

# Combined evaluation of physicochemical parameters and *Daphnia magna* toxicity tests of Gediz River surface water

## Gediz Nehri yüzey suyunun fizikokimyasal parametreleri ile *Daphnia magna* toksisite testlerinin birlikte değerlendirilmesi

Tuna Karaytuğ<sup>1\*</sup> • Ferah Sayım<sup>2</sup>

<sup>1</sup>Department of Biology, Ege University, Institute of Science, 35100, İzmir, Türkiye

<sup>2</sup>Department of Biology, Ege University, Faculty of Science, 35100, İzmir, Türkiye

\*Corresponding author: [tunakaraytu@gmail.com](mailto:tunakaraytu@gmail.com)

Received date: 20.06.2025

Accepted date: 30.10.2025

### How to cite this paper:

Karaytuğ, T., & Sayım, F. (2025). Combined evaluation of physicochemical parameters and *Daphnia magna* toxicity tests of Gediz River surface water. *Ege Journal of Fisheries and Aquatic Sciences*, 42(4), 338-348. <https://doi.org/10.12714/egejfas.42.4.08>

**Abstract:** In this study, pollution in the Gediz River was assessed using *Daphnia magna* toxicity tests. Water samples were collected from five distinct stations during spring and summer. Concentrations of key chemical constituents, including Fe, Mn, Cl<sup>-</sup>, NH<sub>3</sub>-N, S<sup>2-</sup>, K, NO<sub>3</sub>-N, and PO<sub>4</sub><sup>3-</sup>, were determined spectrophotometrically. Acute and chronic toxicity tests were conducted according to OECD guidelines. Acute toxicity was observed at multiple stations in spring and at one station in summer. Conversely, chronic exposure results revealed no significant adverse effects on *D. magna*, despite the known organic and inorganic pollution in the Gediz River. Interestingly, some samples demonstrated stimulation of growth, development, and reproduction in the test organisms, indicating hormetic effects caused by low pollutant concentrations in the water, which are more concentrated in sediments. These findings underscore that *Daphnia*-based water-column toxicity tests alone may not fully capture the combined impacts of organic and inorganic contaminants present in the Gediz River.

**Keywords:** *Daphnia magna*, Gediz River, water pollution, acute toxicity, chronic toxicity, hormesis

**Öz:** Bu çalışmada, Gediz Nehri'ndeki kirlilik, *Daphnia magna* toksisite testleri kullanılarak değerlendirilmiştir. Su örnekleri, ilkbahar ve yaz mevsimlerinde beş farklı istasyondan toplandı. Fe, Mn, Cl<sup>-</sup>, NH<sub>3</sub>-N, S<sup>2-</sup>, K, NO<sub>3</sub>-N ve PO<sub>4</sub><sup>3-</sup> gibi temel kimyasal bileşenlerin konsantrasyonları spektrofotometrik yöntemlerle belirlendi. Akut ve kronik toksisite testleri OECD yönergelerine uygun olarak gerçekleştirildi. Akut toksisite, ilkbaharda birden fazla istasyonda ve yazın bir istasyonda gözlemlendi. Buna karşılık, kronik maruziyet sonuçları, Gediz Nehri'nde bilinen organik ve inorganik kirliliğe rağmen *D. magna* üzerinde anlamlı olumsuz etkiler göstermedi. İlginç bir şekilde, bazı örnekler test organizmalarında büyüme, gelişim ve üremenin uyarıldığını ortaya koydu; bu durum, suda düşük konsantrasyonlarda bulunan ve sedimentlerde daha fazla yoğunlaşan kirlenimlerden kaynaklanan hormetik etkileri işaret etmektedir. Bu bulgular, yalnızca su kolonuna dayalı *Daphnia* toksisite testlerinin, Gediz Nehri'nde bulunan organik ve inorganik kirlenimlerin birleşik etkilerini tam olarak yansıtamayabileceğini ortaya koymaktadır.

**Anahtar kelimeler:** *Daphnia magna*, Gediz Nehri, su kirliliği, akut toksisite, kronik toksisite, hormesis

## INTRODUCTION

Water is essential for life, serving as a medium for biochemical reactions, a habitat for most animals, and a source of groundwater and surface water. Access to freshwater is critical globally, but population growth, urbanization, and industrialization have increasingly made clean water scarce, turning water bodies into waste disposal sites (Gündoğdu and Kocataş, 2006). To protect these resources, water quality is assessed using chemical, physical, and biological methods. While physicochemical parameters provide valuable information on the structure of aquatic ecosystems, they alone cannot fully evaluate pollution impacts. A comprehensive approach, integrating toxicity tests with physicochemical analyses, is needed to assess the effects of toxic chemicals in water (Feiler et al., 2006; Kirsanov et al., 2014; Serpa et al., 2014). Short- and long-term toxicity tests using *Daphnia magna*, a widely recognized environmental indicator, are commonly employed (Barata et al., 2008; Serpa et al., 2014). The Gediz River, the second-largest river in the Aegean Region, is heavily polluted due to untreated industrial discharges (e.g., heavy metals such as lead, cadmium, and mercury), domestic wastewater, and agricultural runoff containing fertilizers and pesticides. This

contributes to a complex mixture of organic and inorganic pollutants that pose significant ecological risks, including bioaccumulation and toxicity to aquatic organisms (Hafizoğlu and Tekin, 2004; Küçüksezgin et al., 2008; Aydın and Küçüksezgin, 2012). Recent studies classify the river's water quality as Class IV (very polluted) (Öner and Çelik, 2011; Ministry of Environment and Urbanization, 2014; Ertaş et al., 2021). Assessing the specific impact of each pollutant is challenging due to their interactions, which vary with environmental conditions (Hertzberg and MacDonell, 2002; Serpa et al., 2014). Therefore, evaluating the combined effects of all organic and inorganic pollutants in unextracted water samples provides a more accurate measure of pollution (Lyu et al., 2013). *Daphnia magna* is widely used in toxicity tests recommended by international organizations (USEPA, EEC, OECD) due to its sensitivity to pollutants, small size, short life cycle, parthenogenetic reproduction, high fecundity, ease of culture, and transparent body, which allows observation of internal structures (Koivisto, 1995; Wojtal-Frankiewicz, 2012; Miner et al., 2012). This study aims to assess Gediz River pollution using standard *D. magna* acute and chronic toxicity tests. The findings

are expected to highlight the river's aquatic ecosystem status, raise awareness among authorities, and support sustainable water resource management.

## MATERIALS AND METHODS

### Water sampling stations

This study was conducted using water samples collected from five stations along the Gediz River. Sampling was

performed twice a year: in spring (March and April 2014) and in summer (July and August 2014). Satellite images of the stations are provided in Figure 1, and their coordinates, determined with a GPS device (Garmin GPSMAP 62s), are listed in Table 1. During fieldwork, measurements of electrical conductivity, pH, dissolved oxygen, and salinity were taken at each station using a Hach Lange portable device. Water temperature was measured separately with a digital thermometer.



Figure 1. Satellite images of the sampling stations

Table 1. The sampling stations and their characteristics

Coordinates	Stations	Station Characteristics
38° 39' 37,7" N 27° 18' 45,8" E	1st station: Muradiye-Gediz Bridge	Main river branch; surrounded by agricultural fields and small settlements; polluted by fertilizers, pesticides, and domestic sources; moderate flow rate allowing some dilution of contaminants.
38° 38' 34,8" N 27° 21' 59,4" E	2nd station: Karacay	Tributary; near agricultural lands and small industrial facilities; potential pollution from agricultural runoff and industrial wastewater; seasonal variability in turbidity and organic matter load; lower flow than the main branch.
38° 38' 35,5" N 27° 26' 33,1" E	3rd station: Nif Stream	Tributary; originates from Nif Mountain; passes Kemalpaşa's semi-urban and industrial areas; affected by agriculture, domestic wastewater, high suspended solids (sediments) in rainy periods; variable seasonal flow.
38° 38' 36,7" N 27° 26' 33,5" E	4th station: Gediz Bridge-Istanbul Road	Main river branch; originates from Nif Mountain; passes through Kemalpaşa's semi-urban and industrial areas; affected by roadway runoff (oil, heavy metals), agriculture, domestic wastewater, and high suspended solids (sediments) in rainy periods; flow rate supports partial dilution.
38° 38' 33,3" N 27° 26' 30,8" E	5th station: Gediz Bridge	Main river branch; near industrial and urban areas; high potential load of heavy metals (Pb, Cd, Hg) and organic pollutants; risk of low dissolved oxygen during dry periods; high flow allows some dispersion of pollutants.

### Setting up *D. magna* culture

Commercial drinking water with known physicochemical parameters was used for *D. magna* culture. The culture was established in a glass aquarium (35 x 15 cm) with a water level of 40-50 cm by placing female individuals carrying eggs. The pH of the *Daphnia* culture was maintained between 7.5 and 8.0, and dissolved oxygen levels ranged from 7.48 to 9.64 mg/L. Water temperature was kept at 20±2 °C, and a photoperiodic lighting schedule (16 hours light, 8 hours darkness) was followed. White fluorescent lamp was used for

illumination, and the culture was continuously aerated by a ventilation motor. Weekly records were kept for pH and dissolved oxygen levels, while water temperature was recorded daily. The population density was set at 2 mL of water per individual. When this density was exceeded, some adolescent individuals were transferred to another aquarium. For feeding the *Daphnia* culture, a specific amount of stock algal culture was taken under aseptic conditions using a burner flame in a sterilized isolation room that had been exposed to

UV light for 30 minutes to prevent contamination. The algal culture was then transferred to a glass beaker and subsequently added to the aquarium containing the *Daphnia* culture with the help of a pipette.

#### Culturing of *Scenedesmus* sp.

*Scenedesmus* sp. was obtained from the algae culture laboratory at the Faculty of Engineering, E.U., Department of Bioengineering. After autoclaving under sterile conditions, the culture was transferred to a medium bottle. Bold's Basal Medium (Bischoff and Bold, 1963) was used for the culture. The medium was prepared with distilled water, adjusted to pH 6.8, sterilized, and autoclaved (Hirayama, HV-50L) for 20 minutes at 121 °C.

A glass pipette fitted with a plastic hose at the tip was placed into the Erlenmeyer flasks used for culturing. The mouth of the Erlenmeyer flask was covered with cotton and aluminum foil. Small portions of cotton were placed inside the pipette and the plastic hose to filter the air. After preparation and autoclaving, the culture was transferred into sterilized Erlenmeyer flasks, where it was added to Bold's Basal Medium at a ratio of 1:10 under aseptic conditions. Following the transfer, the hose tip was connected to a ventilation system, and the culture was incubated under fluorescent light at 26±1 °C for one week. After one week, the culture was concentrated, and fresh sterile medium was added to new Erlenmeyer flasks to ensure the longevity of the culture. Cell density was counted under a 10x magnification light microscope (Prior A216) using a Neubauer hemocytometer. Cultures with a density of 2.5–3 million cells/mL were used to feed *Daphnia*.

#### Chemical analysis of water samples

Water samples were collected from each station at approximately 20 cm below the surface using sterile polyethylene bottles. The samples were transported to the laboratory within 2 hours in insulated coolers with ice packs, maintained at 4 °C. Upon arrival, the samples were stored in a refrigerator at 4 °C prior to analysis. Various chemical parameters of the water samples collected from each station—such as iron, manganese, chloride, ammonia nitrogen, sulfur, potassium, nitrate nitrogen, and phosphate—were analyzed in the laboratory using spectrophotometric techniques with a Hach Lange DR 2800 VIS spectrophotometer.

#### *D. magna* toxicity tests

In the toxicity tests, neonates of *Daphnia magna* Straus 1820 (Crustacea, Cladocera) less than 24 hours old were used. The experimental organisms were maintained under optimal laboratory conditions and obtained from parthenogenetically reproducing *D. magna*. Experiments were conducted at a controlled temperature of 20 ± 2 °C with a 16:8 h light/dark cycle to simulate photoperiodic conditions. Before the acute toxicity tests, range-finding studies were performed for each station to determine the effective dilution range.

#### Sample preparation and dilution of test solutions

Water samples for acute and chronic toxicity tests were diluted with distilled water (% v/v) to achieve specific dilutions according to sampling stations and seasons. In spring, samples from Stations 1–4 were diluted to 30%, 50%, 70%, 90%, and 100%, while Station 5 samples were diluted to 4%, 8%, 12%, 16%, and 20% of the original sample. In summer, due to low toxicity observed in preliminary tests, samples from Stations 1–4 were used undiluted (100%, control equivalent), and Station 5 samples were used at 4%, 8%, 12%, 16%, and 20% of the original sample. Sublethal dilutions for chronic toxicity tests were selected based on prior acute results: in spring, Stations 1 and 2 were 1%, 4%, 8%, 16%, and 32%; Stations 3 and 4, 4%, 8%, 16%, 32%, 64%, and 90%; and Station 5, 1%, 4%, 8%, 12%, and 16%. In summer, Stations 1, 2, and 4 were 10%, 30%, 50%, 70%, and 90%; Station 3, 1.25%, 2.5%, 5%, 7.5%, and 10%; and Station 5, 0.5%, 1%, 2%, 4%, and 8% of the original sample. All dilutions were prepared using calibrated pipettes and volumetric flasks, and gently stirred to ensure uniform contaminant distribution before transfer to test vessels.

#### Conducting acute toxicity tests

Acute toxicity tests were carried out according to the standard protocol (Organisation for Economic Co-operation and Development [OECD], 2004; Test No: 202) for the "*Daphnia* sp. Acute Immobilization Test," using a static system. During the 48-hour test, test solutions were not changed, flasks were not aerated, and organisms were not fed. For each station, the tests were conducted in quadruplicate and included five test groups and one control group, each consisting of five individuals (5 individuals/10 mL test solution). At the 24th and 48th hours of exposure, the number of immobilized individuals and any observable behavioral changes in both test and control groups were assessed using a stereomicroscope. At the end of the test, the 24- and 48-hour EC<sub>10</sub>, EC<sub>50</sub>, and EC<sub>90</sub> values were determined using probit analysis via SPSS statistical software.

Water samples were tested in their unextracted form. Preliminary experiments for acute toxicity involved filtration through 0.20 µm cellulose acetate membrane filters (Millipore, Merck) using a vacuum pump (KNF); however, no toxicity was observed in these filtered samples, so unfiltered water was used for all subsequent acute and chronic tests to reflect the natural state of the Gediz River.

#### Conducting chronic toxicity tests

Chronic toxicity assessments were conducted following OECD Guideline No. 211 (1998), known as the "*Daphnia magna* Reproduction Test," utilizing a semi-static exposure setup. Throughout the 21-day test period, solutions were refreshed twice weekly. On each renewal day, individual organisms received 2 mL of *Scenedesmus* sp. (cell concentration: 3 × 10<sup>6</sup> cells/mL) as food. The pH and dissolved oxygen levels of both fresh and spent media were

monitored during each renewal. Sublethal dilutions of Gediz River water were selected based on prior acute toxicity findings. Ten neonates were allocated to separate 150 mL glass beakers, each filled with 50 mL of test or control solution. The tests were conducted in quadruplicate, with five test groups corresponding to five different sublethal dilutions and one control group. Throughout the test, the total number of live juveniles produced per parent and the day of first reproduction were recorded daily. Juveniles were removed daily from the experimental setup. At the end of the 1st and 3rd weeks, the body lengths of individuals from both control and test groups were measured with a stereomicroscope (OLYMPUS A216) using millimeter paper. Reproductive outputs from both test and control groups were statistically analyzed using one-way ANOVA (IBM SPSS Statistics v21.0). To evaluate group-wise differences in the number of offspring per adult, post-hoc comparisons were performed using Tukey's test.

**Table 2.** Physicochemical parameter values

Parameters	Stations									
	Stn. 1		Stn. 2		Stn. 3		Stn. 4		Stn. 5	
	Spr.	Sum.	Spr.	Sum.	Spr.	Sum.	Spr.	Sum.	Spr.	Sum.
Air temperature (°C)	25	35	25	35	25	35	25	35	25	35
Water temperature (°C)	20.9	25.7	20.2	33.4	19.2	27.4	20.5	27.8	22.6	31.5
pH	7.67	7.94	8.12	8.30	8.12	7.81	7.97	8.17	7.71	7.88
Conductivity (µs/cm)	1139	715	2970	3060	1174	847	1048	1039	919	907
Salinity (‰)	0.57	0.34	1.55	1.6	0.59	0.42	0.51	0.32	0.45	0.44
Dissolved oxygen (mg O <sub>2</sub> /L)	1.33	4.75	7.36	7.55	0.47	1.01	5.43	5.49	0.64	0.24
Iron (mg Fe/L)	0.56	0.57	-	-	1.43	0.91	0.58	0.25	0.06	0.05
Manganese (mg Mn/L)	1.5	0.7	0.7	2.4	4.2	2.3	1.1	3.5	0.8	2.0
Chloride (mg Cl/L)	294.1	32.6	418.5	274.7	179.6	45.4	76.5	27.0	62.4	75.9
Ammonia nitrogen (mg NH <sub>3</sub> -N/L)	4.47	1.37	2.40	1.05	7.48	5.1	0.8	0.31	21.64	17.36
Sulfur (µg S <sup>2-</sup> /L)	7	29	45	33	44	28	17	38	54	189
Potassium (mg K/L)	14.7	1.3	24.6	21.0	17.5	5.9	14	10.0	3.8	12.8
Nitrate nitrogen (mg NO <sub>3</sub> -N/L)	14.9	-	2.0	0.4	0.4	-	3.3	-	0.9	-
Phosphate (mg PO <sub>4</sub> <sup>3-</sup> /L)	-	1.89	10.34	12.85	0.46	2.73	5.32	1.34	9.38	7.77

\* - indicates that the parameter was not detected. (Stn.: Station, Spr.: Spring, Sum.: Summer)

**Table 3.** Acute immobilization test (Stations 1, 2, 3, 4, and 5 - spring)

Dilution (% v/v)	Total Live Organisms	Immobilized Individuals									
		Stn. 1 (24 h)	Stn. 1 (48 h)	Stn. 2 (24 h)	Stn. 2 (48 h)	Stn. 3 (24 h)	Stn. 3 (48 h)	Stn. 4 (24 h)	Stn. 4 (48 h)	Stn. 5 (24 h)	Stn. 5 (48 h)
Ctrl	20	0	0	0	0	0	0	0	0	0	0
15	20	-	-	-	-	-	-	-	-	5	7
25	20	-	-	-	-	-	-	-	-	9	11
30	20	0	5	2	2	0	0	0	0	12	12
50	20	2	9	5	9	0	0	0	0	15	16
70	20	4	12	7	14	0	0	0	0	19	20
90	20	10	15	11	20	0	0	0	0	-	-
100	20	20	20	13	20	0	0	0	0	-	-

The "-" symbol indicates that the dilution was not used at the respective station. (Stn.: Station, Ctrl: Control)

**Table 4.** EC values for station 1 in spring

EC values	24 h	48 h
EC <sub>10</sub>	58.32 (-5.09-72.13)	18.20 (-3.07- 30.60)
EC <sub>50</sub>	81.47 (64.62-101.61)	57.53 (48.41-66.06)
EC <sub>90</sub>	104.62 (90.09-175.32)	96.87 (85.49-115.92)

**Table 5.** EC for station 2 in spring

EC values	24 h	48 h
EC <sub>10</sub>	35.71 (14.81-47.79)	31.43 (18.50-39.24)
EC <sub>50</sub>	83.60 (73.71-97.52)	55.01 (48.54-61.70)
EC <sub>90</sub>	131.49 (113.29-166.56)	78.59 (70.46-92.28)

## RESULTS

### Physicochemical parameters

Table 2 presents the values of selected chemical parameters in water samples collected from the five stations during spring and summer.

### Toxicity test results

#### Acute immobilization test

In the spring period, the number of immobilized individuals at 24 and 48 hours is presented for Stations 1, 2, 3, 4, and 5 in Table 3. No toxic effects were observed in water samples from Stations 3 and 4, even when used undiluted (100%, control equivalent), and no differences in mobility were found between the treatment and control groups. Therefore, no acute toxicity was detected at these stations, and EC values were not calculated. In contrast, 24- and 48-hour EC values for Stations 1, 2, and 5 were determined and shown in Tables 4, 5 and 6.

**Table 6.** EC for station 5 in spring

EC values	24 h	48 h
EC <sub>10</sub>	7.81 (-14.09-17.49)	6.96 (-20.21-16.59)
EC <sub>50</sub>	32.34 (23.84-43.57)	28.28 (19.32-40.48)
EC <sub>90</sub>	56.88 (45.13-86.28)	49.60 (38.28-84.94)

During the summer period, no toxic effects were observed in the 24- and 48-hour acute toxicity tests conducted with water samples from Stations 1, 2, 3, and 4. As no differences in mobility were detected between the treatment and control groups, EC values for these stations were not calculated.

Immobilization, indicating acute toxicity, was only observed at Station 5 (Table 7), and its EC values are presented in Table 8.

**Table 7.** Acute immobilization test (Station 5 - summer)

Dilution (% v/v)	Total Live Organisms	Immobilized Individuals (48 h)
Control	20	0
4	20	0
8	20	3
12	20	5
16	20	8
20	20	10

**Table 8.** The EC for Station 5 in summer

EC values	48 h
EC <sub>10</sub>	8.11 (2.91-10.78)
EC <sub>50</sub>	18.68 (16.10-23.54)
EC <sub>90</sub>	29.24 (24.15-41.45)

When comparing the 48-hour acute toxicity of water samples from the spring period based on EC<sub>50</sub> values, Station 5 was identified as the most toxic, with an EC<sub>50</sub> value of 28.28. The toxicity ranking was as follows: Station 2 (EC<sub>50</sub>=55.01) and Station 1 (EC<sub>50</sub>=57.53). However, since the EC<sub>50</sub> values for Stations 2 and 1 are quite similar, it can be concluded that their toxicity levels are also comparable. In the acute toxicity tests conducted with water samples from the summer period, acute toxicity (EC<sub>50</sub>=18.68) was observed only at Station 5 after 48 hours. No acute toxicity was detected at the other stations.

### Chronic toxicity tests

During the spring period, the average body lengths of individuals in the treatment groups were significantly higher than those in the control group at Stations 1, 3, and 4 for 1st-week measurements, and at Stations 1, 4, and 5 for 3rd-week measurements (Table 9). The first egg formation occurred

significantly earlier in treatment groups at Stations 1, 3, 4, and 5, while the first reproduction occurred earlier at Stations 1, 3, and 5 compared to the control group. Additionally, the average number of offspring per adult in treatment groups at Stations 1 and 5 was significantly higher than in the control group (Table 10). Station 2 was excluded from the tables, as its data did not show statistically significant differences compared to the control.

**Table 9.** Individuals' average body length at weeks 1 and 3 in spring

Stn.	Dilution	Average Body Length (mm)	
		1st Week	3 st Week
Stn. 1	Ctrl	1.24 (1.12-1.37)	2.45 (2.34-2.56)
	1%	1.51* (1.40-1.63)	2.43 (2.34-2.54)
	4%	1.76* (1.65-1.86)	2.49 (2.40-2.58)
	8%	1.88* (1.82-1.93)	2.68* (2.60-2.77)
	16%	1.93* (1.85-2.00)	2.83* (2.74-2.92)
	32%	1.89* (1.80-1.99)	2.76* (2.65-2.87)
Stn. 3	Ctrl	1.60 (1.50-1.70)	-
	4%	1.87* (1.78-1.96)	-
	8%	1.95* (1.86-2.04)	-
	16%	1.84* (1.73-1.94)	-
	32%	1.95* (1.85-2.05)	-
	64%	1.97* (1.91-2.02)	-
	90%	2.02* (1.98-2.06)	-
Stn. 4	Ctrl	1.48 (1.39-1.56)	2.64 (2.50-2.78)
	4%	1.68* (1.60-1.77)	2.71 (2.60-2.81)
	8%	1.79* (1.69-1.90)	2.82 (2.72-2.92)
	16%	1.93* (1.83-2.03)	2.74 (2.62-2.87)
	32%	2.01* (1.92-2.11)	2.82 (2.67-2.98)
	64%	2.06* (2.01-2.10)	2.93* (2.87-2.99)
	90%	2.12* (2.04-2.19)	2.97* (2.93-3.00)
Stn. 5	Ctrl	-	2.71 (2.55-2.87)
	1%	-	2.82 (2.67-2.97)
	4%	-	2.90 (2.85-2.95)
	8%	-	3.10* (3.00-3.20)
	12%	-	3.05* (2.94-3.16)
	16%	-	3.17* (2.98-3.36)

\*Statistically significant difference compared to the control group ( $p < 0.05$ ). The '-' symbol indicates no statistically significant difference compared to the control group. (Stn.: Station, Ctrl: Control)

**Table 10.** Individuals' average first egg formation, reproduction days, and offspring per adult in spring

Stn.	Dilution	Average first egg formation days	Average first reproduction days	Average number of offspring per adult
Stn. 1	Ctrl	13.96 (12.49-15.43)	15.90 (14.50-17.31)	9.05 (14.50-17.31)
	1%	11.50* (10.53-12.47)	14.38 (13.29-15.47)	10.57* (13.29-15.47)
	4%	10.26* (9.59-10.93)	12.35* (11.57-13.13)	12.60* (11.57-13.13)
	8%	9.37* (8.82-9.92)	12.03* (11.47-12.59)	11.10* (11.47-12.59)
	16%	9.76* (9.20-10.31)	12.34* (11.82-11.82)	11.58* (11.82-12.87)
	32%	9.41* (8.76-10.06)	12.30* (11.50-13.11)	12.82* (11.50-13.11)
Stn. 3	Ctrl	12.61 (11.24-13.98)	14.72 (13.59-15.85)	-
	4%	9.65* (8.26-11.03)	12.79 (11.38-14.20)	-
	8%	9.26* (8.41-10.10)	12.00* (11.08-12.92)	-
	16%	8.92* (8.18-9.66)	11.89* (11.16-12.61)	-
	32%	9.44* (8.41-10.47)	12.43 (11.15-13.70)	-
	64%	10.15* (7.94-12.37)	13.80 (10.35-17.25)	-
	90%	8.05* (7.10-9.00)	11.10* (10.15-12.05)	-
Stn. 4	Ctrl	12.12 (11.18-13.06)	-	-
	4%	11.00 (10.06-11.94)	-	-
	8%	10.94 (9.70-12.17)	-	-
	16%	11.29 (10.00-12.59)	-	-
	32%	9.39* (8.84-9.93)	-	-
	64%	9.59* (8.74-10.44)	-	-
Stn. 5	Ctrl	10.19 (9.85-10.52)	13.61 (12.77-14.46)	22.58 (18.50-26.55)
	1%	9.95 (9.09-10.80)	12.20* (11.66-12.74)	28.94 (22.66-35.22)
	4%	8.26 (7.72-8.80)	11.20* (10.67-11.73)	46.26* (38.38-54.15)
	8%	8.49 (7.79-9.19)	11.08* (10.54-11.63)	57.61* (47.98-67.24)
	12%	8.06* (7.65-8.46)	10.76* (10.36-11.17)	80.00* (68.87-91.13)
	16%	8.62* (8.05-9.19)	11.31* (10.72-11.91)	71.97* (61.71-82.23)

\*Statistically significant difference compared to the control group ( $p < 0.05$ ). The '-' symbol indicates no statistically significant difference compared to the control group. (Stn.: Station, Ctrl: Control)

During the summer period, the average body lengths of individuals in the treatment groups were significantly higher than those of the control group at Stations 1, 2, 4, and 5 for 1st-week measurements, and at Station 1 for 3rd-week measurements. Additionally, the first egg formation and first reproduction occurred significantly earlier in treatment groups at Stations 1, 2, and 3 compared to the control group. Moreover, the average number of offspring per adult in treatment groups at Stations 2 and 3 was significantly greater than in the control group. Body length measurements from Station 3 were excluded from Table 11 because they did not show statistically significant differences compared to the control, whereas reproductive metrics showing significant differences are included in Table 12.

**DISCUSSION**

In this study, pollution levels in the Gediz River were assessed using acute and chronic toxicity tests with *Daphnia magna*, a widely used bioindicator organism that reflects water quality through measurable responses such as immobilization, growth, and reproduction. These analyses provide a robust framework to compare pollution levels across different stations, which are impacted by multiple sources, including agricultural runoff (high nitrogen and phosphorus from fertilizers and pesticides), industrial discharges (heavy metals and inorganic pollutants), and domestic wastewater.

**Table 11.** Individuals' average body length at weeks 1 and 3 in summer

Stn.	Dilution	Average Body Length (mm)	
		1st Week	3 st Week
Stn. 1	Ctrl	1.91 (1.83-1.99)	2.58 (2.43-2.74)
	10%	2.09* (2.04-2.14)	2.95* (2.91-3.00)
	30%	2.20* (2.12-2.29)	3.09* (2.99-3.18)
	50%	2.30* (2.15-2.45)	3.11* (3.03-3.19)
	70%	2.24* (2.11-2.28)	3.04* (2.93-3.14)
	90%	2.19* (2.01-2.37)	2.85* (2.71-2.99)
Stn. 2	Ctrl	2.04 (1.99-2.09)	-
	10%	2.42* (2.22-2.61)	-
	30%	2.39* (2.26-2.52)	-
	50%	2.43* (2.28-2.57)	-
	70%	2.36* (2.17-2.56)	-
	90%	2.34* (2.14-2.54)	-
Stn. 4	Ctrl	1.89 (1.81-1.98)	-
	10%	1.86 (1.76-1.96)	-
	30%	2.03 (1.96-2.10)	-
	50%	2.07* (1.96-2.19)	-
	70%	2.05* (1.96-2.15)	-
	90%	2.16* (2.09-2.23)	-
Stn. 5	Ctrl	2.08 (2.04-2.12)	-
	0.5%	2.09 (2.02-2.16)	-
	1%	2.06 (1.98-2.13)	-
	2%	2.02 (1.95-2.09)	-
	4%	1.97 (1.88-2.07)	-
	8%	1.80* (1.65-1.95)	-

\*Statistically significant difference compared to the control group (p<0.05). The '-' symbol indicates no statistically significant difference compared to the control group. (Stn.: Station, Ctrl: Control)

**Table 12.** Individuals' average first egg formation and reproduction days, and offspring per adult in summer

Stn.	Dilution	Average first egg formation days	Average first reproduction days	Average number of offspring per adult
Stn. 1	Ctrl	10.76 (9.86-11.66)	13.17 (12.14-14.19)	-
	10%	8.31* (7.41-9.21)	10.69* (9.75-11.63)	-
	30%	7.33* (6.70-7.96)	9.44* (8.79-10.10)	-
	50%	7.31* (6.54-8.08)	9.93* (10.10-10.30)	-
	70%	8.14* (7.30-8.97)	10.39* (9.48-11.31)	-
	90%	8.08* (7.11-9.06)	10.32* (9.44-11.20)	-
Stn. 2	Ctrl	9.08 (8.58-9.58)	11.51 (10.95-12.07)	25.28 (22.24-28.31)
	10%	6.62* (6.01-7.22)	8.66* (8.12-9.19)	73.36* (63.70-83.03)
	30%	5.44* (4.91-5.91)	7.64* (7.09-8.19)	59.51* (46.45-72.58)
	50%	6.79* (4.60-8.99)	7.79* (7.04-8.54)	70.59* (56.35-84.84)
	70%	6.63* (5.02-8.23)	8.26* (6.93-9.59)	81.50* (63.81-99.19)
	90%	7.63* (5.96-9.29)	9.67* (8.17-11.17)	71.39* (51.96-90.83)
Stn. 3	Ctrl	8.96 (8.35-9.57)	11.54 (10.98-12.09)	18.57 (16.48-20.66)
	1.25%	8.22 (6.88-9.56)	10.52 (9.08-11.97)	20.74 (15.25-26.23)
	2.5%	7.74 (6.93-8.55)	10.30 (9.45-11.14)	21.22 (18.62-23.82)
	5%	7.14* (6.33-7.96)	10.00 (9.10-10.90)	25.11 (22.30-27.92)
	7.5%	5.68* (5.26-6.10)	8.12* (7.61-8.63)	28.36* (23.72-33.00)
	10%	6.13* (5.21-7.05)	9.09* (7.98-10.19)	27.91* (22.64-33.18)

\*Statistically significant difference compared to the control group (p<0.05). The '-' symbol indicates no statistically significant difference compared to the control group. (Stn.: Station, Ctrl: Control)

The 48-hour acute toxicity tests revealed toxicity in water samples from Stations 1, 2, and 5 in spring and Station 5 in summer, consistent with seasonal variations reported in prior studies (Kaza et al., 2007; Hussain et al., 2023; Hassan et al., 2024). These results underscore the dynamic nature of pollution patterns, highlighting the importance of monthly monitoring to capture temporal changes in water quality. Physicochemical parameters provide critical insights into the observed toxicity patterns. Ammonia (NH<sub>3</sub>-N) in Nif Stream

(Station 3) reached 7.48 mg/L in March–April 2014 (Table 13), approximately three times higher than the 2.201 mg/L average reported by Şentürk and Yıldız (2015), indicating intense agricultural fertilizer runoff and contributing to eutrophication risk.

Iron (Fe) concentrations reached 1.43 mg/L at Nif Stream (Station 3) and 0.58 mg/L at Gediz Bridge–İstanbul Road (Station 4) in March–April 2014, and 0.91 mg/L and 0.25 mg/L respectively in July–August, while manganese (Mn) levels rose

to 4.20 mg/L at Station 3 and 3.50 mg/L at Station 4 in March–April, and 2.30 mg/L and 3.50 mg/L respectively in July–August. These values exceed the generalized Gediz River Basin ranges reported by Küçüksezgin et al. (2008) (0.0013–0.687 mg/L for Fe and 0.03–0.17 mg/L for Mn, Table 13), suggesting localized trace metal enrichment. High Fe and Mn concentrations likely had limited toxicity due to environmental factors (e.g., pH, sediment interactions) reducing their bioavailability (Aydın and Küçüksezgin, 2012; Miranda et al., 2021). This comparison is limited by the lack of station-specific data in Küçüksezgin et al. (2008), as noted in the table footnotes.

Conversely, nitrate (NO<sub>3</sub>-N) decreased significantly from previous studies' averages (Şentürk and Yıldız, 2015), reaching 0.40 mg/L in March–April at Station 3 and 3.30 mg/L at Station 4, and was undetectable in July–August at both

Stations 3 and 4 (Table 13). This sharp decline is consistent with reduced agricultural runoff during warmer months, as rainfall-driven fertilizer discharge typically peaks in spring (March–May) due to increased precipitation (Şentürk and Yıldız, 2015). Microbial denitrification processes, enhanced by higher temperatures in summer, may further reduce nitrate levels. At Station 3, high nitrate levels in previous studies (up to 151.965 mg/L in April 2012) were linked to industrial and domestic wastewater inputs from Nif Stream, a major pollutant source contributing to the Gediz River's pollution load. At Station 4, elevated nitrate levels in spring are attributed to fertilizer discharge during rainfall and domestic wastewater inputs via small tributaries near the İstanbul Road–Gediz Bridge, classifying the river as highly polluted (IV. quality class) per the Water Pollution Control Regulation (Şentürk and Yıldız, 2015).

**Table 13.** Comparison of physicochemical parameters measured in 2014 at Nif Stream (Station 3) and Gediz Bridge-Istanbul Road (Station 4) with previous studies (1998–2012)

Parameter	Station	Küçüksezgin et al. (2008)	Şentürk and Yıldız (2015)	This Study
pH	Nif Stream	7.0 (Aug 1998)	7.42±0.38 (6.80–7.93)	8.12 (Mar–Apr) 7.81 (Jul–Aug)
		7.1 (Oct 1998)		
		5.9 (Feb 1999)		
		7.2 (Jun 1999)		
Gediz Bridge-Istanbul Road	-	7.54±0.36 (6.81–8.03)	7.94 (Mar–Apr) 8.17 (Jul–Aug)	
DO (mg/L)	Nif Stream	2.93 (Aug 1998)	3.7±0.86 (2.43–4.79)	0.47 (Mar–Apr) 1.01 (Jul–Aug)
		3.52 (Oct 1998)		
		8.80 (Feb 1999)		
		3.75 (Jun 1999)		
Gediz Bridge-Istanbul Road	-	4.89±0.99 (3.40–6.25)	5.43 (Mar–Apr) 5.49 (Jul–Aug)	
NH <sub>3</sub> -N (mg/L)	Nif Stream	NR	2.201±0.521 (1.012–2.764)	7.48 (Mar–Apr) 5.10 (Jul–Aug)
	Gediz Bridge-Istanbul Road	-	1.401±0.536 (0.057–3.051)	0.80 (Mar–Apr) 0.31 (Jul–Aug)
NO <sub>3</sub> -N (mg/L)	Nif Stream	NR	39.118±52.451 (2.906–151.965)	0.40 (Mar–Apr) ND (Jul–Aug)
	Gediz Bridge-Istanbul Road	-	60.812±34.294 (7.844–126.744)	3.30 (Mar–Apr) ND (Jul–Aug)
Fe (mg/L)	Nif Stream	-	NR	1.43 (Mar–Apr) 0.91 (Jul–Aug)
	Gediz Bridge-Istanbul Road	-	NR	0.58 (Mar–Apr) 0.25 (Jul–Aug)
	Gediz River Basin (general)	0.0013–0.687 (Aug 1998–Jun 1999)	NR	-
Mn (mg/L)	Nif Stream	-	NR	4.20 (Mar–Apr) 2.30 (Jul–Aug)
	Gediz Bridge-Istanbul Road	-	NR	1.10 (Mar–Apr) 3.50 (Jul–Aug)
	Gediz River Basin (general)	0.03–0.17 (Aug 1998–Jun 1999)	NR	-

\*This study: Spring (Mar–Apr 2014), Summer (Jul–Aug 2014). Şentürk & Yıldız (2015): Oct 2011–Sep 2012; values expressed as mean±SD and min–max. Küçüksezgin et al. (2008): Aug 1998, Oct 1998, Feb 1999, Jun 1999. Units converted: µg/L → mg/L (1mg/L=1000 µg/L). NR: Not Reported; ND: Not Detected, -: Indicates data not available for specific stations and parameters.

In contrast, Stations 3 and 4 exhibited no acute toxicity despite varying pollution levels (Table 3), likely due to a combination of environmental conditions, dilution of test waters, and biological adaptations of *Daphnia magna*. At Station 3 (Nif Stream), high ammonia levels (NH<sub>3</sub>-N: 7.48 mg/L in spring, 5.1 mg/L in summer) and low dissolved oxygen (DO: 0.47 mg/L in spring, 1.01 mg/L in summer, Table 2) suggest potential organic pollution from agricultural and domestic sources. Conversely, Station 4 (Gediz Bridge–Istanbul Road) showed low ammonia levels (NH<sub>3</sub>-N: 0.8 mg/L in spring, 0.31

mg/L in summer) and high DO (5.43–5.49 mg/L, Table 2), indicating a less toxic environment.

Dilution of water samples (with distilled water), conducted in accordance with standard toxicity testing protocols, likely reduced pollutant concentrations below levels that could induce acute toxicity in *Daphnia magna*. Additionally, lower water temperatures in spring (19.2 °C at Station 3, 20.5 °C at Station 4, Table 2) and stable pH (7.81–8.12 at Station 3, 7.94–8.17 at Station 4, Table 2) may have decreased the proportion

of free ammonia (NH<sub>3</sub>), further mitigating toxicity (Ip et al., 2001). The optimal pre-test culture conditions (pH 7.5–8.0, DO 7.48–9.64 mg/L, 20±2 °C) and genetic homogeneity of parthenogenetic *Daphnia magna* populations likely enhanced their resilience to short-term hypoxic conditions, particularly at Station 3 (Paul et al., 1998; Garreta-Lara et al., 2018). The potential accumulation of trace metals in sediments could still affect bioavailability, warranting further investigation (Ali et al., 2019; Miranda et al., 2021).

Eutrophication, a major water quality issue in the Gediz River, promotes algal growth and oxygen depletion, contributing to the observed toxicity at Station 5 (NH<sub>3</sub>-N: 21.64 mg/L, DO: 0.64 mg/L in spring; NH<sub>3</sub>-N: 17.36 mg/L, DO: 0.24 mg/L in summer; EC<sub>50</sub>: 28.28–32.34% in spring, 18.68% in summer, Tables 2, 6 and 8). The combination of elevated ammonia, low DO, and nutrient-driven eutrophication, consistent with the high nitrogen and phosphorus inputs from domestic and industrial sources (Şentürk and Yıldız, 2015), explains the observed acute toxicity during both seasons. High NH<sub>3</sub>-N levels inhibit growth, impair physiological functions, and increase mortality in *Daphnia magna* (Serra et al., 2019; Yu et al., 2022; Ma et al., 2024). Station 1 showed acute toxicity in

spring (NH<sub>3</sub>-N: 4.47 mg/L, DO: 1.33 mg/L, EC<sub>50</sub>: 57.53–81.47%, Tables 2, 4), indicating bioavailable contaminants. Station 2 exhibited toxicity in spring (NH<sub>3</sub>-N: 2.40 mg/L, DO: 7.36 mg/L, EC<sub>50</sub>: 55.01–83.60%, Table 5) but not in summer, likely due to reduced agricultural runoff lowering NH<sub>3</sub>-N (1.05 mg/L), salinity (1.55‰), and chloride (418.5 mg/L, Table 2), which may induce osmotic stress (Gonçalves et al., 2007).

To further explore chronic effects, tests revealed hormetic effects, defined as stimulatory responses to low-level stressors, in several stations, including increased body length, early egg formation, early reproduction, and increased offspring numbers (Table 14). These responses, observed at Stations 1, 2, 3, 4, and 5, correspond to exposure to low levels of NH<sub>3</sub>-N, PO<sub>4</sub><sup>3-</sup>, Fe, and Mn (Table 2), suggesting that moderate nutrient and trace metal concentrations can stimulate growth and reproduction in *D. magna*. Compared to Parlak et al. (2010), which reported hormesis in water samples as increased brood production, this study observed a broader range of hormetic effects, including early reproduction and increased offspring numbers, particularly at Stations 1, 3, and 5 in spring and Stations 2 and 3 in summer (Table 14).

**Table 14.** Comparison of toxicological results measured with *D. magna* in Gediz River and Nif Stream (2014) with previous studies (2007–2012)

Reference	Location/Date	Acute Toxicity (EC <sub>50</sub> /LC <sub>50</sub> )	Chronic toxicity (Observed effects)
This study	Gediz River Mar–Apr 2014	Stations 1, 2, and 5 Water EC <sub>50</sub> : 28.28–83.60% ( <i>D. magna</i> , 24–48 h, immobilization)	Water (Non-toxic) Hormesis - Increased body length (Stations 1, 3, 4, 5); early reproduction (Stations 1, 3, 5); increased offspring numbers (Stations 1, 5)
	Gediz River Jul–Aug 2014	Station 5 Water EC <sub>50</sub> : 18.68% ( <i>D. magna</i> , 48 h, immobilization)	Water (Non-toxic) Hormesis - Increased body length (Stations 1, 2, 4, 5); early egg formation (Stations 1, 2, 3); early reproduction (Stations 2, 3); increased offspring numbers (Stations 2, 3)
Parlak et al. (2010)	Nif Stream Apr 2007	Stations 1, 2, 3, 4 Water LC <sub>50</sub> : 6.8–12.67 mg/L Sediment LC <sub>50</sub> : 6.83–38.04 µg/L ( <i>D. magna</i> , 48 h)	Water (Non-toxic) Hormesis - Increased brood production in all stations except Station 2 Sediment (toxic) Low survival, reduced neonate number, and total molts for all stations
Katalay et al. (2012)	Nif Stream Apr 2007	NR	Water (Non-toxic) Hormesis - Increase in algal growth in all stations except Station 1 Sediment (toxic) Inhibition of algal growth in all stations (green algae- <i>Desmodesmus subspicatus</i> )
Boyacıoğlu et al. (2008)	Nif Stream Apr 2007	NR	Water (toxic) All stations toxic; dose-dependent malformations; skeletal malformations up to 63%; blastula/gastrula arrest up to 7% Sediment (toxic) All stations toxic; dose-dependent malformations; embryonic arrest up to 98% (sea urchin- <i>Paracentrotus lividus</i> )

R: Not Reported

Nutrient enrichment from domestic wastewater, particularly in areas like Nif Stream and near the Istanbul Road–Gediz Bridge, may enhance reproduction in cladocerans (Cooman et al., 2005; Hart and Bychek, 2011). Food availability influences body size, which correlates with fecundity (Marques et al., 2012; Bednarska, 2022; Nisbet et al., 2004; Rogalski and

Ferah, 2023). Hormetic effects, particularly those caused by xenobiotics and heavy metals, involve adaptive responses and homeostasis, including intercellular communication, cell proliferation, apoptosis, signal transduction, and DNA damage and repair (Hashmi et al., 2014; Chirumbolo and Bjørklund, 2017). These hormetic responses serve as early indicators of

expected ecological changes under chemical stress, making them valuable tools in toxicity evaluations and ecological risk assessments (Hashmi et al., 2014).

This study highlights the complex interplay between physicochemical parameters, nutrient enrichment, and biological responses in the Gediz River, as evidenced by *D. magna* toxicity tests. Elevated ammonia, low dissolved oxygen, and nutrient-driven eutrophication, particularly at Station 5, explain the observed acute toxicity, while hormetic effects at lower pollutant levels suggest stimulatory responses in *D. magna* (Table 14).

The significant decline in nitrate levels at Stations 3 and 4 during summer, driven by reduced agricultural runoff and microbial denitrification, underscores the seasonal dynamics of pollution (Şentürk and Yıldız, 2015). Compared to previous studies reporting sediment toxicity (Parlak et al., 2010; Katalay et al., 2012), this study emphasizes hormetic effects in the water column, potentially influenced by sediment-water interactions, suggesting a shift in pollutant dynamics. However, the potential role of sediment-water interactions in pollutant bioavailability requires further investigation with dedicated sediment analyses. These findings contribute to understanding the ecological impacts of pollution in the Gediz River, emphasizing the need for continued monitoring and mitigation of nutrient inputs to address eutrophication and protect aquatic ecosystems.

## CONCLUSION

This study assessed pollution in the Gediz River using acute and chronic toxicity tests with *Daphnia magna*. Toxic effects were observed at Stations 1, 2, and 5, with seasonal variations, while Stations 3 and 4 showed no significant toxicity, likely due to pollutant retention in sediments and favourable physicochemical conditions. Hormetic effects, including increased growth, development, and reproduction, were also detected, emphasizing the role of sediment-water interactions in shaping bioavailability and ecological responses. These findings demonstrate that relying solely on water-column toxicity tests may underestimate ecological risk and highlight the importance of integrating sediment analyses, multi-trophic

## REFERENCES

- Ali, H., Khan, E., & Ilahi, I. (2019). Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *Journal of Chemistry*, 1, 6730305. <https://doi.org/10.1155/2019/6730305>
- Aydın, Ş., & Küçüksezgin, F. (2012). Distribution and chemical speciation of heavy metals in the surficial sediments of the Bakircay and Gediz Rivers, Eastern Aegean. *Environmental Earth Sciences*, 65(3), 789–803. <https://doi.org/10.1007/s12665-011-1124-7>
- Barata, C., Alañon, P., Gutiérrez-Alonso, S., Riva, M. C., Fernández, C., & Tarazona, J. V. (2008). A *Daphnia magna* feeding bioassay as a cost effective and ecologically relevant sublethal toxicity test for environmental risk assessment of toxic effluents. *Science of the Total Environment*, 405(1–3), 78–86. <https://doi.org/10.1016/j.scitotenv.2008.06.028>
- Bednarska, A. (2022). Food quantity and quality shapes reproductive

level assessments, and monthly sampling to capture spatial and seasonal pollution dynamics. Overall, the study provides valuable insights into the river's aquatic ecosystem status, supports awareness among authorities, and can inform sustainable water resource management.

## ACKNOWLEDGEMENT AND FUNDING

The authors would like to express their gratitude to Prof. Dr. Meltem Conk Dalay and Dr. Zeliha Demirel for providing the initial algal culture and to Assoc. Prof. Dr. Orkide Minareci for accompanying them during the first fieldwork and sharing her valuable experience.

This study was supported by the Scientific Research Projects Commission of Ege University (Project No: 2013Fen17) and TÜBİTAK-BİDEB (2210-C Domestic Master's Scholarship Program for Priority Areas).

## AUTHOR CONTRIBUTIONS

This study was derived from the M.Sc. thesis of the first author and conducted under the supervision of the second author. All authors contributed to the theoretical design and planning of the study. Data collection and analysis were performed by Tuna Karaytuğ. The first draft of the manuscript was written by Tuna Karaytuğ. All authors read and approved the final manuscript.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ETHICAL APPROVAL

Ethical approval is not required for this study.

## DECLARATION OF AI USE

We have not used AI-assisted technologies in creating this article. All content was originally produced by the authors.

## DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

strategies of *Daphnia*. *Ecology and Evolution*, 12(8), e9163. <https://doi.org/10.1002/ece3.9163>

Bischoff, H. W., & Bold, H. C. (1963). *Some soil algae from enchanted rock and related algal species* (Phycological Studies No. 6318). University of Texas Publications.

Boyacıoğlu, M., Parlak, H., Oral, R., & Çakal Arslan, Ö. (2008). Mutagenicity of sediment and water samples from Nif Brook (Western Turkey). *Fresenius Environmental Bulletin*, 17 (1), 9–15.

Chirumbolo, S., & Bjørklund, G. (2017). PERM hypothesis: the fundamental machinery able to elucidate the role of xenobiotics and hormesis in cell survival and homeostasis. *International Journal of Molecular Sciences*, 18(1), 165. <https://doi.org/10.3390/ijms18010165>

Cooman, K., Debels, M., Gajardo, M., Urrutia, R., & Barra, R. (2005). Use of *Daphnia* spp. for the ecotoxicological assessment of water quality in an

- agricultural watershed in south-central Chile. *Archives of Environmental Contamination and Toxicology*, 48(2), 191-200. <https://doi.org/10.1007/s00244-004-0218-6>
- Ertaş, A., Yaşartürk, M., Boz, T., & Kızılkaya, İ. T. (2021). Evaluation of the water quality of Karabal Stream (Gediz River, Turkey) and comparative performance of the used indices. *Acta Aqualica Turcica*, 17(3), 334–349. <https://doi.org/10.22392/actaqua.819579>
- Feiler, U., Krebs, F., & Heininger, P. (2006). Aquatic plant assay used in the assessment of water quality in German rivers. *Hydrobiologia*, 570(1), 67–71. <https://doi.org/10.1007/s10750-006-0163-7>
- Garreta-Lara, E., Campos, B., Barata, C., Lacorte, S., & Tauler, R. (2018). Combined effects of salinity, temperature, and hypoxia on *Daphnia magna* metabolism. *Science of the Total Environment*, 610, 602-612. <https://doi.org/10.1016/j.scitotenv.2017.05.190>
- Gonçalves, A. M. M., Castro, B. B., Pardal, M. A., & Gonçalves, F. (2007). Salinity effects on survival and life history of two freshwater cladocerans (*Daphnia magna* and *Daphnia longispina*). *Annales de Limnologie – International Journal of Limnology*, 43(1), 13-20. <https://doi.org/10.1051/limn/2007022>
- Gündoğdu, V., & Kocataş, A. (2006). An approach towards the formation of Gediz River basin management plan. *Ege University Journal of Fisheries and Aquatic Sciences*, 23(3-4), 371–378.
- Hafizoğlu, E., & Tekin, F. (2004). Investigation of heavy metal pollution in the Gediz River (Manisa). *Soma Vocational School Journal of Technical Sciences*, 2(2), 41–51.
- Hart, R. C., & Bychek, E. A. (2011). Body size in freshwater planktonic crustaceans: an overview of extrinsic determinants and modifying influences of biotic interactions. *Hydrobiologia*, 668(1), 61–108. <https://doi.org/10.1007/s10750-010-0400-y>
- Hashmi, M. Z., Shen, H., Zhu, S., Yu, C., & Shen, C. (2014). Growth, bioluminescence and shoal behavior hormetic responses to inorganic and/or organic chemicals: a review. *Environment International*, 64, 28–39. <https://doi.org/10.1016/j.envint.2013.11.018>
- Hassan, K. T., Ferdoushi, Z., Rana, M. M., & Alam, M. S. (2024). Assessing the seasonal variability of water quality and heavy metals concentration in sediment, water, and fish muscles of Korotoa River in Bangladesh. *Aquaculture Research*, 2024(1), 5343363. <https://doi.org/10.1155/2024/5343363>
- Hertzberg, R. C., & MacDonell, M. M. (2002). Synergy and other ineffective mixture risk definitions. *Science of the Total Environment*, 288(1-2), 31–42. [https://doi.org/10.1016/S0048-9697\(01\)01113-5](https://doi.org/10.1016/S0048-9697(01)01113-5)
- Hussain, H., Mahmood, S., Khalid, A., Shahzad, K., & Anjum, M. Z. (2023). Seasonal variation in non-point source heavy metal pollution in Satpara Lake and its toxicity in trout fish. *Environmental Monitoring and Assessment*, 195(7), 901. <https://doi.org/10.1007/s10661-023-11498-x>
- Ip, Y. K., Chew, S. F., & Randall, D. J. (2001). Ammonia toxicity, tolerance, and excretion. In P. A. Wright & P. M. Anderson (Eds.), *Fish physiology: Nitrogen excretion* (Vol. 20, pp. 109–148). Academic Press. [https://doi.org/10.1016/S1546-5098\(01\)20005-3](https://doi.org/10.1016/S1546-5098(01)20005-3)
- Katalay, S., Boyacıoğlu, M., Arslan, Ö. Ç., Parlak, H., & Karaaslan, M. A. (2012). Phytotoxicity of water and sediment from Nif Brook (Izmir, Turkey) on green algae *Desmodesmus (=Scenedesmus) subspicatus*. *E*, 21(83), 25–31. <https://doi.org/10.5053/ekoloji.2012.833>
- Kaza, M., Mankiewicz-Boczek, J., Jyzdorzcyk, K., & Sawicki, J. (2007). Toxicity assessment of water samples from rivers in Central Poland using a battery of microbiotests —a pilot study. *Polish Journal of Environmental Studies*, 16(1), 81–89.
- Kirsanov, D., Legin, E., Zagrebina, A., Ignatieva, N., Rybakina, V., & Legin, A. (2014). Mimicking *Daphnia magna* bioassay performance by an electronic tongue for urban water quality control. *Analytica Chimica Acta*, 824, 64–70. <https://doi.org/10.1016/j.aca.2014.03.021>
- Koivisto, S. (1995). Is *Daphnia magna* an ecologically representative zooplankton species in toxicity tests? *Environmental Pollution*, 90(2), 263–267. [https://doi.org/10.1016/0269-7491\(95\)00029-Q](https://doi.org/10.1016/0269-7491(95)00029-Q)
- Küçüksezgin, F., Uluturhan, E., & Batki, H. (2008). Distribution of heavy metals in water, particulate matter, and sediments of the Gediz River (Eastern Aegean). *Environmental Monitoring and Assessment*, 141, 213–225. <https://doi.org/10.1007/s10661-007-9889-6>
- Lyu, K., Cao, H., Chen, R., Wang, Q., & Yang, Z. (2013). Combined effects of hypoxia and ammonia to *Daphnia similis* estimated with life-history traits. *Environmental Science and Pollution Research*, 20, 5379–5387. <https://doi.org/10.1007/s11356-013-1555-7>
- Ma, Y., Liu, Y., Sun, J., Min, P., Liu, W., Li, L., Yi, P., Guo, R., & Chen, J. (2024). Ecological risks of high-ammonia environment with inhibited growth of *Daphnia magna*: disturbed energy metabolism and oxidative stress. *Science of the Total Environment*, 948, 174959. <https://doi.org/10.1016/j.scitotenv.2024.174959>
- Marques, C. R., Gonçalves, A. M. M., Pereira, R., & Gonçalves, F. (2012). Ecotoxicological effects of Mikado and Viper on algae and daphnids. *Environmental Toxicology*, 27(12), 685-699. <https://doi.org/10.1002/tox.20687>
- Miner, B. E., De Meester, L., Pfrender, M. E., Lampert, W., & Hairston Jr, N. G. (2012). Linking genes to communities and ecosystems: *Daphnia* as an ecogenomic model. *Proceedings of the Royal Society*, 279(1735), 1873–82. <https://doi.org/10.1098/rspb.2011.2404>
- Ministry of Environment and Urbanization. (2014). *Gediz Basin water quality monitoring report: spring period*. Ministry of Environment and Urbanization. <https://webdosya.csb.gov.tr/db/ced/editoridosya/2014%20F inal%20Rapor2.pdf>
- Miranda, L. S., Wijesiri, B., Ayoko, G. A., Egodawatta, P., & Goonetilleke, A. (2021). Water-sediment interactions and mobility of heavy metals in aquatic environments. *Water Research*, 202, 117386. <https://doi.org/10.1016/j.watres.2021.117386>
- Nisbet, R. M., McCauley, E., Gurney, W. S., Murdoch, W. W., & Wood, S. N. (2004). Formulating and testing a partially specified dynamic energy budget model. *Ecology*, 85, 3132–3139. <https://doi.org/10.1890/03-0429>
- OECD. (1998). *Daphnia magna* reproduction test (Test No. 211). OECD Publishing. [https://www.oecd.org/content/dam/oecd/en/publications/reports/2012/10/test-no-211-daphnia-magna-reproduction-test\\_g1g24069/9789264185203-en.pdf](https://www.oecd.org/content/dam/oecd/en/publications/reports/2012/10/test-no-211-daphnia-magna-reproduction-test_g1g24069/9789264185203-en.pdf)
- OECD. (2004). *Daphnia* sp. acute immobilisation test (Test No. 202). OECD Publishing. [https://www.oecd.org/content/dam/oecd/en/publications/reports/2004/11/test-no-202-daphnia-sp-acute-immobilisation-test\\_g1gh28f3/9789264069947-en.pdf](https://www.oecd.org/content/dam/oecd/en/publications/reports/2004/11/test-no-202-daphnia-sp-acute-immobilisation-test_g1gh28f3/9789264069947-en.pdf)
- Öner, Ö., & Çelik, A. (2011). Investigation of some pollution parameters in water and sediment samples from the Lower Gediz Basin of the Gediz River. *Ekoloji*, 20(78), 48–52. <https://doi.org/10.5053/ekoloji.2011.788>
- Parlak, H., Arslan, Ö. Ç., Boyacıoğlu, M., & Karaaslan, M. A. (2010). Acute and chronic toxicity of contaminated fresh water and sediment of Nif Brook on *Daphnia magna* (Straus, 1820). *Ege Journal of Fisheries and Aquatic Sciences*, 27(4), 135–141.
- Paul, R. J., Colmorgen, M., Pirov, R., Chen, Y. H., & Tsai, M. C. (1998). Systemic and metabolic responses in *Daphnia magna* to anoxia. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 120(3), 519-530. [https://doi.org/10.1016/S1095-6433\(98\)10062-4](https://doi.org/10.1016/S1095-6433(98)10062-4)
- Rogalski, M. A., & Ferah, U. (2023). Lake water chemistry and population of origin interact to shape fecundity and growth in *Daphnia ambigua*. *Ecology and Evolution*. 13(6), e10176. <https://doi.org/10.1002/ece3.10176>
- Serpa, D., Keizer, J. J., Cassidy, J., Cuco, A., Silva, V., Gonçalves, F., Cerqueira, M., & Abrantes, N. (2014). Assessment of river water quality using an integrated physicochemical, biological, and ecotoxicological approach. *Environmental Science: Processes & Impacts*, 16,1434–1444. <https://doi.org/10.1039/c3em00488k>
- Serra, T., Soler, M., Pous, N., & Colomer, J. (2019). *Daphnia magna* filtration, swimming and mortality under ammonium, nitrite, nitrate and phosphate. *Science of the Total Environment*, 656, 331-337. <https://doi.org/10.1016/j.scitotenv.2018.11.382>
- Şentürk, T., & Yıldız, Ş. (2015). Determination of some physicochemical parameters and inorganic nutrient content of Gediz River (Manisa). *Turkish Journal of Biochemistry*, 40(3), 210-216. <https://doi.org/10.1515/tjb-2015-0003>

Wojtal-Frankiewicz, A. (2012). The effects of global warming on *Daphnia* spp. population dynamics: a review. *Aquatic Ecology*, 46, 37-53. <https://doi.org/10.1007/s10452-011-9380-x>

Yu, B., Lyu, K., Li, J., Yang, Z., & Sun, Y. (2022). Combined toxic effects of nitrite and ammonia on life history traits of *Daphnia pulex*. *Frontiers in Environmental Science*, 10, 1019483. <https://doi.org/10.3389/fenvs.2022.1019483>