ASSESSMENT OF THE TROPHIC STATUS OF MARCHICA LAGOON (NE MOROCCO, MEDITERRANEAN) AFTER RESTORATION ACTIVITIES USING TROPHIC INDEX (TRIX): SEASONAL AND SPATIAL VARIATIONS

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Abstract. In the present study data obtained during the monitoring of the Marchica lagoon (NE-Morocco, Mediterranean) was analyzed, and the trophic index (TRIX) was calculated. The spatiotemporal pattern of TRIX in water from thirteen stations in the Marchica lagoon was studied during the winter, spring, and autumn seasons in 2020. The parameters used for the determination of the trophic index were based on the physicochemical indicators measured. The four state variables were used: Chlorophyll a, dissolved oxygen, dissolved inorganic nitrogen, and total phosphorus. The use of the TRIX index showed that the waters of the lagoon are generally oligotrophic with good to excellent quality. Evaluation of the TRIX index in Marchica Lagoon shows also that point values, exceeding 6 TRIX units, are typical of highly productive coastal waters, where the effects of eutrophication determine frequent episodes of anoxia in the bottom waters and are indicated by high nutrient levels. Watershed effects, anthropogenic pressure from agricultural activities and sewage discharges from wastewater treatment plants, and aquatic hydrodynamics are thought to be responsible for the increase in macronutrients that cause the eutrophication of waters. Management and development strategies for the lagoon are required by local authorities, stakeholders, and communities to prevent the eutrophication phenomenon of the lagoon.

Keywords: chlorophyll a, eutrophication, nitrogen, phosphorus, TRIX

Introduction

Coastal lagoons are composed of a large number of physical and ecological boundaries and gradients; between water and sediment, pelagic and benthic assemblages, lagoon-freshwater and terrestrial systems, and the atmosphere. In addition, the strong dependence of lagoon ecosystems on their catchment areas makes them particularly vulnerable to human impact and to freshwater and terrestrial inputs (Pérez-Ruzafa et al., 2005). The combination of these factors increases the accumulation of organic matter and contaminants in the sediments and makes these systems vulnerable to eutrophication (Pereira et al., 2010). Eutrophication is a global problem characterized by high loads of nitrogen and phosphorus in waters and results in excessive growth of phytoplankton and other aquatic plants (Schindler et al., 2008).

In recent decades, nutrient-rich anthropogenic inputs to coastal ecosystems have led to eutrophication (Smith, 2003). The main sources of nutrients are untreated domestic wastewater, municipal, industrial, and agricultural wastes, fertilizers, and aquaculture activities (Costanzo et al., 2003; Nixon, 1995; Sahu et al., 2013; Vizzini and Mazzola, 2006). The increase in urban pollution has become a major threat to the environmental quality of coastal ecosystems (Gao et al., 2012) which severely affects primary biological productivity, leading to the loss of fisheries, as well as the loss of biodiversity (Amir et al., 2019).

Mediterranean coastal lagoons are often surrounded by densely populated areas and are therefore subject to high levels of anthropogenic pressure, including high levels of domestic wastewater inputs (Souchu et al., 2010; Viaroli et al., 2008; Zaldívar et al., 2008). In general, two main sources contribute most to the nutrient status and balance of coastal areas: (a) loading from diffuse and point sources and (b) internal loading from sediments (Coelho et al., 2004; Paludan and Morris, 1999; Zwolsman, 1994). For several years, the daily discharge of domestic, agricultural, and industrial waste into the Nador lagoon has stimulated the proliferation of algae. This increases the consumption of oxygen, causing an imbalance in this ecosystem, which leads to eutrophication (Ruiz et al., 2006). Since 2010, two wastewater treatment plants have been set up to preserve the biological and ecological qualities and the development of tourism activities around this ecosystem (Nador wastewater treatment plant in 2010 and Arekmane wastewater treatment plant in 2013), the opening of a new pass between the lagoon and the Mediterranean Sea in 2011 to exchange water, which has improved the lagoon (Hilmi et al., 2015). These measures represent the restoration plan for the Nador lagoon. Eutrophication studies were carried out before the restoration of the lagoon (Bloundi, 2005; El Madani, 2012; Ruiz et al., 2006), and other studies on water quality during the post-restoration period, the variation of physicochemical parameters and nutrients in the water was studied (Aknaf et al., 2017; Mostarih et al., 2016a; Oujidi et al., 2020).

The objective of this study is to determine the spatial variation of the Physicochemical parameters of the water and the use of the eutrophication index (TRIX) to highlight the state of the quality of the surface water after ten years of development work in the Marchica lagoon. The Eutrophication Index (TRIX) integrates Chl a, oxygen saturation, dissolved inorganic nitrogen, and dissolved inorganic phosphorus and is scaled from 0 to 10 (Antonio-Robles et al., 2021; Vollenweider et al., 1998) covering a range of four trophic states (high, good, moderate and poor).

Material and methods

Study area

The Marchica lagoon is one of the largest coastal lagoons in the Mediterranean Sea, with a length of 25 km, a width of 7.5 km, and an estimated area of 115 km². It is located on the north-eastern coast of Morocco between the "Cap des trois fourches" and the "Cap de l'eau", more precisely between latitudes $35^{\circ}05$ 'N and $35^{\circ}14$ 'N and longitudes $2^{\circ}44$ 'W and $2^{\circ}56$ 'W (*Fig. 1*). It is separated from the sea by a coastal dune (25 km). To allow a better exchange of the lagoon's waters with the Mediterranean waters, a new inlet was opened in 2010, about 300 m wide and 6.5 m deep.

Sampling and analysis strategy

A total of 13 samples were selected for the study of water quality in the Nador lagoon (*Fig. 1*). The sampling network was established by considering the main anthropogenic pressure (mouths of wadis, wastewater treatment plants outfall, and agriculture): 3 stations in the internal border of the sandbar under marine influence; 2 stations in the center of the lagoon, also under marine influence; 2 stations at the confined zones northwest and southeast of the lagoon; 3 stations under continental influence, including the mouths of the wadis (Cabaillo wadi and Selouane wadi); 1 station at the outfall of the great Nador wastewater treatment plant (WWTP); and another between Bouarg and Arekmane. The samples were taken in 2020 (winter, summer, and autumn) at the level of the surface layer of the water (20 cm). Dissolved oxygen, pH, temperature, turbidity, chlorophyll-a, and salinity, were measured in situ using portable devices with specific probes for each parameter.

The water samples taken were subject to other analyses: ammonium NH_4^+ (NF T90-015), phosphates (PO₄³⁻), total phosphorus (TP), nitrites NO_2^- and nitrates NO_3^- (Rodier et al., 2009) Dissolved inorganic nitrogen (DIN) is the sum of nitrates, nitrites, and ammonium.



Figure 1. Location of the study area and sampling stations

Trophic state assessment

The TRIX values were calculated for each station according to the (Antonio-Robles et al., 2021; Vollenweider et al., 1998). It is a mathematical tool derived from the combination of dissolved inorganic nitrogen, inorganic phosphorus, dissolved oxygen, and chlorophyll a. the TRIX analytical expression is given as follows:

$$TRIX = \frac{\log 10(DIN \times DIP \times | D\% DO | \times chl a) + a}{b}$$
(Eq.1)

where Chl a represents the concentration of chlorophyll-a (in $\mu g/L$), |D%DO| represents the absolute value percentage deviation of the oxygen concentration from saturation conditions (in %). DIN and DIP represent dissolved inorganic nitrogen (in $\mu g/L$) and dissolved inorganic phosphorus (in $\mu g/L$), respectively. The parameters a = 1.5 and b = 1.2 are scale coefficients used to fix the index lower limit and the scale ranges from 0 to10. Values between 0 and 4 correspond to high quality (oligotrophic), 4-6 to good conditions (mesotrophic), 6-8 to moderate conditions (eutrophic), and 8-10 to degraded conditions (hyper-eutrophic) (Antonio-Robles et al., 2021).

Data analysis

The sampling stations were grouped into two groups based on pollution sources: group 1: including stations 1, 7, 8, 9, 10, 11, and 12 (influenced by pollution sources); group 2: stations 2, 3, 4, 5, 6, and 13 (presumed to be distant from pollution). To test the spatial and seasonal effects, significant differences between the mean values of each parameter (nutrients and in situ physico-chemical parameters) among the three seasons and groups of sampling sites were tested using the Kruskal-Wallis; normality was not verified overall (Shapiro-Wilk test, p < 0.05). The Spearman rank coefficient was calculated to test the relationship between the above parameters.

To investigate the relationships between the variables and their possible distribution patterns, principal component analysis (PCA) was used. PCA was applied to identify potential pollution and its characteristic elements. It has been used in several studies (Daniel, 2004; Mendiguchía et al., 2007; Rigollet et al., 2004; Simeonov et al., 2001; Zhou et al., 2007). The TRIX values were interpolated (inverse distance weighting) on the Marchica map to illustrate the spatiotemporal variation of this index along the Marchica lagoon during the study period. The data were processed using SPSS IBM 26 software.

Results

Seasonal variation of physicochemical and nutrient parameters

The results of the physicochemical and nutrient parameters are shown in *Table 1*. For salinity, dissolved oxygen (DO), turbidity, Chl a, total phosphorus (PT), and ammonium no significance was observed (p > 0.05) while for temperature, nitrate, nitrite, and pH; a significance between the seasons was observed (p < 0.05). The results show significant spatial variation for nitrate, nitrite, ammonium, total phosphorus, turbidity, and salinity (p < 0.05) and no significant differences for Chl a, T, and pH (p > 0.05).

In winter, the highest nitrite values were recorded in stations 10, 9, and 8 with a maximum of 0.019 mg/L and the minimum of 0.001 mg/L in station 5. The maximum of nitrates was 0.935 mg/L, recorded at station 10, and the minimum of 0.0045 in stations 1 and 13. Ammonium was recorded only in stations 8, 10, 9, and 12, with a maximum of 0.056 mg/L in station 8 and not detected in the other stations. Total phosphorus was recorded in stations 8, 9, 10, and 12 with a maximum of 0.45 mg/l in station 8, while phosphates (PO4³⁻) were below the detection limit (<0.05 mg/L) in all stations. The maximum temperature (18.2 °C) was recorded at station 2 and the minimum (15 °C) at

station 10. DO values range from 3.35 mg/L at station 8 to 9.90 mg/L at station 1. Salinity and pH were generally homogeneous with a maximum of 39 at station 1 and a minimum of 37.4 at station 9 for salinity, and 8.17 at station 1 and a minimum of 7.62 at station 10 for pH. The maximum turbidity was recorded at station 9 with 60.5 NTU at station 9 and a minimum of 0.37 NTU at station 3. For Chl a, the maximum of 30.57 μ g/L was reported at station 8 and the minimum of 2.44 μ g/L at station 11.

		NO2 ⁻ (mgN/L)	NO3 ⁻ (mgN/L)	NH4 ⁺ (mgN/L)	PT (mg/L)	T (°C)	DO (mg/L)	SAL	pН	Turb (NTU)	Chl a (µg/L)
Winter	Average	0.005	0.096	0.009	0.050	16.7	8.23	38.2	7.99	14.4	6.24
	Min	0.001	0.004	0.000	0.000	15.0	3.35	37.4	7.62	0.37	2.44
	Max	0.019	0.935	0.056	0.450	18.2	9.90	39.0	8.17	60.5	30.5
	Std dev	0.006	0.253	0.017	0.124	0.95	1.81	0.42	0.15	17.3	7.60
Summer	Average	0.001	0.005	0.006	0.015	23.5	7.28	38.4	8.15	3.7	11.73
	Min	0.000	0.000	0.000	0.000	22.8	3.70	38.1	7.90	0.18	3.01
	Max	0.004	0.035	0.053	0.150	24.9	8.60	39.10	8.35	9.80	47.64
	Std dev	0.001	0.010	0.014	0.042	0.71	1.47	0.34	0.13	3.37	13.92
Autumn	Average	0.008	0.023	0.033	0.001	21.9	8.37	38.60	8.23	4.54	8.39
	Min	0.000	0.000	0.000	0.000	21.0	4.51	37.70	7.85	0.18	2.80
	Max	0.055	0.132	0.220	0.015	23.0	10.5	39.90	8.53	16.20	44.03
	Std dev	0.017	0.039	0.065	0.004	0.55	1.48	0.63	0.19	4.81	11.32

Table 1. Results of physicochemical parameters and nutritive salts during the study period

In summer, nitrites were detected only at stations 8, 10, 9, and 7 with a maximum of 0.004 mg/L at station 8 and not detected at the other stations. The same for nitrates was recorded in stations 8, 9, 5, 6 and 7 with a maximum of 0.035 mg/L in station 8 and below the detection limit in the other stations. Ammonium was detected in stations 8, 9, 12, 7, and 1 with a maximum of 0.053 mg/L in station 8 and not detected in the other stations. Phosphorus was only recorded in stations 8 and 9 with a maximum of 0.15 mg/L in station 8 and not detected in the other stations. Phosphorus was only recorded in stations. Phosphates were below the detection limit at all sampling stations. The results of temperature (22.8 °C–24.9 °C), salinity (38.10 -39.10), and pH (7.90-8.35); were generally homogeneous. Station 8 recorded the maximum of turbidity and Chl a (19.8 NTU and 47.6 μ g/L respectively) and the minimum of DO (3.70 mg/L).

In autumn, nutrients were generally recorded at stations with anthropogenic pressure (wastewater treatment plant outfall, mouths of wadis, and agricultural effluents) and not detected at other stations. Station 8 recorded the maximum of nitrates, nitrites, ammonium, phosphorus, Chl a, and turbidity; with values of 0.132 mg/L; 0.055 mg/L; 0.22 mg/L; 0.015 mg/L; 16.8 NTU and 44 μ g/L respectively, and the minimum of DO (4.51 mg/L). The pH (7.85-8.53); temperature (21 °C-23 °C) and salinity (37.7-39.9) were generally homogeneous throughout the lagoon.

Correlations of physicochemical parameters with the sampling stations

In winter, the results of the PCA showed three groups (*Fig.* 2); on PC1 (43.34% of the total variance): the first group constructed by the majority of the stations with a

positive correlation to the parameters oxygen, salinity, and pH, the second group (S9 and S10) with positive correlation to turbidity and DIN. On PC2 (39.19% of the total variance), only one group was identified by station 8 with a positive contribution of total phosphorus and Chl a.



Figure 2. Physicochemical parameters and stations projection on the principal components during the winter

In summer the PCA showed four groups (*Fig. 3*): on PC 1 (55.2% of the total variance) the first group was formed by station 8 with the contribution of PT, DIN, Chl a, and turbidity, the second group is represented by stations S1, S2, S3, S4, S5, and S6 positively correlated with dissolved oxygen and negatively with PT, DIN, Chla and turbidity. On PC2 (32.84% of the total variance) the parameters pH, T, and salinity contribute positively. Two groups of stations were identified; the first one represented by (S11, S12, and S13) and the second group of (S7, S9, and S10) stations.



Figure 3. Physicochemical parameters and stations projection on the principal components during the summer

In autumn, three groups can be distinguished (*Fig. 4*): the first group on PC1 (53.77% of the total variance) formed by the station 8 with contributions of the elements DIN, turbidity, Chl a, PT, and T.

Rahhou et al.: Assessment of the trophic status of Marchica Lagoon (Ne Morocco, Mediterranean) after restoration activities using trophic index (TRIX): seasonal and spatial pattern - 4673 -



Figure 4. Physicochemical parameters and stations projection on the principal components during the autumn

A second group on the same PC1 is formed by the majority of the stations with contributions of salinity and dissolved oxygen. The last group on PC2 (18.17% of the total variance) is formed by stations (S9 and S10).

Eutrophication index (TRIX)

Spatial and seasonal variation of TRIX is shown in the *Figures 5, 6,* and 7. No significance was observed between seasons (P > 0.05). The results showed three groups of trophic status: a high quality group (Oligotrophic) which presents the general state of the lagoon and includes the majority of the sampling stations. A second group with good quality (mesotrophic) is represented by station 12 on the Arekmane side and the third group of moderate quality (eutrophic) is represented by stations 8, 9, and 10.



Figure 5. TRIX projection during winter



Figure 6. TRIX projection in the summer



Figure 7. TRIX projection during autumn

Correlations of physicochemical parameters and TRIX

Table 2 shows the result of physicochemical parameters of coastal waters and TRIX index during the study period. The correlation of TRIX is negative with salinity (p < 0.01), and positive with Turbidity (p < 0.01). For the other parameters, positive

correlations were observed between pH and Temperature (p < 0.01), between pH and Salinity (p < 0.01), and negative correlation was found between salinity and turbidity (p < 0.01).

Table 2. Correlations of physicochemical parameters and TRIX during the period of this study

	Т	Salinity	pН	Turbidity	TRIX
Т	1				
Salinity	0.282	1			
pH	0.422**	0.530**	1		
Turbidity	-0.28	-0.439**	-0.123	1	
TRIX	-0.243	-0.428**	-0.131	0.574**	1

**Correlation is significant at the 0.01 level. *Correlation is significant at the 0.05 level

Discussion

The minimum and maximum values of pH (7.82-8.21), salinity (37.53-39.03), and temperature (20.10-21.6 °C) were homogeneous throughout the lagoon and this is due to the strong hydrodynamics and circulation that homogenizes the lagoon waters with the sea waters after the opening of the new inlet (Maicu et al., 2021), the same for dissolved oxygen except for the stations where there is a strong anthropogenic pressure, notably the wastewater's plants discharges and the agricultural activities. In comparison with the studies carried out on the quality of the lagoon water (Table 3) before the development of the site (Bloundi, 2005; Ruiz et al., 2006; Zerrougi et al., 2013) and the studies after the opening of the new inlet with Mediterranean Sea (Aknaf et al., 2017; Mostarih et al., 2016a; Oujidi et al., 2020), a clear improvement in the concentration of phosphorus was recorded in the lagoon, except for the area receiving wastewater from the Nador wastewater treatment plant. The same for nitrogenous elements, except for the stations with high anthropogenic pressure (Bouareg and Arekmane zones) and stations influenced by the watershed (Oued Seloine). Indeed, after the opening of the new pass in 2011, water exchanges are effectively better between the lagoon and the Mediterranean Sea, not exceeding a few days for the renewal of the lagoon waters (Maicu et al., 2021), also the developments around the lagoon have improved the oxygenation of the surface waters and reduced the overall eutrophication of the lagoon according to (Aknaf et al., 2017; Mostarih et al., 2016a; Oujidi et al., 2020).

On the other hand, the establishment of wastewater treatment plants in the Marchica catchment area has increased the risk of point source eutrophication in the wastewater discharge points, which, raw or treated, are characterized by high nitrogen and phosphorus contents, since the Moroccan standard does not take nitrogen and phosphorus into consideration in the discharge standards. The impact of wastewater has been raised by the presence of low levels of oxygen and high levels of nutrient salts (Oujidi et al., 2020; Ruiz et al., 2006).

TRIX is frequently used to characterize the trophy status of the ecosystem. TRIX has been applied to many ecosystems in coastal marine waters, in the Mediterranean Sea, and lagoon ecosystems (Abidi et al., 2018; Antonio-Robles et al., 2021; Béjaoui et al., 2016; Cañedo- Argüelles et al., 2012; Cevirgen et al., 2020; Christia et al., 2011; Dewi et al., 2019; Giovanardi and Vollenweider, 2004; Morsy et al., 2022; Pérez-Ruzafa et

al., 2005; Salas et al., 2008). The evaluation of the TRIX index in the Marchica lagoon during the three seasons of the study period shows point values exceeding 6 TRIX units, typical of highly productive coastal waters, where the effects of eutrophication determine frequent episodes of anoxia in the bottom waters and are indicated by high phytoplankton and high concentrations of nutrients salts. These values were recorded in the Bouareg-Arekmane area, which is subject to strong anthropogenic pressures, including wastewater discharges from the Nador and Arekmane wastewater treatment plants directly into the lagoon and wastewater discharges at Oued Seloine as well as agricultural activities and the influence of groundwater. The rest of the lagoon is generally oligotrophic with good to excellent quality.

References	Periods	NH4 ⁺ (mg/L)	NO ₂ ⁻ (mg/L)	PO ₄ ³⁻ (mg/L)	PT (mg/L)	NO ₃ ⁻ (mg/L)	O ₂ (mg/L)	рН	Salinity	T (°C)
Zerrouqi et al., 2013	Summer	0.005-0.015	0.014-0.047	0.005-0.162	0.030-0.242	nd	6.6-11.2	8.2-8.84	37.2-37.9	22.1-26.3
Ruiz et al., 2006	Summer	0.001-0.04	0.001-0.024	0.001-0.004	nd	nd	nd	7.95-8.62	37.6-40.9	nd
Bloundi, 2005	Winter, spring and summer	0.018-0.864	nd	0.094-0.376	nd	0.06-1.00	nd	7.27-9.07	35-40.9	13.7-30.5
El Madani, 2012	Summer, spring, winter and autumn	nd	0.01-0.094	0.01-7.77	nd	0.01-0.621	3.66-8.31	7.8-8.24	32.7-40.2	14.3-29.6
Aknaf et al., 2017	Autumn and spring	nd	0.0-0.1	0-0.14	nd	0-2.2	2.5-10.6	7.8-8.64	36.6-39.6	14.5-31
Oujidi et al., 2020	Wet and dry season	0.21-4.54	nd	0-2.75	nd	< 0.5	3.84-13.8	7.73-8.92	27.5-38.6	18.1-29.1
Mostarih et al., 2016b	Spring and wummer	nd	0-0.009	0-0.7	nd	0.007-1.83	5.21-11.83	7.78-8.43	nd	16.6-26.4
This study	Winter, summer and autumn	0-0.22	0-0.055	< 0.05	0-0.45	0-0.935	3.35-10.5	7.62-8.53	37.4-39.9	15-24.9

Table 3. Results of physicochemical parameters and nutrients before and after the openingof the new inlet and development plan

The relationships between the index and physicochemical parameters measured in each station were tested using Spearman correlation coefficients. As a result, in these coastal waters and the research period described, the correlation of TRIX is negative with salinity and positive with turbidity, which implies the effect of the watershed characterized by waters of low salinity, while the waters of the lagoon influenced by exchanges with the Mediterranean Sea are of good quality and therefore of low TRIX. TRIX values below 3 are generally open sea values (Giovanardi and Vollenweider, 2004).

Conclusion

The eutrophication index (TRIX) is a very important tool as an indicator of pollution for the assessment of water quality. Using the eutrophication index, it was found that the water quality in the lagoon of Marchica is generally good with the presence of points subject to strong anthropic pressures characterized by wastewater discharges and agriculture. The present study provided water quality parameters at thirteen stations and the state of eutrophication during 2020. The stations close to the Nador-Bouareg-Arekmane coast were found to be the most affected by eutrophication, which coincides with its position, close to wastewater discharges from wastewater treatment plants, and agricultural activities as well as input from their catchment area (Oued Seloine). Indeed, the establishment of wastewater treatment plants in sensitive wetlands such as the Marchica is a choice to be reviewed, as the Moroccan discharge standard does not take into consideration nitrogen and phosphorus, both of which are the cause of eutrophication. The state of eutrophication has also decreased towards the Mediterranean, which implies the new pass in the improvement of the global quality.

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