



**Makale / Research Paper**

**Biomonitoring of Heavy Metal Levels in Izmit Gulf in Marmara Sea with Biofilm Formation in Plexiglass Substrate**

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**Abstract:** In this study, plexiglass substrates were used for biofilm formation to observe short-term variations in heavy metals in Izmit Gulf. The substrates were inserted and collected along in two weeks period as three times in the autumn season. The averaged accumulation values of metals were decreased in the order of Fe>Mn>Zn>Cu>Pb>Cr>Ni>Cd. The same trend was observed in the first and third samples of the autumn without an exception in the sequence. The order for the second sample was as Fe>Mn>Zn>Pb>Cr>Cu>Ni>Cd. Among the 37 species found, 22 belonged to Bacillariophyceae, 2 species to Cyanophyceae and 13 species to Dinophyceae taxa. While Bacillariophyceae consisted of about 55 % of total colony, 38 % and 7 % composed of species of Dinophyceae and Cyanophyceae taxa, respectively. Total biodensity values are 22362, 23513 and 21348 cell/mL. For various incubation days (15, 30 and 45), it was seen that increasing incubation time would not always result in the increase in densities of all species and total density of biofilms. Metal levels did not show big variations. Low correlations were observed in relative abundances of first three dominant species occupying around 85 % of total algal community with some metals, though the variations are not much significant.

**Keywords:** Biomonitoring, biofilm, heavy metal, Marmara sea.

**Marmara Denizi'nde İzmit Körfezinde Ağır Metal Seviyelerinin, Pleksiglas Substratta Biyofilm Oluşumu ile Biyoizlenmesi**

**Öz:** Bu çalışmada, Biyofilm oluşumu ile İzmit Körfezi'ndeki ağır metallerde kısa süreli değişimleri gözlemlemek için pleksiglas substratlar kullanılmıştır. Substratlar sonbahar mevsiminde yerleştirildi ve üç defada iki haftalık periyot ile toplandı. Metallerin ortalama birikim değerleri, Fe>Mn>Zn>Cu>Pb>Cr>Ni>Cd sırasına göre azalmıştır. Aynı eğilim, istisnasız olarak sonbaharın ilk ve üçüncü örneklerinde de gözlenmiştir. İkinci numunenin sırası, Fe> Mn> Zn> Pb> Cr> Cu> Ni> Cd şeklindedir. Bulunan 37 türün 22'si Bacillariophyceae'ye, 2'si Cyanophyceae'ye ve 13 türü Dinophyceae taxa'ya aittir. Bacillariophyceae toplam koloninin yaklaşık % 55'ini oluştururken, % 38 Dinophyceae ve % 7'si Cyanophyceae taxa türlerinden oluşmuştur. Toplam biyolojik yoğunluk değerleri 22362, 23513 ve 21348 hücre/mL'dir. Çeşitli inkübasyon günlerinde (15, 30 ve 45), artan inkübasyon süresinin her zaman tüm türlerin yoğunluklarında ve biyofilmlerin toplam yoğunluğunda bir artışa yol açmadığı görülmüştür. Metal seviyeleri büyük değişiklikler göstermedi. Varyasyonlar çok anlamlı olmamakla birlikte, bazı metallerle toplam alg topluluğunun % 85'ini kaplayan ilk üç baskın türün göreceli bolluğunda düşük korelasyonlar gözlenmiştir.

**Anahtar kelimeler:** Biyoizleme, biyofilm, ağır metal, Marmara denizi.

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## 1. Introduction

Eventhough low concentrations of heavy metals are usually observed in the aquatic system, anthropogenic origin has raised their concentration and create environmental problems especially in coastal zones. When the tolerable levels are exceeded then species survival and stability of the ecosystem starts to be disturbed. The human population consuming seafood is also exposed to metals. Increased levels of metals of aquatic animals result in greater health risk for human beings [1].

Metals are more effective and important in biological mediums than that of metals in water. Different binding form, long-term effects, and accumulation of metals are among the reasons for this effect. Metals with high bioavailability could be absorbed by organisms. Then, they make harm to organisms and so to human beings. The mean contaminant concentration of seawater cannot be regarded as a reliable measure of that location [2]. Sediment analysis provides total contaminant load instead of direct ecotoxicological relevance [3]. Concentrations of pollutants may not be correlated with data from sediments in the overlying water column. Contamination information cannot also give information about the higher levels of the food chain [4]. An organism living in the area as potentially polluted may be an indicator for the integrated heavy metal bioavailability [5]. Heavy metal monitoring is more suited to the living systems owing to all these reasons. Biomonitoring can be defined as the systematic use of organisms to evaluate changes with the intent of establishing a quality control program in the environment [6].

The environmental changes by natural or anthropogenic phenomena can be revealed in the biomonitoring studies. Using every organism as a biomonitor is not applicable due to some limiting factors. For example, the effects of metal pollutants may be weakened due to the metabolic ability of the exposed macrophyte. Community-level testing is very effective in toxicology. The ecological consequences of pollution exposure may be assessed with a high ecological reliability and realism [7]. With the prevailing advantageous, community-level indicator in biofilm may be the most suitable in the monitoring of metals.

As complex communities, biofilms are accumulating at surfaces of artificial or natural substrata. They are composed of mainly algae (photoautotrophic), bacteria, fungi and protozoa (heterotrophic microorganisms) [8-10]. Algae are composed of a large number of species and the main primary producers in the aqua. They have various ecological preferences [11-13]. So they can be regarded as community-level monitoring systems. Relatively easy collection and rapid response to environmental changes, they are suitable aquatic fauna for monitoring the contamination [14-17].

In our study, the primary objective was to evaluate the accumulation of some essential and non-essential heavy metals in biofilms of the artificial substrate (plexiglass) and investigate the applicability of biofilms as indicators for the biomonitoring of heavy metal contamination in marine environments (Marmara Sea). Biomonitoring with biofilm colonization is relatively hard to be applied in marine environments due to strong storms and medcezir. Therefore, there are few marine-related biomonitoring studies in literature. We also wanted to observe the relation of heavy metal levels with taxonomic diversity as a part of the biomonitoring study. Another aim in the work is to present a consistent data set, for the first time for the area, on biofilm samples for some heavy metals and to focus upon the short-term distributions of the taxonomic composition of the assemblages and heavy metals in the area.

## 2. Experimental Methods

### 2.1. Biofilm sampling and preparation to analysis

The nature of the substratum is greatly important for the colonization of algae. Based on previous researches made in our group, plexiglass slides were chosen as an artificial substrate to collect the periphytic algae for further biological taxonomical examination and counting and chemical analysis. Plexiglass slides were supported by polyvinyl chloride (PVC) holder which is a rectangular frame in shape. Plexiglass slides were attached to the PVC frames with plastic clips (Fig. 1). Each PVC holder contained 10 plexiglass slides for biofilm construction. Dimensions of plexiglass supports were 12 cm in long, 2 cm in wide and 0.5 cm in thickness. The substrate was washed with 2 % nitric acid, cleaned with absolute alcohol and rinsed with deionized water (DW) before attachment onto the PVC frame.

A plastic buoy was used to stabilize the holder 10 meters away from the shore in the Izmit gulf. The buoy was fixed with two rods with 90 degrees to each other, the end of which connected to rocks in the bottom of the sea. Two holes in the upper and bottom part of the holder were opened. Upper part was connected to the buoy with a rod about 40 cm so that the plastic holder and plexiglass supports would be vertically submerged into the water at a depth of 40-50 cm below the water surface. We preferred the vertical position to keep the biofilm slides affected with water movement like plant leaves which are naturally positioned in a vertical orientation.

Izmit gulf in the Marmara Sea was decided as the station to biomonitor the heavy metal pollution. The biofilm substrates inserted in the spring season were collected along in two weeks period. The first collected slides were waited for two-week, the second ones were taken out after four weeks, the third slides were retrieved from marine after six weeks and the last two slides were unfortunately lost with the buoy and polymer holder before collection by strong storm in the Sea.

Plexiglass films were carried to the laboratory in formaldehyde for the analysis. The transferred biofilms were divided into two parts for chemical and biological investigations. One part was dried overnight (24 hours) at 70 °C and were grinded in liquid nitrogen for chemical analysis. The other one was used in biological taxonomy. The activity numbers and species of collected microorganisms on surfaces were determined under an inverted microscope with 400x magnification and given as algae density in cell per liter.

Glass materials was generally avoided to be used for the metal contamination. Polyethylene and micropipettes with plastic tips were used when needed. All materials were cleaned by dilute (1+9) nitric acid at least 1 day. Distilled water was used to wash all materials. They were dried on filter papers.



**Figure 1.** PVC frames with plexiglass slides

## 2.2. Chemicals and analysis

Total heavy metal contents of the dry biofilm and seawater samples from the station collected in the autumn season were analyzed and determined in High Resolution Inductively Coupled Plasma Mass Spectrometer (HR-ICP-MS) after digested the sample in HCl-HNO<sub>3</sub>-H<sub>2</sub>O<sub>2</sub> mixture. Standard and internal standard solutions of heavy metals were used in the calibration studies of HR-ICP-MS. Dimetilglioxim is used to concentrate analytes and to eliminate salt content of seawater samples. All chemicals were of analytical grade or high purity. High quality, concentrated nitric acid (65 % w/v), hydrochloric acid (37 % w/v) and hydrogen peroxide (30 % w/v) were purchased from Merck and Riedel. Reagent dilutions were performed with DW. DW was purified with a Milli-Q water purification system. Calibration standards of metals were from High-Purity Standards. The standard solutions of the metals for calibration and detection were prepared from stock solutions of 1000 mg/L or near 1000 mg/L. They are prepared by successive dilutions with DW. They were prepared in the same conditions with the samples in different analysis steps. The reagent and sample solutions were also diluted if needed with DW and they were prepared daily. Dilutions of the standards were appropriate and useful to calibrate the HR-ICP-MS instrument before the determination of the metals in the samples. For accuracy check, NIST 1573a Tomato Leaves standard reference material was used for heavy metals for biological sample test. NIST Water 1640a standard reference material was used for heavy metals for seawater sample test.

## 2.3. Total heavy metal content in decomposed biofilm samples

Amounts of 0.05–0.20 grams of dry algal samples from any station were accurately weighed and decomposed with a mixture of 65 % HNO<sub>3</sub> (Merck), 35 % H<sub>2</sub>O<sub>2</sub> (Merck) and 37 % HCl (Merck) with the volume percent ratios of 60/20/20, respectively. Digests were quantitatively transferred into volumetric flasks after cooling. They were completed to a final volume of 25 ml with deionized water.

After decomposition, the supernatants were centrifuged for 15 minutes and stored immediately otherwise used in refrigerator at -18 °C for HR-ICP-MS analysis. They were waited to a constant room temperature and then again diluted ten times with DW when measured in the instrument. Blank and standard solutions of the metals were also prepared in the same conditions. Signal of blank, standards, certified standard material and samples were observed and the total heavy metal contents of each sample were determined in HR-ICP-MS instrument.

The instrument was calibrated using multi-element standards solutions prepared by mixing HNO<sub>3</sub>, HCl, and H<sub>2</sub>O<sub>2</sub> and diluting ICP stock solutions of individual elements with the proper ratio of sample content. Aliquots of digests (5 mL) were spiked with internal standard (indium) with a final concentration of 5 µg/L and completed to 10 mL with deionized water. Standard solutions of in 0.01, 0.1, 1.00, 10.00 and 100.00 ng/mL concentrations are prepared from their stock solutions. Indium was also added to these calibration standard solutions as an internal standard to monitor and compensate for the possible instrumental drift. Various isotopes were selected and used for the determination of the related elements.

## 2.4. Heavy metal content in seawater

Water samples were collected in the sampling area at different times in the Marmara Sea. Seawater samples were collected about 50 cm below the sea surface. The containers are used as non-metallic materials. All seawater contact materials were pre-cleaned with 1 N HNO<sub>3</sub>. Bottles were washed with HCl (0.1 N) before use. The water samples filtered through Millipore filters (0.45 µm pore-size). Previously acidified filter papers were also with nitric acid (10 % (v/v)) and rinse out with DW. Seawater samples were acidified with HNO<sub>3</sub> (0.5 %), after filtration. They were stored in dark

at 4 °C until they were analyzed. Metal analyses of water samples are somehow problematic due to the strong matrix effect. Therefore, the dilution technique was applied before analysis in HR-ICP-MS to eliminate matrix, overcome direct nebulization problem due to the high salt levels and measure trace metals.

Standard solutions in 0.01, 0.10, 1.00, 10.00 and 100.00 ng/mL concentrations are prepared from their stock solutions in water. As an internal standard for the possible instrumental drift, indium was added to calibration and standard solutions. They were hundred times diluted. Various isotopes and resolving powers were selected and used for the determination of the related elements in HR-ICP-MS.

## **2.5. Algal communities (species taxa, abundance and diversity)**

Algal species diversity was calculated using the Utermöhl method [18], according to the statistic instructions of Lund et al. [19]. With Utermöhl method, the total number of cells was counted with light microscopy at 400x magnification. It was recorded as cells per unit area (diatom number of cells.cm<sup>-2</sup>). With literature from central Europe, algal species had been identified with individual field scans [20]. Then, theoretical biovolumes were also extracted for each species.

With Utermöhl method, enumeration was done in fixed sample using a Nageotte counting chamber: the total number of cells counted in 10 fields (1.25 ml each, 0.5 mm depth) using light microscopy at 400x magnification was then recorded as cells per unit area of sampled substrate (number of diatom cells.cm<sup>-2</sup>). Taxonomic analysis of diatom assemblages was assigned, too. They were prepared according to ANSP protocols.

## **3. Results and Discussion**

Detection of metal content of biofilm could be done reliably. Heavy metals are reported to accumulate with high levels in natural biofilms in acute contaminated environments [21]. Biofilm as a biological monitor is suggested to be useful for anthropogenic waste [22,23]. The metal content of biofilm was seen as an indication for the bioavailability and ambient concentrations of metals over long periods [24,25].

A suitable solid surface may be a polycarbonate, plexiglass or andesite for artificial biofilm support. In our study, we used plexiglass substrate as biofilm support. The biofilm samples were inserted and collected in the autumn season of 2009 in the Izmit gulf in the Marmara Sea. The substrates were taken out three times in two-weeks period in September and December and the fourth sample was lost due to a strong storm at the end of December. Therefore, the results as three columns belonged to the samples of the autumn season and they will be used to observe short-term variations in terms of metal content in biofilm and compare with the samples from seawater and algal species density and diversity in biofilm.

### **3.1. Short-term variation in metal levels of biofilms in Izmit gulf**

The mean concentrations and standard deviations of heavy metals (Cr, Fe, Mn, Ni, Cu, Zn, Cd and Pb) investigated in this work in the algal biofilm samples collected in autumn season from the gulf of Izmit in Marmara Sea are shown in Table 1. Each element displayed a characteristic pattern of variation during the periodic sampling. Although the concentrations of some of the elements seem relatively constant, certain differences appear on those of many of the heavy metals in the samples in the season. As compared the results of three autumn samples, it was observed that although the difference in chromium (Cr) concentration was not big between the first and second samples, it was about 50 percent higher in the second one than that in the third one. Iron (Fe) had similar

concentration values and the results were not much significant in the three samples. There was not much difference between the first and second sample in terms of manganese (Mn) concentrations, though the second sample was still about twenty percent higher than the third one. The highest value was seen in the second sample in nickel (Ni) concentration, the variation was about twenty percent with other samples. The first sample had the highest values in terms of copper (Cu) and cadmium (Cd) concentrations. Copper concentration in the first sample was about 35 % and 15 % higher than those in the second and third ones, respectively. Cadmium in the first sample was about 50 % higher than that of both second and third ones. Although there were small differences between samples in terms of zinc (Zn) and lead (Pb) concentrations, the differences were insignificant. As a general aspect, the differences between concentrations of metals were not so big that searching the reason for the difference would be needed.

**Table 1.** Concentration of metals (mg/kg dry wt.) in the algal biofilm samples collected with 15-day period in Autumn Season Izmit Gulf in Marmara Sea

Element	1° Sample	2° Sample	3° Sample
Cr	54.0 ± 3.0	63.0 ± 1.5	42.3 ± 2.9
Fe	3888 ± 238	4190 ± 151	3746 ± 228
Mn	1094 ± 31	956 ± 22	1188 ± 91
Ni	16.4 ± 0.3	19.4 ± 0.9	15.4 ± 0.3
Cu	83.7 ± 2.0	60.7 ± 4.8	71.5 ± 7.1
Zn	738 ± 34	689 ± 55	691 ± 22
Cd	6.1 ± 0.5	4.2 ± 0.3	4.0 ± 0.3
Pb	59.1 ± 4.4	65.4 ± 6.3	65.6 ± 3.9

Concentrations are expressed in mg/kg; the uncertainties represent 95% confidence limits (n=3). 100 mg sample was digested with 10 ml of HNO<sub>3</sub>+HCl+H<sub>2</sub>O<sub>2</sub> mixture (Ratio:3/1/1). Digested samples were measured after 100-fold dilution in HR-ICP-MS.

The averaged accumulation values of metals were decreased in the order of Fe>Mn>Zn>Cu>Pb>Cr>Ni>Cd in the gulf of Izmit biosamples. The same trend was observed in the first and third samples of the autumn without an exception in the sequence. Copper was reduced to sixth position in the sequence of the second sample and the order was as Fe>Mn>Zn>Pb>Cr>Cu>Ni>Cd.

These results show that increasing the collection time would not always result in an increase in the content of metal in biofilms. The sorption of metals by algae is highly variable. It depends on the metal, the taxonomy, age of taxon, etc. [26]. They also vary with season [27,28]. We also observed the replacement of some metals examined in the sorption sequence even in short-term monitoring study while observing some factors such as metal content of water, biofilm density, and diversity, affecting metal sorption in biofilm.

### 3.2. Short-term variation in seawater in Izmit gulf

In Izmit gulf, the concentrations of most of the metals demonstrated variations in water samples (Table 2). Industrial metal inputs mostly dependent on discharge. Autumn samples exhibited high levels of metals in dissolved form probably due to high temperature and weak solid adsorption with particulate matter.

Direct input of wastewaters into the waters is one of the variability in metal concentrations. This is the natural result in highly industrialized areas. Trace of metals for industries is seen as the change in the concentrations of As, Cr, Cu, Mn, Ni and Fe. These metals are traces from industries (iron-steel) in the region. They use coal as fuel containing high arsenic content. Other metals such as Cd, Co, Pb, Sn, Zn have similar properties. The concentration distributions are generally in harmony.

They are related as the indicators of paint industries [29,30]. Many of them are present in the studied region.

**Table 2.** Concentration of heavy metals ( $\mu\text{g/L}$ ) in the seawater samples collected with a 15-day period in the Izmit Gulf in Marmara Sea

Element	1° Sample	2° Sample	3° Sample
Cr	$3.60 \pm 0.18$	$4.73 \pm 0.30$	$2.43 \pm 0.21$
Fe	$3.96 \pm 0.07$	$4.242 \pm 0.444$	$3.24 \pm 0.39$
Mn	$5.48 \pm 0.33$	$3.65 \pm 0.24$	$3.15 \pm 0.37$
Ni	$2.24 \pm 0.21$	$3.11 \pm 0.30$	$2.69 \pm 0.14$
Cu	$7.82 \pm 0.76$	$9.81 \pm 0.64$	$6.59 \pm 0.46$
Zn	$15.2 \pm 1.03$	$10.20 \pm 0.46$	$12.9 \pm 1.04$
Cd	$1.64 \pm 0.08$	$1.18 \pm 0.07$	$1.35 \pm 0.14$
Pb	$3.09 \pm 0.17$	$4.29 \pm 0.30$	$4.34 \pm 0.42$

Concentrations are expressed in  $\text{ng/ml}$ ; the uncertainties represent 95% confidence limits ( $n=3$ ). Samples were filtered and measured after 20-fold dilution with HR-ICP-MS.

Metal load in the gulf of Izmit has been attributed largely to metal and paint industries. However, hydrological variations may occur with complex responses in metal concentrations in dissolved form. Dilution effect, various industrial leaching, ground and surface water interactions are assessable among these complex relations.

### 3.3. Short-term variation in colonization in Izmit gulf

Based on the global approach, the relative abundances of the 37 algal species were determined with very high levels of cumulative abundances during the autumn season in the Izmit Gulf in the Marmara Sea. Among the 37 species found, 22 belonged to Bacillariophyceae, 2 species to Cyanophyceae and 13 species to Dinophyceae taxa. While Bacillariophyceae consisted of about 55 % of total colony, 38 % and 7 % composed of species of Dinophyceae and Cyanophyceae taxa, respectively. Biofilms were always dominated by Bacillariophyceae species both in species diversity and in total biodensity at all stages in all samples at the Izmit Gulf station. Total biodensity values are 22362, 23513 and 21348 cell/ml in the autumn samples.

As the results of various incubation days (15, 30 and 45) in the autumn season were compared, it was seen that there was a continuous increase in the relative percent abundance of species, whereas the relationship was not linear. The relative abundance of most dominant species (*Cylindrotheca closterium*) was found at the values of 44, 48 and 49 % of the total communities during 45 days for three sampling in the autumn season (Table 3). Similar to the first one, *Prorocentrum scutellum* the second dominant species also varied greatly with the values of 30, 35 and 37 %. It showed a great increase from first to second sample and then a slight increase was observed in the third one in percent ratio. *Prorocentrum micans* were the third dominant species among the autumn season samples instead of *Thalassiosira* sp. which was the third one in the average of all samples in Izmit gulf. Unlike the first two dominant species, the relative percent abundance of *Prorocentrum micans* showed great decrease as 11, 5 and 4 % from first to second one and a slight decrease in the third one. It should also be noted that while the most dominant species belongs to Bacillariophyceae taxa, second and third dominants come from Dinophyceae.

Species densities are more useful than percent abundance of them due to the comparable characteristics with total cell densities, though percent abundance is sometimes important in the discussion of relative comparisons of species with each other. In terms of species density, the results of first three dominants present a different situation in the autumn. The results were the first markedly increased and then slightly decreased. Density values of *Cylindrotheca closterium* and

Prorocentrum scutellum have similar sequences throughout 45 days as 9800, 11320, 10400 and 6800, 8300, 7800, respectively. At the opposite, density values of Prorocentrum micans were drastically decreased as 2420, 1230, 820. Total algal density values (22362, 23513 and 21348 ) were comparable with densities of some species in the autumn season samples throughout 45 days.

**Table 3.** The colonization density and percent abundance values of three samples from the gulf of Izmit in Marmara Sea, collected with a 15-day period in the autumn season of 2009

Top 10 Dominants	Density (cell/ml)			Percent (%)		
	1°	2°	3°	1°	2°	3°
Cylindrotheca closterium	9800	11320	10400	43,82	48,14	48,72
Prorocentrum scutellum (D)	6800	8300	7800	30,41	35,30	36,54
Thalassiosira sp.	196	255	64	0,88	1,08	0,30
Prorocentrum micans (D)	2420	1230	820	10,82	5,23	3,84
Pseudo-nitzschia sp.	489	506	676	2,19	2,15	3,17
Chaetoceros sp.	624	350	215	2,79	1,49	1,01
Skeletonema costatum	435	164	138	1,95	0,70	0,65
Nitzschia seriata	412	255	205	1,84	1,08	0,96
Nitzschia longissima	116	196	264	0,52	0,83	1,24
Prorocentrum minimum	235	145	108	1,05	0,62	0,51
<b>Total of 10</b>	<b>21527</b>	<b>22721</b>	<b>20690</b>	<b>96,27</b>	<b>96,62</b>	<b>96,94</b>
<b>Total of all</b>	<b>22362</b>	<b>23513</b>	<b>21348</b>	<b>100</b>	<b>100</b>	<b>100</b>

The results in Table 3 show that increasing incubation time would not always result in the increase in densities of all species and total density of biofilms. Even though the most important observation was that variation in total density seems due to variations with positive correlations in the density of first two dominant species. it should also be noticed that Prorocentrum micans species is very sensitive to variations due to negative correlations with total biomass and its density becomes disappeared with time. Another important observation is that biofilms are always dominated by three species and no one in dominant taxa was replaced with another species in terms of density at all stages during periodic sampling within 45 days, even though their density values change. In other words, there was no competition between microorganisms for dominance, however their densities were varied. Although there seems no continuous specific effect for the first two dominant species, there could be a specific effect for the variation in density of the third dominant species. Nonlinear behaviour of abundances of most of the species would be related with a common effect such as nutrition supplies, salt content and light attenuation to the marine environment. Factors resulting in variations in water quality was thought to be changed within the autumn season, more or less.

The composition of periphytic communities in aquatic environments is mentioned as a good indicator for several forms of the presence of excessive concentrations of pollutants both toxic and non-toxic in several studies [31]. Other factors reported were related only to the structural changes within the biofilm.

One of the factors affecting colonization structure of biofilms may be the metal content in water appeared as the waste of industries built near the area of the Izmit Gulf coasts. Despite many factors affecting algal diversity and total biomass, [32] specific pollution index (SPI) is known to be sensitive to micropollutants in some cases, anyway. As a major source of urban pollution, a polluted site is affected by discharges of metal and organic effluents, which can induce a decrease in species diversity as described by [33], and also changes in community for the benefit of the most tolerant taxa [34]. De Jonge et al., [35] arrived at the results that diatom indices are not significantly correlated with metal concentrations in water.

The gradual changes either increase or decrease in the abundances of species and total algal densities could be relatively correlated with a change in some heavy metal content in biofilm. In the present study, metal levels in autumn samples did not show big variations. Relative abundances of first three dominant species occupying around 85 % of total algal community with low correlations of some metals in biofilm were found in Izmit gulf, though the variations are not much significant. Positive correlations are observed among total algal densities, densities of *Cylindrotheca closterium* and *Prorocentrum scutellum* and concentrations of Cr, Fe and Zn in biofilms. Algal densities are negatively correlated with densities of *Prorocentrum micans*. *Cylindrotheca closterium* and *Prorocentrum scutellum* may be tolerant species, whereas, *Prorocentrum micans* would be sensitive to mentioned metals but tolerant to cadmium due to positive correlation between them.

#### 4. Conclusions

As a general result of autumn samples, we observed that variation in metal levels in biofilm samples was not much significant and stayed relatively constant through 45 days which means that at least two weeks are sufficient for saturation of colonization in biofilm and their metal content, though the variation in metal levels in water was big enough to change the biofilm characteristics. Many works demonstrate similar behaviour with our study in biofilm samples. For example, Coste et al., [32] observed similar results but different saturation time. In the work, despite an increase in their densities, the main characteristics and metal content of diatom communities remained relatively stable after 6 weeks at the three levels of contamination. According to Chen et al., [36], correlations of the water quality parameters with biofilms colonization for one-month is higher than accumulation biofilms (colonization time bigger than one-month). It suggests that short type colonization of biofilm was sufficient in determining a seawater quality. They also defend that recent colonization of microorganisms on artificial substrates are relatively active. The water quality directly influences the structures of the species in biofilms. Biggs [37] also claims that 4 weeks are suitable for algal biomass and further confirmed with experiments. Moreover, similar to our results, Hoagland et al., [38] suggests that communities 2 weeks as a shorter period in mature form can be established in equilibrium with the area. We did not have a chance to observe the behaviour of biofilms after 6 weeks due to the lost of the samples in a strong storm in the Izmit Gulf, whereas we suggest that 6 weeks are enough to evaluate the time related variations and no need to last sample.

Protection of the bottom cells by the upper algal cells within the thickness of the biofilms may be the reason for short-term metal saturation and may protect them against pollutants and metal input. We may suggest such an interpretation. More complementary studies and support are needed taking into account the analyses with very short bioaccumulation. A protective role against the metal stress of organic materials in mature communities was expressed in laboratory streams [39]. Although EPS of algae in mature form [40] presents high metal complexation, bioavailability and toxicity of metals reduce in algal cells.

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