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Establishing Boundary Classes for the Quality Classification of Southeastern Black Sea Using Phytoplankton Biomass

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Abstract

Benthic macroinvertebrates, macroalgae and phytoplankton constitute the Biological Quality Elements (BQE) proposed in the Water Framework Directive (WFD, 2000/60/EC) to be used for the classification of the ecological status of a water body. Chlorophyll-a is a usefull expression of phytoplankton biomass and this indicator is an effective and relevant BQE for coastal ecosystems which is universally accepted. In the present study, interpretations of the class boundaries according to normative definitions of WFD, are presented for chlorophyll-a in South Eastern (SE) Black Sea. Water quality classification was determined in five different categories as “high, good, moderate, poor and bad”. The coastal waters of SE Black Sea were classified in 8 different typologies (K1-K8) based on depth, salinity and substratum types. In this study, types K1 and K2 (>17.5 salinity, >30 m depth) were considered because of availability of time-series data for those typologies. Sinop and Sürmene sites were selected due to the best available long-term chlorophyll-a data set, respectively over the period of 2002-2010 and 2001-2011 for chlorophyll-a respectively. Type specific chlorophyll-a (Chl-a) reference and threshold values were determined based on the 90th percentile of the long-term collected chlorophyll data set. Due to the high seasonal variability of phytoplankton biomass, the annual values were not considered adequate and the classification tool was developed on seasonal basis. The High/Good (H/G) and Good/Moderate (GM) boundaries were defined as seasonal from the long term data sets for Sürmene and Sinop sites. All the boundaries were higher at the Sinop site. Ecological quality ratios distributed between 0-1. It would be necessary to underline the fact that these class boundaries might be higher for waters where depths are below 30 m and salinity values are less than 17.5. However, there is not enough data to support this assumption for the near coast waters of the SE Black Sea. Eventhough Chl-a scaling can not be used as a single tool for the ecological quality classification it is a reliable approach to use the obtained boundaries at temporal and spatial scales for the quality classification of SE Black Sea waters above 30 m depth.

Keywords: Chlorophyll-a, water quality, classification, Water Framework Directive, Blacksea.

Güneydoğu Karadeniz’de Su Kalitesinin Fitoplankton Biyokütlesi Kullanılarak Sınıflandırılması

Özet

Bentik makro omurgasızlar, makroalg ve fitoplanktonlar Su Çerçeve Direktifinde (SÇD, 2000/60 / EC) önerilen Biyolojik Kalite Elemanları (BQE) olup, su kütlesinin ekolojik statü sınıflandırılmasında kullanılmaktadırlar. Klorofil-a fitoplankton biyokütle göstergesidir. Bu gösterge evrensel olarak kıyı ekosistemleri için etkili ve uygun bir Biyolojik Kalite Elamanıdır. Bu çalışmada, Güney Doğu Karadeniz için SÇD'nin tanımlamalarına göre klorofil-a sınıf sınır değerleri hesaplanmıştır. Su kalitesi sınıf sınır değerleri " yüksek kalite, iyi, orta, zayıf ve kötü" gibi beş farklı kategoride belirlenmiştir.

Güneydoğu Karadeniz kıyı suları derinlik, tuzluluk ve sediman karakteristiğine göre 8 farklı tipolojide (K1-K8) sınıflandırılmıştır. Bu çalışmada, K1 ve K2 (>17,5 tuzluluk, >30 m derinlik), tip su kütlesi için var olan zaman serisi verileri kullanılmıştır. Sinop ve Sürmene önlerinde sırasıyla 2002-2010 ve 2001-2011 yılları arasında gerçekleştirilen zaman serisi çalışmalarının yüzey klorofil-a verileri kullanılarak ekolojik kalite durumu belirlenmiştir.

Uzun dönemli zaman serisi çalışmalarında klorofil-a nın referans ve sınıf sınır değerleri yüzdebirlik (90^{th} percentale) metodu kullanılarak tanımlanmıştır. Fitoplankton biyokütlesinin mevsimsel farklılıklar göstermesinden dolayı sınıflandırma yıllık olarak yapılmasının yanı sıra mevsimsel olarak ta geliştirilmiştir. Yüksek / İyi (H/G) ve İyi / Orta (G/M) su kalitesi sınıfı sınır değerleri Sürmene ve Sinop bölgeleri için uzun dönemli veri setlerinden mevsimlik olarak belirlenmiştir. Bütün sınıf sınır değerleri Sinop bölgesinde daha yüksek olarak gözlenmiştir. Ekolojik Kalite Oranları (EQR) 0-1 aralığında değişim göstermiştir. Sınıf sınır değerlerinin <30 m ve <17,5 tuzluluğa sahip kıyı bölgelerinde daha yüksek olarak gözleneceği aşikardır. Ancak elimizde bu su kütleleri için veri seti mevcut değildir. Ekolojik kalite sınıflandırılması için sadece klorofil-a nın kullanılması çok geçerli gibi görülmesede yapılan ekolojik kalite sınıflandırmasının güney doğu Karadenizin 30m den derin suları için zamansal ve mekansal açıdan güvenilir bir yaklaşım olduğu değerlendirilmiştir.

Anahtar Kelimeler: Klorofil-a, su kalitesi, sınıflandırma, Su Çerçeve Direktifi, Karadeniz.

Introduction

Man-induced eutrophication started in the 1970's and identified as a key ecological problem for coastal waters of Black Sea especially north-western part subjected to the influence of fresh water inputs (Moncheva, *et al*., 2002; Oleg *et al*., 2007; Oğuz and Velikova, 2010; Vasiliu *et al*., 2012).

Ecological changes in open-sea regions of the Black Sea have received less attention than those of coastal regions. These open-sea areas constitute a partially isolated ecosystem in which water masses are separated from impacted coastal areas by a meandering 'Rim Current' frontal zone (Oğuz *et al*., 1993).

Structural and functional characteristics of the phytoplankton are closely related to the intensity of eutrophication, and can be used as indices for determining the state of the phytoplankton community in the Black Sea (Vinogradov *et al*., 1999). Therefore, tracing temporal variations in chlorophyll *a* (Chl *a*), an acceptable indicator of phytoplankton biomass, is necessary in order to reliably identify changes induced by human activity or by natural fluctuations.

The first measurements of Chl *a* in the Black Sea were made in 1961 (Finenko, 1965). Since then, many studies related to or directly examining monthly, seasonal and interannual changes in Chl *a* levels have been carried out in different regions of the open and coastal Black Sea waters (Vedernikov *et al*., 1983; Yunev *et al*., 1987; Vedernikov, 1989; Yunev, 1989; Krupatkina *et al*., 1991; Finenko and Krupatkina, 1993, Vedernikov and Demidov, 1993; Yilmaz *et al*., 1998; Demidov, 1999; Kopelevich *et al*., 2002; Alkan *et al*., 2013). Despite these numerous measurements, there are no data on long-term variations in chlorophyll in the shallow regions of the Black Sea, especially at the southeastern region. Therefore (for example) any anthropogenic impact on the pelagic ecosystem of the basin would be difficult to discern.

Many studies used Chl-*a* as indicator of eutrophication or water quality (Harding and Perry, 1997; Boyer *et al*., 2009) due to its very simple and integrative analysis. Increase in the phytoplankton biomass can be measured as an increase in the chlorophyll concentrations. Chlorophyll is a useful expression of phytoplankton biomass and is arguably the single most responsive indicator of N and P enrichment in the marine system. (Harding, 1994; Devlin *et al*., 2007). In the Mediterranean Geographical Intercalibration Group meeting (Med-GIG) held in Rome in 2011, Mediterranean member states agreed that: 'Chl-*a* index will be an effective and relevant BQE for coastal ecosystems and this is universally accepted' (MED-GIG Report, 2011).

Phytoplankton is a key biological element taken into consideration by the Water Framework Directive as an indicator of the Water Quality of coastal ecosystems besides benthic invertebrates and macroalgae. Chlorophyll-a has been regarded as a biological quality element in the Water Framework Directive due to the fact that it has been used as an indicator of the biomass of phytoplankton for over 50 years.

The coastal waters covered by the WFD with respect to biological features are generally limited to surface waters one nautical mile from the coast. Open marine waters are not covered, but the WFD is likely to influence management of all marine ecosystems because land-based inputs of pollutants intrudes to the open waters through the coastal zone.

The main objective of the present work is to classify the surface waters of Southeastern Black Sea for assessing ecological quality levels. We present examples of how the ecological status (in terms of phytoplankton biomass) of the Southeastern Black Sea (Sürmene and Sinop), could be classified according to the principles of the WFD and propose an approach to define ecological quality targets for phytoplankton chlorophyll a for the Eastern Black Sea.

Materials and Methods

The southeastern and central Black Sea study sites (Sürmene and Sinop) were located in 1 km and 3.5 km away from the coast at 40°93′7318 N-40°20′1993 E; 40°97′2814 N-40°20′0797 E and 42°00′58 N- 35°15′88 E; respectively (Figure 1). Within 9-10 years, monthly/bimonthly monitoring efforts (2001-2011) were conducted at the surface (0.5 m) layer at 3 stations (Figure 1). Chlorophyll-a was measured spectrophotometrically using the equations of Jeffrey and Humphrey (1975).

Classification of Southeastern and Central Black Sea off-shore (>30 m depth) surface waters has been conducted using the same methodology adopted by all other European countries according to Phytoplankton biomass content used as a Water Framework Directive Biological Quality Element and intercalibration works have been conducted among countries having shores in the same seas (EC/JRC-IES, 2009; MED-GIG 2011).

The method is comprised of 3 steps as follows:

1- Determination of reference conditions
2- Determination of class boundary values
3- Determination of Ecological Quality Ratios (EQR)

Determination of Reference Conditions

The first and most widely used method to determine reference conditions is the use of monitoring data combined with historical data on the system to estimate conditions prior to large-scale disturbance. While there is lack of systematic historical data at the southern part of the Black Sea, there are two time-series monitoring data sets that can

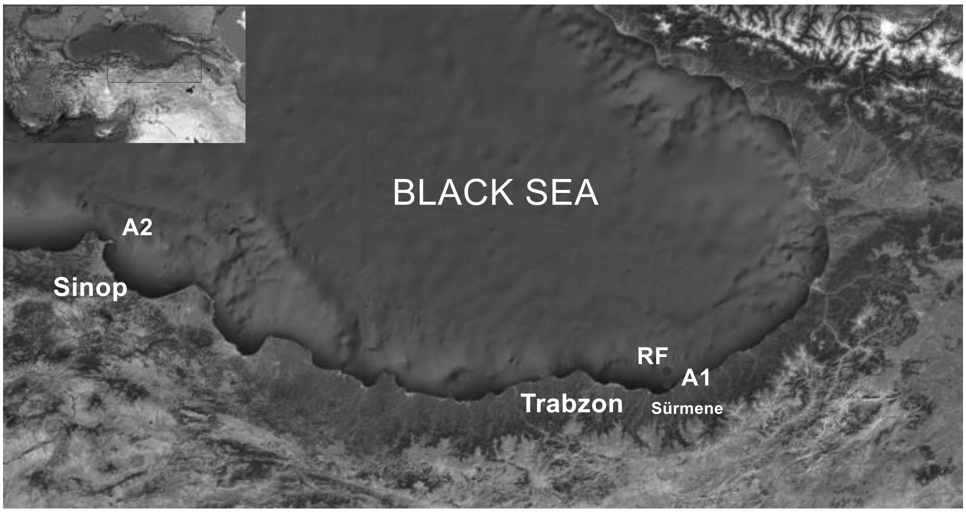

Figure 1. Locations of Sampling Stations in the Southeastern and Central Black Sea Regions.

be mined and used to help the estimation of the reference conditions.

Reference conditions were determined on a data set of a site in the South Central and Southeastern Black Sea with no anthropogenic influence. The station on the map marked RF has been used as a reference for both region. This station is considered as a reference station, i.e., non-impacted by anthropogenic influence, due to its distance from the main pollution sources on land (3.5 km off-shore). This site is also considered to be type K1 and K2 waters (see paragraph below) where the salinity is >17.5 indicating the marine waters (TUBITAK-MRC and MoEU-GDEM, 2014).

The coastal waters of SE Black Sea were classified in 8 different typologies (K1-K8) based on depth, salinity and substratum types (TUBITAK-MRC and MoEU-GDEM, 2014). In this study, types K1 and K2 (>17.5 salinity, >30 m depth) were considered because of availability of time-series data for those typologies and the reference conditions were set for those types.

The 90th percentale method has been used for determining reference conditions. This mathematical calculation applied on a seasonal longterm chlorophyll-a data set. 10 percentile (lower quartile) of the seasonal long-term data sets (time series period 2001-2011 for Sürmene and 2002-2010 for Sinop) was chosen as a reference value.

Determination of Class Boundary Values

The Water Framework Directive (2000/60/EC) requires classification, in terms of ecological status, for all European surface waters. The classification should be based on reference conditions, which are intended to represent minimal anthropogenic impact, and observed deviation from these conditions. Ecological status is to be expressed as a numerical value (the ecological quality ratio) between 1 (high ecological status) and 0 (bad ecological status) with intervals equating to: high, good, moderate, poor and bad ecological status.

It is recommended to use monthly data collected over a period of at least five years for this method (MED-GIG 2011). Furthermore, seasonal data sets including the same periods collected over a longer term can also be used. Each boundary class is determined from 10th, 25th, 50th, 75th, and 90th percentile calculated for each season's data set for the entire period 2001-2011.

In these determined percentage brackets, 90% indicated the worst condition (BAD), 75% indicates a (POOR) condition, 50% indicates a (MODERATE), 25% indicates a (GOOD) and 10% indicates a very good (HIGH) condition.

Determination of Ecological Quality Ratio (EQR)

The Ecological Quality Ratio (EQR) which is a quantitative scale is obtained by dividing the reference value by the value monitored and is always between 0 and 1. When the value is closer to 1, it indicates a good status; when the value is closer to 0, it indicates a bad status. The stages of determination of EQR is depicted in Figure 2.

Results and Discussions

Quality classification of the Southeastern Black Sea with Chlorophyll-a has been made using the data obtained from the studies conducted at Sürmene (>30 m, >17.5 salinity) and at Sinop (>30 m, >17.5 salinity) sampling sites between 2001-2011 and 2002-2010 respectively (Figure 1). The percentile method was used separately on the reference data and also on all the data collected from the stations and the results obtained are presented below.

Table 1 shows the reference values for Chl *a*, for each site of the Black Sea. One reference station was set for both site because of same water type. Using the same reference point has been deemed to be suitable

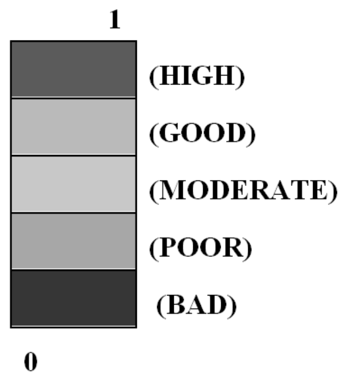

Figure 2. Basic principles for the classification of ecological status based on ecological quality ratios (adapted from Anon, 2003).

Table 1. Reference Values Determined for Chlorophyll-a (μg/L)

Sampling site	Sampling intervals	Percentile 10th	n
Sinop	2002-2010	0.18	51
Sürmene	2001-2011	0.16	58

due to the fact that the depth of the water in both areas is deeper than 30 meters and away from the river inputs. Reference conditions in 10th percentile of Chlorophyll-*a* data were found as 0.16 and 0.18 μg/L respectively for Sinop and Sürmene site of the SE Black Sea (Table 1). Sampling intervals of time series studies are different (Table 1) and reference values were taken into account during these time period.

10 percentile (lower quartile) of the data sets (time series period 2001-2011 for Sürmene and 2002-2010 for Sinop) was chosen as a reference value to test applicability for definition of reference values. Reference values, were defined as 0.18 and 0.16 μg/L chlorophyll-a for Sinop and Sürmene site respectively. Reference values were also defined as seasonal for both sites and given in Table 2. Reference values in winter and autumn were higher than spring and summer.

In areas of southeastern (Sürmene) and central (Sinop) Black Sea, surface chlorophyll-*a* concentrations varied between 0.16-2.75 and 0.18-4.29 μg/L respectively that repeates systematically a seasonal pattern almost each year (Figure 3). The results of the statistical analysis of the data sets are depicted in Table 3. The mean and median values were estimated as 0.86 μg/L at Sürmene and 0.74 μg/L at Sinop.

In the classification analyses conducted for the Central and Southeastern Black Sea, all data (on a monthly/bimonthly basis) have been evaluated and also examined on a seasonal basis. Seasonal classifications have been made, due to the fact that seasonal variations have been determined to be high (Figure 3 and 4).

The highest average Chl-a was found in winter, spring and autumn, which would indicate favourable growth conditions for phytoplankton during these seasons (Table 4).

The boundaries were set applying a statistical approach. The reference and boundary values of chlorophyll-a were determined by calculating 10-90% percentile of all the 9-10 years data and of the seasonal data sets in central and southeastern Black Sea (Figure 3 and 4).

The calculated 10th-90th percentile Chl-a concentrations (class boundaries) for overall data in the southeastern Black Sea for Sinop and Sürmene ranged from 0.34 to 1.71 and 0.23 to 1.17 μg/L respectively (Table 4). Boundary values of chlorophyll-a for ecological status were determined and if chlorophyll-a concentrations was <0.23 μg/L, the status was considered as "high"; if it was between 0.24-0.39 μg/L, 0.4-0.74 μg/L, 0.75-1.17 μg/L, >1.17 μg/L, the status was considered as good, moderate, poor and bad for Sürmene site. Similarly for the Sinop site, if chlorophyll-a concentrations was <0.34 μg/L "high"; between 0.35-0.62 μg/L "good"; between 0.63-1.04 μg/L "moderate"; between 1.04-1.71 μg/L "poor" and >1.71 μg/L "bad". The results obtained in the percentile of Chl-a were not very different for both site of the Black Sea in all boundary classes (Table 5).

The values of the Chl-a metric established as the boundaries between "high–good" and "good–moderate" status were 0.23 and 0.39 μg/L for Sürmene (SE Black Sea) and 0.34 and 0.62 μg/L for Sinop (central part of Black Sea) respectively (Table 5). Class boundary values were also defined as seasonal for Sürmene and Sinop site and results given in Table 6 and Figure 4.

It has been observed that the variations in seasonal class boundary values for both sites are more

Table 2. Seasonal reference values determined (percentale 10 th) for chlorophyll-a (µg/L)

REF	Sürmene	Sinop
Winter	0.34	0.43
Spring	0.10	0.10
Summer	0.11	0.12
Autumn	0.26	0.26

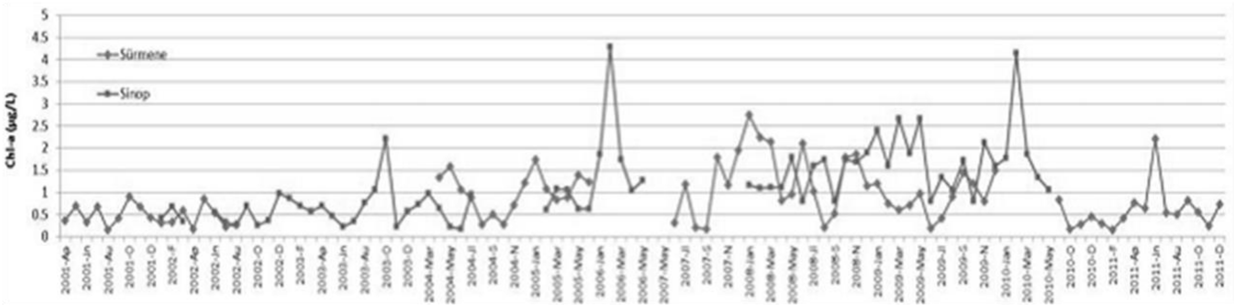

Figure 3. Surface chlorophyll-a variation between 2001-2011 in Sinop and Sürmene area.

Table 3. The results of statistical analysis of Southeastern and Central Black Sea data

	Southeastern BS (Sürmene)	South Central BS (Sinop)
N	79	67
average	0.86	1.18
median	0.74	1.04
Stdev	0.59	0.82
min	0.16	0.18
max	2.75	4.29

Table 4. Seasonal statistics for Chlorophyll-a data sets of 2001-2011

	Sürmene				Sinop				n	n
	spring	summer	autumn	winter	spring	summer	autumn	winter	Sürmene	Sinop
ave	0.88	0.71	0.82	1.08	1.20	0.77	1.14	1.52	17-22	12-22
stdev	0.46	0.58	0.54	0.76	0.67	0.49	0.72	1.13		
min	0.17	0.16	0.17	0.16	0.23	0.18	0.23	0.41		
max	2.14	2.21	1.86	2.75	2.67	1.74	2.20	4.29		

pronounced and that the difference in each class between summer and other season (Table 6 and Figure 4). Class boundary values have varied in 5 classification categories during winter, spring, summer and autumn and it is observed that the values are lower in summer (Figure 4 and Table 6).

These values were used for the assessment of the ecological status of >30 m depth waters of the southeastern and south central parts of the Black Sea based on BQE- phytoplankton biomass data.

EQR has been close to zero where water quality has been determined to be BAD and close to one when it has been determined to be HIGH (Figure 4). EQR is much lower than 1 in spring (EQR:0.25) and higher than 1 (EQR:1.11) in autumn and winter at Sürmene site. EQRs were calculated based on different reference condition for winter, spring, summer and autumn. As mentioned in the MED-GIG report (2011), it is normal to obtain such results (EQR >1) due to the oceanographic conditions of the deep water masses where the stations are located. The selected reference station data are suitable for Sinop site and the EQR has been close to zero where water quality has been determined to be BAD and close to one when it has been determined to be HIGH (Figure 4).

Due to the fact that no such data set is present for areas shallower than 30m to represent the near coastal areas, classification has not been made for these water masses. It is necessary to make plans in order to compensate for the missing data to make more reliable quality classifications in future.

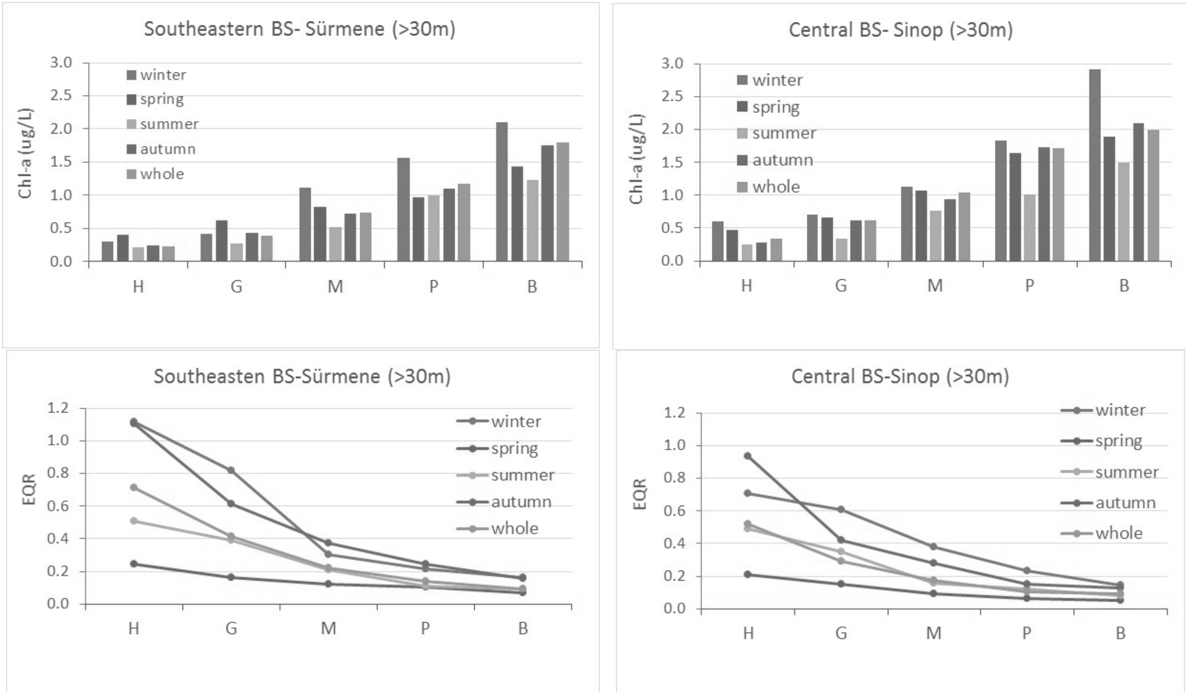

Figure 4. Seasonal Chlorophyll-a Class Boundary Values for Southeastern and Central Black Sea (µg/L) and the EQR values. These values were used for the assessment of the ecological status of >30 m depth waters of the southeastern and south central parts of the Black Sea based on BQE- phytoplankton biomass data.

Table 5. Class boundary values (Chl-a µg/L) for the time-series sites

Site	H-G boundary	G-M boundary	M-P boundary	P-B boundary
Sürmene	0.23	0.39	0.74	1.17
Sinop	0.34	0.62	1.04	1.71

Table 6. Seasonal class boundary values of Chl-a (µg/L) at Sürmene and Sinop sites

Sürmene (>30 m)	H	G	M	P	B
Winter	0.31	0.42	1.11	1.56	2.10
Spring	0.41	0.63	0.82	0.97	1.44
Summer	0.22	0.28	0.52	1.00	1.23
Autumn	0.24	0.44	0.73	1.10	1.75
Sinop (>30m)	H	G	M	P	B
Winter	0.61	0.71	1.13	1.84	2.92
Spring	0.48	0.66	1.07	1.64	1.89
Summer	0.24	0.34	0.77	1.01	1.50
Autumn	0.28	0.62	0.93	1.73	2.09

Discussion

The results of the intercalibration studies (GIG Black Sea, 2011) for the Black Sea conducted within the scope of Water Framework Directive by neighboring countries (Bulgaria and Romania) having coasts in the same seas have been compared to the results obtained from the present study (Table 7).

The data available for the Black Sea is for the Central (Sinop) and Southeastern Black Sea (Sürmene) regions that are deeper than 30 meters. However, the studies conducted in Bulgaria and Romania include coastal areas of inner sections of gulfs that are shallower than 30m. For this reason, the data has been examined to see what type of status is indicated for offshore and coastal waters before a comparison has been made. It has been determined that class boundary values (H/G and G/M) obtained for the offshore areas of Sinop and Sürmene that are deeper than 30 meters are considerably lower than the northwesten shelf areas (Table 7).

Table 7 and Figure 4 have been prepared to compare the H-G, G-M, M-P and P-B values of the class boundaries obtained in Black Sea, to see seasonal variations and make an evaluation of "good environmental status" (GES) as required by Marine Strategy Framework Directive (MSFD) (2008/56/EC).

When the G/M class boundary values obtained by the evaluation of data available for areas deeper than 30 meters at both sites collected over different time periods are taken into consideration, the suitable target GES value for the Southeastern (Sürmene) and Central regions (Sinop) of the Black Sea is determined to be 0.39 and 0.62 μg/L for whole years data respectively (Table 5). The critical value will be the border between good and moderate (G/M) ecological status (Andersen *et al*., 2004). Since the WFD requires that all waters be restored to be above this value, the threshold value between good and moderate status will have significant economic consequences (Andersen *et al*., 2004).

Seasonal classifications have also been made due to the fact that both time series data exhibits seasonal variations. It is obvious that seasonal fluctuations occur, as can be seen in Figures 3 and 4. Class boundary values have varied in 5 classification categories during the all seasons and it is observed that the values are lower in summer (Figure 4 and Table 6).

There is a general agreement about the link between mean value of nutrients and mean values of chlorophyll in the coastal waters (MED-GIG 2011). However, there is no any correlation observed between environmental parameters (nutrients) and phytoplankton abundance with chlorophyll-a at both sites of the Black Sea. This might be due to the multi-factoral interactions of phytoplankton communities and the non-linearity of the responses. Previous studies showed that small-sized microphytoplankton species were dominant in the studied area (Eker *et al*., 1999; Eker and Kıdeys, 2003). This may be attributed to the presence of small cells having problems of microscopic identification and to the difficulty in determination of their biomass (Llewellyn at al 2005).

On the other hand, pressure factors were also tested with chlorophyll as an impact factor based on the use of LUSI/LUSIVal (Land Uses Simplified Index) pressure indeces (Flo *et al*., 2011, Romero *et al*., 2013) where the similar work was done in all Turkish coastal Water bodies (Tan *et al*., 2015 (in preparation), TUBITAK-MRC and MoEU-GDEM, 2014). Figure 5 shows the relationship between the

Table 7. Comparison of high (H) and good (G) class boundary chlorophyll-a values (μg/L)

Type	Site	Chl-a (mg/m^3) Winter		Spring		Summer		Autumn		Ref
		H/G	G/M	H/G	G/M	H/G	G/M	H/G	G/M	
<30 m Mesohaline Mixed substratum exposed	Kaliakra	0.77	2.30	0.79	0.97	0.65	1.12	1.32	1.82	GIG Black Sea 2011
<30 m Mesohaline Mixed substratum exposed	Galata V. Bay	1.4	1.7	1.3	2.2	1.2	2.1	0.8	1.3	GIG Black Sea 2011
<30 m Mesohaline Mixed substratum exposed	Burgas Bay	1.05	1.7	1.4	2.1	0.8	1.2	0.7	1.2	GIG Black Sea 2011
> 30 m salinity >17.5	Sinop	0.61	0.71	0.48	0.66	0.24	0.34	0.28	0.62	Present study
> 30 m salinity >17.5	Sürmene	0.31	0.42	0.41	0.63	0.22	0.28	0.24	0.44	Present study

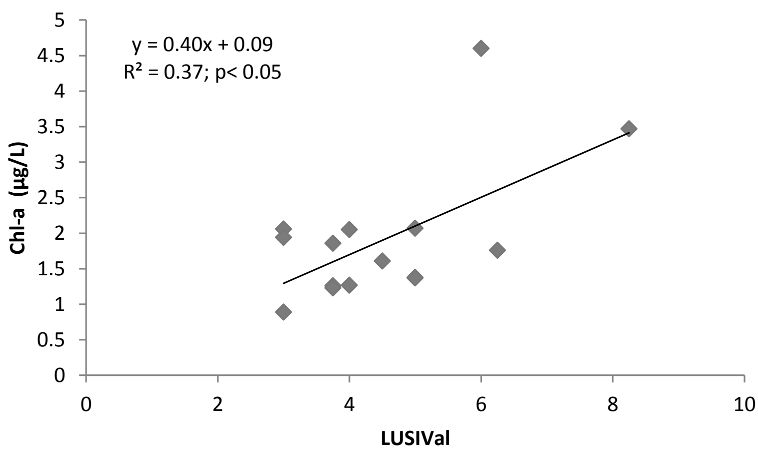

Figure 5. Relationships between pressure (LUSIVal) and impact (90th percentile (μg/L Chl-a) data for the region.

LUSIVal and Chlorophyll-a values is significant for the coastal water bodies of the Black Sea. Based on these results, it might be speculated that nutrient loadings due to different land uses contributes algal growth which may create eutrophication in the coastal water bodies whereas in this study these waters could not be classified.

Scaling of Chl-a concentrations was proposed for the first time in this study for Southeasten Black Sea waters (>30 m depth waters). This scale includes the five levels of ecological status implied by the Water Framework Directive. An ecological quality scale based on Chl-a concentrations was therefore proposed (Table 7). Chl-a scaling can be a promising tool if applied at temporal and spatial structures and therefore, water masses can be identified regarding their trophic state. Reliable assessments based on single variable indicators can be carried out only if long term measurements of chlorophyll concentrations are available at waters of different typologies.

Further testing needs to undertaken over the next five years to compare more complete datasets from a greater number of non impacted and impacted waterbodies. As more information becomes available, boundary conditions will potentially be revised as our confidence in the reference data increases.

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