

**ESTIMATION OF N/P RATIOS LEVELS IN A COASTAL BAY,
SOUTHERN ADRIATIC SEA**

ABSTRACT

The optimal DIN:DIP atomic ratio (N/P ratio) considered optimal for phytoplankton growth is 16:1 (bases on molar concentration) and is called the (Redfield ratio). Significant deviations from 14 at low N/P ratios might indicate potential nitrogen limitation, ratio between 14 and 16 both nitrogen and phosphorus co-limitation and from 16 at high N/P ratios potential phosphorus limitation of phytoplankton primary production. This might affect to biological state of the ecosystem, in particular the phytoplankton biomass, species composition and eventually food web dynamics. NO_3^- and PO_4^{3-} concentrations of surface waters in six station samples were measured with spectrophotometer UV-VIS monthly whereas total Chl *a* using flourometric method from May - October 2011 and from June - October 2012. The data are converted on molar concentration ($\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$) to calculate N/P ratios. Comparing N/P ratios of year 2011 to 2012 in Durres Bay, results that in 2011 both N and P co-limitation, whereas in 2012 N limitation of primary production. Comparing N:P ratio of both 2011 and 2012 years in Durres Bay to Southern Adriatic Sea, it resulted that in Durres Bay had N limitation, whereas in open waters both N and P co-limitation of primary production. 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.

In Durres Bay the concentration of $\text{NO}_3\text{-N}$ & $\text{PO}_4\text{-P}$ were higher than permitted standards for surface waters, while in the Southern Adriatic Sea these concentrations had been presented within standards, show clearly oligotrophic characteristics.

KEYWORDS: N/P ratio, horizontal distribution, Southern Adriatic Sea, coastal waters.

INTRODUCTION

Since the observation of Redfield (Redfield, 1934, 1958) 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 which nutrient restricts eutrophication. If the ratio N: P > 16, phosphorus (P) is a limiting factor for the growth of algae (Redfield *et al.*, 1963; Hodgkiss & Lu, 2004).

If N: P is between 14 and 16, are both N and P limiting factors (Koerselman & Meuleman, 1996; Mills *et al.*, 2008) and if the ratio N: P < 14 indicates that nitrogen (N) is the limiting factor for the growth of algae (Redfield *et al.*, 1963; Hodgkiss & Lu, 2004).

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 (Ryther & Dunstan 1971; Schindler 1975; Nixon 1995; Boesch *et al.*, 2001; Smith & Schindler 2009). Recent analyses of diverse nutrient limitation studies in both freshwater and marine ecosystems indicate that these paradigms may be "eroding" (Elser *et al.*, 2007; Lewis & Wurtsbaugh 2008; Sterner 2008; Conley *et al.*, 2009; Lewis *et al.*, 2011). 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 (Elser *et al.*, 2007;

Lewis *et al.*, 2011). Concurrently, recent estuarine and coastal studies indicated that N and P or P limitation are geographically widespread (Peeters & Peperzak 1990; Elmgren & Larsson 2001; Sylvan *et al.*, 2006; Paerl & Justić 2011; Laurent *et al.*, 2012; Paerl *et al.*, 2014).

Durres Bay is located between Selita Cape (Lagj) in south and Durres Cape in north, that are 20 km far away from each others. 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 there are dominated storage processes, and high coast where are dominated abrasion processes. The first coast covers from Dajlan Bridge to Karpen, whereas the second coast covers the Karpen-Lagj Cape and Currila area (www.updurres.com.al).

The first aim of this study was to estimate limiting nutrient of phytoplankton primary production basing on N/P ratios and studying relationship like *Chl a* to $\text{NO}_3\text{-N}$, *Chl a* to $\text{PO}_4\text{-P}$ and *Chl a* to N/P in surface waters of Durres Bay.

Second aim was comparing average concentrations of $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ of coastal waters to average concentrations of $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ in surface open waters as Southern Adriatic Sea basing on previous study (Manca *et al.*, 2004).

Also another aim was comparing $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ concentrations of Durres Bay with the recommended standard of $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ for surface waters in natural conditions (Chapman, 1996).

MATERIALS AND METHODS

Samples were monthly collected 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 from the water surface. Sea water (~ 2 l) was collected for NO_3^- and PO_4^{3-} , total *Chl a* and other investigation purposes (what are they?). Nitrate NO_3^- and PO_4^{3-} concentrations of surface waters in six station samples were measured with spectrophotometer UV-VIS, whereas total *Chl a* using flourometric method (Idromar sonde) according to APHA *et al.* (2005) and Grasshoff *et al.* (1999). The plastic container was filled up, and preserved in an ice chest. Thereafter, the samples were taken to the laboratory for analyses.



Figure 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).

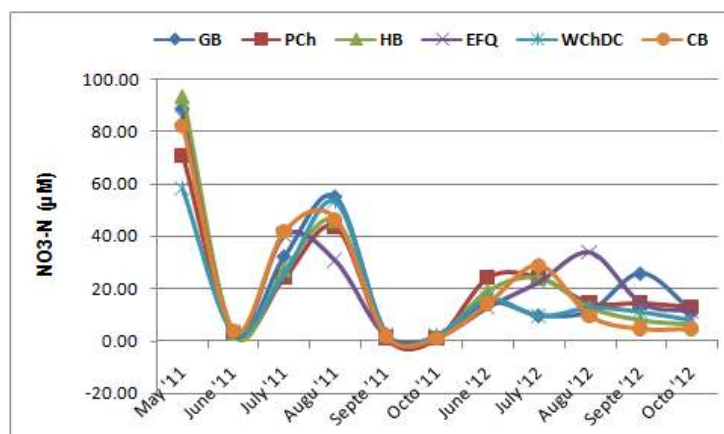
NO_3^- and PO_4^{3-} are reported in $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ in μM , and the N/P ratios was computed, according to ICES (International Council for the Exploration of the Sea; <http://ocean.ices.dk/Tools/UnitConversion.aspx>). Statistical programs were Excel (2007) and MegaStat.xla (2007). Pearson correlation coefficients ($p < 0,05$ and $p < 0,01$) were measured through program MegaStat.xla (2007).

83

84 RESULTS AND DISCUSSIONS

85 3.1 [REDACTED] NO₃-N [REDACTED] estimates

86 Surface water of nitrate (NO₃-N) concentration for all stations for period May - October 2011 and from
 87 June - October 2012 ranged between 0.97 – 93.54 μM, respectively October 2011 in PCh and May 2011
 88 in HB (Fig. 2) (Gjyli & Bacu, 2014; Gjyli *et al.*, 2013a, b; Gjyli & Kolitari, 2011).
 89 In 2011, GB had the highest NO₃-N concentration (30.52 μM ± 35.68) (Tab. 1). Then there were ranked
 90 CB, HB, EFQ, WChDC, whereas the lowest concentration was in PCh (23.91 μM ± 28.62).
 91 Nitrogen values of 2011 were higher than the recommended standard of NO₃-N (≤ 7.14 μM) converted
 92 according ICES for surface waters in natural conditions (Chapman, 1996; Gjyli *et al.*, 2013b; Gjyli &
 93 Bacu, 2014).
 94 Referring 2012, EFQ had the highest NO₃-N concentration (19.03 μM ± 9.36). Then there were ranked
 95 PCh, GB, HB, CB whereas the lowest concentration was in WChDC (11.61 μM ± 3.10).
 96 Nitrogen values of 2012 were higher than the recommended standard of NO₃-N (≤ 7.14 μM) for surface
 97 waters in natural conditions (Chapman, 1996; Gjyli *et al.*, 2013b; Gjyli & Bacu, 2014).
 98 NO₃-N concentration of 2012 was 1.8 times smaller than 2011, improving situation of coastal waters in
 99 Durres Bay.



100

101 **Figure 2:** Nitrogen concentrations as NO₃-N (μM) during period of study according sample stations: GB, ChP, HB,
 102 EFQ, WChDC and CB.

103 If we compare total average of NO₃-N (21.77 ± 23.41 μM) concentration for years 2011 and 2012 of
 104 Durres Bay to NO₃-N (0.57 μM ± 0.47) of surface open waters in Southern Adriatic Sea (Manca *et al.*,
 105 2004) converted according ICES, it resulted that the concentration of nitrogen in Durres Bay was 38 times
 106 higher than in Southern Adriatic Sea and 3 times higher than the recommended standard of nitrogen for
 107 surface waters in natural conditions. Unlike coastal waters, the surface open waters of Southern Adriatic
 108 Sea are approximately 13 times better than the recommended standard of nitrogen for surface waters in
 109 natural conditions (Tab. 1) (Chapman, 1996; Manca *et al.*, 2004). There is a positive correlation (r =
 110 0.412; p-value ≤ 0.01) between total Chl *a* and NO₃-N (Table 2, Fig.4 C). This explains the relationship of
 111 primary production and nitrogen in coastal waters.

112 3.2 [REDACTED] PO₄-P [REDACTED] estimates

113 Surface water phosphate (PO₄-P) concentration for all stations for period May - October 2011 and from
 114 June - October 2012 range between 0.22 – 6.32 μM (Fig. 3) (Gjyli & Bacu, 2014; Gjyli *et al.*, 2013a, b;
 115 Gjyli & Kolitari, 2011).

In 2011, PCh had the highest PO₄-P concentration ($2.11 \pm 2.16 \mu\text{M}$). Then there were ranked WChDC, EFQ, HB, CB, whereas the lowest concentration was in GB ($1.47 \mu\text{M} \pm 1.78$) (Tab. 1). Phosphorus values of 2011 are considered higher than the recommended standard of PO₄-P ($0.16 - 0.65 \mu\text{M}$) converted according ICES for surface waters in natural conditions (Chapman, 1996; Gjyli *et al.*, 2013b; Gjyli & Bacu, 2014). Referring 2012, ChP had the highest PO₄-P concentration ($2.95 \mu\text{M} \pm 0.84$). Then there were ranked WChDC, EFQ, GB, HB whereas the lowest concentration is CB ($2.34 \mu\text{M} \pm 0.32$). Phosphorus values of 2012 were higher than the recommended standard of PO₄-P ($0.16 - 0.65 \mu\text{M}$) for surface waters in natural conditions (Chapman, 1996; Gjyli *et al.*, 2013b; Gjyli & Bacu, 2014). PO₄-P concentration of 2012 was nearly 1.4 times higher than 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 be increased the amount of phosphorus loads in coastal waters bodies.

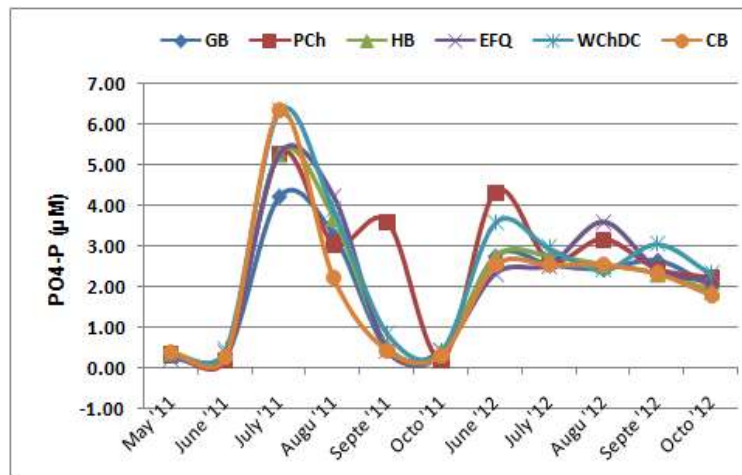


Figure 3: Phosphorus concentrations as PO₄-P (μM) during period of study according sample stations: GB, ChP, HB, EFQ, WChDC and CB.

If we compare total average of PO₄-P ($2.17 \mu\text{M} \pm 1.61$) concentration for years 2011 and 2012 of Durres Bay to PO₄-P ($0.04 \pm 0.03 \mu\text{M}$) of surface open waters in Southern Adriatic Sea (Manca *et al.*, 2004) converted according 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 surface open waters of Southern Adriatic Sea are approximately 4 times better than the lowest level recommended standard of phosphorus for surface waters in natural conditions (Tab. 1) (Chapman, 1996; Manca *et al.*, 2004). In Durres Bay results there is no or negligible relationship ($r = -0.179$) between total *Chl a* and PO₄-P (Table 2, Fig.4 D). This explains that there is no relationship of primary production and phosphorus in coastal waters.

3.3. Estimating nutrient limitation basing on N/P ratio in Durres Bay and relationship of total *Chl a* to NO₃-N, PO₄-P and NO₃-N / PO₄-P

There is a very strong positive relationship between N/P and NO₃-N ($r = 0.774$, $p\text{-value} \leq 0.01$), while a moderate negative relationship between N/P and PO₄-P ($r = -0.359$, $p\text{-value} \leq 0.01$) (Tab. 2; Fig.4 A, B). So N/P ratio is influenced more from nitrogen than phosphorus in Durres Bay (<http://www.solving-math-problems.com/statistics-pearson-correlation.html>).

Basing on the N/P ratios calculated from NO₃-N and PO₄-P values taken in 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 (Redfield *et al.*, 1963; Hodgkiss & Lu, 2004 Koerselman & Meuleman, 1996; Mills *et al.*, 2008).

In 2011, it resulted WChDC and PCh had 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 for co-limitation of N and P of primary production. So in 2011, the stations were divided in all three types of nutrient limitation of phytoplankton primary production.

As it resulted only in WChDC and PCh, nitrogen (N) was the dominant limiting nutrient. As the sewage waters were discharged permanently in WChDC and PCh, here there was brought a good quantity of nutrients loads in these stations. May be this the reason of 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 (Peeters & Peperzak, 1990; Elmgren & Larsson, 2001; Sylvan *et al.*, 2006; Paerl & Justić, 2011; Laurent *et al.*, 2012; Paerl *et al.*, 2014).

In 2011, the mean N/P for all stations was 15.15. So there was a N and P co-limitation of phytoplankton classified in hypertrophic level according total Chl *a* mean in 2011 ($46.04 \pm 19.04 \mu\text{g/l}$). Basing on Håkanson *et al.* (2007) coastal water systems of different trophic levels based on the content of Chl *a* could be classified into four trophic classes: oligotrophic (Chl *a* < $2 \mu\text{g/l}$), mesotrophic ($2-6 \mu\text{g/l}$), eutrophic ($6-20 \mu\text{g/l}$), hypertrophic ($> 20 \mu\text{g/l}$) (Gjyli *et al.*, 2013a, b; Gjyli & Bacu, 2014).

Table 1: NO₃-N (μM) and PO₄-P (μM), N/P and Chl *a* ($\mu\text{g/l}$) ratios according sample stations: GB, ChP, HB, EFQ, WChDC and CB in years 2011, 2012, and Southern Adriatic Sea mean (Manca *et al.*, 2004).

		NO ₃ -N (μM)	PO ₄ -P (μM)	N/P	Limiting factor	Chl <i>a</i> ($\mu\text{g/l}$)
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 2012, ratios N/P in all stations resulted N limitation, as in the more saline downstream estuarine and coastal waters. The highest N/P ratio value was in EFQ (7.35). Then there were ranked ChP, GB, HB, CB, whereas the lowest N/P ratio was WChDC (4.05).

This result is in line with the result reported from Billen *et al.* (1991), which report 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, especially it is evident in WChDC, where there is permanent discharge of wastewater including sewage.

If we compare the total mean N/P of 2011 to 2012, it is reported that this ratio is decreased significantly (from 15.15 to 5.75), passing from co-limitation of N and P, to limitation of N P for primary production.

So in 2012, there was a N limitation of phytoplankton classified in hypertrophic level according total *Chl a* mean in 2011 ($21.82 \mu\text{g/l} \pm 8.99$).

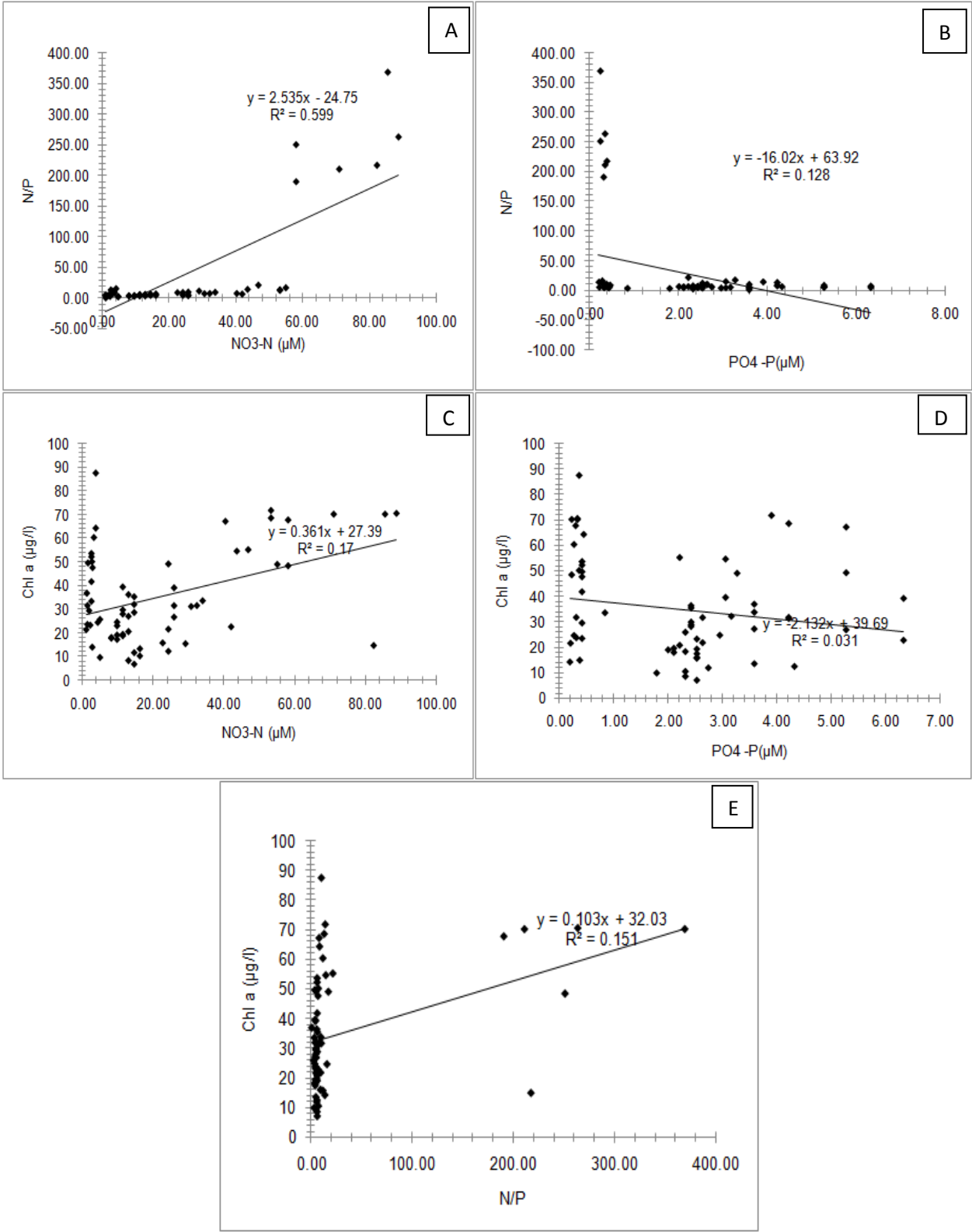


Figure 4: Relationships: A) N/P to NO₃-N (μM); B) N/P to PO₄-P (μM); C) *Chl a* (μg/l) to NO₃-N; D) *Chl a* (μg/L) to PO₄-P; E) *Chl a* (μg/L) to N/P; according data collected in sample stations: GB, ChP, HB, EFQ, WChDC and CB.

Table 2: The correlation matrix, expressing Pearson correlation coefficients between parameters: NO₃-N (μM) and PO₄-P (μM), N/P and *Chl a* (μg/l).

	NO3-N	PO4-P	N/P	Chl a
NO3-N	1.000			
PO4-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)

Comparing total mean of N/P ratio (10.02) of 2011 and 2012 years in Durres Bay to N/P ratio (14.25) of surface open waters in Southern Adriatic Sea (Manca *et al.*, 2004), it is resulted that N had been identified the limiting nutrient in Durres Bay. This result is in line with the result reported from Billen *et al.* (1991), where N/P ratio in nutrient loads from urban areas tends to favoring N limitation in the coastal systems that receive such loads.

Whereas in surface waters of the Southern Adriatic Sea there is co-limitation of N and P (Manca *et al.*, 2004). The offshore central and southern Adriatic, however, show clearly oligotrophic characteristics (Vilicic *et al.*, 1989) 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 (Zavatarelli *et al.*, 2000). 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 (Arrigo, 2005). Similarly results like Southern Adriatic Sea, there are reported for the Eastern Mediterranean (Thingstad *et al.*, 2005) and for the oligotrophic Central North Atlantic (Mills *et al.*, 2008), where phytoplankton community was shown to be N and P co-limited.

We observed a strong positive correlation ($r = 0.412$; $p\text{-value} \leq 0.01$) between total *Chl a* and NO₃-N (Table 2, Fig.4 C), 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 PO₄-P (Table 2, Fig.4 D), 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 ($r = 0.389$; $p\text{-value} \leq 0.01$) between total *Chl a* and N/P (Table 2, Fig.4 E). This of course agrees with Figure 4 C, and confirms the moderate positive relationship of primary production and N/P ratio in coastal waters of Durres Bay.

CONCLUSIONS

Shallow coastal waters of Durres Bay had N limitation of primary production, whereas in open waters were both N and P co-limitation for primary production, basing on N/P ratios calculated on NO₃-N and PO₄-P mean. It was highlighted a strong positive relationship between primary production (total *Chl a*) and N, in coastal waters 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 PO₄-P was showed. In Durres Bay, the concentration of NO₃-N and PO₄-P were higher than permitted standards for surface waters, showing also from *Chl a* levels hypertrophic characteristics, while in the Southern Adriatic Sea these concentrations had been presented within standards, show clearly oligotrophic characteristics. The lowest N/P ratio it was 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 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, process that has begun, and to build plants to process sewage and other polluted waters of city Durres.

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