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RESEARCH ARTICLE

Relationship between dinoflagellate *Alexandrium minutum* (Halim) and environmental factors in Homa Lagoon (İzmir Bay)

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Abstract

Monthly water sampling was conducted in Homa Lagoon situated in Izmir Bay, Turkey, between December 2013 and November 2014 aiming to understand how *Alexandrium minutum* concentration changes due to Chlorophyll-*a*, nutrients such as nitrite, nitrate, ammonium, silicate, phosphate, and physical parameters like temperature, salinity, dissolved oxygen and pH. *A. minutum* concentration was highest in July at Station 4 and it was related to nitrate and phosphate concentrations mostly. No bloom of *A. minutum* was observed during the study.

Keywords: *Alexandrium minutum*, nutrients, lagoon, nitrate, phosphate

Introduction

Harmful algal blooms have a negative effect on human health and tourism activities as well as economic development (Hallegraeff 1993). Toxic or not, the increase of the red-tide events globally from the 1980's (Anderson 1989) has become interesting and consequently the number of researches has increased.

One of the most important red-tide causative organisms is a small species called *Alexandrium minutum* Halim (Balech 1995). *A. minutum* is an armoured, marine, planktonic dinoflagellate which lives mostly in temperate and tropical waters and also in lagoons. It is known that *A. minutum* produces gonyautoxin derivatives called GTX1, GTX2, GTX3, GTX4, neoSTX and STX which cause Paralitic Shellfish Poisoning (PSP) (Mackenzie and Berkett 1997).

A. minutum is a widely distributed species and it was firstly described in the Mediterranean (Alexandria Harbour, Egypt) by Halim (1960) and in Turkey in İzmir Bay by Koray and Büyükışık (1988).

Garces *et al*. (2004), pointed out that *A. minutum* blooms which occur in the restricted waters are related to resting cysts. Nutrient input through water flow has significant effect for harmful algal blooms (Anderson *et al.* 2002). Giacobbe *et al*. (1996) stated if the water has a good mixture and there is not any *A. minutum* cell, dissolved inorganic nitrogen and N:P ratios are high in autumn and winter in Ganziri lagoon (Mediterranean). Beside this, the cell concentrations of *A. minutum* could be directly proportional to the nitrate as well as inversely proportional to the ammonium (Bravo *et al*. 2008). Guisande *et al*. (2002) stated that, the toxin concentration of *A. minutum* increases as phosphate concentration decraeses in the culture conditions.

Nutrient concentrations stimulate the blooms of *A. minutum* but it is still unknown the exact concentrations of nitrogen or phosphate to cause blooms (Maguer *et al*. 2004).

Homa Lagoon (Izmir Bay) is part of Gediz Delta which is very important wetland in terms of biological diversity. Beside hosting many bird species, the delta comprises salt water, fresh water and brackish water ecosystems together. Occasionally dreinage input to the lagoon can be seen. Çamaltı Saltpan located adjacent to Homa Lagoon as a part of Gediz Delta is one of the biggest saltpans in Turkey producing 40% of the salt requirement of the country. Ege University Fisheries Faculty maintaines the management of the lagoon and all fisheries operations. This area has been declared as Menemen-Homa Lagoon Wildlife Protected Area by the Ministry of Forestry in 1994. The area has been also included in International Ramsar Convention since 1998.

In this study, it is aimed to analyze the potential bloom of *A. minutum* and its relationship with the nutrients and physical parameters in Homa Lagoon.

Materials and Methods

Homa Lagoon (38°33′10″N, 26°49′50″E) is located 25 kilometers to the northwest of İzmir Bay and it is 7.4 km long, 3 km wide with the deepest point of 1.5 m (Figure 1).

Monthly sampling was conducted between December 2013 and November 2014 at four stations with 5 liter-capacity plastic bottles for qualitative and quantitative analyses of *A. minutum* and the samples were fixed with standart lugol solution as 10 mL:1L (Koray 2002). Additional water sample was taken with 2 liter-capacity plastic bottles for NO_2-N, NO_3-N, NH_4-N, Si, PO_4-P and chlorophyll-a analysis. Dissolved oxygen and water temperature were measured with HACH HQ30d model oxygen meter *in situ*. pH was measured with WTW 3110 model pH meter *in situ*, as well. Sampling was performed between the hours 09.00-09.30 for station 1, 10.00-10.30 for station 2, 12.00-12.30 for station 3 and 13.00-13.30 for station 4 every month.

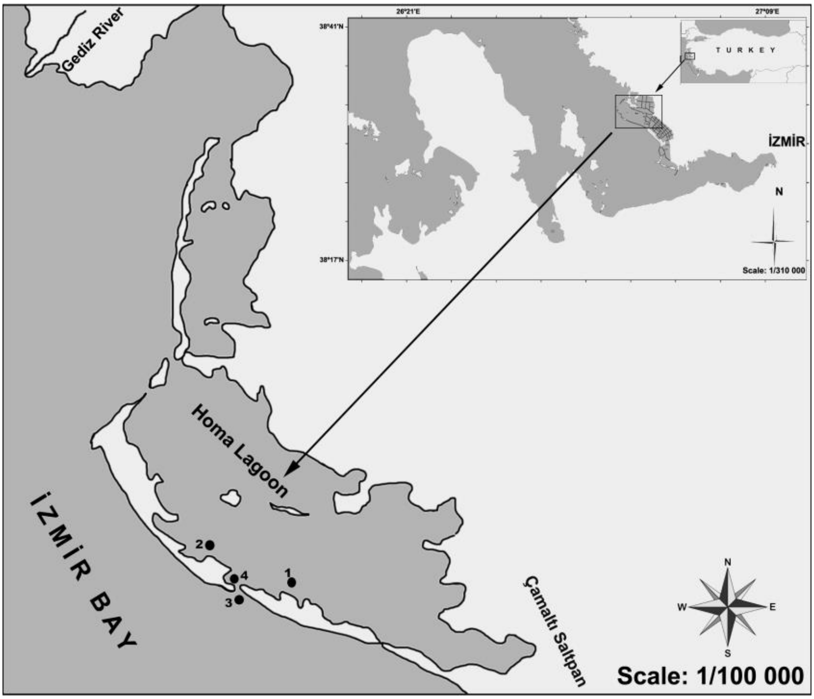

Figure 1. Sampling stations at study area.

Samples taken with 5-liter plastic bottles were brought to the laboratory which was lightened up with fluorescent lamp at 20°C to leave them for sedimentation and at least 48 hours later samples were concentrated by siphoning the top layer of the sample and transfered to 250 ml measuring cylinder. Same procedure was applied at least 48 hours later for concentrating to 25 ml test tube. Before analyzing under the microscope, the sample was concentrated to the 5 ml tubes. These samples were fixed with formaldehyde for preserving to have the final concentration as 4%.

Single drop method was used for counting and phytoplankton cell count results were converted to cell per litre using reverse calculating method (Venrick 1978; Semina 1978). All samples were analyzed using Olympus BX-50 model microscope. Water samples for nutrient analysis were filtered using filtering flask, Whatman GF/C filter papers with 47 mm diameter and 1.2 μ pore size and motor-pomp with 570 mmHg pressure value. Dissolved nitrate (NO_3^--N), nitrite (NO_2^--N), ammonium (NH_4^+-N), silicate (Si), phosphate (PO4-P) and chlorophyll-*a* analysis were conducted in laboratory colorimetrically with HACH DR/4000U spectrophotometer. All analyses were performed in the laboratory conditions at 20°C temperature and 40W fluorescent light using Strickland & Parson's method

(Strickland and Parson 1972). Harvey method was used for salinity analysis in the laboratory (Harvey 1957).

Pearson's correlation analysis was done for the correlation between the number of *A. minutum* and environmental variables. All statistical analysis were performed using IBM SPSS Statistics Version 20 software.

Results

During the sampling period, *A. minutum* concentration was 6546 cell L^{-1} in July at station 4 as the maximum value while the minimum cell concentration was 13 cell L^{-1} in August at station 3. *A. minutum* was counted in all sampling stations in May, June, October and November (Table 1).

Table 1. *A. minutum* concentration (cell L^{-1}) at four stations in Homa Lagoon troughout the sampling period.

	Dec. 2013	**Jan. 2014**	**Feb. 2014**	**Mar. 2014**	**Apr. 2014**	**May 2014**	**Jun. 2014**	**Jul. 2014**	**Aug. 2014**	**Sep. 2014**	**Oct. 2014**	**Nov. 2014**
ST01	0	82	463	275	176	216	154	0	17	188	122	270
ST02	96	0	53	608	129	395	96	0	0	0	296	258
ST03	18	0	459	39	0	16	353	19	13	0	211	288
ST04	75	0	0	0	20	235	810	6546	34	0	158	264

The maximum temperature was measured as 31.3ºC in July at station 3 and the minimum temperature as 6ºC in December at station 1. As the result of temperature measurements from all stations throughout the year, mean temperature value was calculated as 18.17ºC (Table 2).

In terms of salinity, the maximum value is 63.7‰ in January at station 1. Minimum salinity was measured as 35.6‰ in September at station 3. During the sampling period in the lagoon, the mean salinity value was calculated as 44.7‰ (Table 2).

As the level of dissolved oxygen, the maximum value was measured as 16.29 mg L^{-1} in April at station 4 while the minimum value is 5.34 mg L^{-1} in August at station 2. Maximum pH was 8.83 in February at station 4 and the minimum pH was 7.76 in April at station 1 (Table 2).

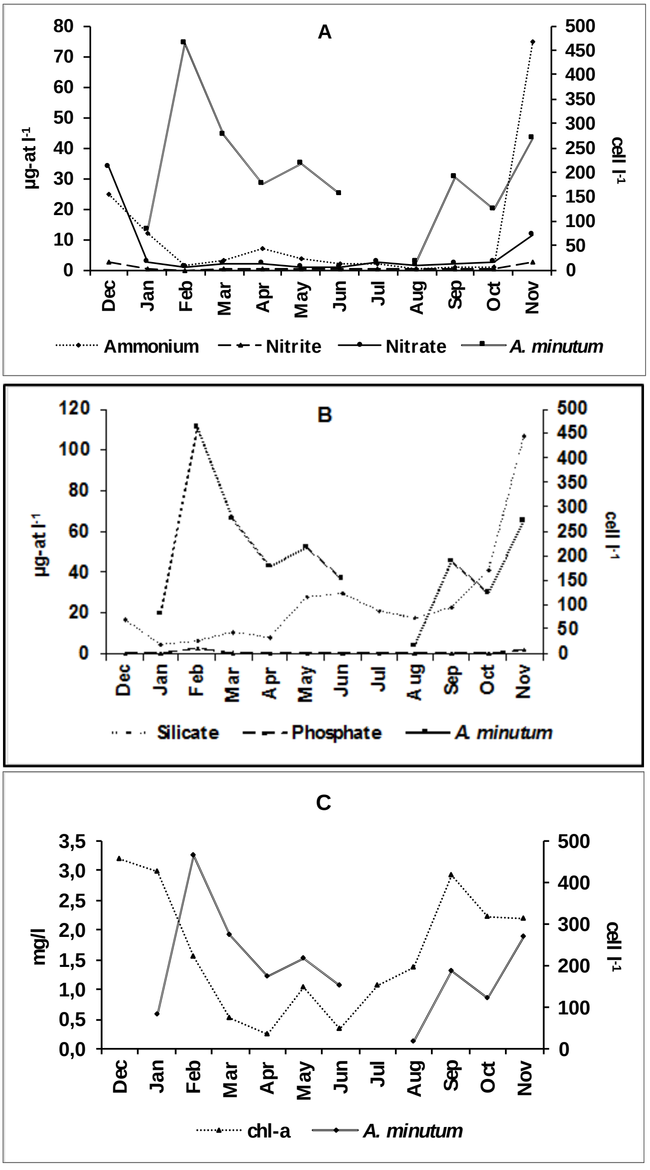

Figure 2. Relationship between ammonium, nitrite, nitrate (A), silicate, phosphate (B), chlorophyll-*a* (C) and *A. minutum* at station 1

Table 2. Physical parameters at all stations through the sampling period

		Temperature (°C)	Dissolved Oxygen (mg/l)	Salinity (‰)	pH
December	ST01	6,0	8,06	53,0	8,178
	ST02	6,4	9,91	53,1	8,297
	ST03	8,5	9,49	54,4	8,399
	ST04	9,4	14,99	53,5	8,261
January	ST01	9,6	8,20	58,4	7,812
	ST02	10,3	8,08	63,7	8,452
	ST03	10,0	8,34	62,3	8,354
	ST04	10,2	8,80	59,7	8,788
February	ST01	11,1	10,57	45,2	8,451
	ST02	10,6	9,31	43,8	8,382
	ST03	12,6	9,68	41,1	8,165
	ST04	14,2	12,04	39,8	8,832
March	ST01	11,0	9,51	41,1	8,072
	ST02	13,1	9,13	38,5	8,191
	ST03	14,6	8,09	37,1	7,990
	ST04	15,7	9,63	39,8	8,340
April	ST01	18,7	8,19	41,1	7,760
	ST02	18,4	8,67	42,4	7,809
	ST03	21,4	9,09	38,5	8,299
	ST04	25,3	16,29	41,1	8,287
May	ST01	20,9	7,02	42,4	8,000
	ST02	21,1	6,92	41,1	8,282
	ST03	21,9	7,10	43,8	8,028
	ST04	22,9	10,73	42,4	8,489
June	ST01	25,8	7,89	45,1	8,056
	ST02	26,5	6,45	42,4	8,313
	ST03	27,5	9,71	45,2	8,322
	ST04	29,6	13,77	43,8	8,524
July	ST01	29,0	6,73	46,0	8,128
	ST02	30,0	6,92	48,0	8,216
	ST03	31,3	7,72	47,0	8,324
	ST04	31,0	8,27	46,0	8,241
August	ST01	24,2	7,40	42,4	8,661
	ST02	24,4	5,34	42,6	8,557
	ST03	26,3	7,59	39,8	8,514
	ST04	26,3	10,36	41,3	8,641
September	ST01	18,7	7,66	37,3	7,960
	ST02	20,0	10,70	38,4	8,080
	ST03	22,7	8,51	35,6	8,120
	ST04	21,6	12,22	37,2	8,130
October	ST01	15,3	6,81	41,0	8,390
	ST02	15,4	8,86	40,0	8,513
	ST03	17,1	7,80	40,0	8,335
	ST04	17,5	13,96	39,0	8,552
November	ST01	10,5	7,95	39,7	8,145
	ST02	10,7	8,34	45,0	8,216
	ST03	12,6	9,17	37,1	8,311
	ST04	14,3	12,60	41,4	8,593

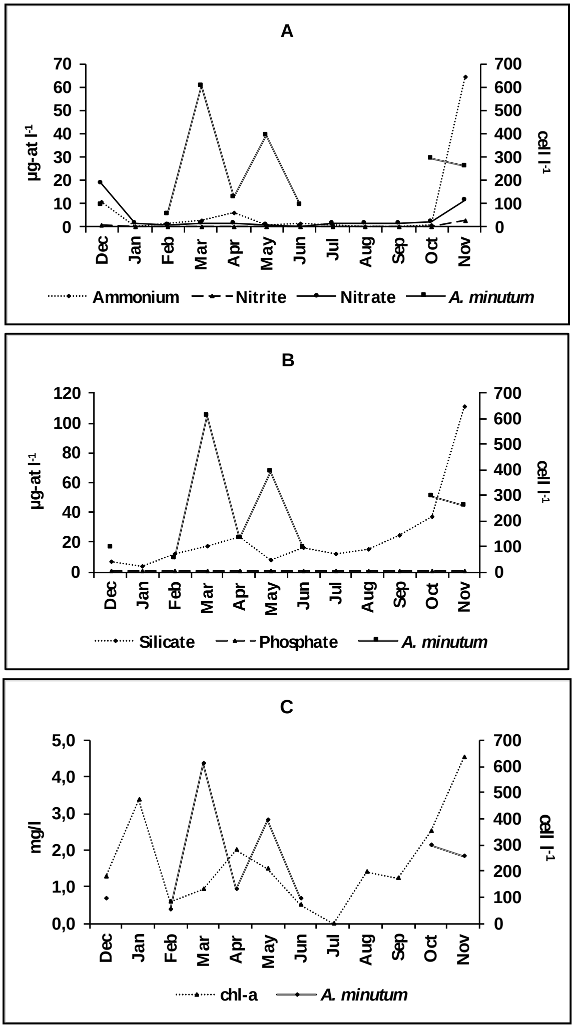

Figure 3. Relationship between ammonium, nitrite, nitrate (A), silicate, phosphate (B), chlorophyll-*a* (C) and *A. minutum* at station 2.

In nutrient values, the maximum ammonium was 74.55 µg-at.N L^{-1} in November at station 1 (Figure 2) and the minimum was 0.18 µg-at.N L^{-1} in August at station 4 (Figure 5). Nitrite was 3.29 µg-at.N L^{-1} as the maximum value in November at station 4 (Figure 5), but in February no nitrite was recorded at station 2 (Figure 3). The maximum amount of nitrate was measured in December at station 1 as 33.45 µg-at.N L^{-1} (Figure 2), the minimum was 0.47 µg-at.N L^{-1} in June at station 4 (Figure 5). Silicate peaked in November and it was 118.37 µg-at.Si L^{-1} as the maximum value, 1.35 µg-at.Si L^{-1} was the minimum which was mesured in May at station 4 (Figure 5).

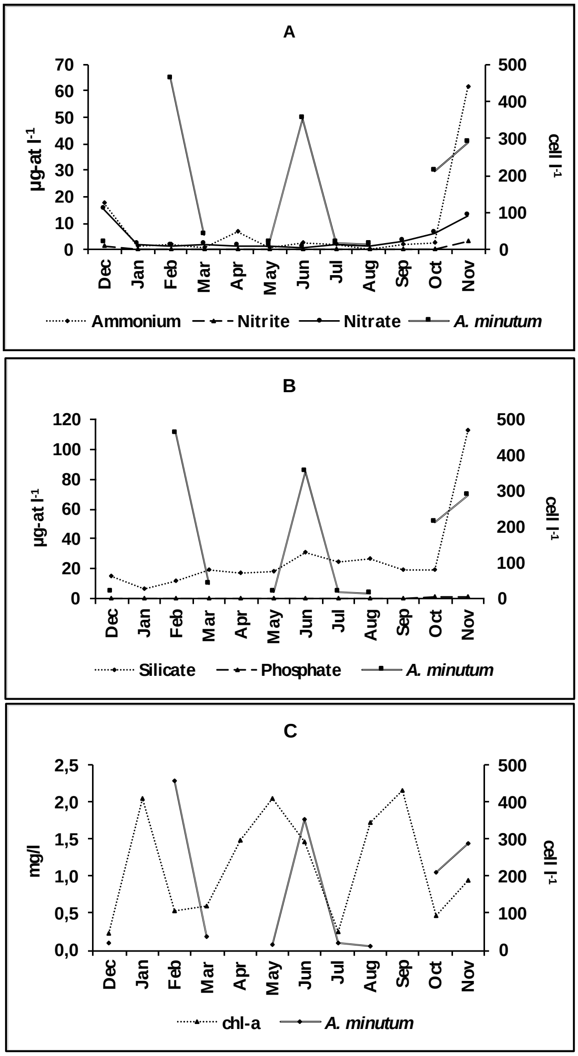

Figure 4. Relationship between ammonium, nitrite, nitrate (A), silicate, phosphate (B), chlorophyll-*a* (C) and *A. minutum* at station 3.

While February showed the maximum value for phosphate at station 1 (Figure 2) with 2.66 µg-at.P L^{-1}, the minimum amount of phosphate was in February at station 2 (Figure 3), station 3 as 0.04 µg-at.P L-1. As a result of chlorophyll-*a* measurements, the maximum value was 12.154 mg L^{-1} in May at station 4 (Figure 5). In July at station 2, chlorophyll-*a* value was none (Figure 3).

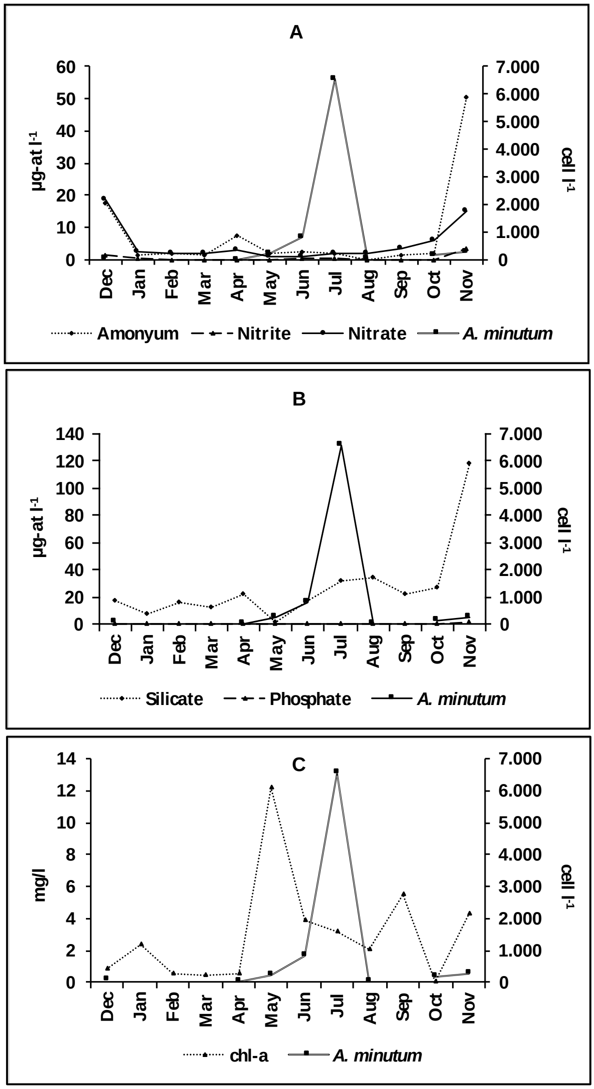

Figure 5. Relationship between ammonium, nitrite, nitrate (A), silicate, phosphate (B), chlorophyll-a (C) and A. minutum at station 4.

As a result of Pearson's correlation analysis between the number of *A. minutum* and environmental factors, the former positively correlated with PO_4 (r=0.67; p<0.05) at station 1 and the dissolved oxygen at station 1 (r=0.71; p<0.05) and station 3 (r=0.58; $p<0.05$). There was no significant correlation between the number of *A. minutum* and other environmental factors at the other sampling stations.

Discussion

Homa Lagoon is shallow and its physical and chemical characteristics could be much variable. Occasionally nutrient values could be high in the lagoon. Egemen *et al.* (1999) indicated that high nutrient values in Homa Lagoon which is located in the middle bay of İzmir could be due to pollutants coming from the inner bay and the Gediz River.

Salinity values mostly increase in summer due to shallow water and excessive evaporation, but in this study, maximum salinity values were measured in January and February. Kutlu and Büyükışık (2007) mentioned that salinity change maybe affected by the climatic factors due to the shallow water of the lagoon. As in Sabancı and Koray (2012), Sabancı (2014), evaporation and less rainfall maybe the reason of high salinity in the lagoon. Comparably, it is conceivable that the climatic changes and shallow waters could be the reason of high salinity in this study. pH values was not unordinary throughout the year in the lagoon. Maximum pH values in this study was seen usually at station 4 (Table 2).

Kutlu and Büyükışık (2007) stated that ammonium and nitrate concentrations in the lagoon were related with the rainfall and fertilized soil of the agricultural areas. In the same study it is mentioned that phosphate and nitrate concentrations could decrease due to the phytoplankton activity. Accordingly, although not yet certain, a possible rainfall or fertilized soil reaching into the lagoon could increase the ammonium and nitrate concentration in the November-December period in this study.

The results of Pearson's correlation analysis shows us *A. minutum* is positively correlated with PO4 at station 1 (r=0.68) and the dissolved oxygen at station 1 (r=0.73) and station 3 (r=0.58) while the other parameters, particularly ammonium, was not significantly correlated. Giacobbe *et al.* (1996) indicated that *A. minutum* quantity was negatively correlated with ammonium concentrations, but orthophosphate has considerably an effect on *Alexandrium*. Selli *et al.* (1992) stated that an increase of phosphate concentrations is directly proportional with activity of nitrate reductase for *Alexandrium*.

Koray and Büyükışık (1988) indicated that *A. minutum* blooms have occured in the temperature range between 14.5-21.2°C. *A. minutum* appearance was recorded between 6.4-31°C in this study.

Even though *A. minutum* concentrations were not at the bloom level of 10^6 cell l^{-1} as it had previously been occured in İzmir Bay (Koray and Büyükışık 1988), due to the fisheries activity in Homa lagoon, it must be considering that *A. minutum* can produce PSP toxins and in case of blooming it could be dangerous for human health. Further studies should be conducted and the situation of the lagoon should be periodically monitored for this purpose.

Homa Lagünü (İzmir Körfezi)'nde dinoflagellat *Alexandrium minutum* (Halim) ve çevresel faktörler arasındaki ilişki

Özet

Bu çalışmada *Alexandrium minutum* konsantrasyonunun klorofil-a ile nitrit, nitrat, amonyum, silikat ve fosfat gibi nutrientlere ve sıcaklık, tuzluluk, çözünmüş oksijen ve pH gibi fiziksel parametrelere bağlı olarak nasıl değiştiğini görmeyi amaçlayarak Aralık 2013 ve Kasım 2014 arasında aylık örnekleme yapılmıştır. *A. minutum* analizi için 5 litre kapasiteli şişelerde su örnekleri ve nutrient ile klorofil-a analizleri için 2 litre kapasiteli şişelerde su örneği alınmıştır. *A. minutum* konsantrasyonunun Temmuz ayı, 4 no'lu istasyonda en yüksek değerde olduğu ve bunun çoğunlukla nitrat ve fosfat konsantrasyonu ile ilişkili olduğu görülmüştür. Herhangi bir *A. minutum* patlaması görülmemiştir.

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