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Assessment of water quality with emphasis on trophic status in bathing areas from the central-southern coast of Cuba

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ABSTRACT

Bathing water quality has become a matter of concern due to health risks. This study sought to assess water quality in bathing areas from the southeastern coast of the Cienfuegos province, Cuba. Certain physical, chemical, and biological parameters (salinity, temperature, pH, oxygen saturation, $N\text{-}NH_4^+$, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$, $P\text{-}PO_4^{3-}$, COD, BOD_5, fats and oils, chlorophyll-*a*, thermotolerant and total coliforms, and phytoplankton) were measured on five beaches during 2019-2020 in both rainy and dry seasons. A water quality index (WQI) was calculated using the the results of an eutrophication index (EI) and subsequently analyzed. Cluster analysis (CA) and principal components analysis (PCA) were conducted to interpret water quality variations. The WQI values ranged between fair and good, with significant differences between seasons. Multivariate analyses demonstrated the influence of river contributions on water quality indicators based on the results of the CA and the first principal component (PC1) correlated with Sal, DO, total and thermotolerant coliforms, and certain nutrients. PCA also reflected the increase in BOD_5 levels during the summer based on PC2, while the PC3 correlated with COD and response parameters (chlorophyll-a and phytoplankton) were linked to the trophic status. The assessment of trophic status showed non-eutrophic conditions. The abundance and diversity of phytoplankton in these beaches was low (128.5×10^3 cells L^{-1}, total mean value), following the normative definitions of the water framework directive. Harmful algal species occurred in low numbers (1.88×10^3 cells L^{-1}, total mean value), but certain species could represent a risk of dermatitis to bathers. The application of different classification schemes allowed for a comprehensive assessment of water quality in this coastal zone, helping to identify the need for monitoring and further ecological study on harmful algae species.

Descriptors: Beaches, Eutrophication, Phytoplankton, Indices, Season.

INTRODUCTION

The development of marine environment monitoring programs is a starting point for an accurate diagnosis and thus for effective coastal zone management. As such, the surveillance of environmental quality in aquatic systems through monitoring programs receives considerable attention. Coastal water quality assessment requires the integration of chemical, physical, and biological studies (GEOHAB, 2006).

The Bathing Water Directive (BWD) from the European Union seeks to promote safe recreational aquatic environments globally. The BWD's focus on waterborne bacteria supplements the monitoring of nutrients and chemicals as required under the Water Framework Directive (WFD), with the aim of improving all aspects of water quality (EEA, 2020).

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In Cuba, the standard NC.22:1999 (ONN, 1999) is established for assessing water quality in bathing areas. However, this standard lacks quantitative criteria for nutrients, as it only establishes if concentrations are eutrophic. Further, it also lacks a classification scheme for water quality assessments such as those of water quality indices (WQI), even though these are considered ideal to diagnose the state of a water system (Avila et al., 2011).

The application of eutrophication indices (EI) has been very useful to assess nutrient levels (Karydis et al., 1983; Vollenweider et al., 1998; Coelho et al., 2007, Primpas et al., 2009). Since eutrophication indices can reflect the anthropogenic influence on water quality and the ecological functioning of aquatic systems (Cunha et al., 2013), certain ones proposed by Karydis et al., (1983), Lin (1996), and Vollenweider et al. (1998) have been used to assess trophic status in Cuban coastal zones (Betanzos-Vega et al*.,* 2012; Montalvo et al., 2014; Seisdedo et al., 2014). However, there has been limited study on classification schemes related to water quality indices (WQI) on Cuban beaches (Miravet et al., 2009), despite their significance in supporting environmental management (Seisdedo et al., 2016).

Strong relationships have been observed globally between the increase in nutrient loading and the proliferation of algae in aquatic ecosystems, known as eutrophication. Phytoplankton show rapid responses to altered nutrient levels through changes in both biomass and composition (Reynolds, 2006). Increases in nutrient loading in marine ecosystems have also been associated with algal blooms, leading to anoxia and even toxic impacts on fisheries, human health, or recreation (Anderson et al., 2002). Phytoplankton has been used as an indicator of nutrient loading (e.g. nitrogen and phosphorus) in marine water; their assessment is considered in a range of legislation, including the European WFD (Garmendia et al., 2013). Harmful Algal Blooms (HABs) influence both water quality and coastal aesthetics, affecting tourism, local economies, and ecosystem health (GEOHAB, 2006; Affe et al., 2021). But HABs also represent a threat to human health, through the transfer of algal toxins into the human food chain via seafood products, by direct contact with bloom or toxin contaminated seawater, or through exposure to marine aerosols containing toxic cells and/or their released contents during blooms (Berdalet et al., 2016).

Exposure to aerosolized algal toxins and dermal contact with HABs have been a matter of increased concerns to human health in recent years. One example is a three decade, multi-institutional, and multidisciplinary initiative in the Gulf of Mexico and the coast of Florida to study how the inhalation of aerosols containing brevetoxins during high-biomass, toxic *Karenia brevis* blooms can cause respiratory symptoms (e.g. Fleming et al., 2005, 2007, 2011).

Beaches from the southeastern coast of the Cienfuegos province are among the most beautiful landscapes in Cuba. Prior studies of these beaches have shown low impacts on physical and chemical parameters and trophic status (Seisdedo, 2008; Seisdedo et al., 2010). Though socioeconomic activities in this area have not notably changed since prior studies, it is necessary to execute an integrated and updated assessment of water quality for tourist-recreational purpose through the application of different classification schemes (water quality and eutrophication indices) together with biological parameters, such as phytoplankton composition and abundance. This work seeks to assess water quality with an emphasis on trophic status in bathing areas from the southeastern coast of the Cienfuegos province, central-southern Cuba.

METHODS

STUDY AREA AND SAMPLING

This study was conducted on five of the most used beaches of the southeastern coast of the Cienfuegos province, central-southern Cuba. Bathing areas are between Rancho Luna beach and the mouth of the Yaguanabo River (Figure 1). Rivers flowing into this coastal region include Arimao, San Juan, Gavilanes, and Yaguanabo. However, the studied beaches do not see a fluvial influence, with the exception of station B1, which receives direct discharges from the Yaguanabo river, and station B5, which receives indirect

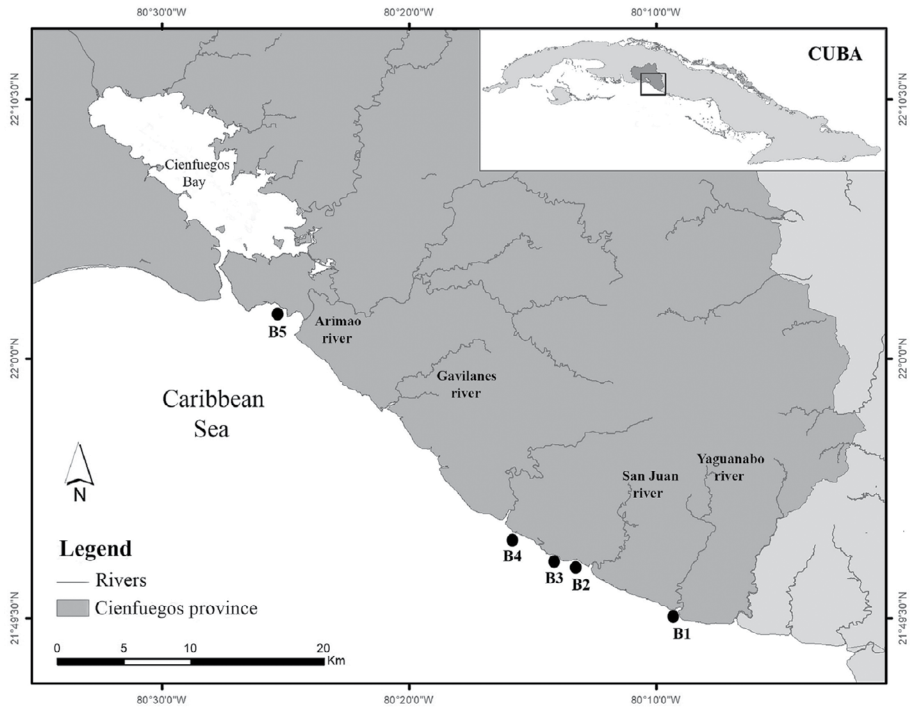

Figure 1. Survey bathing areas in the eastern coast of the Cienfuegos province (sampling stations, B1:Yaguanabo, B2: Playa Fría, B3: Playa Inglés, B4: Tatahagua, B5: Rancho Luna).

discharges from the Arimao river. The marine currents in this study area are weak tidal with average speeds of 0.03 m s^{-1} and a trajectory from east-southeast towards west-northwest (Lamas, 2019).

The primary uses of the studied coastal areas are tourist-recreational and recreational fishing. Fishing is carried out in boats and underwater, and is fundamentally pelagic and non-profit. The objective is family consumption, which requires a permit allowing for catches of up to 33 kilos per day (Jiménez, 2018). Tourism is the primary economic activity, centered on the Rancho Luna and Faro Luna hotels, the Villas of Rancho Luna and Yaguanabo, and rental houses. There are several population settlements of under 500 inhabitants, such as Rancho Luna, Camilo Cienfuegos, San Juan, Caleta Muñoz, and Yaguanabo.

The two seasonal periods are rainy (May to October), which captures approximately 80% of the annual rainfall, and dry (November to April). The mean annual accumulated rainfall in Cienfuegos is 1,256 mm, of which 1,014 mm occurs during the rainy season. The driest and most evaporated areas in the province are in the coastal zone (Barcia et al., 2011).

Four sampling campaigns were conducted (April and September 2019, June and November 2020), two during each season. The seasonal influence on water quality (MIZC, 2010) was considered, as in prior studies (Seisdedo et al., 2014; Bustamante et al., 2016). Using a 20L jar, four water samples were collected at each beach every 20-30 m along the coastline, at an approximate depth of 1 m, for a total of 40 samples per season. Sampling and preservation protocols followed APHA specifications (1998); when required, samples were kept cold prior to analysis. The DO samples were the first to be collected. These were carefully extracted until the bottle was filled, avoiding bubbles. The bottle was then covered and kept in a dark, cool area until fixed with the reagents to determine DO, within six hours. For quantitative sampling of phytoplankton, a 1L water sample was collected at each sample point from the 20L jar using a plastic bottle, preserved with Lugol's solution and stored in the dark until analysis. For taxonomic purposes, a vertical and horizontal tow (during 5 min.) was carried out with a 20 µm simple conical plankton net (20 cm mouth diameter and 60 cm net body, with a rigid cod end), at each local point. The concentrated samples were fixed with Lugol's solution.

WATER QUALITY INDICATORS

Water quality parameters were determined using various methods from the Procedures Manual of the Environmental Assays Laboratory, corresponding to the Centro de Estudios Ambientales de Cienfuegos (CEAC). Dissolved oxygen (DO) and Biochemical Oxygen Demand (BOD_5) were measured using the Winkler chemical method, with five days of incubation at 20°C for BOD_5. The Chemical Oxygen Demand (COD) was analyzed by the permanganate method (APHA, 1998). Salinity (Sal.) and temperature (Temp.) were recorded in situ using a YSI-30 model digital probe, and the pH analysis used a HANNA digital pH meter. To measure salinity and pH, the measuring equipment was allowed to equilibrate for at least one minute prior to recording any values. The concentrations of $N\text{-}NH_4^+$ and $P\text{-}PO_4^{3}$ were determined using the methodologies described by Koroleff (1983) and Grasshoff et al. (1983) respectively, while the concentrations of $N\text{-}NO_3^-$ and $N\text{-}NO_2^-$ were quantified according to Strickland and Parsons (1972), all using high-precision spectrophotometry since variation coefficients of these methods were less than 3%. Qualitative filters were used to filter samples for dissolved nutrients. Thermotolerant (TT Colif.) and total coliforms (T Colif.) were determined using the multiple tube fermentation technique, per APHA (1998). Fats and oils (FO) were analyzed using the gravimetric method (APHA, 1998), in which 1 L of sample is acidified with HCl to pH<2, and three extractions are carried out with n-hexane in a separation funnel. The extract is then dried with sodium sulfate, the solvent evaporated by distillation and drying, and gravimetric determination is performed. Quality control procedures were carried out in order to ensure the quality of the data. The oxygen saturation (OS, in %) was obtained by applying the empirical equation proposed by Weiss (1970).

To assess water quality for each beach, we used a Water Quality Index (WQI) considering the criteria for the indicators (BOD_5, COD, OS, Sal., TT Colif., T Colif. and nutrients) established in the Cuban standard for bathing (NC. 22, 1999), as well as the equations proposed by CCME (2001):

$$WQI = 100 - \left(\frac{\sqrt{F1^2 + F2^2 + F3^2}}{1.732} \right) \quad \text{Eq. (1)}$$

Where:

F1- represents the percentage of variables that do not meet their objectives or quality criteria ("failed variables"), relative to the total number of variables measured.

F2- represents the percentage of individual tests that do not meet objectives or quality criteria ("failed tests")

F3- represents the amount by which failed test values do not meet their objectives or quality criteria and it is calculated in three steps.

$$F3 = \left(\frac{nse}{0.1nse + 0.01} \right) \quad \text{Eq. (2)}$$

The collective amount by which individual tests are out of compliance is calculated by summing the excursions of individual tests from their objectives and dividing by the total number of tests (both those meeting objectives and those not meeting objectives). This variable, referred to as the normalized sum of excursions, or *nse*, is calculated as:

$$nse = \frac{\sum_{i=1}^{n} excursion_i}{Number\ of\ tests} \quad \text{Eq. (3)}$$

The number of times by which an individual concentration is greater than (or less than, when the objective is a minimum) the objective is termed an "excursion" and is expressed as follows.

$$excursion_i = \left(\frac{FailedTestValue_i}{Objective_j} \right) - 1 \quad \text{Eq. (4)}$$

For the cases in which the test value must not fall below the objective.

$$excursion_i \left(\frac{Objective_j}{FailedTestValue_i} \right) - 1 \quad \text{Eq. (5)}$$

The 1.732 divisor normalizes the result to a range of 0-100, which is divided into five quality categories: poor (0-44), marginal (45-64), fair (65 -79), good (80-94), and excellent (95-100).

Though the CCME proposal (2001) requires a minimum of four sampling campaigns to determine WQI, this tool was adapted by converting from a temporary to a spatial approach, thuds considering number of sampling points in lieu of campaigns (Seisdedo et al., 2016).

The eutrophication index (EI) proposed by Karydis et al. (1983) was used, as the NC.22 (1999) standard calls for non-eutrophic concentrations of nitrogen and phosphorus. This index was calculated for dissolved inorganic nitrogen (DIN) from the sum $N\text{-}NH^{+}_{4}$ + $N\text{-}NO_{2}^{-}$ + $N\text{-}NO_{3}^{-}$ and dissolved inorganic phosphorus (DIP) by considering $P\text{-}PO_{4}^{3-}$, based on the following equation:

$$EI = \frac{C}{C - \log X_i} + \log A \qquad \text{Eq. (6)}$$

where:

EI: eutrophication index per DIN and DIP at each beach.

A: number of samples during the study period (herein, four per beach)

C: the logarithm of the total concentration of the nutrient during the study period, that is, the sum of concentrations xij of the nutrient obtained in each Ai station, per campaign.

The following classification scheme for the EI was used: EI <3 (oligotrophic conditions), 3 ≤ EI ≤ 5 (mesotrophic conditions) and EI> 5 (eutrophic conditions). For the calculation of the WQI, we considered the quality good when EI ≤5, corresponding to non-eutrophic conditions.

In addition to WQI, chlorophyll-*a* levels were analyzed by the fluorimetric method, using methanol extraction (APHA, 1998). We considered the classification scheme proposed by Contreras et al. (1994) to assess the trophic status based on concentrations of chlorophyll-*a*.

PHYTOPLANKTON ANALYSIS

For quantitative analysis, samples were settled using sedimentation Utermöhl chambers of 25 mL, with a settling time of 18 h. Phytoplankton cells were counted under 200X magnification on an inverted MOTIC microscope (Utermöhl, 1958), and their populations reported as cells L^{-1}. Phytoplankton identification was done to the lowest possible taxonomic level following the usual literature, mainly Tomas (1997), Hallegraeff et al. (2003), Lassus et al. (2016), and Aké-Castillo et al. (2018). Microalgal cells, both alive or fixed in Lugol's solution, were photographed under an Olympus BH-2 light microscope.

Phytoplankton abundance was classified as eutrophic when any single species exceeds 250,000 cells L^{-1} and total cell counts of a sample are over 10^6 cells L^{-1}, and non-eutrophic otherwise (WFD, 2000).

STATISTICAL ANALYSES

After applying a normality test (Shapiro-Wilk), a non-parametrical test (Wilcoxon) was used to assess the significance of differences in WQI and phytoplankton abundance between seasons.

Multivariate statistical analysis has been proposed as ideal to analyze temporal and spatial variations and complex interactions between various parameters caused by natural and anthropogenic factors (Shrestha and Kazama, 2007; Gupta et al., 2009; Fataei et al., 2011; Gonçalves and Alpuim, 2011). As such, we applied Principal component analysis (PCA) and Cluster analysis (CA). The data set suitability for PCA was assessed using Kaiser–Meyer–Olkin (KMO) and Bartlett's tests. A measure of sampling adequacy, KMO values above 0.5 indicate that PCA can be performed. The Bartlett's test of sphericity assesses the null hypothesis that the variables in the population correlation matrix are uncorrelated, and that the correlation matrix is an identity matrix; the null hypothesis is rejected if the observed significance level is <0.05. In this study, PCA was conducted on the standardized data sets by scaling by (value - mean) / SD, as it is preferable that the PCA be independent of scaling, and because this permits values within a specific range. The dissimilarity between stations was determined using a Cluster analysis (CA) based on euclidean distance. The statistical software OriginPro 2018 was used for the statistical analyzes.

RESULTS

WATER QUALITY ASSESSMENT

The mean values of the WQI ranged between fair and good, and the Wilcoxon test showed significant differences ($p<0.05$) between seasons. All stations were classified as good during the dry period, while quality ranged between good and fair during the rainy season. The lowest mean value was measured in station B1 (Yaguanabo beach) during the rainy period, while the highest was recorded in station B5 (Rancho Luna beach) during the dry period (Figure 2).

The mean values of certain indicators ($N\text{-}NH_4^+$, COD, BOD_5, TT Colif., T Colif. pH, and Temperature) were slightly higher during the rainy period. However, other indicators ($N\text{-}NO_2^-$, $P\text{-}PO_4^{3-}$, and FO) showed values lower than the quantification limits in both seasons. The mean salinity concentrations were slightly higher during the dry period (Table 1).

Salinity values ranged between 32.2 and 36.0, with the highest during the dry period and the lowest and widest range of values during the rainy period (Table 1). The maximum value was from station B4, while the minimum was from station B1 (Figure 3). Temperature varied between 25.3 and 32.7 °C, with the widest range during the dry period. The highest value was recorded at station B5 during the rainy period, while the lowest corresponds to station B1 during the dry period. The pH values ranged between 7.1 and 8.9 units, with the maximum at station B5 during rainy period and the minimum at station B3 during the dry period. Oxygen saturation ranged between 106.5% and 140.9%, with a maximum at station B3 during the dry period and a minimum at station B1 during the rainy period. The ranges of pH and oxygen saturation were similar in both seasons.

Chemical Oxygen Demand (COD) average values were between 2.98 and 3.75 mg L^{-1}, and the widest range of results was registered during the rainy period (Table 1). The highest value

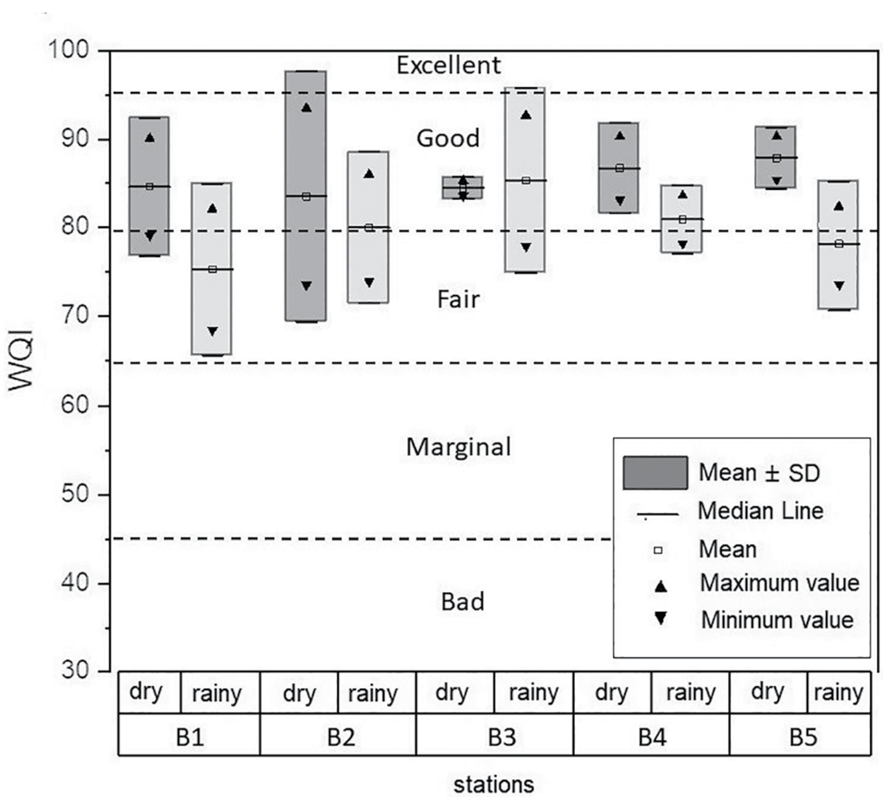

Figure 2. Box plot of WQI values in the studied bathing areas (stations) from the southeast coast of Cienfuegos for each seasonal period (rainy and dry).

Table 1. Results of water quality indicators in the studied area for each seasonal period (rainy and dry). SD=standard deviation. Underlined indicators showed significant differences between seasonal periods ($p<0.05$).

Indicators	Rainy season			Dry season			Quality criteria (NC.22, 1999)
	Mean	Range	SD	Mean	Range	SD	
Temp. (°C)	31.0	29.8 - 32.7	0.91	28.47	25.3 - 31.0	5.20	(*)
pH (unit)	8.4	7.6 - 8.9	0.52	8.1	7.1 -8.9	0.53	6.1 - 8.9
Sal. (psu)	34.5	32.2 - 35.5	1.35	35.2	33.7 - 36.0	0.71	≤ 36
OS (%)	132.4	111.3 - 135.7	10.8	142.1	106.5 - 140.9	10.4	≥ 70
PO_4^{3-} (μmol L^{-1})	1.38	< 1.29 - 4.72	0.50	< 1.29	-	-	NE (**)
NH_4^+ (μmol L^{-1})	1.33	0.64 - 6.93	1.27	0.85	0.64 - 5.4	0.86	NE (**)
NO_3^- (μmol L^{-1})	1.10	0.93 - 4.9	0.75	1.00	0.93 - 4.8	0.66	NE (**)
NO_2^- (μmol L^{-1})	< 0.29	-	-	< 0.29	-	-	NE (**)
COD (mg L^{-1})	3.75	2.40 - 5.55	2.10	2.98	1.60 - 3.75	0.79	≤ 2.0
BOD_5 (mg L^{-1})	2.57	1.95 - 3.1	0.49	1.28	1.12 - 1.48	0.65	≤ 3.0
FO (mg L^{-1})	0.45	< 1.0 - 1.7	0.22	0.46	< 1.0 - 1.7	0.26	≤ 0.5
TT Colif. (MPN/100mL)	2.84	< 1.8 - 2.58	1.05	1.97	< 1.80 - 2.58	0.06	≤ 200
T Colif. (MPN/100mL)	5.64	< 1.8 -26.6	3.26	2.40	2.30 - 2.58	0.23	≤ 1000
Chla (μg L^{-1})	0.14	0.20 - 1.33	0.31	0.13	0.20 - 0.85	0.17	(***)

(*)_ considered for determining OS

NE_ Non-eutrophic conditions

(**)_ considered from results of EI < 5

(***)_Standards do not establish criteria for this indicator

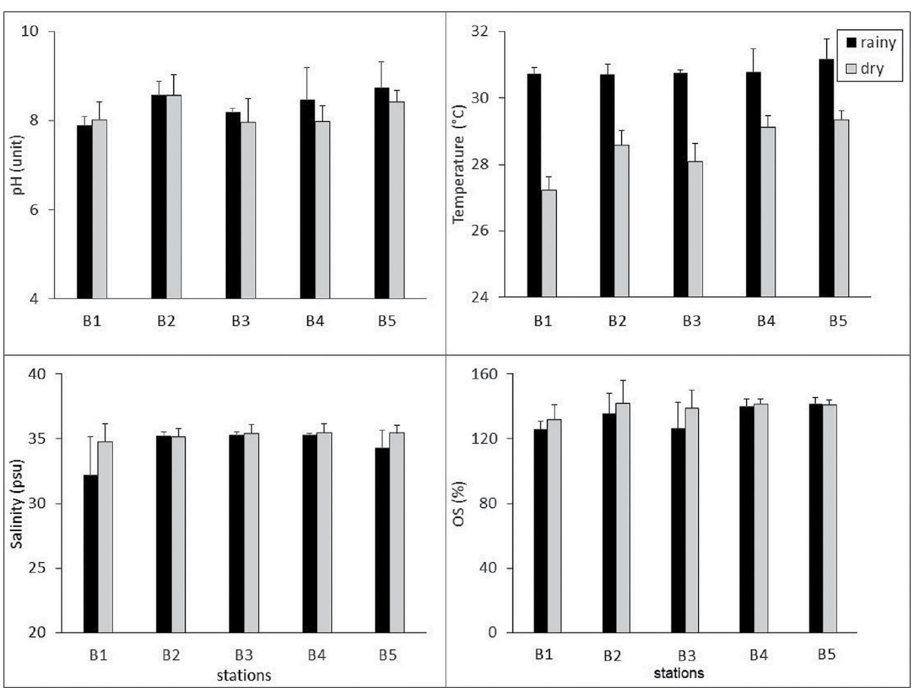

Figure 3. Distribution of pH, temperature, salinity and oxygen saturation values (mean + standard deviation) in the studied bathing areas from the southeast coast of Cienfuegos for each seasonal period (rainy and dry).

was presented in station B2 during the rainy period, with the lowest at station B3 during the dry period. BOD_5 concentrations varied between 1.60 and 5.55 mg L^{-1}, with maximum and minimum values obtained at station B4 during the rainy and dry periods, respectively (Figure 4). Concentrations of Fats and oils were lower than the quantification limit (1 mg L^{-1}), and the same range was noted during both seasons (Table 1). However, certain points showed high values with respect to the NC.22:1999 criteria (ONN, 1999), mainly at station B1 during the rainy period and at stations B2 and B3 during the dry period.

With respect to nutrients, concentrations of $P\text{-}PO_4^{3-}$ were mostly below the quantification limit (1.29 µmol L^{-1}) during both seasonal (Table 1). The concentrations of ammonium were between 0.64 and 6.93 µmol L^{-1}, with the maximum registered at station B3 and the widest range observed during the rainy period. The nitrite results were below 0.02 µmol L^{-1} during both seasons. Nitrate values varied between 0.93 and 4.9 µmol L^{-1}, with similar ranges during both seasons and a maximum recorded at station B3.

The results of thermotolerant coliforms ranged between below the quantification limit (1.8 MPN/100 mL) and 16.2 MPN/100 mL, while total coliform concentrations ranged between below 1.8 and 240 MPN/100 mL. These parameters showed the widest ranges and highest values at station B1 during the rainy period (Figure 4).

MULTIVARIATE STATISTICS

The KMO measure of sample adequacy was 0.52, and the significance level of Bartlett's sphericity test was <0.05. PCA of the entire data set

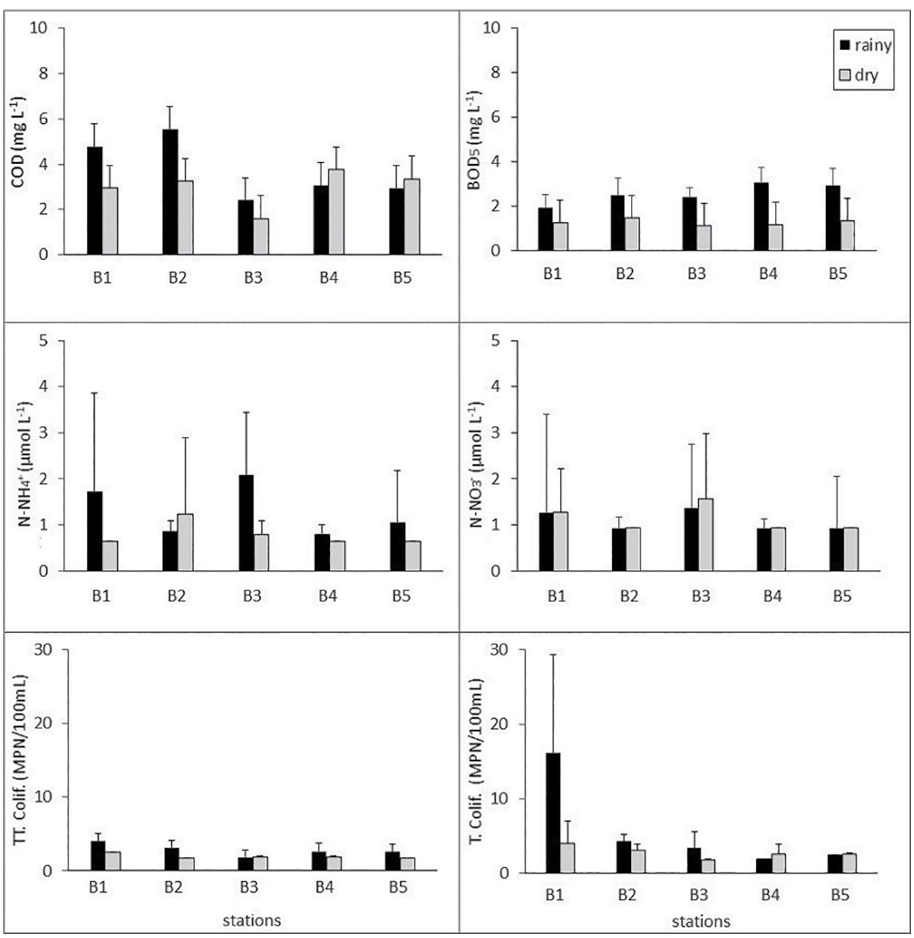

Figure 4. Distribution of values of COD, BOD_5, $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, thermotolerant and total coliforms (mean + standard deviation) in the studied bathing areas (stations) from the southeast coast of Cienfuegos for each seasonal period (rainy and dry).

yielded three components that together account for 61.93% of the variability in the study area. Table 2 summarizes the PCA results comprised of loading indicators on three principal components. The first component (PC1) explained 36.55% of the total variance and was slightly correlated with Sal, DO, TT Colif, T Colif, N-NH_4^+, and N-NO_3^-. The second (PC2) explained 14.03% of the total variance and was correlated with temperature and BOD_5 (Supplementary material 1). The third (PC3) explained 11.35% of the variance and was correlated with COD, chlorophyll-*a,* and phytoplankton.

Table 2. Loadings of indicators on three principal components.

Indicators	Coefficients		
	PC1	PC2	PC3
pH	-0.046	-0.065	0.042
Temp.	0.060	**0.559**	0.185
Sal.	**-0.402**	0.155	-0.082
COD	-0.263	-0.023	**0.523**
TT. Colif.	**0.410**	0.119	0.233
T. Colif.	**0.417**	0.134	0.178
DO	**-0.360**	0.065	0.118
N-NH_4^+	**0.365**	0.335	-0.026
N-NO_3^-	**0.383**	-0.254	-0.205
BOD_5	-0.120	**0.646**	0.120
Chla	0.135	-0.081	**0.526**
Phyto	-0.046	-0.153	**0.435**

The cluster analysis based on the results of analyzed parameters showed a dendrogram (Figure 5), grouping four bathing areas (B2-B5) into one cluster, with station B1 separate from the group.

TROPHIC STATUS ASSESSMENT

Considering that the P-PO_4^{3-} concentrations were mostly below the quantification limit (QL =1.29 µmol L^{-1}), this value was assumed to determine the EI (DIP). Oligotrophic conditions were obtained for this nutrient throughout the studied beaches. In the case of the EI (DIN), the mean values showed the predominance of oligotrophic conditions, although mesotrophic and eutrophic conditions were detected in at least one of the four points analyzed in bathing areas during both seasons (Figure 6).

On the other hand, chlorophyll-*a* values varied between 0.10 and 0.33 µg L^{-1}, with means of 0.14 µg L^{-1} during the rainy period and 0.13 µg L^{-1} during the dry period. In accordance with the scheme proposed by Contreras et al (1994), all stations were classified as 'α oligotrophic' during both seasons. There were no significant differences for the chlorophyll-*a* and EI (DIN) values measured during the two seasonal periods ($p>0.05$).

PHYTOPLANKTON ASSEMBLAGES AND ABUNDANCE

A total of 32 taxa were identified consisting of 18 diatoms, 12 dinoflagellates, and 2 cyanobacteria. The real number could be greater , as certain taxa were difficult to identify. As such, identification of certain taxa was done at the genus higher taxonomic level in the case of small species of diatoms, chlorophytes, and cryptophytes.

Overall, phytoplankton densities were low-moderate during the study period, with no distinct monthly or seasonal variation between beaches. Mean values for total phytoplankton abundance in the sampling units (months/stations) ranged between 24.5 x 10^3 cells L^{-1} (November 2020, station B3) and 367.6 x 10^3 cells L^{-1} (July 2020, station B3) (Figure 7). However, total phytoplankton abundance was slightly higher during the rainy period (146.9 x 10^3 cells L^{-1}) than during the dry period (109.0 x 10^3 cells L^{-1}). The abundance of phytoplankton in all beaches is consistent with good ecological status, per the Water Framework Directive (WFD, 2000).

Diatoms were the dominant taxa in all beaches and months, accounting for at least 90% of total cell density (mean cell density of 116.5 x 10^3 cells L^{-1}). Dinoflagellates were the next most abundant taxa with low densities (mean value of 7.9 x 10^3 cells L^{-1}), contributing 6% to total density. Other taxonomic groups, such as Cryptophytes (3.0 x 10^3 cells L^{-1}), Cyanobacteria (0.4 x 10^3 cells L^{-1}), and Chlorophytes (0.1 x 10^3 cells L^{-1}) were recorded at low abundances, which represented approximately 1.7%, 0.4%, and 0.06% of total cell density, respectively (Figure 7, Supplementary material 2). Dinoflagellates, Cryptophytes, Cyanobacteria, and Chlorophytes were more abundant during rainy than dry periods. There were no significant

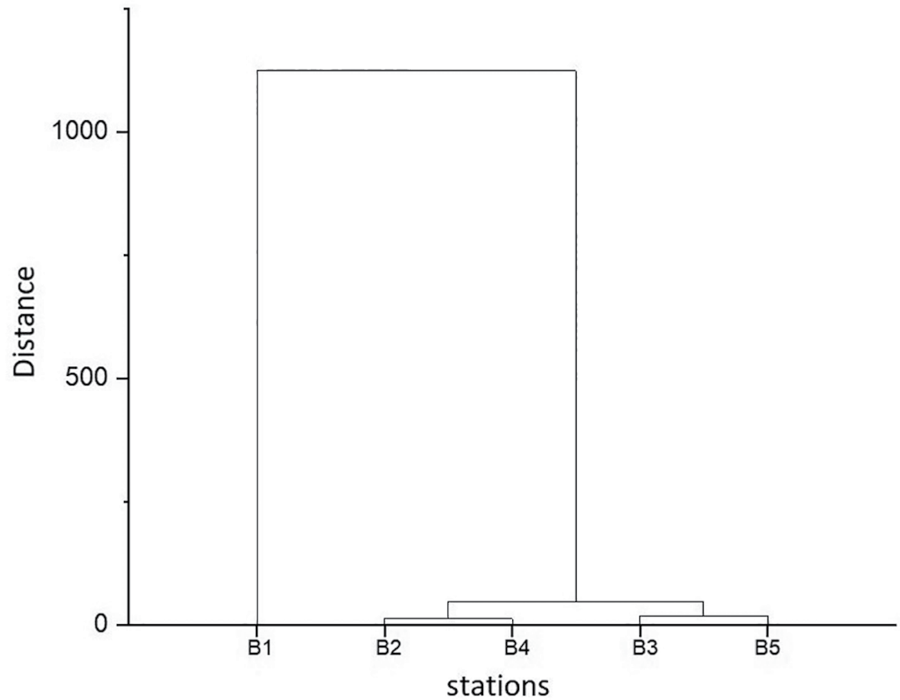

Figure 5. Dendrogram of cluster analysis for the studied bathing areas (stations) from the results of WQI parameters.

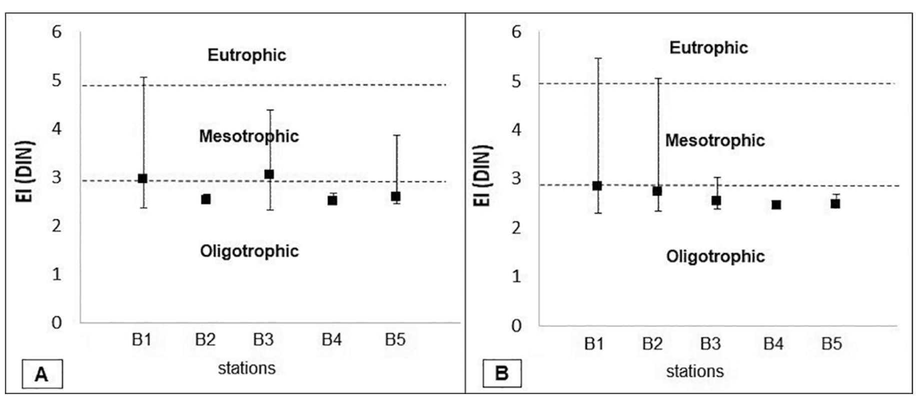

Figure 6. Results of Eutrophication Index for dissolved inorganic nitrogen (mean and range) in the studied bathing areas (stations) for each the seasonal period: A) dry and B) rainy.

differences among total phytoplankton densities measured during the two seasonal periods ($p>0.05$).

The most common and dominant taxa, appearing at all beaches throughout the investigation period, were the pennate diatoms *Thalassionema nitzschiodes* (17.9 x 10^3 cells L^{-1}, mean value for all beaches), *Licmophora* sp. (16.7 x 10^3 cells L^{-1}), *Navicula* sp. (12.2 x 10^3 cells L^{-1}), *Gyrosigma/Pleurosigma* sp. complex (10.5 x 10^3 cells L^{-1}), *Nitzschia longissima* (9.9 x 10^3 cells L^{-1}), and *Diploneis* sp. (7.2 x 10^3 cells L^{-1}). The centric diatom *Thalassiosira* sp. was recorded only in November 2020 at station B1, with high densities (144.0 x 10^3 cells L^{-1}). Dinoflagellates were most commonly represented by *Blixaea quinquecornis* (3.7 x 10^3 cells L^{-1}), *Gymnodinium* sp. (1.5 x 10^3 cells L^{-1}), and *Protoperidinium pellucidum* (1.1 x 10^3 cells L^{-1}) (Supplementary material 2).

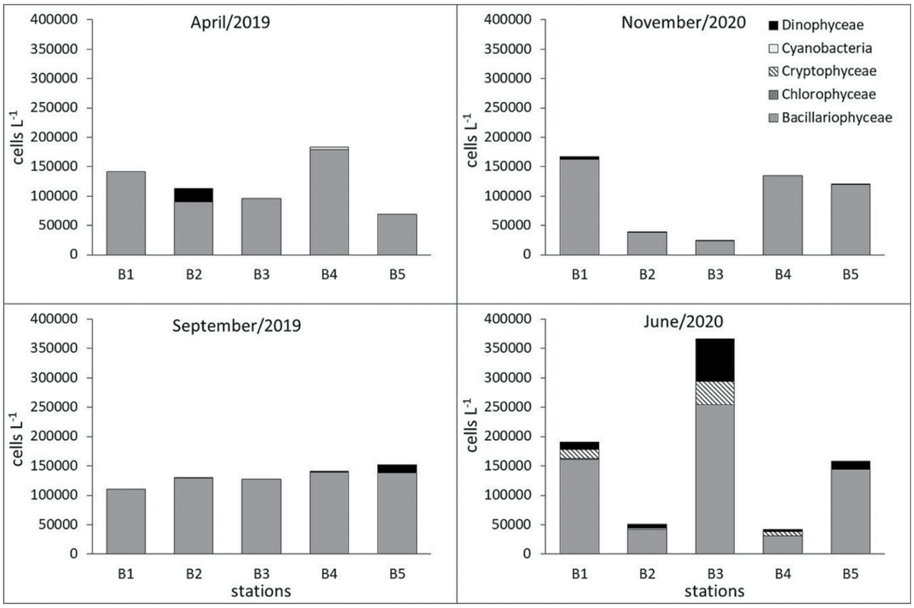

Figure 7. Results of phytoplankton abundance for each bathing area (station) considering taxonomic groups and sampling campaigns.

Six toxic or potentially toxic species were identified: four dinoflagellates (*Ostreopsis* cf. *ovata*, *Prorocentrum hoffmannianum*, *P. lima*, *P. rhathymum*) and two cyanobacteria (*Lyngbya majuscula*, *Oscillatoria* sp.). Toxic species were detected at low densities (less than 1.88 x 10^3 cells L^{-1}), representing less than 1% of total phytoplankton cell density. There was clear seasonality, however the highest densities of some toxic species (e.g. *Ostreopsis* cf. *ovata*, *Lyngbya majuscula*) was observed during the rainy period at beaches with less river input (stations B2, B4) (Supplementary materials 2 and 3). The abundance of nuisance or toxic species was low (< 10^3 cells/L in all beaches), which is deemed good ecological status per the Water Framework Directive (WFD, 2000).

DISCUSSION

Most studied beaches displayed 'good' quality status, not uncommon for open beaches in the Cuban marine environment (Bustamante et al., 2016). However, the concentrations of certain indicators such as BOD_5 and TT Colif. are higher in semi-closed ecosystems of south-central Cuba and other coastal areas worldwide (UNEP/FAO/WHO, 1996, Flores et al., 2011, Seisdedo et al., 2012, Wu et al., 2015). For example, certain tourist beaches of Puerto Colombia recorded maximum values of 13.1 mg L^{-1} BOD_5 and 555 MPN/100 mL of TT Colif. These bathing areas have a greater anthropogenic impact from contamination (organic and microbiological) and the visitor influx in excess of carrying capacity (Salcedo, 2016).

There was no clear seasonality in the trophic status indicators, consistent with a study carried out over ten years ago based on the analysis of certain dissolved inorganic nutrients (Seisdedo et al., 2010). However, the significant differences in water quality for both seasonal periods can be understood given that not all indicators in the water quality index (WQI) are related to the eutrophication process.

Although WQIs are widely used tools to ensure the health of swimmers, calculations methods differ. Almeida et al. (2012) developed a recreational water quality index in Argentina (RWQI) using physical, chemical, and microbiological parameters. To calculate the RWQI mathematically, different rating curves were obtained and the opinion of 17 experts was employed by the Delphi method. Also, Miravet et al (2009) proposed and applied a qualitative numerical index to assess the quality of coastal waters for recreational purposes in the north coast of Havana City and in the south of Havana province, respectively. This index was based on a mathematical formula that weighs quality from nine variables (eight physico-chemical, one microbiological) and classifies water quality into four categories: poor, fair, good, and excellent. However, it has been recognized that assigning specific weights to parameters risks introducing subjectivity into the evaluation (Ott, 1978). In addition, only the quality criteria established in NC 22 (1999) for thermotolerant coliforms were considered for this qualitative numerical index; the importance of trophic status indices and microalgae analysis in the assessment of water quality for recreational purposes was ignored.

In this study, more signs of water quality parameter deterioration were observed during the rainy period, consistent with the results of previous studies both in this coastal zone and in the neighboring Cienfuegos Bay (Seisdedo, 2008; Seisdedo et al., 2021). Certain physical and chemical parameters such as COD, pH, and DIN were slightly higher than threshold concentrations (ONN, 1999) and higher during the rainy season (Table 3), resulting in lower WQI values during this season. The influence of freshwater discharges on physical and chemical parameters, evidenced by the decline in salinity and increase in dissolved inorganic nitrogen, was more pronounced at station B1. This station receives input from the Yaguanabo river in a different way than station B5, which indirectly receives discharge from the Arimao river, as its mouth is approximately 1 km from the station. Similar results, leading in most to eutrophic conditions, have been previously reported in other marine ecosystems (Moreira et al., 2014; Fataei et al., 2011; Montalvo et al., 2017).

Table 3. Percentage of non-compliance with the Good quality criteria established in the NC 22: 1999 (ONN, 1999) with respect to the total results of each indicator during each seasonal period analyzed (N = 40).

Indicators	Rainy period (%)	Dry period (%)
pH (unit)	33	8.0
Sal. (psu)	48	23
OS (%)	0	0
EI (DIN)	3.0	3.0
EI (DIP)	0	0
COD (mg L^{-1})	88	50
BOD_5 (mg L^{-1})	0	0
FO (mg L^{-1})	5.0	13
TT Colif. (MPN/100mL)	0	0
T Colif. (MPN/100mL)	0	0
Total	18	10

Even when the proposals used to assess trophic status were based on both causal (DIN and DIP) and response (chlorophyll-a and abundance of phytoplankton) indicators, the results were consistent. Nitrogen has been recognized as the limiting nutrient in marine waters (Mooret et al., 2013; Bristow et al., 2017). As such, it is thus the most important nutrient to assess trophic status of marine water due to its influence on biological response (Loza et al., 2013, Moreira et al., 2014). The predominance of non-eutrophic conditions in the studied bathing areas may be due to the limited influence of anthropogenic activities and hydrodynamic characteristics, since these beaches are considered “open systems” where coastal transport is active.

Similar assessments of trophic status have been reported from other Cuban coastal areas such as Ana María, Guacanayabo, and Batabanó gulfs as well as from beaches in Eastern Havana (Betanzos et al., 2012; Bustamante et al., 2016; Montalvo et al., 2017). The PCA and CA results also revealed riverine influence on the water quality of the studied beaches (Table 2, Figure 5).

The PCA yielded three data sets explaining 61.93% of the total variance (Table 2). PC1 was linked to river contributions that receive discharges of domestic wastewater or sewers that influence the decrease in salinity in some areas and the increase of TT Colif., T Colif, and nutrient concentrations, leading to the reduction of dissolved oxygen levels. PC2 reflected the pressure on coastal zones in terms of BOD_5 levels during the rainy period, when waters are warmer and there is a greater influx of bathers, consistent with a study by Salcedo (2016). PC3, explaining 11.35% of the variance, is less significant and could be linked to the trophic status of the waters because it is correlated with COD and response indicators (chlorophyll-*a* and abundance of phytoplankton), reflecting the influence this organic matter indicator can have on the biological response. This is consistent with an index of trophic status applied in tropical and subtropical coastal regions of the Pacific Ocean, which relates COD, total nitrogen, total phosphorus, and chlorophyll-*a* (Lin, 1996).

Overall, the abundance and diversity of phytoplankton in the studied beaches was low compared with semi-enclosed seas such as the Mediterranean, that receive anthropogenic perturbations (Alprol et al., 2021), and beaches influenced by natural availability of nutrients, such as Amazon sandy beaches in northern Brazil (Costa et al., 2013). This result reflects the oligotrophic status of water from the beaches on the eastern coast of Cienfuegos province, central-southern Cuba (Seisdedo, 2008). During this study, densities of individual and total microalgal taxa did not exceed the thresholds for eutrophic conditions (WFD, 2000). Moreover, the structure of phytoplankton communities in the studied beaches was dominated by low densities of diatoms, which are typically the primary producers of the base of the marine food web and have generally been indicators of a good marine ecosystem health (Ignatiades et al., 2009). Furthermore, the high bioavailability of silica (silicate) along the coastal area of the studied beaches are favorable to the dominance of diatoms (Álvarez-Cóngora et al., 2006; Seisdedo et al., 2010).

Conversely, harmful algal species (e.g. cyanobacteria, dinoflagellates) occurred in low numbers in all studied beaches, reinforcing the good quality status observed in this coastal area. No nuisance nor toxic algal blooms were reported, unlike other beaches worldwide such as the toxic dinoflagellate blooms in the semi-closed beaches from the Mediterranean Sea (Vila et al., 2001; Tichadou et al., 2010).

Six toxic or potentially toxic species were found on the beaches, four dinoflagellates (*Ostreopsis* cf. *ovata*, *Prorocentrum hoffmannianum*, *P. lima* and *P. rhathymum*) and two cyanobacteria (*Lyngbya majuscula* and *Oscillatoria* sp.). Most of these species are known to be epiphytic, which are resuspended in the water column of these beaches/shallow systems. Although species of the genus *Oscillatoria* can be both benthic and pelagic. Marine cyanobacteria, including *Lyngbya* and *Oscillatoria*, produce several cyanotoxins that have been associated with epidemics of acute contact dermatitis in tropical and subtropical areas worldwide (Carmichael et al., 1992; Osborne et al., 2001).

On the other hand, Ostreopsis ovata is known to produce palytoxin and its analogues, which are potent biotoxins that have been associated with dermatitis and eye and respiratory irritations in tourists and workers at beaches in the Mediterranean Sea (Funari et al. 2015). Benthic Prorocentrum such as Prorocentrum hoffmannianum, P. lima, and, to a lesser extent, P. rhathymum are known to produce okadaic acid (OA) and its analogs, the toxins responsible for diarrheic shellfish poisoning (DSP) (Reguera et al., 2014). Diarrheic shellfish poisoning is only associated with the consumption of shellfish and not with direct exposure to contaminated water in recreational areas (Reguera et al., 2014).

Although dermatitis events and other health problems have not been reported in these beaches and potentially toxic microalgae occurred in low concentrations, the risk associated with their presence should be emphasized in future studies. For example, certain harmful algae such as cyanobacteria and dinoflagellates benefit from the increase of temperature and nutrient loading (Anderson et al., 2002; Paerl, 2009; Hallegraeff, 2010).

Measure management should be conducted in this coastal area to prevent increased eutrophication and to avoid the proliferation of nuisance/toxic algal blooms. Beach monitoring is important to develop a complete distribution of nuisance or toxic species. It is interesting to note that a dinoflagellate (*Vulcanodinium rugosum*) bloom was linked with acute dermatitis cases in bathing areas from the neighboring semi-enclosed coastal zone Cienfuegos Bay during the summer of 2015, under extreme weather conditions (persistent drought and high temperatures), causing social and public health alarms (Moreira-González et al., 2021).

CONCLUSIONS

The combination of eutrophication and water quality indices, complemented with the analysis of phytoplankton, contributed to a more complete and integrated assessment of the study area with tourist-recreational use. Average assessments of water quality between fair and good corresponded to non-eutrophic average assessments obtained through different classification schemes.

Multivariate analyses showed the influence of river contributions on water quality indicators, distinguishing Yaguanabo beach from the rest. In addition, the increase in BOD_5 levels during the summer and the link between COD and biological response were aspects identified by the PCA, allowing us to understand the variations of water quality in the studied area.

Overall, the abundance and diversity of phytoplankton in the studied bathing areas was low, reflecting their oligotrophic status. Harmful algal species occurred in low numbers in all studied beaches, reinforcing the good quality status observed in this coastal area. However, some potentially toxic microalgae such as *Lyngbya majuscula*, *Oscillatoria* sp., and *Ostreopsis* cf. *ovata* represent a risk to bathers in the analyzed beaches, despite their low concentrations. Consequently, the need to monitor these beaches for harmful algae species is evident.

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AUTHOR CONTRIBUTIONS

M.S.L: Conceptualization, Methodology, Statistical analyzes, Writing - original draft, Writing - review & editing

A.R.M.G.: Conceptualization, Methodology, Formal analysis, Writing - review & editing

D.C.H.: Methodology and Writing - review & editing.

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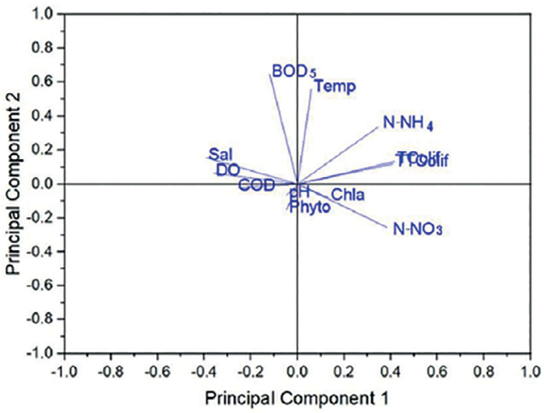
Supplementary material 1



Appendix A. Loading plot of Principal Component Analysis (PCA) with the first two principal axes.

Supplementary material 2

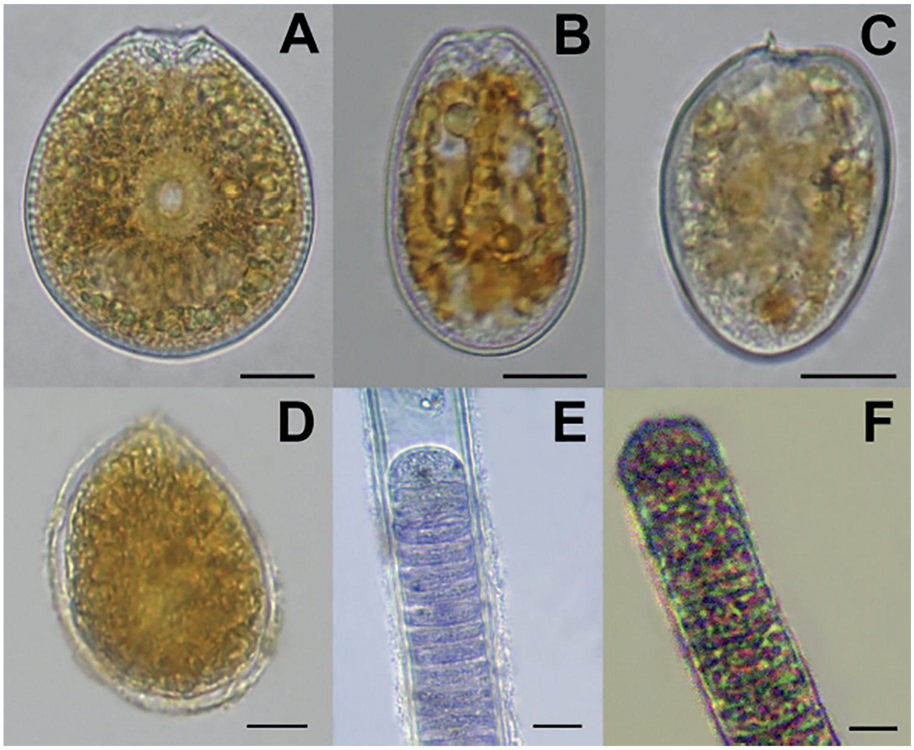
Appendix B. List of phytoplankton species found at five beaches along the eastern littoral of Cienfuegos province, their mean and maximum (between brackets) abundance (x 10^3 cells L^{-1}), and the relative abundance (RA) values (%). April/19 = April/2019, Nov/20= November/2020, Sept/19= September/2019, Jun/20= June/2020.

	Dry period				Rainy period			
	April/19		Nov/20		Sept/19		Jun/20	
DIATOMS	Mean (Max.)	RA (%)	Mean (Max.)	RA (%)	Mean (Max.)	RA (%)	Mean (Max.)	RA (%)
Amphora sp.							0.5 (2.7)	0.3
Aulacoseira granulata							0.7 (3.4)	0.4
Cocconeis sp.	9.4 (17.0)	7.9	3.2 (10.0)	2.3	7.6 (15.0)	5.8	6.3 (26.1)	3.8
Coscinodiscus sp.			1.0 (5.0)		0.7		0.5 (2.6)	0.3
Cylindrotheca closterium	3.8 (19.0)	3.3	3.7 (9.5)	2.7	5.60 (14.0)	4.3	1.7 (3.2)	1.1
Diploneis sp.	4.2 (21.0)	3.6	4.0 (10.0)	2.9	8.8 (16.0)	6.7	11.6 (52.2)	7.1
Gomphonema sp.	2.8 (14.0)	2.3			2.6 (13.0)	1.8		
Gyrosigma/Pleurosigma sp. complex	8.4 (15.0)	6.9	13.3 (35.4)	9.0	17.8 (20.0)	13.5	3.3 (5.6)	2.1
Licmophora sp.	17.4 (22.0)	14.4	27.0 (80.0)	19.6	21.4 (24.0)	16.2	1.1 (3.2)	0.7
Navicula sp.	36.6 (79.0)	30.3	0.4 (2.0)	0.3	11.4 (22.0)	8.7	0.5 (2.4)	0.3
Nitzschia longissima	5.6 (15.0)	4.6	6.0 (9.0)	4.4	8.6 (24.0)	6.5	19.7 (78.4)	12.1
Nitzschia sp.			0.4 (1.5)	0.3	3.8 (19.4)	2.9	1.0 (3.0)	0.6

Continued **Appendix B.**

Odontella aurita	2.2 (11.0)	1.8						
Pseudo-nitzschia sp.							0.5 (2.5)	0.3
Striatella unipunctata	6.2 (16.0)	5.1	0.3 (1.5)	0.2			0.5 (2.5)	0.3
Thalassionema nitzschiodes	13.2 (21.0)	10.9	3.1 (5.5)	2.3	17.0 (20.0)	12.9	38.2 (110.0)	23.5
Thalassiosira sp.			28.8 (144.0)	21 .1				
Ulnaria ulna							0.4 (2.15)	0.3
Small pennate diatoms	5.4 (16.0)	4.5	5.5 (10.0)	4.0	25.2 (26.0)	19.1	38.7 (146.0)	23.8
Small pennate diatoms							0.6 (3.1)	0.4
DINOFLAGELLATES								
Amphidiniopsis sp.							0.4 (2.2)	0.3
Blixaea quinquecornis							14.6 (73.1)	9.0
Gymnodinium sp.	4.6 (23.0)	3.8	1.0 (5.0)	0.7			0.4 (2.2)	0.3
Gyrodinium sp.							0.4 (2.2)	0.3
Hermesinum adriaticum			0.2 (1.0)	0.1			0.6 (2.9)	0.4
Heterocapsa sp.							2.2 (6.5)	1.4
Ostreopsis cf. *ovata*					0.2 (0.9)	0.1	0.5 (1.4)	0.3
Prorocentrum hoffmannianum	0.2 (1.0)	0.2						
Prorocentrum lima							1.1 (3.2)	0.7
Prorocentrum rhathymum							0.5 (2.7)	0.3
Protoperidinium pellucidum			0.5 (1.5)	0.4	2.8 (14.0)	2.1	1.1 (2.8)	0.7
Tripos furca							2.3 (11.3)	1.4
CHLOROPHYTES								
Small unidentified chlorophyte							0.4 (2.2)	0.3
CRYPTOPHYTES								
Small unidentified cryptophyte							12.2 (39.2)	7.5
CYANOBACTERIA								
Lyngbya majuscula					0.1 (0.5)	0.1		
Oscillatoria sp.	0.8 (4.0)	1			0.2 (0.8)	0.1	0.5 (2.4)	0.3

SUPPLEMENTARY MATERIAL 3



Appendix C. Light microscopy microphotographs of potentially toxic microalgal species found in the beaches: (A) *Prorocentrum hoffmanianum*; (B) *Prorocentrum lima*; (C) *Prorocentrum rathymum*; (D) *Ostreopsis* cf. ovata; (E) *Lyngbya majuscula*; (F) *Oscillatoria* sp. Scale bars: 10 μm.