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Araştırma Makalesi / Research Article

# The Effects of Different Malathion Concentrations on Algal Growth in Cultural Conditions

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## Abstract

Malathion is one of the insecticides commonly used to control hazelnut pests in hazelnut orchards in Giresun region. This insecticide pollutes lakes, rivers and sea waters by drifting from the soil with rain, flood and snow waters. In this study, *Scenedesmus* sp. cultures were prepared in BG-11 medium for use in laboratory experiments. It was aimed to determine the change in algal growth due to the increase in malathion concentration applied to these cultures. Growth of strains in cultures treated with malathion at doses of 0.05 mg/L, 0.5 mg/L, 1 mg/L, 5 mg/L and 10 mg/L were compared with those grown in non-malathion cultures. In addition, pH was measured and chlorophyll-*a* values were also calculated for the control group and the cultures to which malathion was added during the study. Cell number showed different changes over time according to pesticide concentrations. The highest number of cells was $3.61x10^6$ cells/ml at 10 mg/L dose at the end of 24th hour and the lowest number of cells was $2.05x10^6$ cells/ml at 10 mg/L dose at the end of 48th hour. pH values did not fluctuate much and generally decreased at the end of 96th hour. The lowest chlorophyll-*a* was calculated as 0.35 µg/L at 96th hour. As a result, it was determined that the doses studied negatively affected algal growth, although not too much.

**Keywords:** Stream, green algae, algal growth, pesticide, malathion.

# Farklı Malathion Konsantrasyonlarının Kültürel Koşullarda Alg Büyümesi Üzerine Etkileri

## Öz

Malathion, Giresun yöresindeki fındık bahçelerinde fındık zararlılarını yok etmek için yaygın olarak kullanılan insektisitlerden biridir. Bu insektisit yağmur, sel ve kar suları ile topraktan sürüklenerek göl, nehir ve deniz sularını kirletmektedir. Bu çalışmada, laboratuar deneylerinde kullanılmak üzere BG-11 besiyerinde *Scenedesmus* sp. kültürleri hazırlanmıştır. Bu kültürlere uygulanan malathion konsantrasyonundaki artışa bağlı olarak alg gelişimindeki değişimin belirlenmesi amaçlanmıştır. 0.05 mg/L, 0.5 mg/L, 1 mg/L, 5 mg/L ve 10 mg/L dozlarında malathion uygulanan kültürlerdeki suşların büyümesi, malathion olmayan kültürde yetiştirilenlerle karşılaştırılmıştır. Ek olarak kontrol grubu ve çalışma sırasında malathion eklenmiş kültürlerin çalışma süresince pH ölçümleri yapılmış ve klorofil-*a* değerleri de ayrıca hesaplanmıştır. Hücre sayısı pestisit konsantrasyonlarına göre zamanla farklı değişimler göstermiştir. En fazla hücre 24. saat sonunda 10 mg/L dozunda $3,61x10^6$ hücre/ml; en az hücre ise 48. saat sonunda yine 10 mg/L dozunda $2,05x10^6$ hücre/ml olarak ölçülmüştür. pH değerlerinin çok fazla dalgalanma göstermediği, 96. saat sonunda genel olarak düştüğü belirlendi. En düşük klorofil-*a* ise 96. saatte 0,35 µg/L olarak hesaplanmıştır. Sonuç olarak çalışılan dozların alg gelişimini çok fazla olmasa da olumsuz etkilediği belirlenmiştir.

**Anahtar Kelimeler:** Nehir, yeşil alg, algal büyüme, pestisit, malathion.

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## 1. Introduction

The increasing need for foodstuffs in parallel with the increase in the world population has made the use of fertilizers and pesticides inevitable in order to obtain maximum yield from agricultural areas (Chen et al., 2017; Storck et al., 2017; Doğan and Karpuzcu, 2019). Pesticides have been used for a long time in pest control (Solmaz et al., 2010). Intensive spraying activities seriously affect freshwater ecosystems (Öztürk and Fakıoğlu, 2023). Pesticides accumulate in water and sediment, damaging the ecological structure and disrupting its normal functioning. It is seen that studies on aquatic organisms are mostly focused on organisms representing the top step of the food chain (Cessna, 2009). Phytoplanktonic organisms, which form the first link of the food chain in aquatic ecosystems, are among the first groups of organisms affected by pesticides used in agricultural activities reaching surface waters. Excessive accumulation of pesticides also leads to a decrease in the diversity and numbers of phytoplanktonic organisms and the fish and other organisms that feed on them. Determining the responses of phytoplanktonic organisms to pesticides is effective in revealing pesticide effects in aquatic ecosystems (Tunca et al., 2021).

Microalgae have the capacity to bioaccumulate pesticides and bio-transform some environmental pollutants (Guanzon et al., 1996). Studies on the effects of pesticides on freshwater algae have mostly used green algae such as *Chlorella*, *Scenedesmus* and *Chlamydomonas* (Tadros et al., 1994). *Chlamydomonas reinhardtii* (Chlorophyceae) is another green alga frequently used in toxicology studies (Zhang et al., 2011). In this study, the effect of malathion on *Scenedesmus* sp., a member of the Chlorophyta family dominant in Turkish fresh waters, was investigated.

Pesticide use is still the most preferred method of agricultural control of pests and diseases, despite having long-lasting devastating effects on the environment, non-target organisms and humans (Karahan et al. 2018). Pesticides can be transported over long distances by atmospheric precipitation, agricultural land, sewage in various centers, hazardous waste disposal waters, and even by air (Tankiewicz et al., 2010). Pesticides cause environmental problems by drifting to other places with factors such as wind and rain in the areas where they are used. Some of them are photochemically decomposed into toxic or non-toxic substances, some of them evaporate and cause permanent accumulation of toxic substances in the atmosphere. Some of them are washed away from the soil by rain, floods and snow water and end up in rivers, lakes and sea waters. The use of pesticides in agriculture pollutes air, soil and water over time. The impact of pollutants on communities varies depending on many factors such as pollutant concentration, duration and number of exposures (Relyea and Diecks, 2008). Malathion is widely used in agriculture and may have harmful effects on aquatic organisms even at trace levels (Dash and Osborne, 2023). Malathion is a broad-spectrum organophosphorus pesticide used to control insects in fields. Recent studies have shown that

malathion has a wide range of effects. These include hepatotoxicity (Josse et al., 2014; Mesnage et al., 2014), genetic damage and disruption of normal hormone activity (Taxvig et al., 2013).

Standardized test methods for toxicity to freshwater algae were regularly applied until the early 1970s. Studies to determine the effects of pesticides on algae started to increase after 2000s. However, most of these studies have focused on investigating the effects of herbicides on algae. The continuation of such studies plays an important role in revealing the effects of pesticide pollution on aquatic ecosystems, especially on algae. Recently, studies on the inhibitory effects of insecticides on algal cultures have increased (Öterler and Albay, 2016). Although there are studies on the effects of pesticides on various algal species in our country (Ağırman and Çetin, 2012; Öterler and Albay, 2016; Yılmaz and Taş, 2021) there are not many studies on the effects of malathion on phytoplankton. There are some important studies on this subject abroad (Ghadai et al., 2010; Ibrahim and Essa, 2010; Ibrahim et al., 2014). It is aimed that this deficiency will be overcome with this project.

Malathion is a pesticide frequently used after harvest for hazelnut sprout moth disinfestation in hazelnut orchards in the Black Sea Region (Demirbaş, 2010).The aim of this study is to determine the effect of malathion, on cultures of *Scenedesmus* sp, one of the green algae species isolated from rivers and grown under culture conditions. The growth of the species grown in cultures treated with 0.05 mg/L, 0.5 mg/L, 1 mg/L, 5 mg/L and 10 mg/L malathion was compared with the species grown in cultures without malathion. It was aimed to determine how and to what extent algal growth in cultures changes depending on the increase in malathion concentration and to contribute to scientific data that can be used in future planning studies with the data obtained.

## 2. Material and Method

### 2.1. Description of the Research Area and Sampling Stations

Water samples were taken for algal culture from Batlama, Büyük Güre and Aksu Streams located within the borders of Giresun province in the Eastern Black Sea Region. Büyük Güre Stream is 3 kilometers away from Giresun province within its borders. Batlama Stream arises from Bektaş Plateau in the south of the western slope of Çaldağ and empties into the sea in the west of the central district. Its length is 40 km. Aksu Stream originates from Karagöl on the border of Giresun, Ordu and Sivas and flows into the Black Sea, passing through the villages of Kızıltaş, Sarıyakup, Pınarlar and Güdül. Aksu Stream is approximately 100 m wide and 60 km long. Its depth varies according to the seasons, but it is around 3 m in average. For pure cultures can be obtained from water samples taken from Aksu Stream, the study continued on this stream.

### 2.2. Algological Features

#### 2.2.1. Sampling and Diagnosis

Surface (0-20 cm) water samples were performed from the determined streams in spring using a one-litre water sampler for algae culture. For phytoplankton analysis, after the water samples were shaken well, sub-samples were prepared with 10 and 20 ml measuring tapes and a few drops of lugol (I-KI) and a drop of 40% formol were added to them and left to stand for 24-48 hours for the phytoplankton to settle to the bottom. After the waiting period, the clear upper part of the measuring tube was emptied by siphoning and the remaining part was shaken well and transferred to Utermohl counting tubes (Utermöhl, 1958). These temporary preparations were examined with the Leica invert microscope and the algae were identified.

For phytoplankton species identification, the identification keys of Huber-Pestalozzi et al., (1982, 1983); Hustedt (1985); Geitler, (1925); Kelly (2000); Komarek and Anagnostidis (2008), John et al., (2003) were used.

#### 2.2.2. Cultural Conditions

In our study, the *Scenedesmus* sp. species, which is a cosmopolitan species commonly found in freshwater habitats and dominant in our country's waters, was used.

Preparation of BG 11 medium used in the study is as in Table 1 (Sukatar, 2002).

**Table 1.** Preparetion of BG11 medium.

| **Stock solution** | **g 1000/mL $H_2O$** | **Trace Elements** | **g 1000/mL $H_2O$** |
|---|---|---|---|
| $NaNO_3$ | 15.0 | $ZnSO_4.7H_2O$ | 0.0022 |
| $K_2HPO_4$ | 4.0 | $H_3BO_3$ | 0.286 |
| $MgSO_4.7H_2O$ | 7.5 | $MnCl_2.4H_2O$ | 0.181 |
| $CaCl_2.2H_2O$ | 3.6 | $Co(NO_3)_2.6H_2O$ | 0.002 |
| $C_6H_8O_7$ | 0.6 | $CuSO_4.5H_2O$ | 0.00004 |
| $(NH_4)_5[Fe(C_6H_4O_7)_2]$ | 0.6 | $NaMoO_4.\ 5H_2O$ | 0.0039 |
| $Na_2EDTA$ | 0.1 | | |
| $Na_2CO_3$ | 2.0 | | |
| Trace Elements | | | |
| Optimize the above compounds by adding them to 829 mL of pure water and optimizing the pH to 7.1 with 1M NaOH and HCl. As some algae require vitamin B12, 1µg is added to the final solution. If solid media is desired, add 15 g/L agar. | | | |

Water samples for algae culture were taken from streams in spring. Spraying with malathion for the control of hazelnut sprout moth starts after harvest (Demirbaş, 2010; Uzundumlu et al., 2017).

In the preservation of the cultures, in the climate cabinet is 60% humidity ± 10 %, 23 $^{o}$C temperature ± 2 $^{o}$C, 8000 lux light intensity ±500 lux, 18 µwat $cm^{-1}nm^{-1}$ light brightness, PPF (Photosynthesis Photon Light Distribution) 53 µmol $m^{-2}s^{-1}$ and 12/12 night/day lighting setting (Sabater and Carrosa, 2001; Lockert et al., 2006; Hong et al., 2008). They were maintained during the day/night lighting period until the time of the experiment (Figure 1).

Pre-cultures in 250 mL flasks containing 100 mL medium were plated at 4000-5000 cells/mL under the specified acclimatization conditions (International Standard ISO 8692, 1989).

Cultures that reached the rapid growth phase (15-25 days) were homogenized by shaking in an automatic shaker for 10 minutes and were divided into subcultures as 100 ml cultures in 250 ml flasks. Approximately 5 days after the cultures entered the exponential growth phase, experiments were started by treating them with pesticides at determined concentrations (Burkiewicz et al., 2005).

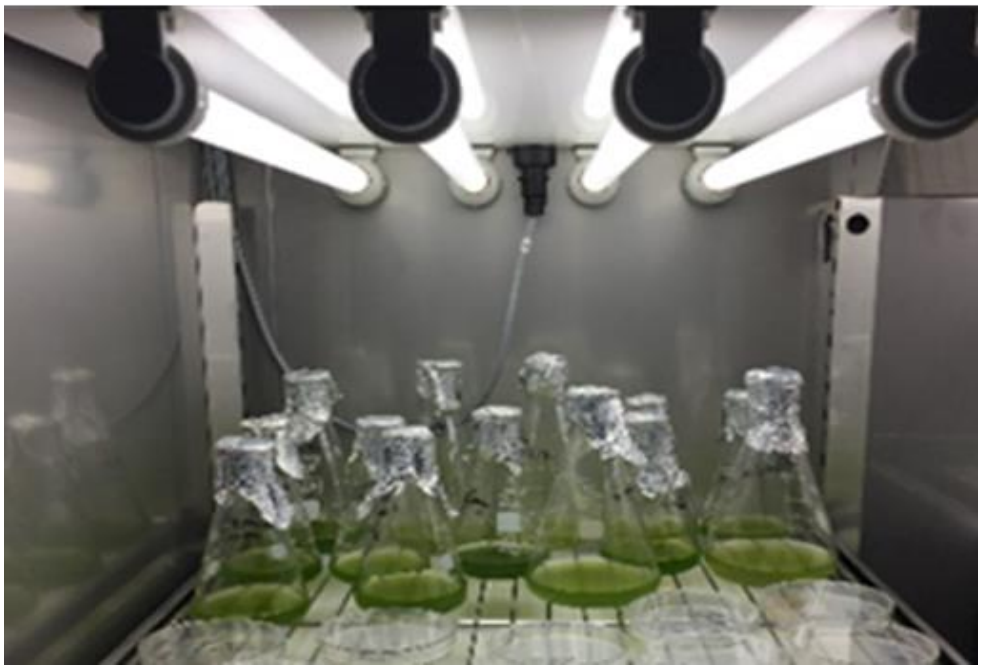

**Figure 1.** Algal samples grown in the culture cabinet.

### 2.2.3. Cell Count

Spectrophotometric methods are generally used to determine cell concentrations in microbiological studies, cell culture and similar applications. However, these methods are not very effective in determining whether cells are alive or dead.

Counting chambers are used to determine the number of cells in a given volume. Cell counts were made with a Sedgewick-Rafter counting chamber. The Sedgewick-Rafter counting chamber consists of 50 columns and 20 rows, a total of 1000 squares. The volume of each square is 1 µl. The

evaluation is performed by counting the randomly selected columns (LeGresley and McDermott, 2010).

The media for the continuity of cultures and experiments were prepared with 100 mL medium in 250 mL flasks for algal species as specified in the standards (ISO-8692, 1989). In the experiments, 5 different doses of malathion (0.05, 0.5, 1, 5 and 10 mg/L) determined according to similar studies were applied to algae cultured in 100 mL bottles (İbrahim et al., 2014; Öterler and Albay, 2016). The experiments were initiated by treating the cultures with pesticides at predetermined concentrations approximately 5 days after the cultures entered the exponential growth phase. The experiment was carried out in 2 different stages.

In the first stage, cell counts of malathion-treated samples were performed for 5 days at 0, 24, 48, 72 and 96 hours using a Sedgewick-Rafter counting chamber.

In the second stage, spectrophotometric growth rates of malathion-applied samples and absorbance values at 500, 665 and 750 nm wavelengths were measured and chlorophyll-*a* calculations were made (Nusch, 1980).

$$\text{Chl-}a\ (\mu g l^{-1}) = 29.6 \times (BA_{665} - BA_{750}) - (AA_{665} - AA_{750}) \times v/V \times L \quad (1)$$

BA: Spectrophotometer Value Before Acid,
AA: Spectrophotometer Value After Acid,
v : Volume of ethanol (ml)
V : Volume of sample (L)
L : Diameter of tub (1 cm)

In addition pH of the control group and the cultures after dosing were made during the study (Figures 3).

### 2.2.4. Pesticide Analysis

*Scenedesmus* sp. culture, which was pre-cultured in large volumes and whose development was regularly monitored, was divided into 100 ml in sterile conditions in 250 ml flasks between the 15th and 21st days when they reached a sufficient number of cells and entered the rapid growth phase. 20 ml of culture sample was taken from each experimental set under sterile conditions at 0th hour. To find the spectral growth rates, absorbance values at 500, 665 and 750 nm were measured, followed by pH measurement and cell counting. Then, 10 ml of the culture was filtered with a filtration set through Whatmann GF/C filter paper with a diameter of 4.7 mm. The filtered filter paper was taken to the deep freezer for chlorophyll_a analysis and chlorophyll analysis was performed the next day. The experiments were completed in accordance with ISO 8692 standards by applying 5 different

pesticide concentrations: 0 hour, 12 hour, 24 hour, 48 hour, 72 hour and 96 hour (ISO-8692, 1989). The same procedures were repeated at the 12th hour, 24th hour, 48th hour, 72nd hour and 96th hour of the experiments.

## 3. Results and Discussion

In order to determine the effect of malathion on the growth of *Scenedesmus* sp., a green algae isolated from rivers, under culture conditions, cultures were treated with 0.05 mg/L, 0.5 mg/L, 1 mg/L, 5 mg/L and 10 mg/L malathion. The growth of algae grown in cultures was compared with those grown in non-malathion culture. With this research, it was aimed to determine the change of algae growth in cultures depending on the increase in malathion concentration.

In our study, an increase was observed in the number of organisms in the control group at the $96^{th}$ hour according to the organism number-dose graph. At the end of the $96^{th}$ hour, the number of organisms was determined as $3.025 \times 10^6$ cells/mL. It was observed that the increase in the number of organisms continued when a dose of 0.05 mg/L was applied compared to the control group. It was determined that this dose amount did not adversely affect algae growth. Likewise, it was determined that the highest dose of 10 mg/L reduced algae growth to the lowest number of organisms in 48 hours, but increased again after this period (Figure 2). Ibrahim et al. (2014) reported that at low malathion concentrations (0.02 -20 ppm) the total cell number of *A. oryzae* and *N. muscorum* cultures increased by 41% and 75%, respectively, within 24 days. At the same time, it was determined that different malathion concentrations reduced the growth of *S. platensis* culture, compared to the control group, the total cell number decreased by 19%. In another study with malathion, it was determined that malathion had an inhibitory effect on the development of *Chlorogloea fritschii*, a blue-green algae, and that growth was suppressed permanently at a dose of 200 mg/L (Lal and Lal, 1988). Yeh and Chen (2006) investigated the effects of atrazine, parathion, dichlorvos, malathion, fenthion, 2-methyl-4chlorophenoxyacetic acid and pentachlorophenol on the growth of *Pseudokirchneriella subcapitata* and revealed that these pesticides affect the growth of non-target organisms. In addition, some studies have shown that low concentrations promote algal growth in terms of cell number, while high concentrations reduce algal growth (Ghadai et al., 2010; Ibrahim and Essa, 2010). In our study, it was observed that low concentrations stimulated algal growth. In a study aiming to characterize the genetic and enzymatic components responsible for the use of malathion and other organophosphorus pesticides, they stated that the number of cells may increase due to the fact that the algae species used biodegrade malathion and use it as a phosphorus source (Ibrahim et al., 2014). This situation is in parallel with our study.

As a result of the measurements, the lowest pH value at the beginning of the experiment (0. time) was 6.4 in the 5 mg/L and 10 mg/L dose groups at the 96$^{th}$ hour, and the highest pH value at the 0$^{th}$ hour for the 1 mg/L dose, and at the 24$^{th}$ hour for the 10 mg/L dose. It measured 6.9. It was determined that the pH values did not fluctuate much between the days, and decreased in general at the end of the 96$^{th}$ hour (Figure 3).

As a result of the measurements, the highest chlorophyll-*a* was measured as 1.48 µg/L at the 0$^{th}$ hour and the lowest chlorophyll-*a* was measured as 0.35 µg/L at the 96$^{th}$ hour in the control group. During the study, the highest chlorophyll-*a* was 2.27 µg/L at the 48$^{th}$ hour at a dose of 0.05 mg/L, and the lowest chlorophyll-*a* was determined as 0.5 mg/L and 0.11 µg/L at 10 mg/L dose at the 24$^{th}$ hour (Figure 4). Growth, photosynthesis, chlorophyll values and other parameters reflect the toxic effects of pollutants on microalgae (Cid et al., 2012). Pigments are used as one of the biomarkers of exposure to pesticides in plants and generally in algae (Couderchet and Vernet, 2003). The most important photosynthetic pigments found in green algae are chlorophylls and carotenoids. Chlorophyll measurement is one of the most widely used parameters to evaluate the effects of pesticides on algae growth. As a result of our studies, it was determined that chlorophyll-*a* values increased during the first 48 hours at a dose of 0.05 mg/L in *Scenedesmus* cultures containing malathion and then decreased. As the exposure time to pesticides in algae increased, higher doses were found to reduce photosynthetic capacity.

Although cell numbers did not change much throughout all measurements, chlorophyll-*a* generally increased (except for the control group and the 5 mg/L dose). When algal cells and pesticide concentrations are at different levels, their functions have different dominance. Therefore, different effects and functions are exhibited. Similarly, it has been determined that many pesticides have no effect on growth in cyanobacteria, or even have a growth-accelerating effect, but can affect various physiological processes such as nitrogen activity, photosynthesis, carbon fixation, and assimilate nitrate reduction and ammonia assimilation enzymes in cyanobacteria.

For the survival of algal species, they must maintain a high rate of photosynthesis when exposed to pesticides (Eullaffroy and Vernet, 2003). Mayer and Jensen (1995) reported examples of triazine-induced increase in algal chlorophyll content in the *Selenastrum capricornutum* species. This can be interpreted as a tolerance mechanism (Francois and Robinson, 1990). This process may result from a homeostatic mechanism triggered by exposure to herbicides (Yılmaz and Taş,2021).

Öterler and Albay (2016), as a result of their study on algae and pesticides, reported that Parathion-Ethyl was the pesticide that most affected the growth of this organism in low pesticide applications in *Scenedesmus quadricauda*. They reported that other pesticides, including malathion, decreased the amount of algae continuously for the first 72 hours and increased slightly between 72nd and 96th hours, probably due to the absorption of the pesticides in the culture by the algae in the

environment. In our study, it is thought that malathion may have been removed from the environment over time due to its absorption by algae.

In addition to all these, abiotic factors (temperature, light, etc.) also affect the toxicity of pesticides (Polat and Çetin, 2020). The rate of degradation of malathion varies depending on micro-environmental conditions such as humidity, pH, organic matter, temperature, salinity. Moreover, degradation of malathion under acidic conditions and oxidation with molecular oxygen is very slow (Kumar et al., 2019). In our study, pH increased by 24 hours in the culture containing 10mg/L dose and then acidity increased again (Figure 3). This decrease in pH also slowed down the degradation of malathion, so the cell number and amount of chlorophyll-*a* increased over time at the same dose (10mg/L).

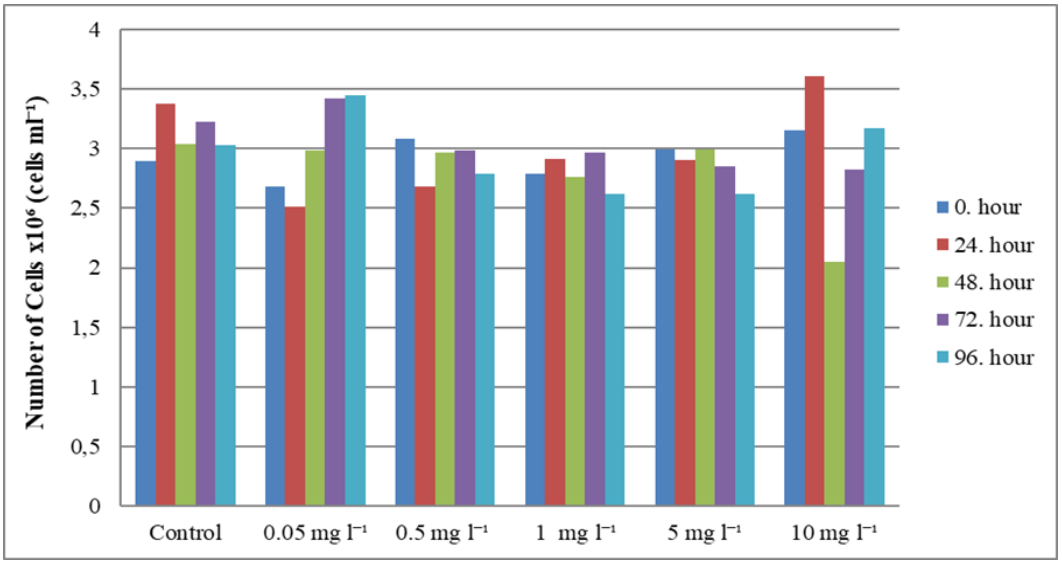


**Figure 2.** Time-dependent variation of the data obtained as a result of counting in the control group and different malathion doses.

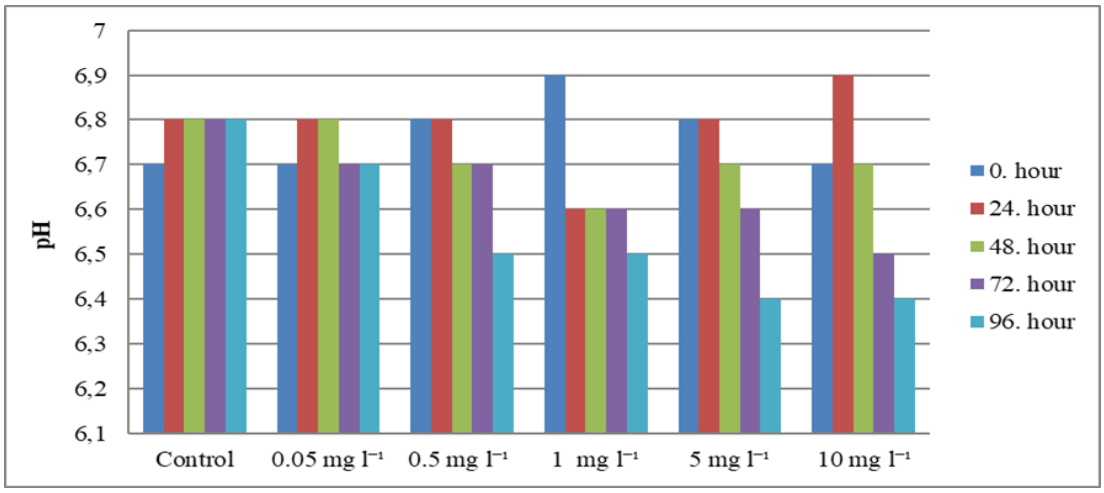


**Figure 3.** pH change in control group and different doses of malathion.

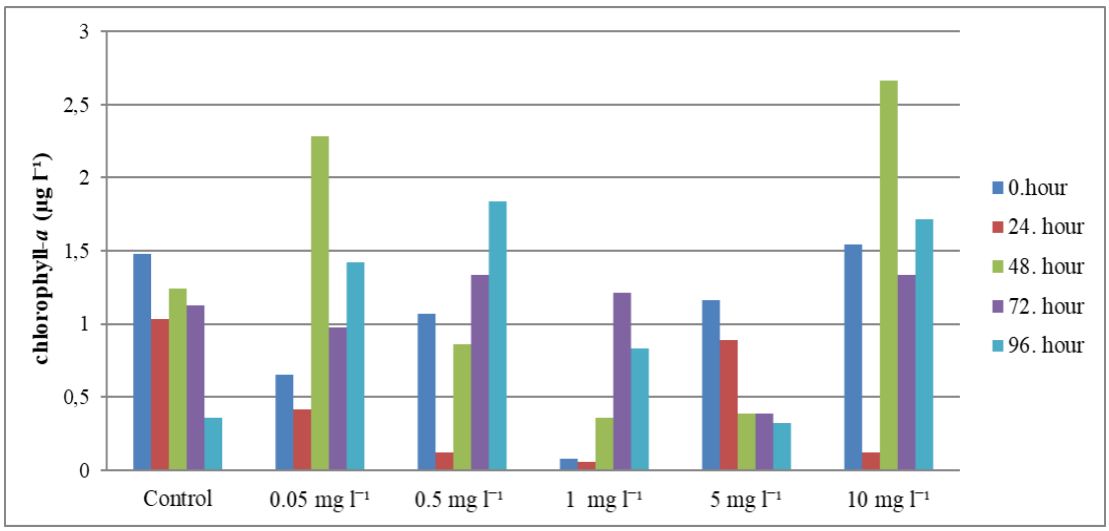


**Figure 4.** Change of chlorophyll-*a* in control group and different doses of malathion

## 4. Conclusion

As a result of the study, it was determined that the doses used in the study affected the growth of algae negatively, but there was no expected decrease in the number of organisms as a result of the counting made in the cultures. However, considering the whole study, it was determined that the low dose amount applied did not affect the algal growth too much.

The amount of pesticides used in Turkey is below the world average, but due to intensive hazelnut cultivation in the Black Sea Region, pesticide use is higher than in other regions. Although pesticides at low concentration levels do not have a significant impact on the aquatic environment, their long-term accumulation will cause pollution of the aquatic environment and adversely affect aquatic life. Studies and this research also support this situation (Ağırman ve Çetin, 2012; Yılmaz ve Taş, 2021; Koçyiğit ve Sinanoğlu, 2019).

The wide range of uses of malathion, with depending on different application periods in agriculture, causes it to be persistent in the environment and to be detected continuously (Deknock et al., 2019). Pesticide residues, even at very low levels, accumulate in living organisms and seriously affect human and animal health as well as the environment. Today, it is aimed to use pesticides effectively and without any problems. This will be possible by establishing a good control mechanism starting from the licensing stage and raising awareness of all institutions and individuals related to pesticides (Altıkat et al., 2009). Due to their effects on the environment and human health, new projects should be developed on the use of pesticides and water resources should be protected against pesticide pollution. The results of this study will be taken into consideration by the relevant local governments and organizations and will be useful in taking the necessary steps to raise public awareness.

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**Contribution Rate Statement Summary of Researchers**

The authors declare that they have contributed equally to the article

**Conflict of Interest**

The Authors declare that there is no conflict of interest.

**Statement of Research and Publication Ethics**

The authors declare that this study complies with Research and Publication Ethics.

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