J. Black Sea/Mediterranean Environment
Vol. 19, No. 3: 299-311 (2013)

RESEARCH ARTICLE

Determination of limited nutrients in the Turkish coastal waters of the Mediterranean and Aegean Seas

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Abstract

Increased human activities, rapid and uncontrolled industrial development, intensive urbanization have polluted and hence drastically modified the Turkish coastal water ecosystem of North East (NE) Mediterranean and Aegean Seas. Assessments of nutrient budgets and limiting nutrients in aquatic systems are of wide interest because of their fundamental importance in understanding carbon fixation, coastal eutrophication and wastewater management. In this study, the relative importance of nitrogen and phosphorus as potential limiting factors for primary producers during spring and summer seasons of 2009 was investigated in the near surface water along the Turkish coasts of the NE Mediterranean and Aegean Seas by using the ^{14}C bioassay technique. The results of bioassays and nutrient concentrations (DIN, PO_4, Si) indicate that phosphorus is primarily limiting factor in the less contaminated, nutrient-depleted coastal waters of NE Mediterranean (including the Aegean Sea). However, nitrogen appears to be potential limiting element in heavily polluted semi-closed coastal zones (Izmir and Edremit Bays of the Aegean Sea) due to large organic and nutrient loads from industrial and domestic sources in the last decades and consequent occurrence of intense denitrification in the elongated Izmir Bay, reducing the N/P ratio in the shallow water column.

Keywords: Eutrophication, nutrients, limiting nutrient, phytoplankton.

Introduction

The coastal areas of NE Mediterranean and Aegean Seas are of considerable socio-economic importance for Turkey since they are engaged in large scale tourism, fishing and port activities. Therefore, wastewaters of industrial, agricultural and domestic activities, which are the main sources of nutrients and organic pollutants, are discharged to the nearshore waters. Terrestrial phosphate input into the oligotrophic Mediterranean Sea has increased considerably since 1960 (Karafistan *et al.* 2002). The eastern Mediterranean Sea including Turkish coastal waters is known to be one of the oligotrophic seas in the world due to the limited nutrient inputs from external (riverine, atmospheric) (Koçak *et al.* 2010) and internal sources and hydro-chemical characteristics of water masses with high N/P ratio (>25) in the phosphate-depleted deep waters (Krom *et al.* 1991; Yılmaz and Tugrul 1998).

Growth of algae in aquatic ecosystems is frequently limited by the availability of nutrients. Historically, nitrogen and phosphorus (Schindler 1977; Wynne and Berman 1980; Birch *et al.* 1981; Lean and Pick 1981) are believed to be the major potential nutrients commonly limiting algal production in marine and fresh-water ecosystems (Hecky and Kilham 1988; Dodds *et al.* 1993). Reactive phosphorus is the potential limiting factor in some marine ecosystems such as the North Pacific Sub-tropical Gyre (Karl 1999) and some regions of the eastern Mediterranean Sea (Krom *et al.* 1991, 2004). The ability to identify limiting nutrients is of considerable importance to our understanding of the ecology of algae and for guiding water management practices in eutrophic coastal waters.
Eutrophication is defined as an increase in the rate of supply of nutrient compounds to aquatic ecosystems (Nixon 1995). Its main cause is a high nutrient enrichment of water, especially nitrogen and/or phosphorus, enhancing phytoplankton growth. Thus increasein the organic production in the surface water can lead to a reduction of dissolved oxygen in the water column and to hypoxia in extreme cases as experienced in the Baltic Sea and the polluted bays of Izmit and Izmir (Bonsdorff *et al.* 1997; Morkoç *et al.* 1997 and 2007; Balcı *et al.* 1995; Küçüksezgin *et al.* 2001).

The coastal regions of the eastern Mediterranean Sea, one of the most oligotrophic seas with low levels of nutrients and primary production (Azov 1991; Krom *et al.* 1991; Ediger *et al.* 1999; Yılmaz and Tugrul 1998), are very sensitive to land-based pollution. Particularly semi-enclosed shallow coastal areas receiving wastewater and riverine discharges are the potential regions for eutrophication due to their limited wastewater assimilation capacity by physical and biochemical processes (Izzo and Pagou 1999). For example, Izmir, Mersin and Iskenderun Bays are highly influenced by terrestrial inputs, leading to the formation of eutrophic conditions in the nearshore zones whereas the offshore waters still have oligotrophic properties (Tugrul *et al.* 2011; Doğan-Sağlamtimur and Tuğrul 2004, 2008; MEDPOL 2008). Mersin Bay is situated on the wide and shallow shelf zone of the Cilician Basin, NE Mediterranean Sea. Therefore, the inner bay is isolated from the general circulation patterns of the NE Mediterranean Basin as well as having limited water exchange with the open sea (Yumruktepe 2011). The nearshore waters of the bay receive large loads of nutrients and organic pollutants from the major rivers and by direct discharges of partly treated municipal wastewater. Thus, the nearshore waters become eutrophic, persisting during the year. However, the offshore waters (depth>50m) still remain oligotrophic with low concentrations of nitrate and phosphate in the euphotic zone (Doğan-Saglamtimur and Tugrul 2004, 2008; Ediger *et al*. 1999).

Located in the northern corner of the eastern Mediterranean, Iskenderun Bay adjoins one of the biggest continental shelf areas in this region. The coasts of Iskenderun Bay are heavily populated and industrialized. Environmental perturbations resulting from human activities cause drastic changes in the nearshore ecosystem. The Ceyhan River is one of the major rivers on the NE Mediterranean coast of Turkey, discharging nutrient-rich freshwater into the outer bay. In addition, maritime traffic is heavy in the bay due to industrial facilities, crude oil pipelines and loading facilities. In spite of these effects, the bay water has not reached eutrophic conditions because of sufficient ventilation of the central and outer bay waters by general circulation patterns of the NE Mediterranean (Özsoy 1989; Polat 2002).

Izmir Bay (western Turkey) is one of the great semi-enclosed bays of NE Mediterranean. The heavy urbanization, industrial development and maritime traffic have resulted in increased pollution in the bay; thus the inner bay is heavily polluted by nutrients and organic substances. Izmir Bay is impacted by sewage and other eutrophication sources, leading to the formation of hypoxia in the inner bay with lower DIN/PO4 ratios (Bizsel *et al.* 2001; Kontas *et al.* 2004). Phosphate, which originates from detergents, is an important source for eutrophication in the enclosed bays receiving large loads of domestic discharges as observed in Izmir Bay for years. In early 2000, a Wastewater Treatment Plant (WTP) was established to treat domestic and industrial wastes of Izmir. Although the WTP capacity has been sufficient for removal of nitrogen from wastes, it could partly remove phosphate of domestic origin, keeping the DIN/PO4 ratio low (<10) in the treated wastewater. Eutrophication is still a serious problem in the inner bay throughout the year and red tides have been seen frequently in the last decade (MEDPOL 2008; Kontaş *et al.* 2004).

The aim of this study was to understand which nutrient elements primarily control primary production at selected hot points and in less contaminated sensitive areas along the NE Mediterranean and Aegean coasts. In this study, we conducted a series of nutrient manipulation bioassays to assess the relationship between the increased inorganic nutrient concentrations in seawater and carbon uptake rates of phytoplankton communities.

Materials and Methods

Bioassay studies were performed at 10 locations selected as hot points and sensitive areas along the NE Mediterranean and eastern Aegean Seas (6 stations in the Mediterranean coasts, 4 stations in the Aegean coasts). In addition, a total of 7 reference points, approximately 1 nautical mile offshore were defined for each hot point to determine potential limiting nutrients in the least contaminated water body of the study region (Figure 1). The samples for limiting nutrient studies were collected from 2 m depth. Subsamples after filtration through a 200 m net were enriched with defined amounts of nutrients either single or in combination. They were then incubated for 24 hours at a constant temperature and illumination. Samples were spiked with a known amount of $NaH^{14}CO_3$ solution and incubated. After inoculation with 2 µCi of ^{14}C radioactive standard, they were illuminated at constant temperature for 2 hours (Maestrini *et al.* 1984). Then, phytoplankton cells in the samples were collected on membrane filters. The filters were degraded in scintillation liquid. The activity of each sample was counted using liquid scintillation technique (Packard Tri-Carb 1550 Model low level LSC). The ^{14}C uptake of the nutrient enriched sample was compared with the control and those values were taken as a measure of the photosynthetic ability of the samples.

Nutrients (Silicate, NO_3+NO_2-N, ammonia and o-PO_4) were analyzed using a Brann-Luebbe (Technicon) Autoanalyzer II following slightly modified standard methods (Grasshoff *et al.* 1983; APHA, AWWA and WPCF 2005). Samples for Chl-*a* analysis were filtered through GF/F filters. The filters were extracted in 90% acetone in the dark for a night. Following the extraction, absorbance was measured by fluorometric method according to Holm-Hansen *et al.* (1978).

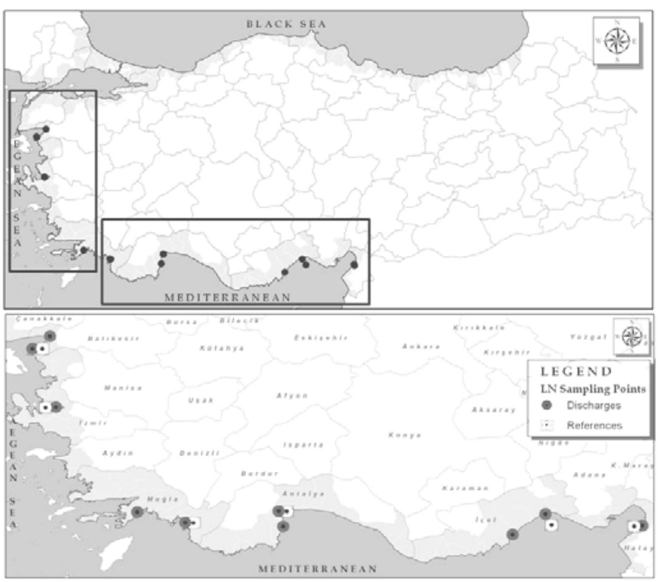

Figure 1. Sampling locations of the nutrient enrichment experiments in the NE Mediterranean and Aegean coastal areas.

Table 1. Sampling points in NE Mediterranean and Aegean Seas, final nutrient concentrations in the samples before 24 hr incubation period

SAMPLING POINTS	Final concentrations of nutrients in the spiked surface waters										
	May 2009			**August 2009**			**October 2009**			**Station Coordinates**	
	NO_3-N µM	PO_4-P µM	Si µM	NO_3-N µM	PO_4-P µM	Si µM	NO_3-N µM	PO_4-P µM	Si µM	N	E
MEDITERRANEAN SEA											
Iskenderun discharge	12,1	0,33	4,2	8,4	1,05	34,87	5,25	1,05	3,90	36°35'48.24"	36°09'13.81"
İskenderun Reference Point	12,1	0,33	3	6,72	1,05	39,6	4,2	1,05	27,28	36°36'36.91"	36°08'33.84"
Mersin Discharge	5,8	2,75	25,8	52,5	1,05	58,3	26,25	1,05	34,65	36°37'13"	34°45'2"
Mersin Reference Point	4,4	0,55	2,4	4,2	1,05	24,2	4,2	1,05	27,28	36°45'34"	34°39'7"
Silifke Kız Kalesi (Maiden’s Castle in Silifke)	8,8	0,66	6	6,5	1,3	14,45	5,2	1,30	24,98	36°27'13.23"	34°08'32.36"
Antalya Discharge	16,5	0,44	22,2	39	1,04		26,25	1,05	23,10	36°49'42.08"	30°37'01.29"
Antalya Reference Point	11	0,44	16,2	26	1,04	36,45	6,5	1,30	20,93	36°49'12.86"	30°38'03.86"
Kemer Discharge	7,7	0,44	12	18,2	1,3	27	4,2	1,05	13,75	36°36'01.59"	30°34'59.67"
Fethiye Discharge	13,2	0,66	32,28	31,2	1,56	72,63	5,25	2,10	56,1	36°38'50.05"	29°06'49.12"
Fethiye Reference Point	3,85	0,44	18	9,1	1,3	40,5	4,62	1,05	41,25	36°39'11.68"	29°05'42.12"
AEGEAN SEA											
Marmaris Discharge	4,4	0,55	1,5	10,4	1,3	20,25	24,20	3,52	24,60	36°48'45.8"	28°18'49.24"
Marmaris Reference Point							5,2	1,3	19,58	36°47’86.3”	28°19’36.5”
Izmir Discharge	6,6	10,56	6,9	2,75	8,25	27	17,6	5,28	27,6	38°27'15.71"	27°00'39.35"
Izmir Reference Point	8,8	10,34	10,8	2,2	0,44	10,2	8,4	1,26	17,6	38°26'18.6"	27°00'53.74"
Ayvalik Discharge	3,3	0,33	6	7,8	1,3	13,5	4,2	1,05	18,7	39°21'07.18"	26°41'20.13"
Edremit Discharge	2,75	0,77	7,98	6,5	1,82	17,96	4,2	1,68	21,01	39°33'24.56"	26°56'52.32"
Ayvalik-Edremit Reference Point	2,2	0,44	4,8	5,2	1,3	10,8	5,25	1,05	18,7	39°21'57.98"	26°40'43.22"

Results

The limiting nutrient stations shown in Figure 1 were visited seasonally in May, August and October, 2009. The experiments were performed at both the discharge zones (about 500 m off the hot points and sensitive areas), and reference points (approximately 1 nautical mile offshore) to assess potential limiting nutrient(s) in the selected coastal regions of the NE Mediterranean and Aegean Seas (Figure 1, Table 1). The bioassay results in Figures 2 and 3 indicate that phosphorus is generally a potential limiting element in both seas with the exception of two hot points (the semi-enclosed bays of Izmir and Ayvalık in the Aegean Sea) heavily polluted by domestic/industrial wastewater discharges. In addition, various combinations of phosphorus, nitrogen, silicate, reduced iron and other trace metal mixtures were also observed to further increase carbon uptake by algae in dry period (summer-autumn) when nutrient concentrations were depleted in the surface waters of the studied sites.

In the inner region of Iskenderun Bay, which is located on the wide shelf zone of NE Mediterranean and receives considerable amount of terrestrial inputs, phosphorus (P) was observed to be a potential limiting factor at the discharge and reference points. In summer-autumn (dry) period, however, the additions of silica, Fe and metal mixtures to the P-enriched subsamples further increased primary production (Figure 2).

Along the coasts of Mersin Bay, three points were selected as the sewage discharge zone, the reference point of Mersin discharge and off Kız Kalesi (Maiden’s Castle in Silifke) in the western bay (Figure 1). While P was expectedly limiting at the Mersin discharge zone, off Kız Kalesi and theirs reference point, weak limiting effects of Si and N can be inferred from the increased ^{14}C uptakes in the (P+Si and N+P) spiked samples (Figure 2).

Antalya Bay receives the large amount of treated domestic waste discharge, surface runoff and riverine input. Bioassays were conducted at three points, Antalya and Kemer discharge zones and a joint reference point between the two discharge points. According to the results of the bioassays, the limiting nutrients at the Antalya discharge point were both P and Si while P was the only limiting nutrient at the reference point (Figure 2).

In the semi-enclosed bay of Fethiye, P and N were both potential limiting factors at the discharge point, whereas the different combinations of P, N and Si further enhanced carbon uptake at the reference point. However, limiting effect of N became apparent in spring but almost disappeared in summer. Addition of metal mixtures also enhanced primary production in autumn even if this effect was limited (Figure 2).

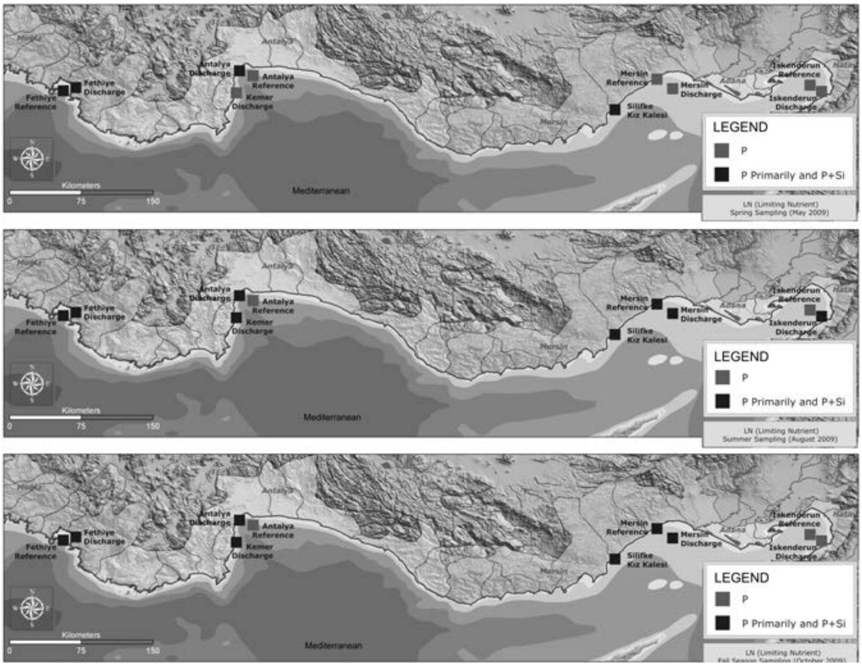

Figure 2. Seasonal variation of nutrient enrichment bioassays in the Mediterranean coasts

The Aegean coasts of Turkey with their natural and cultural heritage are visited by large number of tourists every year, leading to great increases in population especially in summer months. The undulating coast has different sizes of enclosed bays allowing to recreational activities, fishing, yachting and fish farming. The Aegean open sea displays oligotrophic properties. However, eutrophication risk has developed in some semi-enclosed bays of the NE Aegean Sea, such as Izmir and Edremit Bays, which are subject to large loads of domestic and industrial wastewaters.

In the Marmaris discharge zone, different combinations of P, N, Si and trace metal mixtures were observed to enhance primary production in the nutrient-depleted surface waters of the region. However, at the reference station, P and Si both appeared to be potential limiting elements during the dry period (Figure 3).

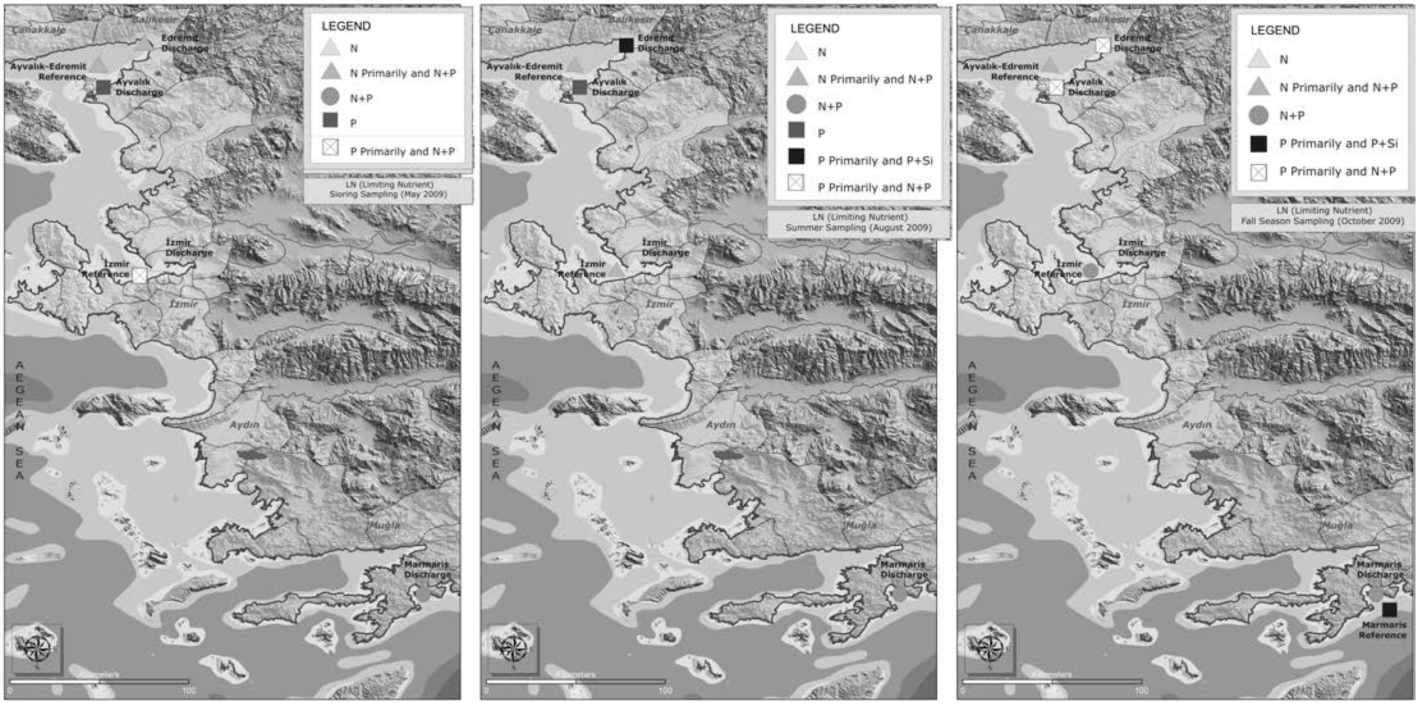

Figure 3. Seasonal variation of nutrient enrichment bioassays in the Aegean coast

The limiting effect of N was seen primarily in the polluted bays of Izmir and Edremit having low ratio of dissolved inorganic nitrogen (DIN: NO_3+NO_2+NH_4-N) to dissolved inorganic phosphorus (DIP) in the surface water. However, the combinations of N, P, Si and metal mixtures further increased the ^{14}C assimilation of the phytoplankton compared to single nutrient addition only. In the summer survey, N, Si and trace metals were all potential limiting elements at the discharge point whilst N was the major limiting factor at the reference point selected in Izmir Bay, followed by P, Si and the mixture of metals (Figure 3).

The coastal waters of Ayvalık Bay are polluted by wastewater discharges from domestic sources and olive processing plants. The bioassay results of Ayvalık, Edremit discharges and the reference point were similar to those obtained in Izmir Bay. It was noted that especially at the Edremit discharge N and trace metals were highly critical elements in summer and autumn and they enhanced carbon uptake, whereas P and Si were be slightly limiting. At the reference point within the bay, the results were almost similar to those observed at the Ayvalık discharge point where the additions of P, N and Si increased inorganic carbon uptake by algae compared to the control (Figure 3). These results imply that they were all potential limiting elements, enhancing algal production in the region.

The nutrient concentrations were also determined to assess spatial variation in the coastal surface waters of the oligotrophic NE Mediterranean and Aegean coasts during the bioassay studies. Reactive phosphate concentration was as low as 0.04-0.05 µM from east to west in dry season, rising to levels of 0.28-1.25 µM in polluted sites in wet period (spring), due to increasing effect of land-based inputs. In May, for example, large loads of phosphorus were introduced to the coastal waters by major rivers of the region (Ceyhan, Seyhan, Berdan) and discharges of partly treated domestic wastewaters of the big cities (Izmir, Iskenderun, Mersin, Antalya, Marmaris).

In the Mediterranean coastal waters, the DIN/DIP (N/P) molar ratios were generally high due to high levels of nitrate concentrations in the NE Mediterranean rivers (Koçak *et al.* 2010). In the Aegean coasts, N/P ratio generally ranged from 7 to 6 in less contaminated, phosphate depleted coastal waters in 2009, decreasing to below 5 in the polluted sites having much higher concentrations of nutrients. This supports the results of N-controlled algal production in the polluted sites. At other points seasonal variations were observed, and it was seen that co-existence of N and P or only P was limiting.

Conclusions

The present bioassay results indicate the occurrence of P-limited algal production in the NE Mediterranean and Eastern Aegean coastal waters, excluding the polluted enclosed bays with low N/P ratios in the water column. These findings are consistent with the N/P ratios of Eastern Mediterranean deep waters and limiting nutrient experiments (Ediger *et al*. 1999; Krom *et al*. 2004) and external sources (Koçak *et al*. 2010). The present biochemical properties of the coastal waters and the bioassay results point out the crucial importance of phosphorus inputs from the terrestrial sources. In other words, organic and inorganic phosphorus loads entering especially semi-enclosed bays and coastal waters should be controlled to prevent eutrophication. For this purpose, necessary administrative and technical measures should be taken to reduce P discharges to the sea and thus increase N/P ratios in wastewater of different origins. In building wastewater treatment plants in the region, priority should be given to applicable treatment technologies that would have high removal rates of organic and inorganic P substances and, thereby, increase N/P ratio in treated water.

Acknowledgements

This study was supported by the Turkish Scientific and Technological Research Council (TUBITAK) within the framework of the national program -Public Research Grant Committee (KAMAG)- through the project no 106G124. The authors would like to thank the scientists and technicians of the METU Marine Science Institute, the captains and crews of RV “BİLİM”, who made concerted efforts during field sampling. We also would like to thank to Erdal Kara, Yiğit Konya, Mehtap Okan, Gülümser Yakupoğlu, Hakan Atabay, Mustafa Mantıkçı and İbrahim Tan for laboratory and field study. Authors wish to Aslı Süha Günay and Onur Akyol for preparation of the maps for limiting nutrients.

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Received: 05.01.2013
Accepted: 01.10.2013