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Estimation of N/P Ratios Levels in a Coastal Bay, Southern Adriatic Sea

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Authors' contributions

This work was carried out in collaboration between all authors. Author LG designed the study, performed the statistical analysis, wrote the protocol and first draft of manuscript. Author AB contributed in analysis and report writing. Authors JK and SG contributed in trial establishment, monitoring and data collection. Author AT performed native English language. All authors read and approved the final manuscript.

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ABSTRACT

The DIN:DIP atomic ratio (N/P) considered optimal for phytoplankton growth is 16:1 (Redfield ratio). Significant deviations from 14 to low N/P ratio might indicate potential nitrogen limitation, the ratio between 14 and 16 indicates both nitrogen and phosphorus co-limitation, whereas from 16 to high N/P ratios indicates potential phosphorus limitation of phytoplankton primary production. This might affect the biological state of the ecosystem, in particular the phytoplankton biomass, species

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composition and food web dynamics. NO_3^- and PO_4^{3-} concetrations in surface waters in six station samples were measured monthly by using spectrophotometer UV-VIS, whereas total *Chl a* was measured using flourometric method, from May - October 2011 and from June - October 2012. The data are converted on molar concentration (NO_3-N and PO_4-P) in order to calculate N/P ratios. The comparison of N/P ratios from 2011 to 2012 in Durres Bay, results in both N and P co-limitation for 2011 and N limitation of primary production in 2012. Comparing N:P ratio in Durres Bay to Southern Adriatic Sea for both 2011 and 2012, resulted in N limitation for Durres Bay and both N and P co-limitation of primary production in open waters. There is a strong positive relationship between primary production (total *Chl a*) and nitrogen in coastal waters and a moderate positive relationship of primary production and N/P ratio in coastal waters of Durres Bay.
The concentrations of NO_3-N and PO_4-P in Durres Bay were higher than permitted standards for surface waters, while in the Southern Adriatic Sea these concentrations resulted within standards, showing clearly oligotrophic characteristics.

Keywords: N/P ratio; horizontal distribution; Southern Adriatic Sea; coastal waters.

1. INTRODUCTION

Since the observation of Redfield [1,2] that marine phytoplankton contains a molecular C:N:P ratio of 106:16:1 (50:7:1 by weight), the use of elemental ratios has become widespread in marine and freshwater phytoplankton studies.

Ratio N/P (called "Redfield ratio") is an important indicator that serves to specify which nutrient restricts eutrophication. If the ratio N: P > 16, phosphorus (P) is a limiting factor for the growth of algae [3,4].

If N: P is between 14 and 16, both N and P are limiting factors (Koerselman & Meuleman, 1996; Mills *et al.,* 2008) and if the ratio is N: P < 14, it indicates that nitrogen (N) is the limiting factor for the growth of algae [3,4].

The dominant nutrient limitation (of primary production) paradigms applied to this continuum for more than one-half a century, were that phosphorus (P) availability controlled primary production in freshwaters, while nitrogen (N) was the dominant limiting nutrient in the more saline downstream estuarine and coastal waters [5-9]. Recent analyses of diverse nutrient limitation studies in both freshwater and marine ecosystems indicate that these paradigms may be “eroding” [10-14]. Increasingly, incidences of N and P “co-limitation”, i.e., the stimulation of primary production by the addition of N and P in combination, where N or P alone stimulate production far less, have been reported [10,14]. Concurrently, recent estuarine and coastal studies indicated that N and P or P limitation are geographically widespread [15-20].

Durres Bay is located between Selita Cape (Lagj) in the south and Durres Cape in the north. These points are 20 km far from each other. The coastline of Durres Bay is 7 km in the east and the highest width is in Golem Beach. In the west of the coastline the depth is more than 10 m. In this bay are known two types of coasts: the low coast where predominate the storage processes, and the high coast where predominate the abrasion processes. The first coast covers the area from Dajlan Bridge to Karpen, whereas the second coast covers the Karpen-Lagj Cape and Currila area [21].

The first aim of this study was to estimate the limiting nutrient of phytoplankton primary production based on N/P ratios and to study the relationship of *Chl a* to NO_3-N, *Chl a* to PO4-P and *Chl a* to N/P in the surface waters of Durres Bay.

The second aim was to compare average concentrations of NO_3-N and PO_4-P of coastal waters to average concentrations of NO_3-N and PO_4-P in the open water surface of Southern Adriatic Sea based on previous studies [22].

Also, another aim was to compare NO_3-N and PO_4-P concentrations in Durres Bay with the recommended standard of NO_3-N and PO_4-P for surface waters in natural conditions [23].

2. MATERIALS AND METHODS

Samples were collected monthly from May - October 2011 and from June - October 2012 in the following stations: Golemi Beach (GB), Plepa Channel (PCh), Hekurudha Beach (HB), Ex-Fuel Quay in Marine Durres Harbour (EFQ), Water

Channel of Durrës City (WChDC) and Currila Beach (CB), at 1 meter depth. Sea water (~ 2 l) was collected for NO_3^-, PO_4^{3-} and total *Chl a* measurements. NO_3^- and PO_4^{3-} concentrations of surface waters in six station samples were measured with spectrophotometer UV-VIS, whereas total *Chl a* was measured using flourometric method (Idromar sonde) according to APHA et al. [24] and Grasshoff et al. [25]. The plastic container was filled up, and preserved in an ice chest. Thereafter, the samples were taken to the laboratory for analyses.

NO_3^- and PO_4^{3-} are reported in NO_3-N and PO_4-P in µM, and the N/P ratios was computed, according to ICES [26]. Pearson correlation coefficients ($p < 0,05$ and $p < 0,01$) were measured through program MegaStat.xla (2007).

3. RESULTS AND DISCUSSION

3.1 NO_3-N Estimates

The nitrate (NO_3-N) concentration in the surface water for all stations from May - October 2011 and from June - October 2012 ranged between 0.97 – 93.54 µM, respectively October 2011 in PCh and May 2011 in HB [27-30].

In 2011, GB had the highest NO_3-N concentration (30.52±35.68 µM) (Table 1, Fig. 2). Further on are ranked CB, HB, EFQ, WChDC. However, the lowest concentration was in PCh (23.91±28.62 µM).

Nitrogen values in 2011 were higher than the recommended standard of NO_3-N (≤ 7.14 µM) converted according ICES for surface waters in natural conditions [23,27,29].

Referring to 2012, EFQ had the highest NO_3-N concentration (19.03±9.36 µM). Then were ranked PCh, GB, HB, CB. However, the lowest concentration was in WChDC (11.61±3.10 µM).

Nitrogen values in 2012 were higher than the recommended standard of NO3-N (≤ 7.14 µM) for surface waters in natural conditions [23,27,29].

NO_3-N concentration in 2012 was 1.8 times smaller than in 2011, improving the situation of coastal waters in Durres Bay.

If we compare total average of NO_3-N (21.77±23.41 µM) concentration for 2011 and 2012 in Durres Bay to NO_3-N (0.57±0.47 µM) of open water surface in Southern Adriatic Sea [22] converted according to ICES, it resulted that the concentration of nitrogen in Durres Bay was 38 times higher than in Southern Adriatic Sea and 3 times higher than the recommended standard of nitrogen for surface waters in natural conditions. Unlike coastal waters, the open water surface of Southern Adriatic Sea are approximately 13 times better than the recommended standard of nitrogen for surface waters in natural conditions (Table 1) [22,23]. There is a positive correlation ($r = 0.412$; p-value ≤ 0.01) between total *Chl a* and NO_3-N (Table 2, Fig. 4C). This explains the relationship of primary production and nitrogen in coastal waters.

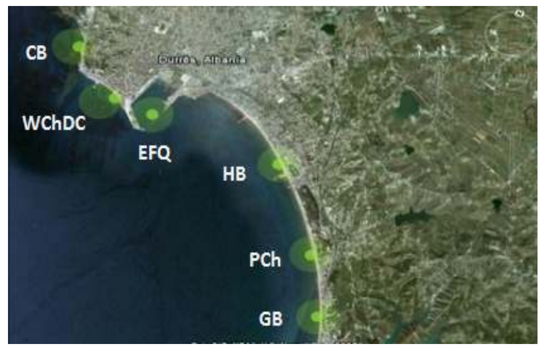

Fig. 1. Locations of sampling stations at Durres Bay (from right to left Golemi Beach (GB), Plepa Channel (PCh), Hekurudha Beach (HB), Ex-Fuel Quay in Marine Durres Harbour (EFQ), Water Channel of Durrës City (WChDC) and Currila Beach (CB). (Google Earth, 2014).

3.2 PO_4-P Estimates

Surface water phosphate (PO_4-P) concentration for all stations for the period May - October 2011 and June - October 2012 ranges between 0.22 – 6.32 µM [27-30].

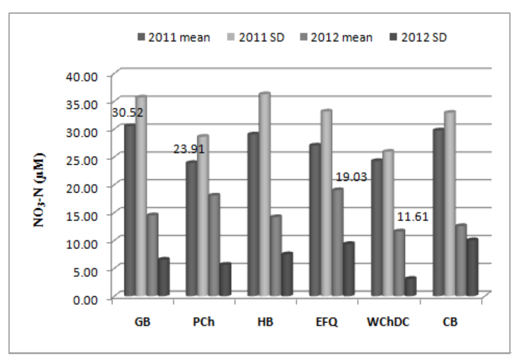

Fig. 2. Nitrogen concentrations as NO_3-N (mean ± standard deviation, µM) during the period of study, according to sample stations: GB, ChP, HB, EFQ, WChDC and CB

In 2011, PCh had the highest PO_4-P concentration (2.11±2.16 µM). Then, were ranked WChDC, EFQ, HB, CB, whereas the lowest concentration was in GB (1.47 µM ±1.78) (Table 1, Fig. 3).

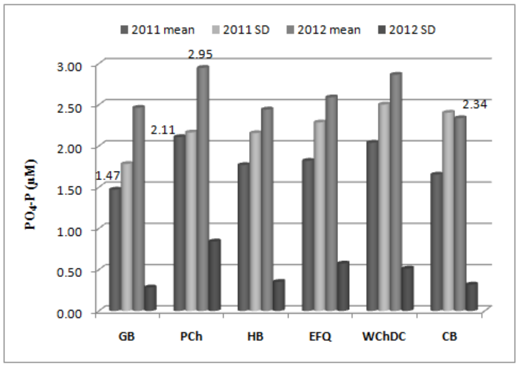

Fig. 3. Phosphorus concentrations as PO_4-P (mean ± standard deviation, µM) during the period of study according to sample stations: GB, ChP, HB, EFQ, WChDC and CB

Phosphorus values in 2011 are considered higher than the recommended standard of PO_4-P (0.16 - 0.65 µM) converted according to ICES for surface waters in natural conditions [23,27,29].

Referring to 2012, ChP had the highest PO_4-P concentration (2.95 µM ±0.84). Then were ranked WChDC, EFQ, GB, HB. CB resulted with the lowest concentration (2.34 µM ±0.32).

Phosphorus values in 2012 were higher than the recommended standard of PO_4-P (0.16 - 0.65 µM) for surface waters in natural conditions [23,27,29].

PO_4-P concentration in 2012 was nearly 1.4 times higher than in 2011, reporting an increase in phosphorus loads of coastal water bodies. It is probable that wastewater discharges, like sewage, especially from PCh and WChDC channels, may have increased the amount of phosphorus loads in coastal water bodies.

If we compare total average of PO_4-P (2.17±1.61 µM) concentration for 2011 and 2012 in Durres Bay to PO_4-P (0.04 ± 0.03 µM) in open water surface in Southern Adriatic Sea [22] converted according to ICES, it resulted that the concentration of phosphorus in Durres Bay was 54 times higher than in Southern Adriatic Sea, and 3.5 times higher than the highest level recommended standard of phosphorus for surface waters in natural conditions. Unlike coastal waters, the open waters surface of Southern Adriatic Sea are approximately 4 times better than the lowest level recommended standard of phosphorus for surface waters in natural conditions (Table 1) [22,23]. The result in Durres Bay shows that there is no or negligible relationship (r = -0.179) between total *Chl a* and PO_4-P (Table 2, Fig. 4 D).

3.3 Estimating Nutrient Limitation Based on N/P Ratio in Durres Bay and the Relationship of Total *Chl a* to NO_3-N, PO_4-P and NO_3-N / PO_4-P

There is a very strong positive relationship between N/P and NO_3-N (r = 0.774, p-value ≤ 0.01), and a moderate negative relationship between N/P and PO_4-P (r = -0.359, p-value ≤ 0.01) (Tab. 2; Fig.4 A, B). This shows that N/P ratio is influenced more from nitrogen than phorosphorus in Durres Bay [31].

Based on the N/P ratios calculated from NO_3-N and PO_4-P values taken from Table 2, stations of Durres Bay, Durres Bay mean 2011, Durres Bay mean 2012, Durres Bay mean 2011 and 2012, and Southern Adriatic Sea mean are classified in N limitation, P limitation or both N and P co-limitation of phytoplankton primary production [3,4,32,33].

In 2011, it resulted that WChDC and PCh had the lowest N/P ratios, respectively 11.9 and 11.34. So there was N limitation of primary production growth in WChDC and PCh.

GB and CB had highest ratios N/P respectively 20.75 and 18.00. So there was P limitation of phytoplankton growth in these coastal water bodies, especially in GB. Whereas HB and EFQ had ratios N/P respectively 16.41 and 14.68, showing co-limitation of N and P of primary production. So in 2011, the stations were divided in three types of nutrient limitation of phytoplankton primary production.

It was found that only in WChDC and PCh, nitrogen (N) was the dominant limiting nutrient. As the sewage waters were discharged permanently in WChDC and PCh, a good quantity of nutrients' loads was brought in these stations. May be this explains the lowest ratios N/P, favoring N limitation in WChDC and PCh.

The results in GB and CB and in HB and EFQ, respectively P limitation and co-limitation of N and P of primary production, are in line with recent estuarine and coastal studies [15-20].

Table 1. NO_3-N (µM) and PO_4-P (µM), N/P and *Chl a* (µg/l) ratios according to sample stations: GB, ChP, HB, EFQ, WChDC and CB in 2011, 2012, and Southern Adriatic Sea mean [22]

		NO3-N (µM)	PO4-P (µM)	N/P	Limiting factor	*Chl a* (µg/l)
2011	GB	30.52±35.68	1.47±1.78	20.75	P	47.22±17.45
	PCh	23.91±28.62	2.11±2.16	11.34	N	41.14±21.15
	HB	29.04±36.26	1.77±2.16	16.41	N & P	49.72±17.22
	EFQ	27.04±33.16	1.82±2.29	14.86	N & P	56.28±20.46
	WChDC	24.26±25.93	2.04±2.50	11.90	N	53.11±16.76
	CB	29.76±32.93	1.65±2.40	18.00	P	28.75±14.08
	Mean	27.42±30.03	1.81±2.07	15.15	N & P	46.04±19.04
2012	GB	14.51±6.55	2.46±0.28	5.89	N	21.91±8.51
	PCh	18.06±5.63	2.95±0.84	6.13	N	24.47±9.31
	HB	14.19±7.51	2.44±0.35	5.80	N	21.27±10.10
	EFQ	19.03±9.36	2.59±0.57	7.35	N	20.55±7.31
	WChDC	11.61±3.10	2.86±0.51	4.05	N	26.44±11.30
	CB	12.58±10.03	2.34±0.32	5.38	N	16.26±8.20
	Mean	15.00±7.28	2.61±0.52	5.75	N	21.82±8.99
2011 & 2012	Total mean	21.77±23.41	2.17±1.61	10.02	N	35.03±19.47
	Southern Adriatic Sea	0.57±0.47	0.04±0.03	14.25	N & P	

In 2011, the mean N/P for all stations was 15.15. This clearly shows, the existence of N and P co-limitation of phytoplankton classified in hypertrophic level according to total *Chl a* mean in 2011 (46.04±19.04 µg/l). Based on Håkanson et al. [34] coastal water systems of different trophic levels based on the content of Chl a could be classified into four trophic classes: oligotrophic (Chl a < 2 µg/l), mesotrophic (2-6 µg/l), eutrophic (6-20 µg/l), hypertrophic (> 20 µg/l) [27-29].

In 2012, the ratios N/P in all stations resulted in N limitation, as in the more saline downstream estuarine and coastal waters. The highest N/P ratio value was in EFQ (7.35). Then, were ranked ChP, GB, HB, CB. The lowest N/P ratio was WChDC (4.05).

This result is in line with the result reported from Billen et al. [35], who reports that the N/P ratio in nutrient loads from urban areas tends to be quite low, further favoring N limitation in the coastal systems that receive such loads, it is especially evident in WChDC, where there is a permanent discharge of wastewater including sewage.

If we compare the total mean N/P of 2011 to 2012, it is reported that this ratio has decreased significantly (from 15.15 to 5.75), passing from co-limitation of N and P, to limitation of N for primary production. So in 2012, there was a N limitation of phytoplankton classified in hypertrophic level according to total *Chl a* mean in 2011 (21.82 µg/l ±8.99).

Total mean of N/P ratio of 2011 and 2012 (10.02) indicated that N had been identified as the limiting nutrient in Durres Bay. This result is in line with the result reported from Billen et al. [35], where N/P ratio in nutrient loads from urban areas tends to favor N limitation in the coastal systems that receive such loads. Whereas N/P ratio (14.25) of open water surfaces in Southern Adriatic Sea [22] shows that there is co-limitation of N and P.

The offshore central and southern Adriatic, however, show clearly oligotrophic characteristics [36] and the primary production cycle is regulated by the nutrient supply to the euphotic zone from the deep part of the water column by different upwelling and mixing processes [37]. In oligotrophic environments where ambient nutrient concentrations are extremely low, the paradigm of a single nutrient limiting phytoplankton may not always be valid, and the autotrophic community can be co-limited by multiple nutrients [38]. Similar results, like the one about Southern Adriatic Sea, are reported for the Eastern Mediterranean [39] and for the oligotrophic Central North Atlantic [33], where phytoplankton community was shown to be N and P co-limited.

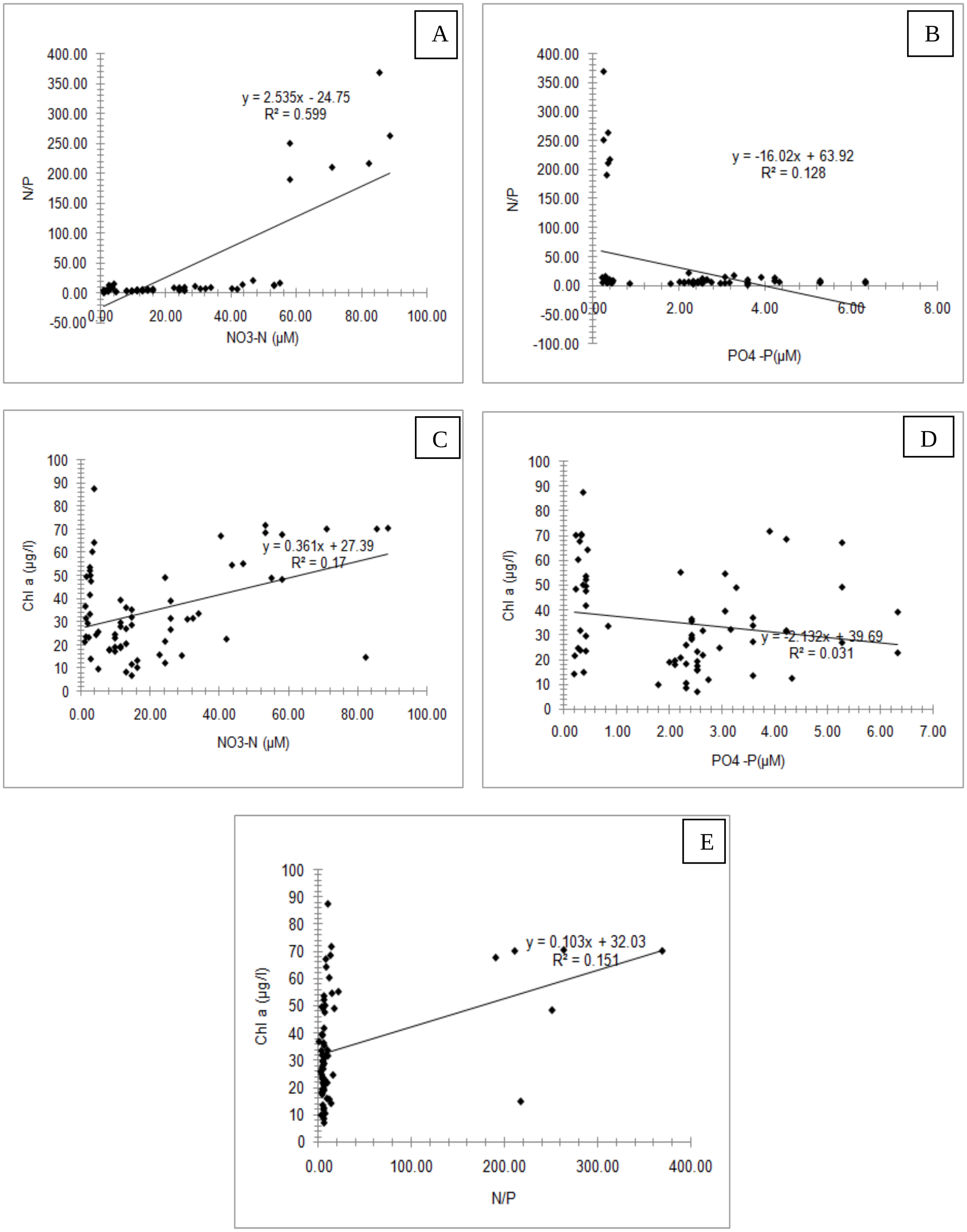

Fig. 4. Relationships: A) N/P to NO_3-N (μM); B) N/P to PO_4-P (μM); C) *Chl a* (μg/l) to NO_3-N; D) *Chl a* (μg/l) to PO_4-P; E) *Chl a* (μg/l) to N/P; according to data collected in sample stations: GB, ChP, HB, EFQ, WChDC and CB

Table 2. The correlation matrix, expressing Pearson correlation coefficients between parameters: NO_3-N (µM) and PO_4-P (µM), N/P and *Chl a* (µg/l)

	NO3-N	***PO4-P***	***N/P***	***Chl a***
NO_3-N	1.000			
PO_4-P	.137	1.000		
N/P	.774	-.359	1.000	
Chl a	.412	-.179	.389	1.000
Legend:	66	sample size		
	± .242	critical value .05 (two-tail)		
	± .315	critical value .01 (two-tail)		

We observed a strong positive correlation (r = 0.412; p-value ≤ 0.01) between total *Chl a* and NO_3-N (Table 2, Fig. 4C), that explains the strong positive relationship of primary production and nitrogen in coastal waters. On the other hand, there is no or negligible relationship (r = -0.179) between total *Chl a* and PO_4-P (Table 2, Fig. 4D), from which it is inferred that there is no relationship of primary production and phosphorus in Durres Bay coastal waters. Finally, there is a moderate positive relationship (r = 0.389; p-value ≤ 0.01) between total Chl a and N/P (Table 2, Fig. 4E). This of course agrees with Fig. 4C, and confirms the moderate positive relationship of primary production and N/P ratio in coastal waters of Durres Bay.

4. CONCLUSIONS

Shallow coastal waters of Durres Bay had N limitation of primary production, whereas open waters had both N and P co-limitation for primary production, based on N/P ratios calculated on NO_3-N and PO_4-P mean. It was highlighted a strong positive relationship between primary production (total *Chl a*) and N, and a moderate positive relationship between primary production and N/P ratio in coastal waters of Durres Bay, while no relationship between total *Chl a* and PO_4-P was shown. In Durres Bay, the concentration of NO_3-N and PO_4-P was higher than permitted standards for surface waters, indicated even from *Chl a* levels for hypertrophic characteristics, while in the Southern Adriatic Sea these concentrations had been presented within standards and show clearly oligotrophic characteristics. The lowest N/P ratio was found in WChDC, as wastewaters like sewage were discharged frequently. This discharge favors a quite low N/P ratio. This distribution pattern may be attributed mostly to the anthropogenic influence of the surface waters in high N and P loads, which makes the N/P ratio values differ; easily passing from P to N limitation or N and P co-limitation of primary production in marine coastal waters of Southern Adriatic Sea, like Durres Bay. Excessive loads of nutrients can cause the eutrophication of coastal waterways. Therefore it is recommended to build sanitation, especially in Golem area, a process that has already begun, and to use plants in order to process sewage and other polluted waters from city Durres.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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