities such as aquaculture, tourism and agriculture.

Sampling Sites

Water samples and live bivalves (mussels and oysters) were analysed. Water samples were collected at seven different stations along the Lagoon (Fig. 1). The stations were selected according to the risk of contamination that might exist (wastewater discharge, grazing, etc.). Bivalves were collected at two stations. Mussels were collected near the main pass. The oysters were obtained from an oyster farm operating in the Lagoon.

Sampling

For each station, seawater samples were collected using sterile 1 L plastic (polyethylene) bottles. Samples were collected below the water surface. Water samples were stored at 4 °C in a cooler until arrival at the laboratory. Mussel samples were obtained by hand from the rocks near S7 at low tide. Oysters were obtained directly from the oyster farm at station 5. Bivalves were collected, taking into account the weight required for analysis, that is, 25 g and 100 g for Salmonella and Escherichia coli respectively. Live bivalve samples were stored at 4 °C prior to bacteriological analysis in the laboratory.

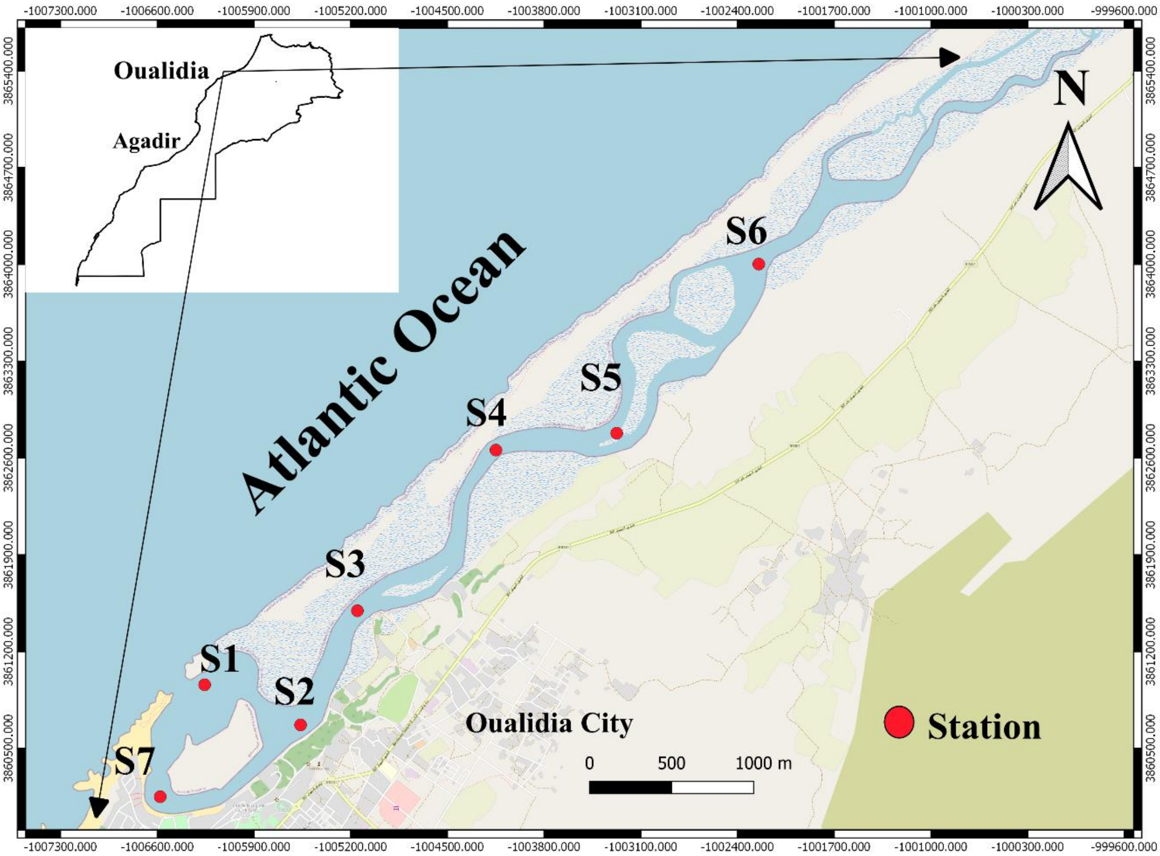


Figure 1. Location of sampling stations in the Oualidia Lagoon, Morocco.

Microbiological Analyses

Microbiological Water Quality

Four microbiological parameters were considered for water analysis: Escherichia coli [CFU/100 mL], intestinal enterococci [CFU/100 mL], Salmonella [absence/1 L], Vibrio [absence/1 L]. Identification of these microbiological parameters involved membrane filtration (0.45 µm), followed by agar plating; Chromogenic Agar for coliform bacteria (CCA) and Bile, Esculin and Azide Agar (BEA) for Escherichia coli and intestinal enterococci respectively. Escherichia coli enumeration was carried out in accordance with NM ISO 9308-1:2019 [11], and intestinal enterococci enumeration was carried out in accordance with NM ISO 7899-2:2007 [12]. For Salmonella and Vibrio identification, after filtration, the membrane was cultured in liquid culture media, namely peptone water (EPT) and saline peptone water (EPSA) for Salmonella and Vibrio respectively. The presence of these two microbiological parameters in our samples was confirmed by a biochemical test using an API 20E gallery. Salmonella detection was carried out in accordance with NM ISO 19250:2012 [13].

Microbiological Quality of Bivalves

Live bivalve molluscs were analysed for two microbiological parameters: Escherichia coli and Salmonella. Escherichia coli was enumerated by the MPN (Most Probable Number) method

[CFU/100 g] in accordance with NM ISO 7251:2012 [14]. Identification of Escherichia coli in live bivalves was carried out using two liquid media (single concentration and double concentration glutamate) followed by culture in tryptone-bile-glucuronate (TBX) agar. Salmonella [absence/25 g] was enumerated according to NM ISO 6579-1:2021 [15].

Physicochemical Analyses

In situ parameters (temperature, salinity, conductivity and pH) were measured using a multiparameter probe. Sulphates**,** nitrates, orthophosphates and chemical oxygen demand (COD) were determined using the spectrophotometric method with reagents specific to each parameter. The volumetric method was used to determine chloride levels. Atomic absorption spectrometry was used for heavy metals. Analyses were carried out according to the protocols described by Rodier [16].

Statistical Analyses

Statistical analyses were performed using XLSTAT software. The Pearson correlation test was used to determine significant relationships between indicators of faecal contamination (Escherichia coli and intestinal enterococci**)** and the various physicochemical parameters measured (pH, salinity, nutrients, etc.). The tests were applied with a 95% confidence interval ($p < 0.05$).

Results

Microbiological Contamination

Figure 2 shows the results of Escherichia coli and intestinal enterococci analyses at the various stations in the Oualidia Lagoon during the winter, spring and summer 2023 seasons. The bacterial load ranged from 0 to 120 CFU/100 mL for Escherichia coli, and from 0 to 50 CFU/100 mL for intestinal enterococci**.** The Lagoon becomes more loaded with faecal contamination bacteria (Escherichia coli and intestinal enterococci) during the summer period, which is characterized by an increase in visitors. In winter, bacteria were only present near stations adjacent to agricultural land (S5 and S6). In addition**,** the microbial load increases in summer downstream of the Lagoon, marked by bathing activities at this level. This leads us to link the presence of faecal contamination indicators in winter to rainwater drainage within the Lagoon. In summer, the increase in faecal contamination downstream of the Lagoon may be linked to tourist activity. The latter may be accompanied by the activation of certain septic tanks discharging into the Lagoon, leading to an increase in faecal contamination of the Lagoon waters.

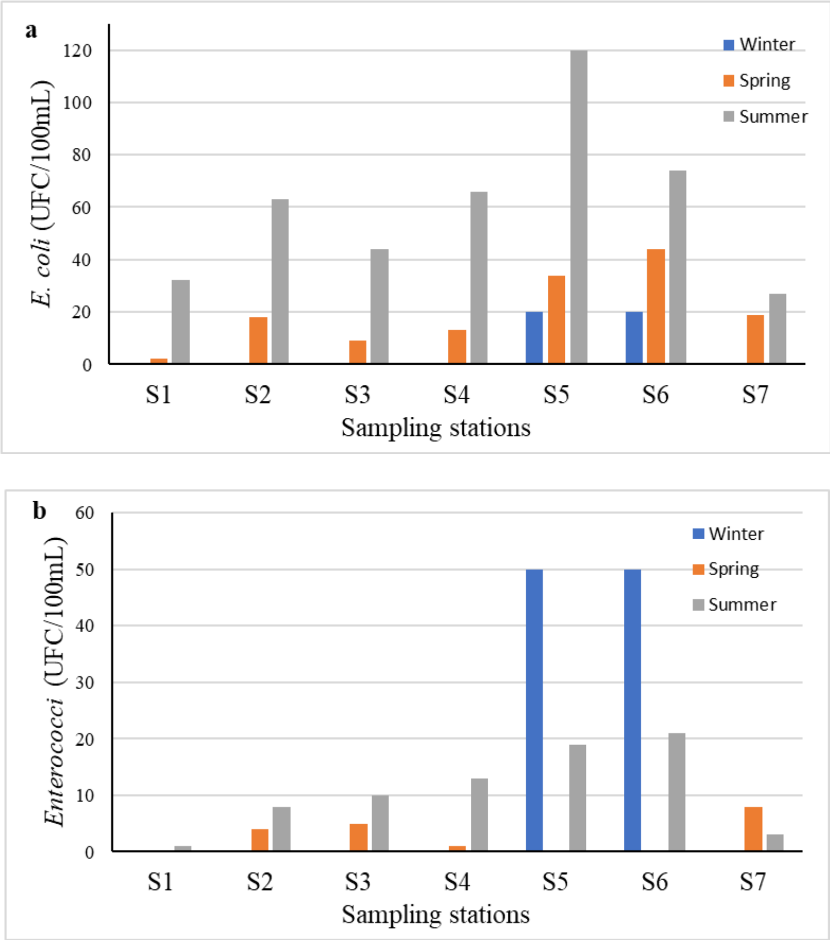


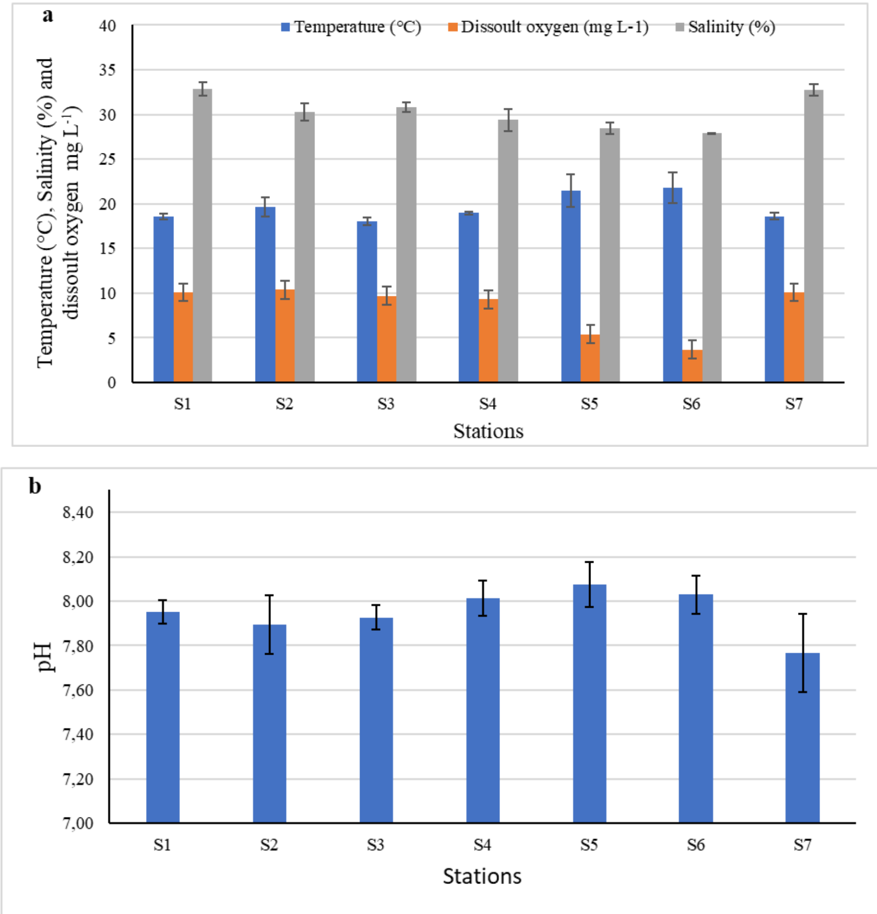
Figure 2. Spatio-temporal variation in *E. coli* (a) and *Enterococci* (b) contamination of Oualidia Lagoon waters.

The highest load of intestinal enterococci was recorded in winter [50 CFU/100 mL]. For Escherichia coli, the highest level was recorded in summer [120 CFU/100 mL] (Fig. 2). This suggests that faecal contamination is mainly of animal origin in winter. In summer, faecal contamination of the Lagoon is mainly of human origin. Microbiological analyses showed a total absence of Salmonella during the different sampling periods. This might be explained by the low resistance of Salmonella to the salinity of marine water [17]. Conversely, the halophilic Vibrio bacterium was only detected during the first survey, at station S1 located near the main channel. This station is characterized by high salinity, which is a major requirement for the Vibrio bacterium. After biochemical identification using the API 20 gallery, the Vibrio species detected was Vibrio alginolyticus. This bacterium belongs to the autochthonous marine flora [18,19]. This explains its presence only near the main pass, which is marked by a permanent exchange with marine water. The live bivalves analysed were of satisfactory quality, not exceeding the thresholds required by Moroccan decree ARR.293-19 for Escherichia coli and Salmonella [20]. Bivalves sampled near the main pass showed a zero bacterial load over the different sampling periods. This can be explained by the continuous renewal of the water at this location.

Physicochemical Pollution

Figure 3 shows the average of the three measurement campaigns for parameters measured in situ in the Lagoon 's waters at the seven sampling stations. Water temperature varies between 18.2 °C and 23.7 °C. It decreases slightly from upstream (S5 and S6) to downstream (S1 and S7) (Fig. 3a). Salinity values are higher upstream of the Lagoon. The salinity level is almost similar to that of marine water upstream, with a maximum of 33.5 % noted at S1 and S7 (Fig. 3a). Dissolved oxygen is normal throughout the Lagoon, with the exception of stations S5 and S6, where it is below 5 mg L^{-1} (Fig. 3a). This indicates strong eutrophication activity at this level.

The pH is weakly alkaline, varying between 7.8 (minimum observed at S7) and 8.1 (maximum observed at S5) (Fig. 3b). Electrical conductivity (Fig. 3c) peaks at S1 and S7 (around 50,000 µS cm^{-1}). It decreases further away from the main pass, where the minimum was recorded at S5 and S6 (around 43,000 µS cm^{-1}).



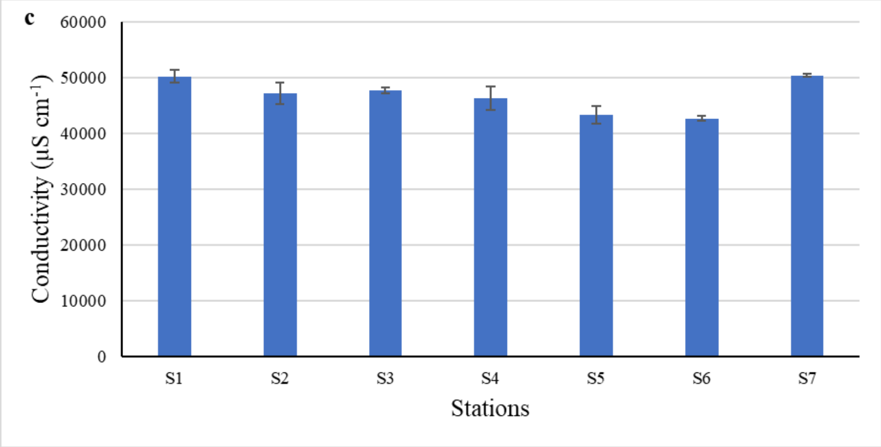


Figure 3. Variation in parameters measured in situ in the channel of the Oualidia Lagoon. (a) Temperature (°C), salinity (%), and dissolved oxygen (mg L^{-1}); (b) pH; (c) electrical conductivity (µS cm^{-1}).

Figure 4 shows the evolution of chemical oxygen demand (COD) measured at the seven sampling stations over the three sampling campaigns. COD increased from winter to summer, showing that in summer the Lagoon becomes more enriched with organic matter. S2 showed the highest COD content of around 2,320 mg L^{-1} during the last sampling campaign.

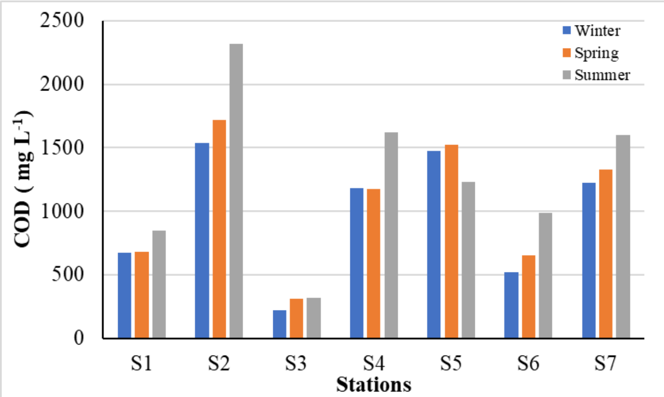


Figure 4. Spatio-temporal variation of COD in the channel of the Oualidia lagoon, Morocco.

Figure 5 shows the results for the various chemical elements (chlorides, nitrates, orthophosphates and sulfates) measured in the Lagoon waters. With the exception of orthophosphate ions, the chemical elements measured in our study showed significant fluctuations over the three sampling campaigns conducted.

Chloride levels were very high, reaching a maximum of 28,400 mg L^{-1} at station S5. Low chloride levels were measured in winter, with a minimum of 639 mg L^{-1} at S6 (Fig. 5a). A maximum of 54.1 mg L^{-1} was recorded for nitrates at S2 during the first season (Fig. 5b). Sulfates showed a decreasing gradient from the first season (winter) to the third season (summer). They peaked at 205.2 mg L^{-1} during the first sampling near S3 (Fig. 5c).

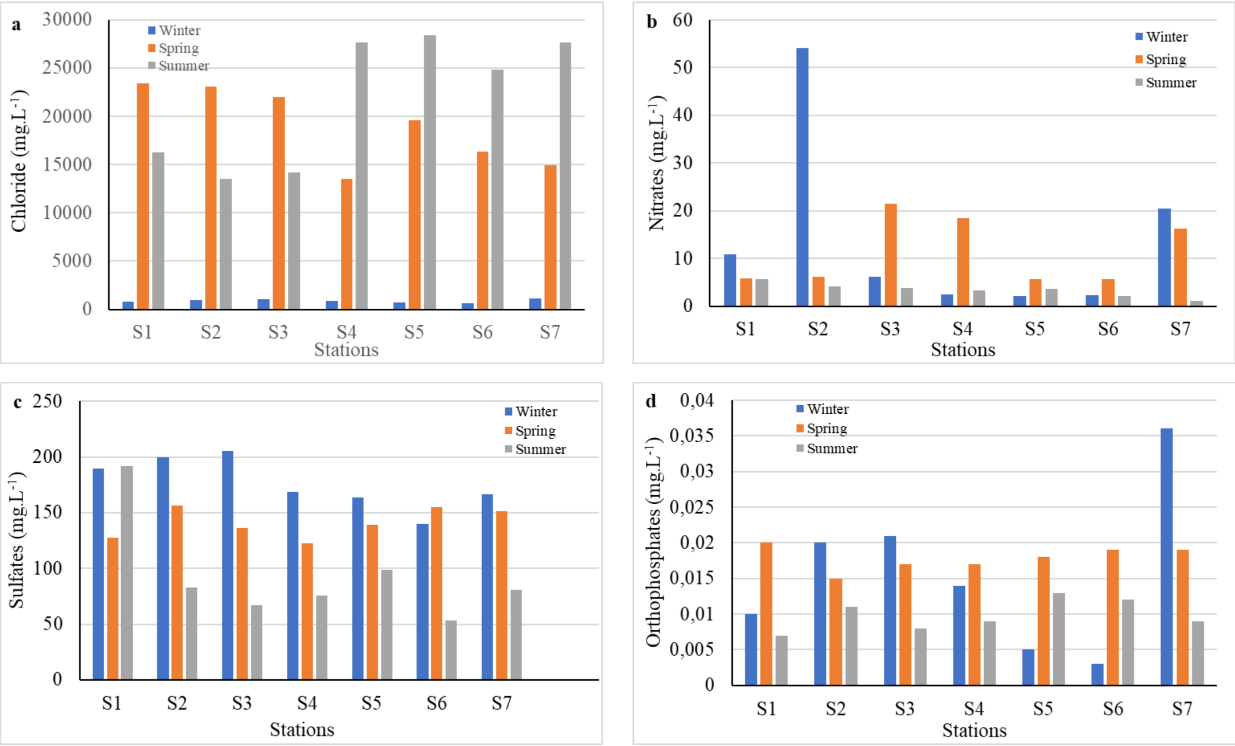


Figure 5. Spatio-temporal variation of chemical parameters in the channel of the Oualidia lagoon a) chlorides, b) nitrates, c) sulfates and d) orthophosphates.

Heavy metal concentrations in the Lagoon water were low (Fig. 6). Lead showed the highest levels, with a maximum of 0.47 mg L^{-1} recorded at S7 during the second season. Iron levels varied slightly at around 0.33 mg L^{-1}. Cadmium showed the lowest concentrations (maximum = 0.01 mg L^{-1}). Copper was only recorded at the first four stations, with a maximum of 0.13 mg L^{-1} at S1. Chromium was not detected during any of the three sampling periods.

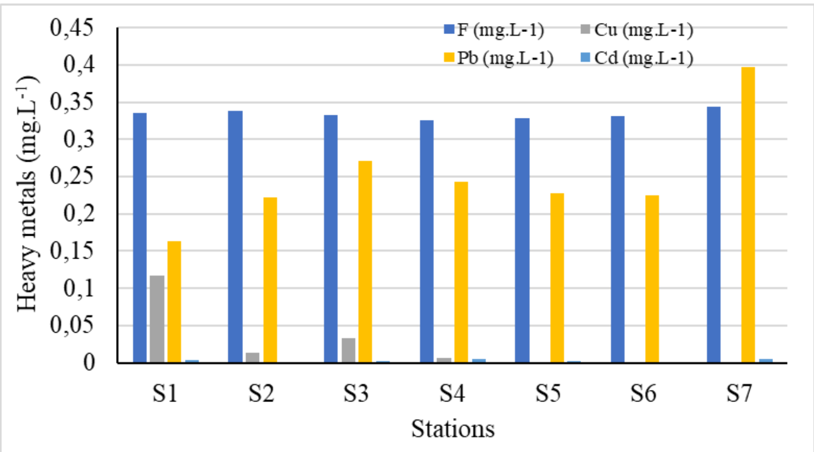


Figure 6. Fluctuation in iron (F), copper (Cu), lead (Pb) and cadmium (Cd) levels in the channel of the Oualidia lagoon

Table 1 shows correlations between some of the parameters studied. A correlation test demonstrated dependent and independent relationships. Nevertheless, a correlation coefficient of r = 0.944 recorded between salinity and conductivity shows that these two parameters are highly dependent on each other. A fairly high correlation (r = 0.792) was also found between temperature and Escherichia coli. This indicates that these two parameters move in the same direction. This leads us to conclude that temperature favours the evolution of Escherichia coli bacterial load. A negative correlation was found between faecal contamination indicator bacteria (Escherichia coli) and intestinal enterococci, with correlation coefficients of r = –0.442 and r = –0.433 respectively. This finding might indicate that salinity acts as a growth-inhibiting factor for faecal contamination germs. In particular, S1, which is marked by the highest salinities, had the lowest germ concentrations. This was true throughout the different sampling periods.

Table 1. Correlation between microbiological and physicochemical parameters.

Variables	pH	T	Salinité	Conductivité	E. Coli	Ent. Intestinaux	Chlorures	Nitrates	Orthophosphates	Sulfates	DCO	F (mg/l)	Cu (mg/l)	Pb (mg/l)	Cd (mg/l)
pH	**1**														
T	0,264	**1**													
Salinité	**-0,498**	**-0,607**	**1**												
Conductivité	**-0,586**	**-0,698**	**0,944**	**1**											
E. Coli	0,112	**0,792**	**-0,442**	**-0,549**	**1**										
Ent. Intestinaı	0,265	0,405	**-0,433**	**-0,448**	0,266	**1**									
Chlorures	-0,154	0,376	-0,062	-0,080	**0,608**	-0,162	**1**								
Nitrates	-0,215	-0,357	0,000	0,106	-0,397	-0,312	-0,310	**1**							
Orthophospha	**-0,492**	-0,253	0,157	0,228	-0,338	**-0,577**	-0,129	**0,478**	**1**						
Sulfates	0,078	**-0,467**	0,240	0,265	**-0,675**	-0,207	**-0,670**	0,415	0,320	**1**					
DCO	-0,072	0,231	-0,047	-0,033	0,257	-0,001	0,188	0,060	-0,023	-0,220	**1**				
F (mg/l)	-0,414	-0,049	**0,520**	0,408	-0,141	-0,290	-0,043	0,040	0,272	0,180	0,313	**1**			
Cu (mg/l)	-0,048	-0,380	**0,571**	**0,517**	-0,276	-0,306	0,016	-0,003	-0,107	0,270	-0,412	-0,106	**1**		
Pb (mg/l)	-0,425	-0,173	0,304	0,282	-0,051	-0,082	0,143	0,041	0,185	-0,239	0,161	**0,490**	**-0,480**	**1**	
Cd (mg/l)	-0,190	**-0,497**	**0,570**	**0,567**	-0,252	-0,289	0,135	-0,012	0,033	0,006	0,088	0,122	0,159	**0,479**	**1**

Les valeurs en gras sont différentes de 0 à un niveau de signification alpha=0,05

Discussion

All the physicochemical parameters studied showed slight spatio-temporal variations. The slight fluctuations in Lagoon water physicochemistry were explained by the duration of water renewal, which decreases from upstream to downstream of the Lagoon. They can also be attributed to the depth of the water layer in the Lagoon channel. According to Chaouti *et al.* [21], the richer the water in ionized mineral salts, the higher the conductivity. This finding concurs with the results of this study, which demonstrated a significantly positive correlation between salinity and electrical conductivity (r = 0.944). The alkaline pH was explained by the strong influence of seawater, which ensures continuous renewal of the Lagoon's waters [1,9]. The decreasing salinity gradient measured from downstream to upstream of the lagoon can be explained by rainfall, which dilutes salinity upstream of the Lagoon. This is particularly true during the winter period. Alternatively, it may be explained by water renewal, which decreases from downstream to upstream of the Lagoon [22].

The low concentrations of heavy metals can be explained by their binding to suspended matter and adsorption by sediments [23]. The increase in chemical oxygen demand from winter to summer results from the increase in organic matter. This may be the result of the activation of certain wastewater discharges in summer. In addition, the high nitrate peak recorded near S2 may be linked to the presence of a wastewater discharge loaded with organic matter near this station. According to Anhichem *et al*. [24], high nitrate levels result from the nitrification of nitrogen contained in organic matter by bacteria in the presence of oxygen.

The faecal coliform content showed a spatio-temporal variation, in line with the results of Hassou *et al.* [1]. This variation may be explained by physico-chemical conditions, which can exert a bactericidal action, and salinity is a very important stress factor for faecal bacteria [10,25].

The season can also affect the temporal variation in bacterial load. Rainwater runoff, for example, can contribute to an increase in germ content at stations adjacent to agricultural land. Nevertheless, the increase in summer visitors can also lead to a rise in faecal bacteria, especially where bathing activities take place. The increase in bacterial load upstream of the Lagoon can be explained by low water renewal [26]. Turbidity is another factor contributing to the increase in bacterial load. Turbidity favours bacterial survival by preventing the effect of environmental factors such as light. In this respect, the study by Hassou *et al.* [1] showed a significantly positive correlation between turbidity and bacterial load. In fact, sedimentology differs between upstream and downstream Lagoon areas. Sediments are coarser at the bottom and finer at the surface. As a result, the sedimentation rate is very low in the upstream part compared with the downstream part [3]. As the upstream part of the Lagoon is more turbid, the load is higher there.

The absence of faecal bacteria in oysters may be explained by the limited survival of these germs in marine water. These germs can be inhibited by environmental factors before they are filtered out by the bivalves. Bivalves contaminated with bacteria can then be purified. Purification refers to the ability of bivalve molluscs to clean themselves. This was reinforced by the study of Bouchriti *et al.* [27].

Overall, the quality of the Lagoon remains excellent compared to its condition during the study carried out by Hennani *et al*. [28], who demonstrated poor Lagoon quality during the various phases of their work. In contrast, our study showed that the Lagoon 's oysters were of good quality during the various campaigns conducted. The Lagoon water analysed in the work by Bouchriti *et al.* [27] showed a load of up to 10^3 CFU/100 mL for intestinal enterococci. Thus, oysters harvested from this Lagoon may present a public health risk at any season of the year. This result contradicts our own findings, which showed satisfactory oyster quality in accordance with Moroccan decree ARR no. 293-19 and suitable for human consumption. The improvement in water and bivalve quality in the Oualidia Lagoon can be attributed to the Lagoon's solid and liquid sanitation efforts and the water monitoring programme.

Conclusion

Anthropogenic pressures and the development of multiple activities on Lagoons often give rise to conflicts of interest and uses, which contribute to their vulnerability. This is why the conservation of Lagoon ecosystems and the management of their uses with a view to sustainable development are essential, not only for the preservation of biodiversity, but also for the maintenance of the goods and services they provide to human society.

The upstream part of the Lagoon is more vulnerable than the downstream part. This can be explained by the upstream–downstream variation in environmental conditions, as well as by the Lagoon's hydrological functioning. The difference in water renewal duration between stations is noteworthy. Nevertheless, Lagoon disruption also potentially depends on adjacent anthropogenic activities. Agricultural and urban pollution are the main sources of contamination for this ecosystem. Instability in the natural conditions of the Lagoon 's waters has been observed. Indeed, their composition varies over time and space. These fluctuations are evident between stations. Nevertheless, these changes are much more pronounced between seasons.

A link established between microbiological parameters and certain physico-chemical factors suggests that bacterial survival is highly dependent on natural conditions. These are non-native bacteria, generally originating from faecal contamination of the water. These microorganisms can be inhibited by certain factors such as salinity. Conversely, other parameters tend to favour the survival of these germs, such as temperature, which allows the development of Escherichia coli.

In fact, to ensure the sustainability of this Lagoon, preserve its biodiversity and benefit from its ecosystem goods and services, we must contribute to the protection of these waters. This can be achieved by establishing an appropriate protection system and ensuring the development of these activities and the continuous renewal of the waters of the Oualidia Lagoon by ocean waters.

Authors' Contributions

Moujahed Yousra: Conceptualisation, Experimental work, Investigation, Data analysis and Writing-Original draft preparation.

El Hissoufi Abdelhamid: Sampling, Experimental work and data analysis

Ennassiri Houda: Experimental work and data analysis

Bazine Siham : Experimental work and data analysis

Samah Ait Benichou: Methodology, Validation, Formal analysis, Writing and Review.

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Ethical Approval

This study was conducted in full accordance with ethical principles.

Disclosure Statement

No potential conflict of interest was reported by the authors.

Data availability Statement

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

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