



## Assessment of Temporal Ecosystem Responses to Phytoplankton via Photosynthetic Pigments under a Potential Oil Spill Event in Iskenderun Bay

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### Abstract

Ecosystem risk assessments after oil spills need at least one biological component's response to have some degree of understanding of the risks associated with the spill. In this study, crude oil spill impacts were studied under a spill scenario in Iskenderun Bay, which is a highly prone area to oil tanker accidents due to the high levels of transpassing. The study evaluated phytoplankton communities' response in 4 different seasons. Photosynthetic pigments data, obtained by HPLC, was used to assess community shifts of phytoplankton under different doses of the crude oil. It was found that the time of year of incidence is critical to understanding the consequences because a highly significant response difference was detected, which is more than an order of magnitude between winter and fall seasons. Due to physical conditions of seawater and initial phytoplankton compositions, the ecosystem showed more tolerance to the crude oil in winter than other seasons. EC<sub>50</sub> values varied between 11.5 and 122.3 µg/L total petroleum hydrocarbon (TPH) concentrations for all seasons. It is shown that the time of the year and known TPH concentrations of the seawater can possibly tell us about the potential impact of an oil spill in this region.

**Keywords:** Oil spill, Iskenderun Bay, phytoplankton, ecotoxicology, pigment.

### Introduction

Petroleum hydrocarbons (PHs) are present in marine environments worldwide. Oil spills are one of the major causes of marine pollution that might have devastating impacts on living species. According to the National Research Council (Board, Board & NRC, 2002) report in 2002, oils enter the ocean from a variety of sources: natural sources (natural seeps; 46%), and human-induced (accidental spills from ships; 12%). These two sources account for a large part of the total annual release of oil into the marine environment. The estimate of the average total worldwide annual release of petroleum ranges widely, from 470,000 tons to a possible 8.4 million tons per year. Accidental or deliberate, operational discharges and spills of oil from ships, especially tankers, offshore platforms, and pipelines, are the most obvious and visible unnatural causes of oil pollution of the marine environment.

Since 1970, there have been almost 1400 spills from oil tankers that ranged from 7 tons to 700 tons. These are classified as medium-scale oil tanker spills. Larger vessels, bigger than 700 tons, have cumulatively reached 455 spills. In the six-year period

including the years from 2010 to 2015, there have been 42 spills of 7 tons and more, resulting in 33,000 tons of oil lost; 86% of this amount was spilled in just 10 incidents (ITOPF, 2017).

The severity of the environmental impact typically depends on many parameters, including the quantity and type of oil spilled, the physical characteristics of the affected area, weather conditions and season, the ambient conditions, and the sensitivity of the affected organisms and their habitats to the oil. Ecosystem responses have been assessed worldwide (Nomura *et al.* 2007; Adenkule, Ajijo, Adcofun, and Omoniyi, 2010; Gilde & Pinckney, 2012; Gonzalez Fernández, Figueiras, & Varela, 2013; Ozhan, Miles, Gao, & Bargu, 2014a) using different crude oils and different organisms. Since phytoplankton at the base of marine food web, their response to oil spill potentially will impact the entire marine ecosystem.

British Petroleum's Baku-Tbilisi-Ceyhan (BTC) pipeline takes oil from the Caspian Sea to the Mediterranean instead of using tanker transport along the Black Sea and the highly congested Bosphorus strait. BTC's throughput capacity is currently 1.2 million barrels per day. The BTC pipeline is located in Iskenderun Bay, where many oil tankers transpass

every day, making the region very susceptible to tanker accidents. Additionally, the semi-enclosed nature of the bay have potential to elevate the likelihood of these impacts. Previous studies conducted (Corps 2002; Chilongo, Guliyev, & Durgut, 2013) in the region did not go beyond the modelling of physical distribution and area likely to be affected by a potential oil spill.

This study aims to conduct the first ecotoxicology study and assess the first ecosystem response of the phytoplankton community to a potential oil spill that could be caused by an oil tanker accident in Iskenderun Bay.

## Materials and Methods

The study assessed the toxicity of the water-accommodated fractions (WAFs) of Azeri crude oil considering their seasonal distinct community compositions and using natural phytoplankton communities isolated from Iskenderun Bay during 4 different seasons. Controlled laboratory microcosm studies were conducted using standard static non-renewal exposure toxicity tests. Changes in the phytoplankton community composition were quantified by the ratios of the concentrations of specific diagnostic pigments and  $EC_{50}$  values were reported.

Samples of phytoplankton were collected from Iskenderun Bay on the route of oil tankers (Figure 1). The field samples were filtered through a 110- $\mu$ m mesh filter to separate zooplankton, then acclimated to ambient laboratory conditions prior to use in the experiments.

Non-weathered Azeri petrol was provided by BOTAS and stored at 4 °C. The WAF was prepared according to the method described in The Chemical Response to Oil Spills: Ecological Research Forum (Aurand & Coelho, 2005). The WAF mixtures used in the algae toxicology tests were prepared with 0.22  $\mu$ m-filtered and autoclaved Mediterranean seawater in a 5-L Klimax, valved-outlet reservoir bottle. Loading of 100 g Azeri petrol in 4 L seawater is known to

result in 20%–25% headspace by volume. The stirring rate was adjusted to 160 rpm to prevent microparticulate settlement at the bottom. After 24 hours of mixing, a settling time of 6 hours was applied. Samples from the WAF were withdrawn through a valve located at the bottom of the bottle to avoid disturbing the water/oil interface. Serial dilutions (10%, 20%, 40%, and 80%) of the water phase from each test medium yielded concentrations of 29, 58, 115, and 231  $\mu$ g/L total petroleum hydrocarbon (TPH), respectively, that were used in the experiments. In addition to these treatments, a control treatment (no crude oil) was prepared. Upon preparation of all control and treatment solutions, the same amount of seawater containing natural phytoplankton communities from the field were added in each flask to initiate experiments with same level of phytoplankton. Diluted nutrients (10 times from the original recipe), from the f/2 culture (Guillard & Ryther, 1962) medium kit, were added in each flask to prevent additional stress that might result from nutrient deficiency for the phytoplankton. The experimental flasks were kept at 25 °C in the temperature-controlled room on a 12:12 hour light: dark cycle with cool-white fluorescent lights at an irradiance of 180  $\mu$ E·m<sup>-2</sup>·s<sup>-1</sup> for 4–6 days. Flasks volume were 2 L. Water samples of approximately 400 mL were taken from each flask throughout the experiment to determine daily changes in phytoplankton pigment composition.

Photosynthetic pigment concentrations were quantified using high performance liquid chromatography (HPLC) for samples from the microcosm setup. Samples taken at days 0, 2, 4, and 5 were filtered through GF/F filters under a low vacuum and immediately stored at -20 °C. According to the analytical method of Barlow, Cummings & Gibb (1997), samples were extracted in 90% acetone using sonication. Extracted samples were kept overnight in the refrigerator and centrifuged before the analysis. The centrifuged samples then were kept in glass vials until injection. All injections were done in an auto sampler, and samples were mixed with 1:1 ion pairing

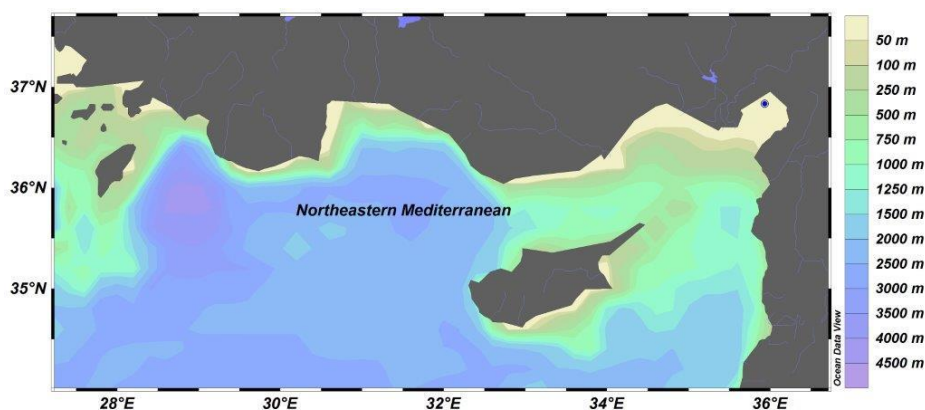


Figure 1. The sampling location in the Northeastern Mediterranean Sea.

1M ammonium acetate solution before injection (Mantoura & Llewellyn, 1983).

Two eluents were used in the mobile phase: the primary eluent (A) consisting of methanol and 1M ammonium acetate (80:20 v/v), and a secondary eluent (B) consisting of 100% methanol. Pigments were separated at a flow rate of 1 ml/min by a linear gradient programmed as follows (minutes; % solvent A; % solvent B): (0;75;25), (1;50;50), (20;30;70), (25;0;100), (32;0;100). The column was then reconditioned to its original conditions over a further 7-min period (Barlow *et al.*, 1997).

Pigment concentrations were calculated by 'external standard' equation (Jeffrey *et al.*, 1997). The standards used are chlorophyll a (CHL-A) (Sigma Chemical Co.), chlorophyll c3 (C3), chlorophyll c2 (C2), peridinin (PER), 19 butanoyloxyfucoxanthin (BUT), fucoxanthin (FUC), 19-hexanoyloxyfucoxanthin (HEX), diadinoxanthin (DIAD), alloxanthin (ALLO), zeaxanthin (ZEA), lutein (LUT), chlorophyll-b (CHL-B), divinyl chlorophyll-a (DIV-A) and  $\beta$ -carotene (B-CAR) (VKI, Denmark). Based on retention time in the column, pigments sorted sequentially and absorbed at 440 nm was calculated in accordance with the following standards.

$$C_p = A_p \times V_{\text{ext}} \times 10 / B \times V_{\text{filt}} \times V_{\text{inj}} \times 1000 \times R_f$$

Where;

$C_p$  ( $\mu\text{g L}^{-1}$ ): concentration of a particular pigment  
 $A_p$  (mAU\*s): peak area of the eluting pigment  
 $R_f$  (ng mAU $^{-1}$ ): the slope of the calibration curve (ng column $^{-1}$ )  
 $V_{\text{filt}}$  (l): the volume of filtered seawater  
 $V_{\text{ext}}$  (ml): the solvent used for the extraction;  
 $V_{\text{inj}}$  ( $\mu\text{l}$ ): the solvent injected to the chromatographic system and  
 B: the buffer dilution factor

After measuring the concentrations of the diagnostic pigments, all concentration levels were converted to % different phytoplankton contribution groups based on the method described by Hirata *et al.* (2011). The following diagnostic pigments were used to identify seven phytoplankton groups: fucoxanthin (Fuco), peridinin (Peri), alloxanthin (Allo), 19'-hexanoyloxyfucoxanthin (Hex), 19'-butanoyloxyfucoxanthin (But), zeaxanthin (Zea), and total chlorophyll-b (TChl-b, i.e. the sum of monovinylchl-b, chl-b, and divinylchl-b (div-b)). According to Hirata *et al.* (2011) the weighted relationships of these diagnostic pigments (DPw) were calculated using multiple regression analysis as follows:

$$\% \text{ diatoms} = 100 * (1.41 \text{ Fuco}) / \text{DPw}$$

$$\% \text{ dinoflagellates} = 100 * (1.41 \text{ Peri}) / \text{DPw}$$

$$\% \text{ haptophytes} = 100 * (1.27 \text{ Hex}) / \text{DPw}$$

$$\% \text{ chrysophytes} = 100 * (0.35 \text{ But}) / \text{DPw}$$

$$\% \text{ cryptophytes} = 100 * (0.6 \text{ Allo}) / \text{DPw}$$

$$\% \text{ chlorophytes} = 100 * (1.01 \text{ TChl-b}) / \text{DPw}$$

$$\% \text{ all cyanobacteria} = 100 * (0.86 \text{ Zea}) / \text{DPw}$$

where DPw = 0.86 Zea + 1.01 TChl-b + 1.27 Hex + 0.35 But + 0.6 Allo + 1.41 Fuco + 1.41 Peri.

Chl-a was used to represent biomass in this study. Chl-a values vs. time growth curves were plotted. The common way of calculating specific growth rate from the slope of each exponential growth phase of the growth curve did not work well in this study because of irregularities in exponential growth phases among the different treatment setups: some had an exponential phase, whereas others had no exponential growth. Instead, algal growth was calculated using the area under the growth curve, which is equal to the total increase in biomass.

$$A = \frac{(N_1 - N_0) \cdot t_1}{2} + \frac{(N_1 + N_2 - 2N_0) \cdot (t_2 - t_1)}{2} + \dots + \frac{(N_{n-1} + N_n - 2N_0) \cdot (t_n - t_{n-1})}{2}$$

$\mu = \ln(N_{t_2}/N_{t_1}) / (t_2 - t_1)$  where  $N_{t_2}$  and  $N_{t_1}$  are chl-a concentrations at time  $t_2$  and  $t_1$ , respectively.

The inhibition rates of different treatments were calculated based on the following formula: I (%) =  $(A_c - A_t) / A_c \times 100$ , where  $A_c$  and  $A_t$  are the area under the growth curve of control group and the treatment, respectively.

Approximately 750 mL WAF aliquot was placed in a separatory funnel and internal standards (nC19-d40 and cadalene) were added. Next, the samples were extracted (liquid-liquid extraction, 3 x 80 mL) into 240 mL hexane. The extracts were then reduced to approximately 10–15 mL on a rotary evaporator. The reduced extracts were transferred to graduated flasks and reduced to the desired volumes under nitrogen gas. A 1-mL sample of the resulted crude was transferred to the instrument. Hydrocarbon analysis was carried out using a gas chromatograph (Agilent 6890N) equipped with a FID detector (GC-FID) and a HF-5 phenylmethylsiloxane capillary column (30 m x 0.25 mm i.d.).

Instrument response factors were calculated using a series of external standard solutions provided by IAEA that contained saturated alkanes in the range of nC10 through nC35 and polycyclic aromatic hydrocarbon combined with the internal standards (nC19-d40 and cadalene.)

Column: 30 m x 0.25 mm, 0.25 mm film thickness.

Carrier gas: Nitrogen; flow rate: 1 mL/min

Temperature program setting: Temperature was

kept at 50 °C for the first 30 minutes and then increased to 120 °C at 1°C increments per minute. Then, the temperature was increased to 240 °C, at 2°C increments per minute, then the final temperature 300 °C was reached by 30 °C increments per minute; analysis was continued isothermally for 15 minutes.

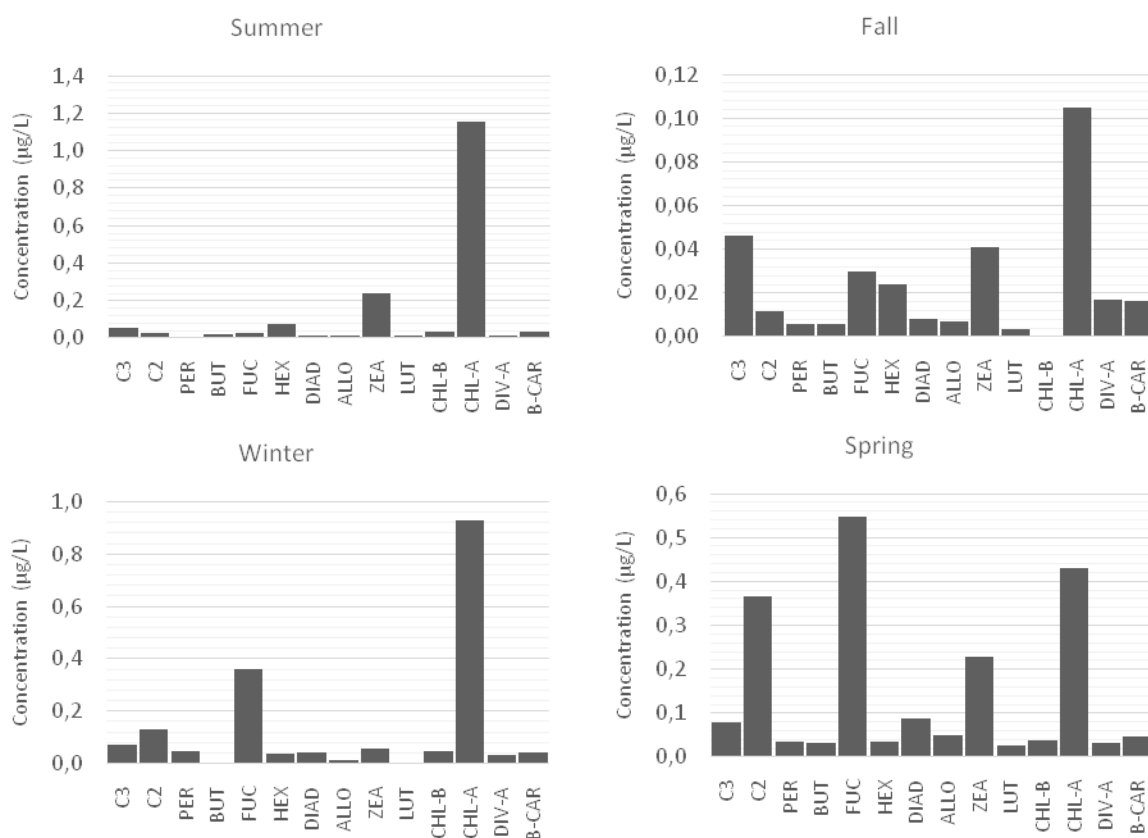
## Results

The measured seawater parameters (Table 1) for each season reflect typical Northeastern Mediterranean seawater values: high salinity and low nutrient content. Summer chlorophyll-a concentrations were an order of magnitude greater than those during the fall season (Table 1). The initial phytoplankton photosynthetic pigment compositions showed significant differences across

the four seasons (Figure 2). Total photosynthetic pigment concentrations were 1.70, 0.32, 1.79, and 2.03 µg/L for summer, fall, winter, and spring, respectively. The pigments % contribution for each season varied greatly. In summer, Chl-a (68%) was the prevailing pigment followed by Zea (14%) and Hex (4.4%). In fall, the phytoplankton abundance was quite low compared to summer phytoplankton abundance. The dominance of a few pigments was moderate this season compared to summer pigments. Chl-a (32%) dominance was followed by C3 (14%), and Zea (13%) in the fall. These 3 pigments made up about 60% of the entire community. In winter, chl-a (52%) dominance was followed by fuc (20%) and C2 (7%). Unexpectedly, in spring, chl-a (21%) was not the most dominant pigment in the phytoplankton community. Fuc (27%) concentration was higher than

**Table 1.** Measured seasonal seawater parameters

	Temperature (°C)	Salinity (PSU)	Chl-a (µg/L)	PO <sub>4</sub> (µM)	NO <sub>3</sub> +NO <sub>2</sub> (µM)	NO <sub>2</sub> (µM)	NH <sub>4</sub> (µM)	Si (µM)
Summer	30,6	38,4	1,15	0,05	0,04	0,03	0,09	3,09
Fall	24,7	38,2	0,10	0,09	0,96	0,19	0,34	2,20
Winter	16,6	38,8	0,92	0,14	1,31	0,37	0,40	3,15
Spring	22,5	38,4	0,43	0,28	3,04	1,56	2,52	3,20



**Figure 2.** The photosynthetic pigment concentrations at day 0.

chl-a, which was followed by C2 (18%) in the spring.

As it is mostly evaluated from dose response toxicology tests, higher dose levels cause greater adverse effects on organisms. In this study, this trend was observed in both the summer and fall seasons, and the lowest dose (29 µg/L) showed no significant impact compared with the control groups at the end of the experiments (Figure 3-Figure 7). However, the winter study told a different story. The control group, 29 µg/L, 58 µg/L, and 115 µg/L crude oil showed a similar pattern: it increased in abundance for the first two days, then declined in abundance until the end of the experiment. The highest crude oil dose did not show any biomass increase (in this study represented as chl-a concentrations) throughout the experiment in winter. In spring, by day 2, there was no significant difference among the treatments; however, at day 4 the control, 29 µg/L, and 58 µg/L treatments showed growth that did not differ significantly from each other. The other two higher doses caused growth inhibition on phytoplankton. The next day, the lowest two doses, 29 µg/L and 58 µg/L, indicated a greater biomass increase compared with the control group.

Effective concentrations that caused 50% growth inhibition (EC<sub>50</sub>) on phytoplankton communities reflected some seasonal distinctions (Figure 8). The highest EC<sub>50</sub> values indicate that phytoplankton are the most tolerant to crude oil, and they were observed in winter and gradually decreased towards the fall. The main difference between the winter and fall seasons was the contribution of other pigments in addition to fuc and chl-a pigments. While fuc was the most predominant pigment after chl-a in the winter, other pigments were also coexisting abundantly in the fall. Apparently, the presence of a high fuc diatom diagnostic pigment provided an advantage to the community in terms of an elevated resistance to the crude oil pollution.

## Discussions

Phytoplankton photosynthetic pigments are used as a tool in marine and freshwater pollution studies (Pinckney, Örnólfssdóttir & Lumsden, 2002; Paerl *et al.* 2003; Tamm, Freiberg, Tönno, Nöges & Nöges, 2015). Most of these studies used CHEMTAX as an analysis tool to determine the concentration of individual species. However, a thorough knowledge of the specific pigment composition of different species is required to incorporate pigment concentration data with community composition (Örnólfssdóttir, Pinckney & Tester, 2003). In reality, those ratios differ by region (Descy, Sarmento & Higgins, 2009). Investigations of the reliability of CHEMTAX emphasize the prerequisite to adapt procedures to the targeted area by searching for the dominant species, their pigment content, and environmental conditions such as light availability and nutrient status (Wright & Jeffrey, 2006). Because these studies and specific pigment compositions of the

Northeastern Mediterranean Sea phytoplankton have not been adequately documented, Hirata *et al.* (2011) used multiple regression analysis instead of CHEMTAX to determine the phytoplankton community composition. Additionally, in this toxicology study, there was a concern about the alteration of pigment contents during the crude oil exposure. A question was raised: "Can toxic behavior of the crude oil cause any changes on phytoplankton pigment contents?". Unfortunately, testing this phenomenon is quite complex in phytoplankton community studies. Even if the quantification of changes in each individual pigment content during the exposure is done, due to other parameters involved such as competition and their growth phase, it is hard to allocate these changes only to the crude oil exposure. When the phytoplankton group composition is talked about in this paper, the assumption of the insignificant changes in their pigment composition during the crude oil should not be forgotten.

A previous study (Ozhan & Bargu, 2014) showed the importance of nutrient condition during algal ecotoxicology tests. In the present study, rather than exposing the phytoplankton to additional stress during ecotoxicology tests due to nutrient deficiency, nutrients were replenished at the beginning of the experiments to ensure that the phytoplankton would not suffer from deficiency.

The expected pigment compositions were detected in each season in the Northeastern Mediterranean Sea. High diatom dominancy was observed in the productive winter and spring seasons while high cyanobacterial abundance in the field was observed in the less-productive summer and fall seasons. However, unexpectedly high chl-a concentrations in summer could be associated with a local bloom or a post-bloom event; low N concentrations in summer support this hypothesis. The initial phytoplankton compositions showed big differences over 4 seasons (Figure 9). Summer-fall and winter-spring separation in terms of the phytoplankton composition was observed. While cyanobacteria were the predominant group in summer, this dominance diminished through winter and spring; diatoms became the most abundant phytoplankton group making up over 60% of the total community. In fall, there was no chlorophytes, in winter, there was no chrysophytes were detected. Dinoflagellate abundances were relatively similar across the seasons (Figure 9).

In later days (days 2, 4, and 5), the phytoplankton compositions were calculated under the assumption that there were no significant changes in pigment content during the crude oil exposure. Regarding individual phytoplankton groups' tolerances, dinoflagellates showed higher tolerance to the crude oil in summer as the dose of crude oil increased (Figure 10). While the relative abundance of cryptophytes became higher in the control, the increasing crude oil level suppressed the relative

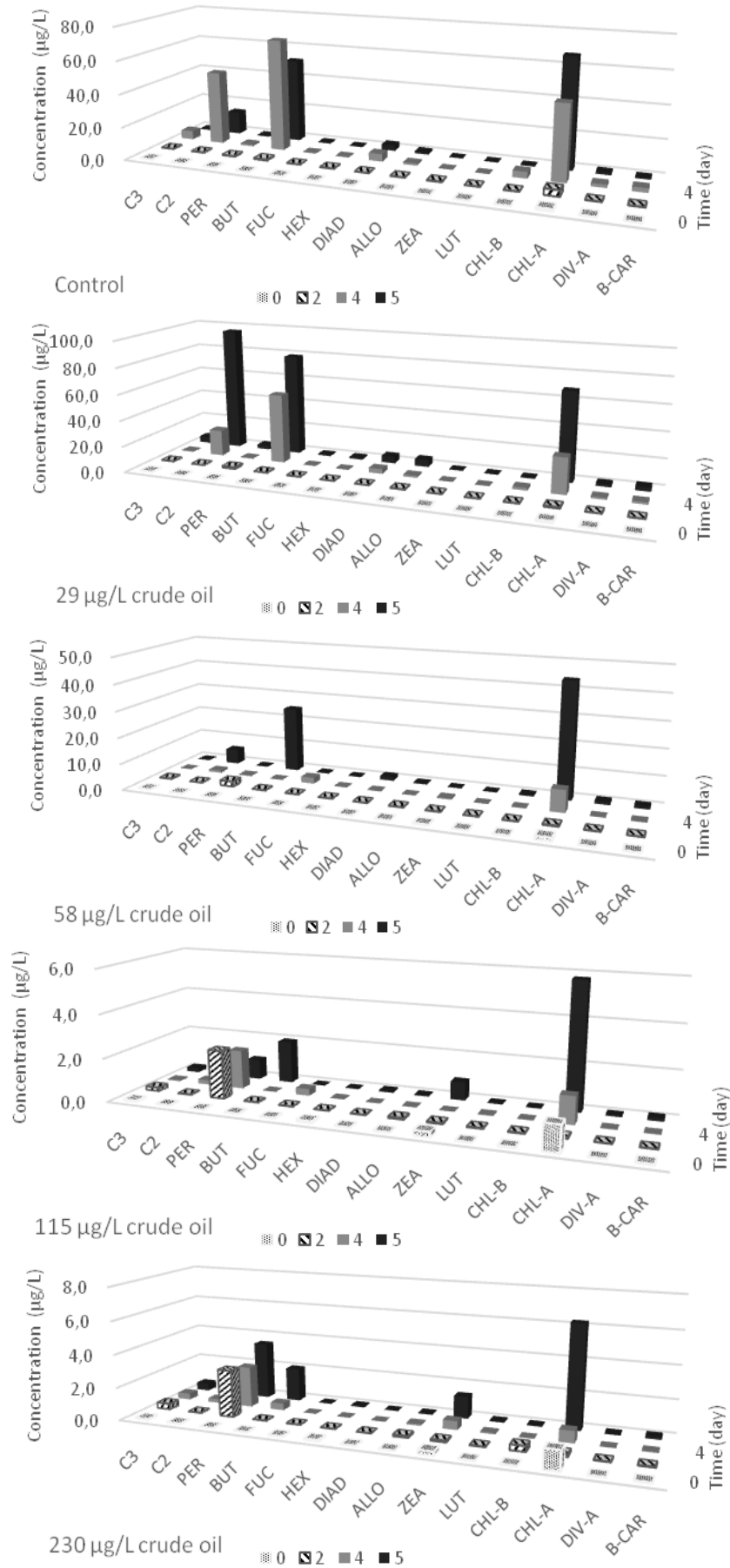
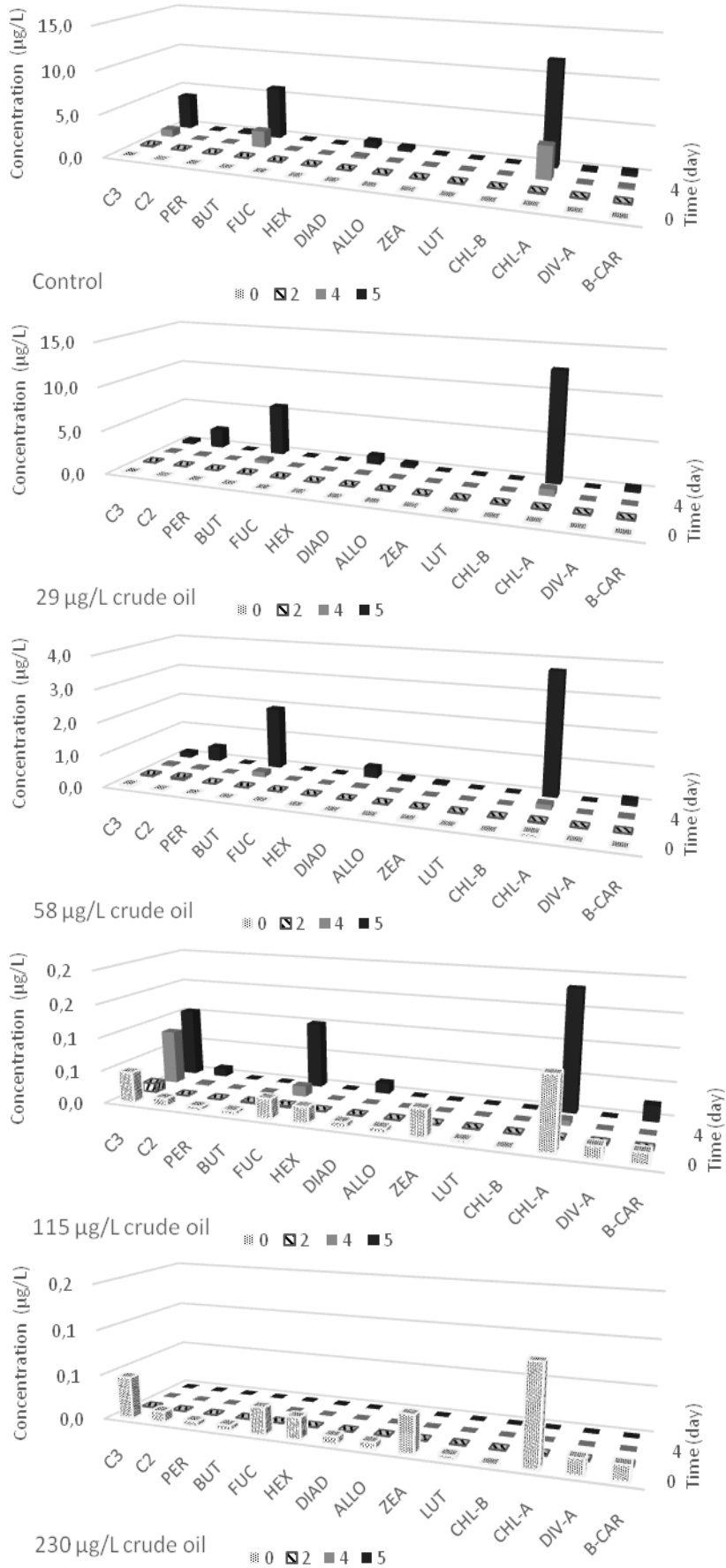
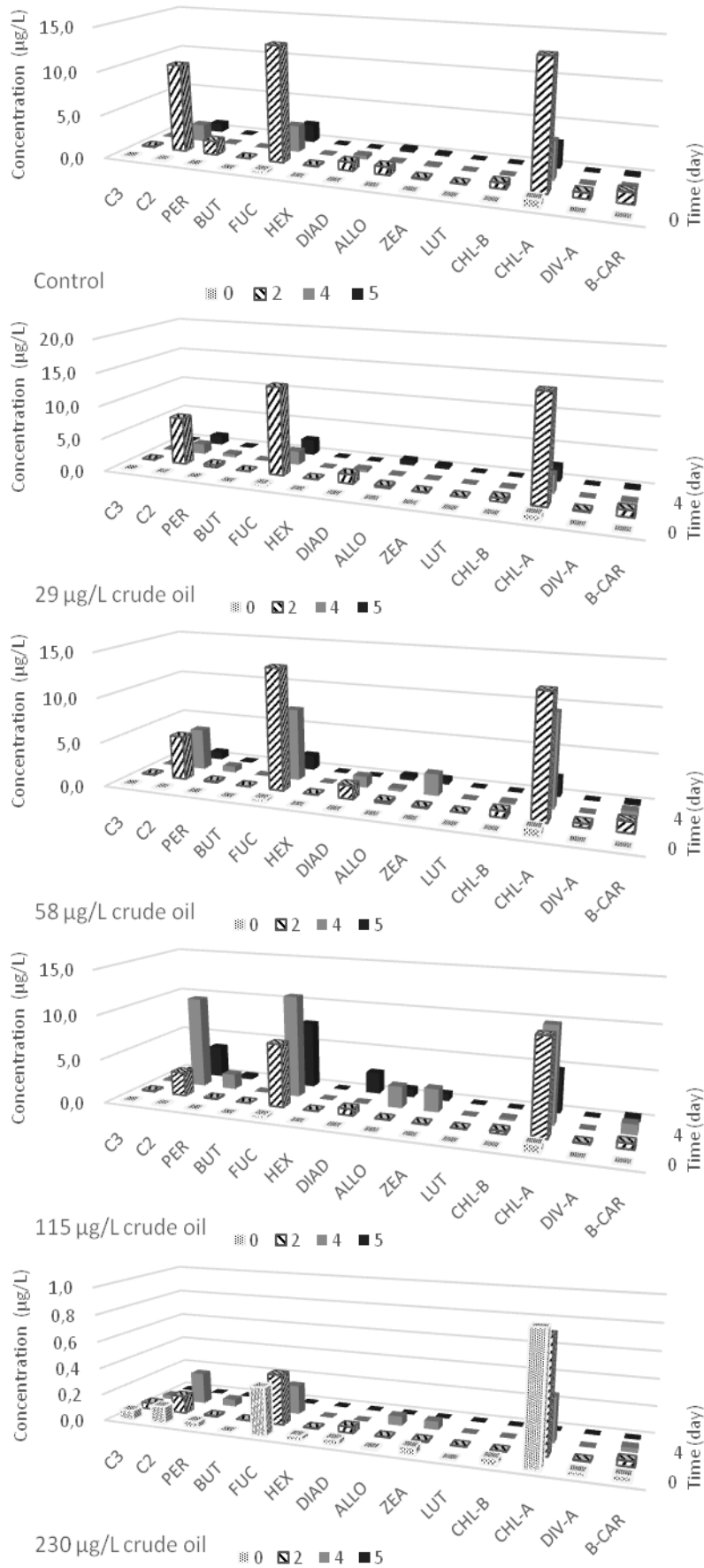


Figure 3. The photosynthetic pigment concentration changes during the crude oil exposure at day 0, 2, 4 and 5 in summer experiments.



**Figure 4.** The photosynthetic pigment concentration changes during the crude oil exposure at day 0, 2, 4 and 5 in fall experiments.



**Figure 5.** The photosynthetic pigment concentration changes during the crude oil exposure at day 0, 2, 4 and 5 in winter experiments.



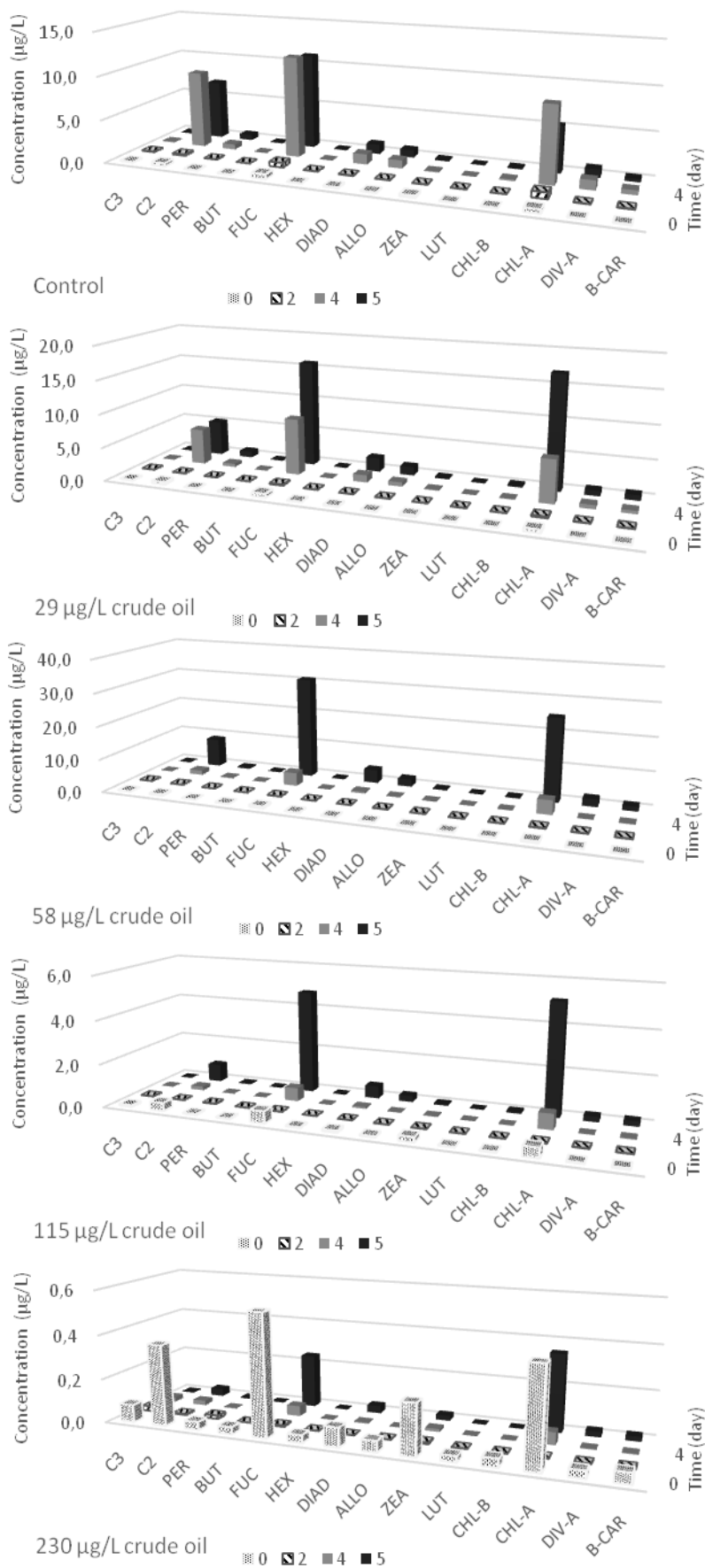
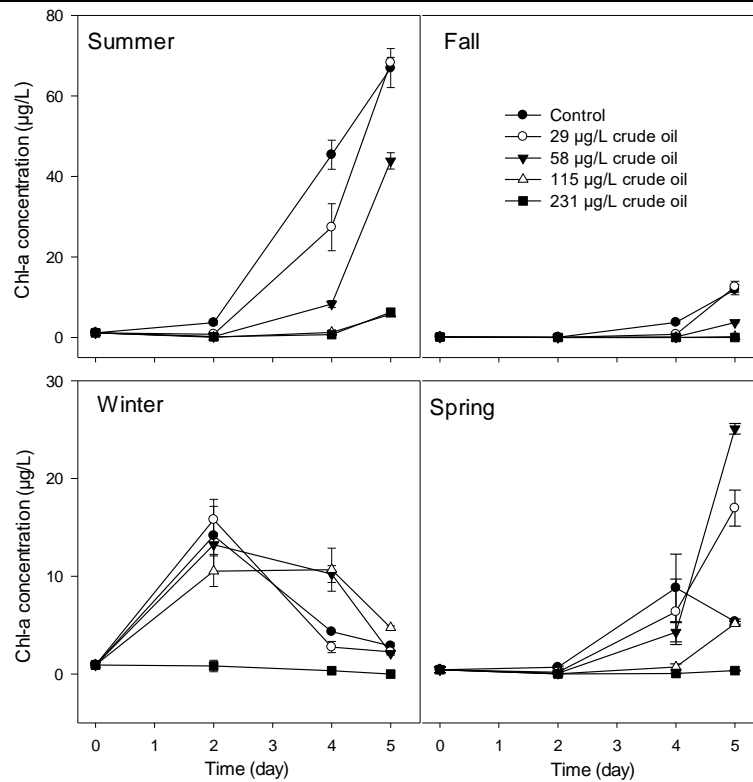
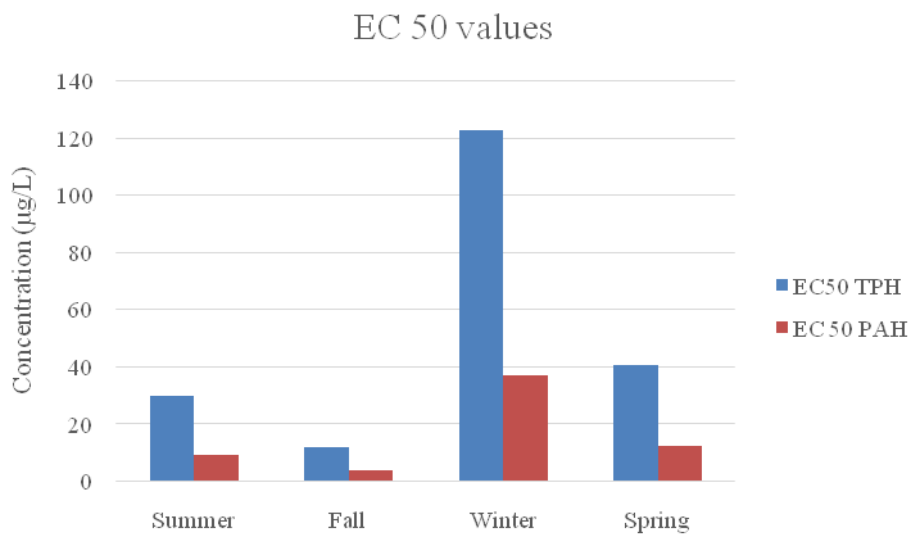


Figure 6. The photosynthetic pigment concentration changes during the crude oil exposure at day 0, 2, 4 and 5 in spring experiments.



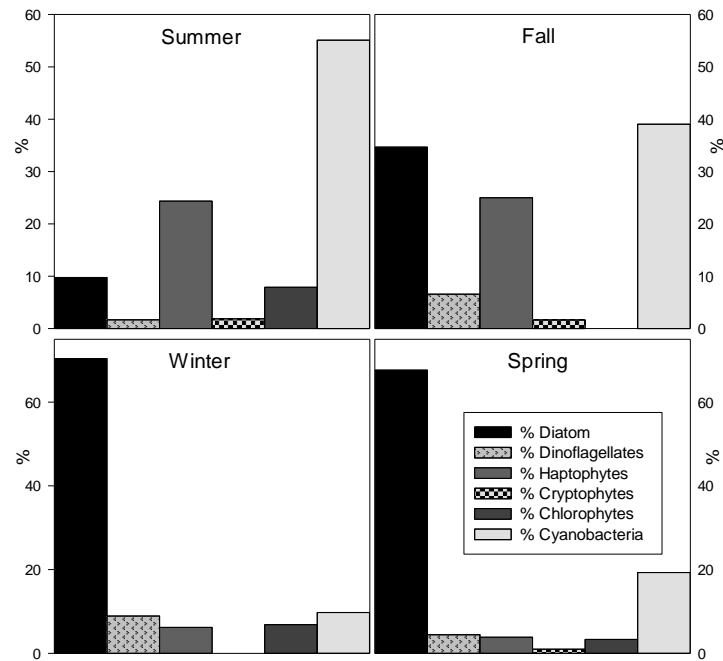
**Figure 7.** Chlorophyll-a concentrations throughout the experiment under different crude oil concentrations.



**Figure 8.** Crude oil EC<sub>50</sub> values for phytoplankton in each season in terms of total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbon (PAH) concentrations.

abundance of this group. In fall, the highest crude oil treatment caused complete crush for all phytoplankton groups. Even after the two lowest crude oil treatments, 29 µg/L and 58 µg/L, only two phytoplankton groups survived: diatoms and cryptophytes. When the concentration of crude oil was increased to 115 µg/L, the cryptophytes survived only until Day 2, and thereafter the entire community

was dominated by diatoms. In spring, in the control group, diatoms outcompeted greatly the other phytoplankton groups. To the increasing crude oil concentrations, two groups, dinoflagellates and cyanobacteria, showed high resistance until Day 2; after that, the relative abundance of diatoms dropped by up to 25%. Afterwards, diatoms bounced back to 80% levels until the end of the experiment.



**Figure 9.** The initial phytoplankton composition in each season.

Different final compositions of phytoplankton after exposure showed that the consequences of an oil spill for phytoplankton depend on the initial phytoplankton composition. Additionally, the biomass was more than an order of magnitude greater in summer compared to fall, causing higher tolerance in the phytoplankton community although the compositions were similar (Figure 9). The highest oil dose (231  $\mu\text{g/L}$ ) caused the complete collapse of the phytoplankton community in the fall; however, summer samples, particularly dinoflagellates, showed a higher tolerance to this oil dose (Figure 10). A previous study (Ozhan & Bargu, 2014) also revealed that a diatom-dominated phytoplankton community showed a high tolerance to oil exposure. Apparently, both the initial compositions and biomass of phytoplankton have effects on the consequences of the oil spill. The present study can be complemented determining currently unknown recovery rates of different phytoplankton species, the length of exposure impact on recovery rates, potential development of any physiological adaptation to crude oil, and the functional equivalence of shifted phytoplankton groups, which may allow for more pertinent extrapolation to real-world conditions. Furthermore, in the real environment, the grazing pressure on phytoplankton will be another parameter that will contribute to the response and abundance of the groups during the exposure.

Previous individual- and community-level oil exposure studies emphasize that certain groups have a greater sensitivity to crude oil (Gilde & Pinckney 2012; González *et al.* 2013; Ozhan, Parsons & Bargu, 2014b). However, due to inconsistent reports, it remains difficult to conclude directly that a certain

group is more resistant to oil than other groups. Even though some studies underlined the high tolerance of diatoms to oil; for example, the suppression of diatom growth and the rise in dominance of flagellates have been observed following oil spills and in laboratory experiments (e.g., Lee *et al.* 1977; Elmgren *et al.* 1980; Harrison *et al.* 1986), other groups (e.g. Ozhan & Bargu, 2014) reported the opposite results. In this distinction, Si concentration, which can suppress diatom growth in the marine ecosystem, might be one of the most important features that need to be addressed in future studies. Although distinction between this diatom and other groups' tolerance can be seen best in fall experiments in the present study, it is not so simple to directly state that diatoms are more resistant to oil than other groups. In the fall, although the control group was dominated by cryptophytes toward the end of the experiment, treatments containing crude oils reflect the dominance of diatoms, particularly at a 115  $\mu\text{g/L}$  crude oil treatment.

The present study also emphasized that the initial phytoplankton composition drives the response of phytoplankton community to crude oil. Different phytoplankton initial compositions produced different responses and dominance of different groups at the end of the experiment. Seasonal  $\text{EC}_{50}$  values decreased from winter to fall. The biggest difference between fall and winter experiments was an almost 10-fold greater biomass and more prominent diatom abundance in winter. The values of  $\text{EC}_{50}$  ranged from 11.51 to 22.3  $\mu\text{g/L}$  for TPH concentration, and from 3.5 to 36.6  $\mu\text{g/L}$  for PAH concentrations in this study. These  $\text{EC}_{50}$  values appear low compared with other literature values (Ozhan *et al.* 2014a) that generally

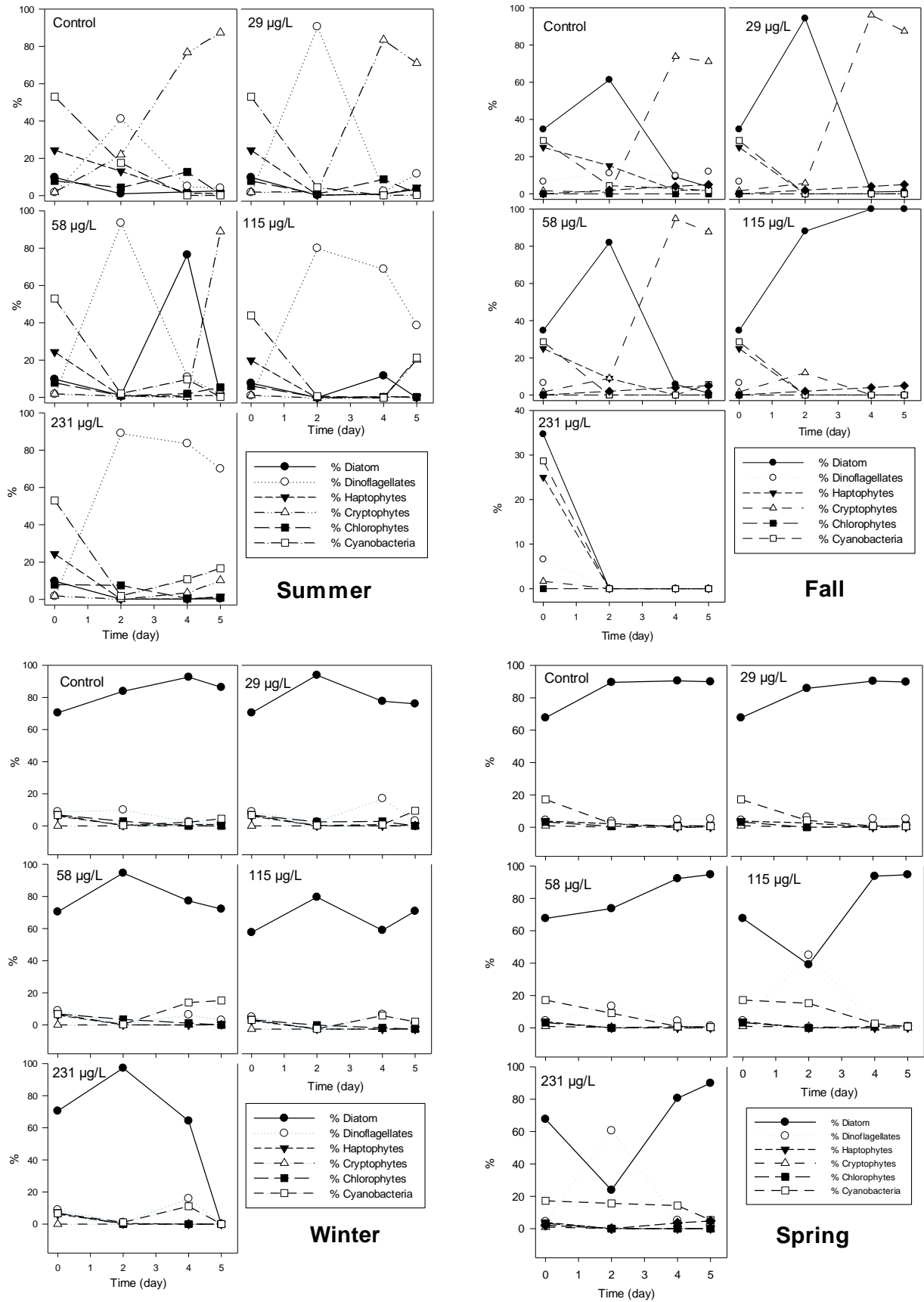


Figure 10. Changes of % composition of each phytoplankton group throughout the experiments for each season.

lay between 1 and 50 mg/L for TPH concentrations. It is an indication of the highly toxic nature of Azeri crude oil compared with other crude oils that have already been studied. This is the first report about the toxic effect of Azeri crude oil indicating that a potential spill impact on the marine ecosystem can be greater than what is expected from other oil types. The present study shows that both spill time and oil type have great effects on the response of ecosystems. This multidimensional effort to predict ecosystem response under an oil spill requires an integration of multidisciplinary research. Thus, not only ecotoxicological studies, but all physical aspects should also be evaluated for ecosystem response assessments after oil spills.

## Conclusion

This study is the first published piece of work investigating the oil spill response of an ecosystem that is highly prone to tanker accidents and accompanying oil spills. The study concluded that with EC<sub>50</sub> values ranging between 11.5 and 122.3 µg/L TPH concentrations, the season is very important for the response of the phytoplankton community to crude oil exposure. The highest EC<sub>50</sub> value, 122.3 µg/L, obtained in winter, could be considered as a highly toxic dose for the phytoplankton community in the region. The system is more prone to the spill in fall and the most tolerant in winter due to the most likely the existence of different phytoplankton community compositions. Knowing about concentrations that do not cause any significant change in the phytoplankton community can be crucial during the ecosystem response assessments of a spill. Further investigations in other food web components will be required to have a complete answer of the ecosystem response to the crude oil spill in the region.

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