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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

A preliminary investigation of the effects of surface waters of the Bakırçay River on the growth of green algae *Scenedesmus dimorphus*

Bakırçay nehri yüzey sularının *Scenedesmus dimorphus* yeşil algi gelişimi üzerine etkileri hakkında bir ön çalışma

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Abstract: Bakırçay River, one of the important rivers of the Aegean region, has a length of 129 km and an approximate catchment area of 3160 km^2. Bakırçay River, which carries agricultural drainage and polluted wastewater with high nitrogen and phosphorus content, is polluted due to domestic and agricultural resources. The water of the Bakırçay River is used for irrigation in agriculture and fishing from the dam lakes on the river for feeding purposes also negatively affects the health of the people in the vicinity. In the Bakırçay River, there is widespread pollution caused by mining areas as well as domestic, industrial and agricultural pollution. For this purpose, the “Algal Growth Inhibition Test, OECD 201” was applied to the water samples obtained from 10 stations on the Bakırçay River. *Scenedesmus dimorphus* (Turpin) Kützing, 1834 green algae culture, accepted as the primary trophic level representative for the " Algal Growth Inhibition Test, OECD 201" test, one of the short-term phytotoxicity test methods, was used in this test. Water samples were tested at five different dilutions (40, 60, 80, 100 %). To determine the effects on the growth of *Scenedesmus dimorphus*, the cells were counted by fluorimeter (Turner design) daily at the same time and the test duration was 72 hours. In conclusion, the highest level of toxicity was found at Stations 1, 9, 10,13,14, and 15. The reason for this was that there was a coal facility near Station 9 and that all branches of the Bakırçay River joined near Station15 and then drained into Çandarlı Bay.

Keywords: Bakırçay River, algal growth inhibition test, pollution, freshwater, *Scenedesmus dimorphus*

Öz: Ege bölgesinin önemli akarsularından biri olan Bakırçay’ın uzunluğu 129 km ve yaklaşık havza alanı 3160 km^2’dir. Tarımsal drenaj ve yüksek azot ve fosfor içerikli kirli atık suları taşıyan Bakırçay, evsel ve tarımsal atıklara bağlı olarak sürekli kirlilik yükü altında kalmaktadır. Bakırçay Deresi’nin suyu tarımsal olarak sulama amacıyla kullanılmakta ve Bakırçay’ın üzerindeki baraj göllerinden beslenme amacıyla balık avlanması Bakırçay çevresinde yaşayan halkın sağlığını olumsuz etkileyerek halk sağlığı açısından risk oluşturmaktadır. Bakırçay’da evsel, endüstriyel ve tarımsal kirliliğin yanı sıra maden alanlarının neden olduğu yaygın kirlilik de söz konusudur. Bu amaçla, Bakırçay Nehri üzerinde bulunan 10 istasyondan temin edilen su örneklerine “Algal Büyüme İnhibisyon Testi, OECD 201” uygulanmıştır. Kısa zamanlı fitotoksisite test yöntemlerinden olan “Algal Büyüme İnhibisyon Testi, OECD 201” testi için birincil trofik seviye temsilcisi olarak kabul edilen *Scenedesmus dimorphus* (Turpin) Kützing, 1834 yeşil alg kültürü bu testte kullanılmıştır. Su örnekleri beş farklı dilusyonda (% 40, 60, 80 ve 100) test edilmişlerdir. *Scenedesmus dimorphus*’un üremesi üzerindeki etkileri belirlemek için hücreler 72 saat boyunca her gün aynı saatte florometre (Turner tasarımı) kullanılarak sayılmıştır. Sonuç olarak en yüksek toksisite düzeyleri İstasyon 1, 9, 10, 13,14 ve15’ te saptanmış olup bunun sebebi 9 nolu istasyonun yakınlarda bir kömür tesisinin bulunması ve Bakırçay Nehrinin bütün kollarının 15 nolu istasyonda birleştikten sonra Çandarlı Körfezi’ne dökülmesidir.

Anahtar kelimeler: Bakırçay, alg büyüme inhibisyon testi, kirlilik, tatlısu, *Scenedesmus dimorphus*

INTRODUCTION

Bakırçay River is among most the important sub-basins in the North Aegean Basin. Its length is about 129 kilometres. Joining with its most crucial branch Yağcılar Brook near Kınık County, Bakırçay River goes through Bergama and then drains to Aegean Sea near Çandarlı County. Contained in the basin are Manisa and Izmir Cities. Being one of the most important rivers in North Aegean Basin (see Figure 1), the river is under the pressure of domestic and agricultural pollution. Coal mining in Soma County of Manisa may be considered the most important industrial activity for environmental pollution. Soma County Harbors Management of Aegean Lignite Business and many private mining plants. Olive and several fruits and vegetables are widely cultivated in the basin. Quarries and tomato paste factories exist in Bergama County. Soma County hosts a thermal power plant. Based on the results of water quality for the summer season of 2015, the river appears to have *Class IV* water quality in the context of the Regulation on Management of Superficial Water Quality. The most polluted locations were found in Soma County (ÇŞB, 2016).

Our country is not rich enough in soil and water sources. Soil and water pollution represents a significant environmental problem with a steadily increasing extent. Soil and water are the most important strategic sources in the 21st century during which famine and hunger threaten the world. Agricultural pollutants, industrial and domestic wastes, excessive use, and lack of planning water sources complicate solutions to maintain and sustain the ecosystem (Tomar, 2009).

The pollutants are considered to be PAH, pesticides and domestic wastes. Carcinogenic and mutagenic substances from industrial and agricultural activities reach the lakes and the seas via terrestrial drainage and rivers. It has been reported that a remarkable increase in the number of fish with several tumours might have been caused by pollution, caused by the mutagenic and carcinogenic xenobiotics in the environment (De Flora et al., 1991).

Recently, water sources have been of steadily increasing importance all around the world. In near-east region including Turkey, this is of much more interest; additionally, the region's potential for water sources is low. Rapidly increasing population, technological advances, and increasing quality of life on the region's countries of the region, on the other hand, significantly increase need for water. In the context of technical and economic conditions, annually consumable -under and above-ground water potential of our country is average 112 billions m^3 (Tomar, 2009). It appears possible that Turkey will be one of the countries experiencing water scarcity. Thus, it is hard to claim that Turkey is rich in water. The usage of water potential in our country has reached 40% of economically consumable water potential (Tomar, 2009).

Investigating the effects of environmental pollutants is crucial for ecosystem health. It can be done in many ways such as determining the number of pollutants or detecting their effects on organisms by bioassays. Algae play critical roles in ecosystems; thus, they have been used widely to improve health status of many ecosystems worldwide. The effects of the chemicals can also be detected by bioassays using the algae. For a number of researchers all around the world, algal growth inhibition test is the most commonly used and standardized method for this type of bioassay (OECD, 2011). Algae have been reported to be equally or more sensitive organisms than animals and have been used very commonly in toxicity assays (Ferreira and Graca, 2002).

Currently, aquatic toxicity test data are routinely used to evaluate the risks associated with discharge of effluents into water bodies and sediments (Üstün, 2011). Algal bioassay is a test method increasingly used to detect the toxic potential of chemicals, xenobiotics, and most environmental samples from several sources of discharge. Toxicity Test Methods for freshwater algae are intended to be used on environmental chemicals, industrial and municipal effluents, drugs for human and animal use, freshwater dredge material, contaminated sediment/elutriates, hazardous chemical wastes, and groundwater contamination. Examples of the contaminants with recently determined toxicity on the algae include several dyes, chemicals for fire control, fuel oil, oil refinery chemicals, manure runoff, metals, herbicides/pesticides, PCBs, waste dump leachates, sediment, wastewaters, and landfill leachate (Hoffman et al., 2003).

Cheung et al. (1993) investigated toxic effect of the landfill leachate on microalgae and found important results. According to the results there were differential sensitivities to leachate exhibited by the tested algal species. Susceptibility to leachates in terms of cell number was in the ascending order of *Chlorella pyrenoidosa, Scenedesmus* sp., *Chlorella vulgaris* and *Dunaliella tertiolecta*.

Several toxicity tests were conducted with freshwater microalgae species including *Selanastrum capricornatum* (Blaise et al.,1986); *Scenedesmus subspicatus* Chodat (OECD., 1984); Scenedesmus quadricauda (Turp.) Breb (EPA, 1985); *Chlorella vulgaris* Beij., *Scenedesmus* sp. (Cheung et al., 1993). This test is also an easy method with its advantages including saving time, being economic, and its easy implementation. These tests usually use *Scenedesmus subspicatus, Scenedesmus (=Desmodesmus) dimorphus, Chlorella spp. and other species (Selenastrum capricornatum, S. quadricauda* (Turp.) Breb. *Scenedesmus sp. Navicula pelliculosa, Anabaena flos-aquae, Synechococcus leopoliensis* (Hoffman et al., 2003).

The present study aimed to assess the effects of surface waters of the Bakırçay River on the growth of green algae *Scenedesmus dimorphus* as the representative of the first trophic level by Algal growth inhibition test (OECD, 2011).

MATERIALS AND METHODS

Water samples were collected from 15 stations located on the Bakırçay River between 17 and 19 April 2018 within the context "Limnofauna of Bakırçay River Basin (16/SÜF/038)", (Figure 1, Table 1). In our study, Algal Inhibition Assay was performed on the water samples collected from Stations 1, 2, 5, 6, 8, 9,10,13, 14, to 15. Briefly, all samples were transferred to the laboratory under ice-cold conditions as soon as possible. Prior to the experiment, all water samples were filtered using filters of 0.45 µm and 0.22 µm and then added to the media directly. The samples from ten stations on the Bakırçay River were tested in set of four dilutions (40, 60, 80, and 100 % (v:v)). Control series including only the test medium were also prepared. All of the test series were replicated three times in accordance with by the guideline "Freshwater Algal and *Cyanobacteria* Growth Inhibition Test" (OECD, 2011).

Freshwater algae species *Scenedesmus dimorphus* (UTEX1237) was cultured in the stock solution of OECD TG 201 medium (enhancement medium) in sterilized glass balloon after it was obtained in pure form. Four days before the test, a pre-culture was set up and incubated at 21±2°C and pH 8,1. Cell count was set by means of culture medium so that the cell count of *Scenedesmus dimorphus* would be 2-5 x10^3 cell/ml from the stock culture solution of 4-7 days. Test tubes were held on a shaker at 100 rpm under constant illumination at approximately 2000 lux. The final volume was 6 milliliters in the test tubes. The cells were counted by fluorimeter (Turner design) daily at the same hour of the day and the test duration was 72 hours. The relative inhibition of growth rate was determined as the reduced cell number of the treated samples relative to the controls. K_2HPO_4 solution was used as positive control. Controls were studied in 6 replicates and then their average was calculated.

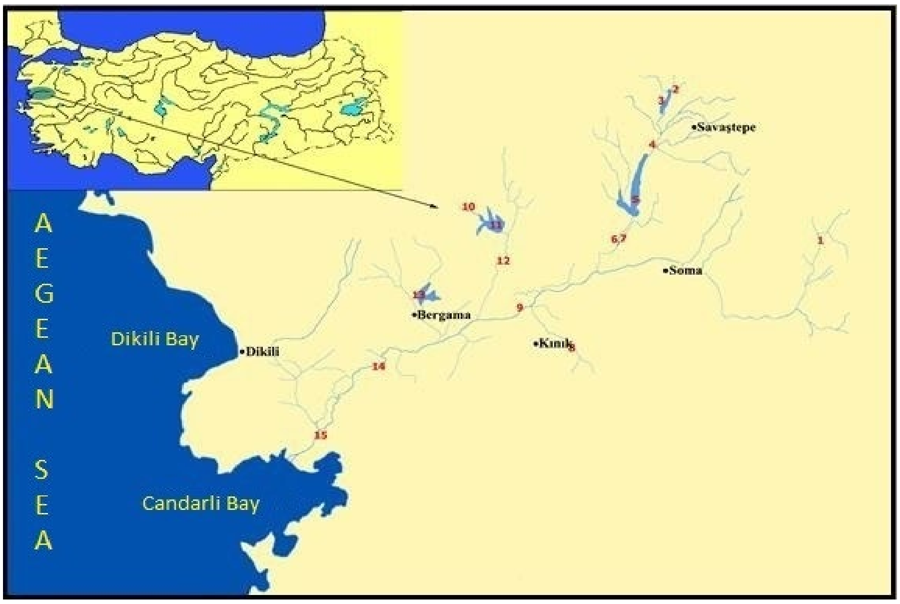

Figure 1. Sampling Sites

Table 1. Data on the stations from which the water samples were taken and their coordinates

St.	Location	Coordinates
1	Koca Creek(Kırkağaç-Manisa)	39°13'39"N - 27°51'30"E
2	Kuzulu Dede Creek (Savaştepe-Balıkesir)	39°26'21"N - 27°23'34"E
3	Sarıbeyler Dam Lake (Savaştepe-Balıkesir)	39°24'42"N - 27°36'38"E
4	Büyük Creek (Savaştepe-Balıkesir)	39°21'22"N - 27°35'39"E
5	Sevişler Dam Lake (Soma-Manisa)	39°15'59"N - 27°33'23"E
6	Yağcılı Creek (Soma-Manisa)	39°11'17"N - 27°29'16"E
7	Bakırçay River (Soma-Manisa)	39°10'41"N - 27°28'55"E
8	Karadere Creek (Kınık-İzmir)	39°05'23"N - 27°25'19"E
9	Bakırçay River (Kınık-İzmir)	39°06'44"N - 27°16'26"E
10	Çınarlıdere Creek (Bergama-İzmir)	39°15'30"N - 27°18'53"E
11	Çaltıkoru Dam Lake (Bergama-İzmir)	39°14'15"N - 27°18'15"E
12	İlyasdere Creek (Bergama-İzmir)	39°12'59"N - 27°20'02"E
13	Kestel Dam Lake (Bergama-İzmir)	39°08'33"N - 27°11'49"E
14	Bakırçay River (Bergama-İzmir)	39°03'11"N - 27°06'40"E
15	Bakırçay River (Dikili-İzmir)	38°57'09"N - 27°00'35"E

Algal growth inhibition test

The green algae *Scenedesmus dimorphus* (Turpin) Kützing 1834: 608, was used as the test organism. The stock algal culture was maintained in an algal medium according to OECD 201. The pH of the medium after equilibration with air was approximately 8. An Algal Bioassay was carried out according to the OECD 201 Standard for algal growth inhibition assay (OECD 2011; Katalay et al., 2012). The endpoints were evaluated based on cell count data and calculated growth rate (0 to 72h) as described in the standard protocols (OECD, 2011) from the mean cell counts of each test series.

The average specific growth rate (μ) for exponentially growing cultures was calculated as follows:

μ0- j= lnxj- lnx0/tj-t0 (day-1)

μ0- j: growth rate,

X0: nominal number of cells / m at time t_0,

Xj: measured number of cells/ml at t_j,

Tj: time of first measurement of after beginning of test.

The percentage of inhibition of the cell growth (Ir %) of each test substance concentration is calculated as the difference between the control growth curve (μc) and the growth curve at each test substance concentration (μt) as:

Ir % = μc -μr /μc x 100.

Inhibition percentage were estimated by comparing the growth with the controls

Ir %: Percent inhibition in average specific growth rate;

μC: Mean value for average specific growth rate (μ) in the control group;

μT: Average specific growth rate for the treatment replicate.

The percentage of growth inhibition was calculated by probit analyses. The Statistica-6.0 software was used for probit analysis and the statistical significance of the data on growth rates was compared with controls using t-test.

RESULTS

Results of the Algal Growth Inhibition Test we performed on superficial water samples from the Bakırçay River using *S. dimorphus* are given as exponential and bar graphics in the Figure 2, 3 and 4. Table 2 shows a comparison of each station with control using Student's T-test. All stations were statistically significantly different from the control group ($p < 0.05$).

The highest level of toxicity was found at Stations 1, 9, 10, 13, 14 and 15 (Figure 2, 3 and 4). For this reason, there was a coal facility near to a Station 9 and that all branches of the Bakırçay River joins near Station15 and then drains into the Çandarlı Bay as one river. Along the river, there are many active agricultural zones, domestic wastes and many wastewater discharges drain into the river. Wastewaters originating from these areas reach to the Bakırçay River and finally discharge to the Çandarlı Bay (Aliağa-İzmir) (Ortabük, 2007).

No inhibition was observed on Stations 2, 5, 6, and 8 (Figure 2, 3); in contrast, hormesis was observed on dilutions of the samples from these stations due to increased concentration (the biological response to low exposures to toxins and other stressors is generally favorable. In toxicology, hormesis is a dose-response phenomenon to xenobiotics or other stressors characterized by a low-dose stimulation, with zero dose and high-dose inhibition).

Table 2. Comparison of each station with control ($p < 0.05$)

Stations	P values
St.1	0.00145
St.2	0.03630
St.5	0.02540
St.6	0.00108
St.8	0.03464
St.9	0.00526
St.10	0.00688
St.13	0.00365
St.14	0.01426
St.15	0.00945

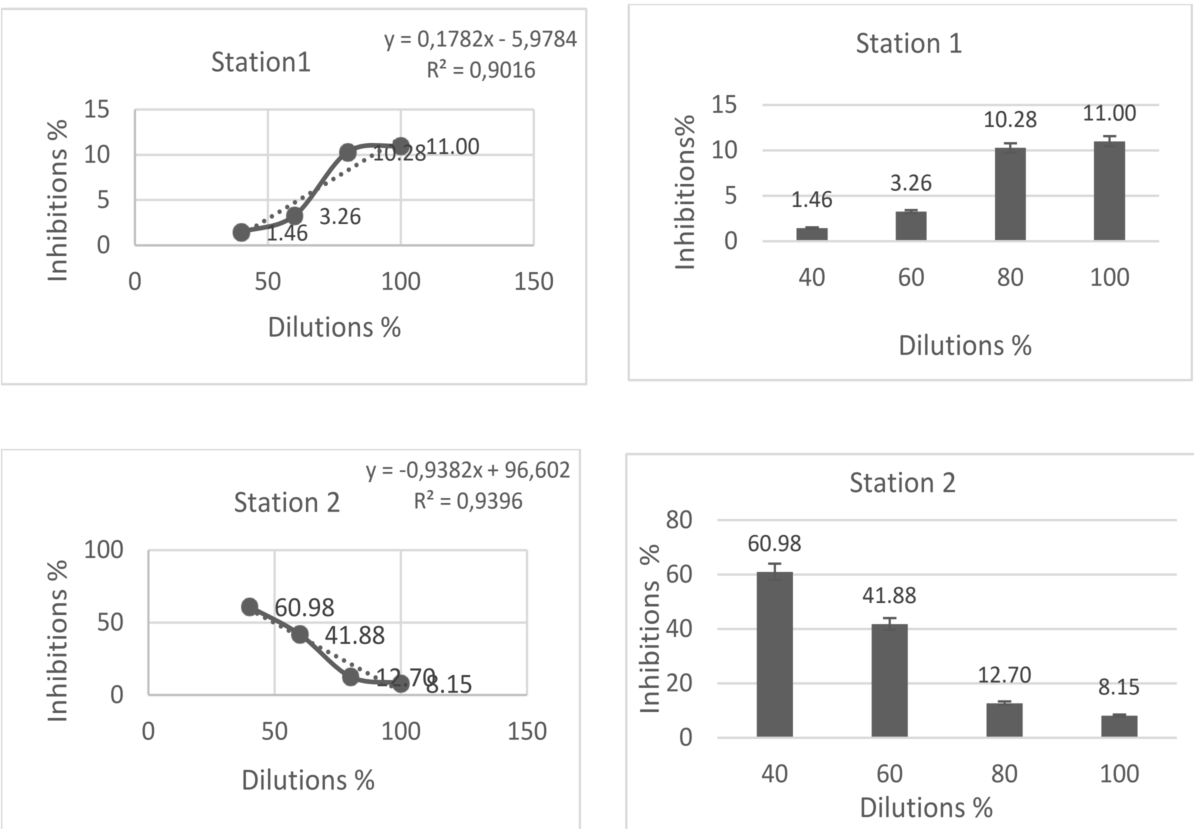

Figure 2. Growth rate of *S. dimorphus* at 72h exposure period

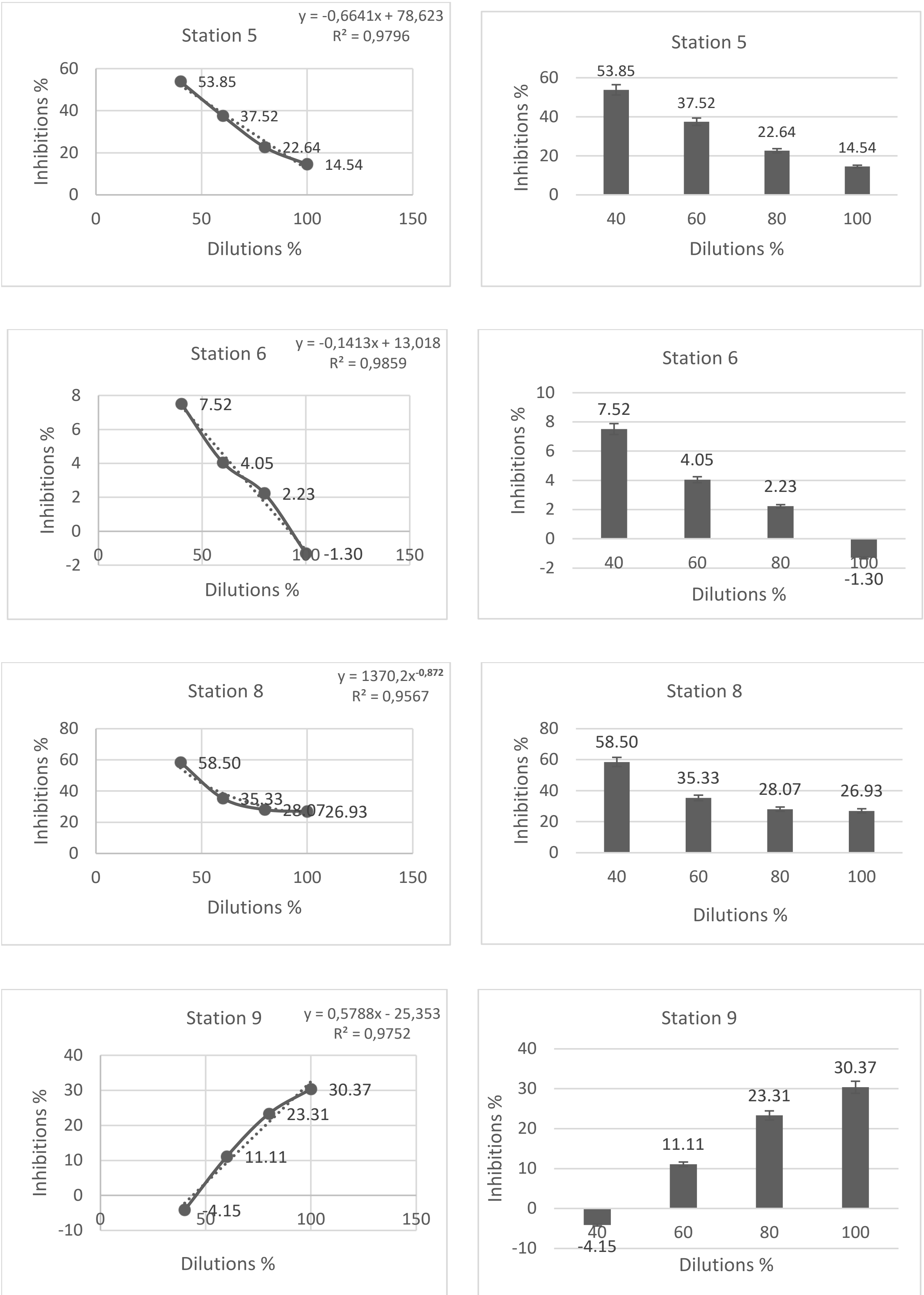

Figure 3. Growth rate of *S. dimorphus* at 72h exposure period

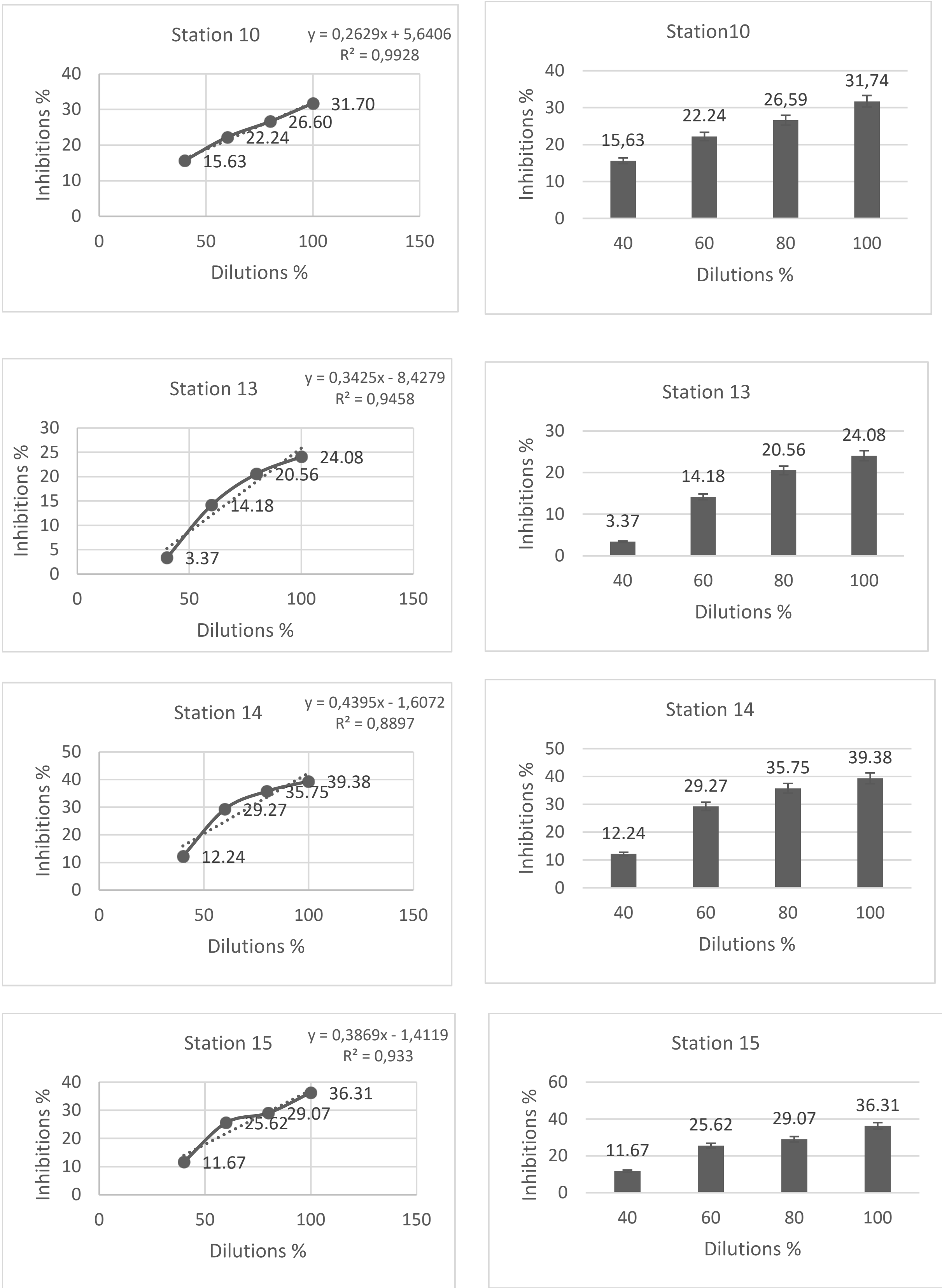

Figure 4. Growth rate of *S. dimorphus* at 72h exposure period

DISCUSSION

Mixing of the drainage waters polluted by the fertilizers and drugs used for agricultural activities with erosion material carried by rainfall increases the pollution burden in the Bakırçay River. Furthermore, water from olive cultivation activities in the region creates a significant problem in the river because Çandarlı Bay is surrounded by crowded settlements, agricultural fields, and industrial areas, Bakırçay River carries wastewater from these regions to Çandarlı Bay (Kaymakçı Başaran, 2004).

Gündoğdu and Turhan (2004) reported that pollution in the Bakırçay Basin was mainly originated from water from processing and cooling activities in Soma Thermal Power Plant, domestic wastes, industrial activities, mining and agricultural activities, and that Bakırçay River had water quality of Class IV according to the Water Quality Control Regulations. According to Kaymakçı Başaran (2004) one of the most important threats to the water quality of Bakırçay River is the presence of the Soma Thermal Power Plant. Additionally, drainage of the wastewater from olive oil plants and other industrial facilities in the region and increased drainage of the fertilizers and drugs used in agricultural activities causes an increased pollution burden in the river. According to Tomar (2009), on the other hand, widespread pollution caused by mine areas exists on the Bakırçay Basin in addition to domestic, industrial, and agricultural pollution.

Department of Laboratory Measurement and Monitoring on the General Directorate of EIA Permission and Surveillance of Ministry of Environment and Urbinazation has been performing surveys on the physicochemical parameters and heavy metals since 2015. In the comparison of summertime results from 2011 to 2015, a decrease was observed in many parameters while an increase was observed in Pb, Cu, total Cr, and Zn parameters. The river was usually detected annually to have Class IV water quality. Being one of the most important rivers of the North Ege Basin, Bakırçay River is under the pressure of domestic pollution (ÇŞB, 2016). Wastes from Soma Thermal Power Plant located on the Bakırçay Basin and domestic wastes have been increasing due to population growth in the region are discharged into the Bakırçay River and its branches with inadequate or no treatment (Kaymakçı Başaran, 2004).

Many studies have been performed on Bakırçay Basin especially focusing on water quality and criteria but no studies exist involving biological tests for the toxic pollutants. Considering the fact that the toxic and mutagenic pollutants are discharged combinedly to the environment without being treated or they pollute the environment indirectly, it will be of great importance to know existence of these pollutants or to gain insight on their concentration in the environment. Thus, biological tests such as the “Algal Growth Inhibition” test are becoming important.

De Liguoro et al. (2010) used *S. dimorphus* as test organism in their study on aquatic toxicity level of Sulfaquinoxalines (SQOs) and Sulfaguanidines (SGDs) and obtained important results.

Arensberg et al. (1995) conducted a study which was similar to ours. A simple mini scale (approx. 1- 2.5 ml) toxicity test procedure with the freshwater green algae *Selenastrum capricornatum* described. The procedure fulfils the validity criteria of the IS0 (International Association for Standardization) standard test protocol. Practically identical concentration-response curves were obtained with the IS0 standard test and the minitest for potassium dichromate and 3,5dichlorophenol (Arensberg et al., 1995).

Katalay et al. (2012) used standard test protocol (Algal Growth Inhibition test, OECD 201) with freshwater algae *Desmodesmus (= Scenedesmus) subspicatus* to determine short-term toxicity and tested water and sediment samples using several dilutions.

Another study was performed on sediment and water samples from Gölcük Lake; in that study, *S. dimorphus* was used in “Algal Growth Inhibition Assay” (OECD, 2011) to determine phytotoxic effect (Boyacıoğlu et al., 2017).

Based on the “Algal Growth Inhibition” test we performed on S. *dimorphus* in the water samples from ten locations on the Bakırçay River, the most toxic and growth-inhibiting samples were those from Soma and those located on the main line draining into Çandarlı Bay.

CONCLUSION

In conclusion, toxicity was detected at Stations 1, 9, 10, 13, 14 and 15 according to the phytotoxicity assay we conducted on ten stations on the Bakırçay River. Most importantly, a phytotoxicity test was done on the Bakırçay River for the first time.

We believe that the present study will be a basement for future studies and guide the multidisciplinary studies. We also hope that many establishments and units will be informed on results of the present study and maybe action will be taken to make necessary measures as a consequence of publication of this study.

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AUTHORSHIP CONTRIBUTIONS

Meltem Boyacıoğlu: Conceptualization, methodology, resources, investigation, writing-reviewing and editing. Cem Aygen: Conceptualization, funding acquisition, project administration, investigation, formal analysis, visualization. Muhammet Ali Karaaslan: Conceptualization, methodology, investigation. Didem Özdemir Mis: Resources, investigation. Özlem Çakal Arslan: Conceptualization, resources, investigation.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest or competing interests.

ETHICS APPROVAL

No specific ethical approval was necessary for this study.

DATA AVAILABILITY

All relevant data is inside the article.

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