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Determination of the effects of environmental pollution on the Balkan terrapin, *Mauremys rivulata* (Valenciennes, 1833)

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NURCİHAN HACIOĞLU DOĞRU, ÇİĞDEM GÜL, NURŞEN ÇÖRDÜK* and MURAT TOSUNOĞLU

Department of Biology, Faculty of Sciences, Çanakkale Onsekiz Mart University, Terzioğlu Campus, 17100, Çanakkale, Turkey

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RESEARCH ARTICLE

ABSTRACT

The effects of environmental pollution on three populations of the Balkan terrapin [*Mauremys rivulata* (Valenciennes, 1833)] from the Bozcaada, Gökçeada and Dardanos regions were evaluated. The morphological parameters of Balkan terrapins collected on each site were measured and blood samples were taken for haematological analysis and micronucleus detection. The physicochemical, microbiological and microelement analyses of the water samples from each region were conducted by standard methods. The highest red blood cell, white blood cell and mean corpuscular haemoglobin concentration values were seen in the samples from Gökçeada. The highest haemoglobin value was found in the samples originating from Bozcaada, whereas the highest haematocrit and mean corpuscular volume values were found in the animals from Dardanos. Based on the microbiological analysis of the water samples, the most polluted site was Gökçeada. The microelement contents of the water and blood samples were different at the three sites, the lowest being in the Gökçeada area. It was revealed that the percentage of red blood cell micronuclei and other nucleus abnormalities in the *M. rivulata* blood samples was the lowest also in the animals living in the region of Gökçeada.

KEYWORDS

Balkan terrapin, haematology, microbial pollution, micronuclei, water quality

INTRODUCTION

Natural processes, such as precipitation, erosion and weathering, as well as anthropogenic activities, such as agriculture, industry and urbanisation, affect water quality and determine its use for different purposes (Bhat et al., 2014). Evaluation of freshwater quality is a complex process because many parameters affect it and, thus, need to be considered (Radu et al., 2014). These microbial and physicochemical parameters can be influenced by various external and internal factors (Hacıoğlu Doğru et al., 2019).

Environmental pollutants can damage the genetic material of living organisms. As a result of unrepaired damage, a series of biological consequences occur at cellular, organ, animal and finally at community and population levels (Lee and Steinert, 2003). For this reason, it is of utmost importance to assess, monitor and investigate the effects of pollutants on the organisms exposed to them in the given ecosystem. Turtles differ from other animal species in their long life span (Gibbons, 1987) and long generation times (Hailey, 1990) that ensure the sustainability of the population. Nonetheless, as long as factors such as fire (Hailey, 2000), loss of habitat (Hailey, 2000) or uncontrolled hunting and trade (Brooks et al., 1991) continue to occur repeatedly, these animals will experience problems until their extinction. The longevity of turtles makes them a good indicator for monitoring heavy metal and other anthropogenic contamination in aquatic ecosystems (Yu et al., 2011; Adel et al., 2017;

*Corresponding author.
E-mail: nursencorduk@gmail.com

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de Oliveira et al., 2020). There are studies examining the pollution rate of freshwater turtles living in waters affected by various human activities, and such studies are very important for the protection of these animals (Yu et al., 2011; Latorre et al., 2015).

Pollution of freshwaters with faecal bacteria and heavy metals from industrial processes might have significant effects on aquatic organisms (turtles, etc.) that are important for the health of the aquatic habitat and community. Monitoring of bacteria, such as faecal coliforms and faecal streptococci, which are not primary infectious agents, in water sources gives an idea about the groups of pathogenic microorganisms (*E. coli* O157:H7, *Cryptosporidium* spp., etc.) (Derose et al., 2020). It is known that there is a significant correlation between faecal pollution in waters and the presence of pathogens, which are an important threat to human and animal health. Heavy metals accumulated in nature as a result of different human activities also pollute water resources (Bahnasawy et al., 2009). Studies on heavy metals such as cadmium, mercury and lead in water resources and wildlife are important for monitoring the toxicity and human health effects of these metals (Kalantzi et al., 2013). Determination of the health status of turtles is important for their conservation and management, and is an important point of discussion for the use of turtles in the biomonitoring of local freshwater ecosystems (Chaffin et al., 2008).

Haematological studies are used to diagnose diseases of turtles and to determine their physiological and health status (Christopher et al., 2003; Joyner et al., 2006; Zhang et al., 2011). The haematological parameters of turtles are affected by factors such as the season, the geographical regions they live in, their age, health status, breeding, and sex (Dickinson et al., 2002; Jacobson, 2007). There are many studies that determine whether freshwater turtles are affected by the characteristics of the wetland they live in, through the examination of their haematological parameters (Tosunoğlu et al., 2011; Latorre et al., 2015; Çördük et al., 2019). It has been reported previously that some haematological parameters in freshwater turtles varied according to the quality and metal contents of the water (Tosunoğlu et al., 2011; Yu et al., 2011).

Environmental pollutants may cause damage to the genetic material of living organisms. Micronucleus frequency in animals exposed to contaminants has been used in many wildlife ecotoxicological studies for monitoring damage to the genetic material (Swartz et al., 2003; Matson et al., 2005). There have been many studies in which the DNA damage was measured to determine the effect of pollution on reptiles (Matson et al., 2005; Josende et al., 2015; Zapata et al., 2016; Çördük et al., 2019; de Oliveira et al., 2020).

The aim of this study was to determine the effect of different environmental pollutants on populations of the Balkan terrapin at three sites (Bozcaada, Gökçeada, Dardanos) by comparing the water quality, the haematological status of the animals and the occurrence of signs indicative of genotoxic effects.

MATERIALS AND METHODS

Study sites and sample collections

The study area included three sites as presented in Fig. 1. Bozcaada has an intense tourism potential in the summer, and one third of the island consists of agricultural areas. There is no natural water source, and small streams are formed in the spring. The Azmak stream located at Çayır (35S417612; 4410562) is a stagnant water body at sea level (Gül et al., 2014). In some valleys of Gökçeada, there are many freshwater streams, and these water resources are rich in animal and plant species. Human impact on nature is great, and this is expected to cause a loss of habitat (Perçin Paçal et al., 2017). The Büyükdere creek in Kaleköy (35T0405784; 4453706), where the specimens were caught, is at sea level and has summer houses and agricultural areas around it. Dardanos is located in the city centre of Çanakkale where agricultural production is carried out and which has a wide tourism capacity with its rich ecosystem. The site (35T445711; 4438009) where the specimens were caught is at sea level and is located within the Kepez Delta.

The field studies were carried out between April and July 2019, and 5 animal samples were collected from Bozcaada, 9 from Dardanos and 10 from Gökçeada with the help of a net. The sample collection was performed according to the rules, and with the permission (2018/09-06) of, the Animal Experiments Ethics Committee at Çanakkale Onsekiz Mart University. All the sampled animals were returned to the area where they were collected. Sampling for the analysis of water parameters was carried out once at the three sites. Within the scope of morphological analysis, sex was determined, and body measurements (carapace length, carapace width, plastron length, plastron width) and body weights (with scales of 0.001 g precision) were taken. The samples having a similar size were used to standardise other analyses.

Determination of physicochemical and microbiological parameters of water samples

The samples for water quality parameters were taken from the study area where the turtle samples were collected. Water temperature (T), pH, electrical conductivity (EC) and dissolved oxygen (DO) were estimated at the spot with the ecological kit (Hach–Lange, USA). Aluminium (Al), barium (Ba), cadmium (Cd), calcium (Ca), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), manganese (Mn), nickel (Ni), iron (Fe), sodium (Na), phosphorus (P), potassium (K), magnesium (M) and zinc (Zn) contents were analysed in each water sample using a PerkinElmer Optime 8000 ICP-OES instrument. Limits of detection (LOD) were 0.0025 ppm for Cd, Zn, Cu, Cr, Co, Al, Ni, Mn and Ba, 0.005 ppm for Pb and 0.025 ppm for Fe, Na, P, Mg, Ca and K. The eventual presence of residues of nine pesticides, including alpha benzene hexachloride (BHC), beta BHC, gamma BHC, delta BHC, heptachlor, malathion, parathion, endosulfan and carbophenothion in the water samples was tested by gas chromatography.

Fig. 1. Map showing the three study sites (Bozcaada, Gökçeada and Dardanos) from where the samples were collected

The microbiological parameters of water quality [total coliforms (TC), faecal coliforms (FC) and faecal enterococcus (FE)] were determined in the laboratory. TC, FC and enterococcus were determined by the standard Most Probable Number (MPN) method (Finstein, 1972). The MPN technique involves making replicate dilutions of samples in an appropriate liquid growth medium and incubating for growth.

Haematological parameters

For haematological analysis, 2 mL of blood was taken from the dorsal caudal vein of each animal with the aid of a 5-needle syringe with a diameter of 21 pins (Thrall et al., 2012). Within the scope of haematological analyses, red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin (Hb) and haematocrit (Hct) value, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined according to Gül et al. (2015).

Microelement and pesticide analysis in blood samples

The concentrations of the above-listed microelements (Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, M, Mn, Na, Ni, P, Pb and Zn) in the blood samples of three individuals from each site were also analysed by a PerkinElmer Optime 8000 ICP-OES instrument. The LOD values were identical with those in the water analyses. Also, detection of the residues of the nine pesticides in the blood samples was performed by gas chromatography.

Measurement of micronuclei and other nuclear abnormalities

Peripheral blood smears from each specimen were prepared, fixed with methanol for 15 min and dyed with Giemsa stain. Micronuclei and other nuclear abnormalities such as blebbed, kidney-shaped, lobed and notched nuclei were identified according to the protocol reported previously (Çördük et al., 2019).

Statistical analyses

In order to perform parametric tests, data should show normal distribution and be homogeneous. In the haematology and morphology analyses, Mann–Whitney *U* test applied to descriptive statistics and non-parametric and non-normally distributed data was performed using the SPSS 20.0 program (IBM). The data of micronucleus and other nuclear abnormalities, expressed as percentages, and the levels of significance in populations in the three sites were analysed. Tukey's comparison test was performed by the use of one-way analysis of variance (ANOVA) on SPSS 20.0 version for Windows software. In all cases, $P \leq 0.05$ values were considered to be statistically significant.

RESULTS

Water quality assessment

The physicochemical and microbiological quality parameters of water from the three sampling sites and their quality classes according to the Turkish Legislation Official Gazette [Water Pollution Control Regulation (WPCR)] (Anonymous, 2004) are presented in Table 1. In Turkey, the inland waters are divided into four groups in terms of water quality: high-quality waters (Class I), moderate-quality waters (Class II), polluted waters (Class III) and highly polluted waters (Class IV). It is seen that, in terms of the physico-chemical parameters, the waters of the three sites belonged to Class I–II, (pH, T, EC and DO), and that while Bozcaada and Gökçeada were Class II in terms of temperature, Dardanos was Class III. While all three regions are Class I in terms of electrical conductivity, Bozcaada and Dardanos are Class I while Gökçeada is Class IV in terms of dissolved oxygen (Table 1). The concentrations of the microelements were within the acceptable limits at all sites except for aluminium, sodium and phosphorus (Table 1). Sodium (except in Gökçeada) and phosphorus had a higher concentration at all sites. As a result of pesticide analysis, no pesticide residue was found in any of the water samples. In the microbiological analyses, regarding the mean values obtained for TC, FC and TE, respectively, the Gökçeada samples showed the highest values compared to the other two sites. This situation shows that Gökçeada is the most polluted region with the highest anthropogenic pressure in terms of the above three values. Also, there are no data about faecal streptococci (FS) in the WPCR. However, FC/TE ratios are used to determine the source of contamination. An FC/TE > 4 is indicative of faecal contamination of human origin, while an FC/TE < 0.7 suggests faecal contamination of animal origin (Djuikom et al., 2008). The FC/FS ratios in this study indicated pollution of both human and animal origin in Gökçeada and pollution of animal origin in Bozcaada and Dardanos. These findings show that the three sites were exposed to human-induced faecal contamination. In this sense, our findings are similar to those reported previously (Hacioglu and Dulger, 2010).

Blood sample analyses

Comparing the Dardanos and Bozcaada samples in terms of haematological parameters, a significant difference was detected in haemoglobin and MCHC values ($P \leq 0.05$), and it was determined that these parameters were lower in the Dardanos samples. Besides, a significant difference was found between the Dardanos and Gökçeada samples in terms of RBC count, WBC count, haemoglobin, haematocrit as well as MCV and MCHC values ($P \leq 0.05$). It was found that the RBC count, WBC count, haemoglobin and MCHC values were lower in the Dardanos samples and that MCV and haematocrit values were lower in the Gökçeada samples (Tables 2 and 3).

Table 1. Quality parameters of the water samples from the three study areas

Parameter	Sites		
	Bozcaada	Gökçeada	Dardanos
pH	8.11 (I–II)	7.54 (I–II)	8.02 (I–II)
T (°C)	25.5 (II)	25.7 (II)	**29.8 (III)**
EC (μS/cm)	3.37 (I)	987 (I)	3.01 (I)
DO (mg/L)	7.43 (I)	**0.13 (IV)**	7.82 (I)
Heavy metals	Bozcaada	Gökçeada	Dardanos
Pb	–	–	–
Cd	–	–	–
Zn	–	–	–
Cu	0.0037 (I)	0.0036 (I)	0.0029 (I)
Cr	–	–	–
Co	–	–	–
Al	**0.83 (II–III)**	0.23 (I)	0.11 (I)
Ni	–	–	–
Mn	0.22 (I)	0.62 (I)	0.15 (I)
Ba	0.05 (I)	0.09 (I)	0.10 (I)
Fe	0.21 (I)	0.68 (I)	0.12 (I)
Na	**861.5 (IV)**	57.1 (I)	**580.5 (IV)**
P	**1.8 (IV)**	**1.1 (IV)**	**0.3 (III)**
Mg	116.4 (I–II)	33.5 (I)	84 (I)
Ca	209.0 (I)	75.7 (I)	64.5 (I)
K	34.4 (II)	3.6 (I)	40.4 (II)
TC (MPN/100 mL)	4×10^2 (I–II)	2×103 (I–II)	0 (I)
FC (MPN/100 mL)	9×10^1 (I–II)	**9×10^2 (II–III)**	0 (I)
TE (MPN/100 mL)*	0	**9×10^2**	3×10^2

*not given in Turkish legislation criteria; '-' means not detected. Roman numbers in brackets (I to IV) show the class assignment according to the Turkish legislation. T: Temperature, EC: Electrical conductivity, DO: Dissolved oxygen; TC: Total coliforms, FC: Faecal coliforms, TE: Total enterococcus; MPN: Most Probable Number method

Table 2. Results of the haematological analyses of populations at the different sites

	N	Minimum	Maximum	Mean	SE	SD
BOZCAADA						
RBC (mm^3)	5	360,000	660,000	500,000	67,823.2998	151,657.5088
WBC (mm^3)	5	1,400	6,000	3,380	969.74223	2,168.40956
Hb (g dL^{-1})	5	5.80	9.80	7.4200	0.65605	1.46697
Hct (%)	5	17	34	24	2.81069	6.28490
MCV (μ^3)	5	257.58	739.13	519.5433	87.58622	195.84874
MCH (pg)	5	104.55	213.04	158.1643	22.09704	49.41048
MCHC (%)	5	26.36	40.59	31.6205	2.46156	5.50421
GÖKÇEADA						
RBC (mm^3)	10	520,000	880,000	617,000	36,637.8674	115,859.1098
WBC (mm^3)	10	3,600	8,000	5,620	458.45150	1,449.75094
Hb (g dL^{-1})	10	6	8.60	6.9000	0.21344	0.67495
Hct (%)	10	12	24	19	1.33333	4.21637
MCV (μ^3)	10	197.37	423.08	316.5436	27.71402	87.63944
MCH (pg)	10	77.27	134.38	114.5466	5.98730	18.93351
MCHC (%)	10	27.83	58.33	37.9153	2.82997	8.94914
DARDANOS						
RBC (mm^3)	9	220,000	640,000	400,000	39,299.4204	117,898.2612
WBC (mm^3)	9	2,000	5,000	3,170	273.78012	821.34037
Hb (g dL^{-1})	9	3.50	7.00	5.0111	0.40976	1.22927
Hct (%)	9	17	38	26.5556	2.28589	6.85768
MCV (μ^3)	9	391.30	1,272.73	718.6917	95.10914	285.32741
MCH (pg)	9	76.09	300	136.9974	21.94767	65.84301
MCHC (%)	9	12.57	26.92	19.4118	1.47701	4.43104

N: sample number, SD: standard deviation, SE: standard error, RBC: red blood cell count, WBC: white blood cell count, Hb: haemoglobin, Hct: haematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration

Table 3. Statistical comparison of the haematology results at the three sites

	Mann–Whitney U	Wilcoxon W	Z	P*
DARDANOS-BOZCAADA				
Hb (g dL^{-1})	3.500	48.500	–2.536	0.011
MCHC (%)	1.000	46.000	–2.867	0.004
DARDANOS –GÖKÇEADA				
RBC (mm^3)	7.500	52.500	–3.067	0.002
WBC (mm^3)	5.500	50.500	–3.231	0.001
Hb (g dL^{-1})	10.000	55.000	–2.877	0.004
Hct (%)	13.500	68.500	–2.583	0.010
MCV (μ^3)	4.000	59.000	–3.348	0.001
MCHC (%)	0.000	45.000	–3.676	0.000

* Only parameters that differ statistically significantly ($P \leq 0.05$) are provided

Table 4. Microelement content (mg L^{-1}) of the blood samples collected from the three sites

	Bozcaada	Gökçeada	Dardanos
Lead (Pb)	–	–	–
Cadmium (Cd)	–	–	–
Zinc (Zn)	1.26	1.55	1.75
Copper (Cu)	0.33	0.77	0.39
Chromium (Cr)	–	–	–
Cobalt (Co)	–	–	–
Aluminium (Al)	–	–	–
Nickel (Ni)	–	–	–
Manganese (Mn)	–	–	–
Barium (Ba)	–	–	0.22
Iron (Fe)	10.54	3.78	1.80
Sodium (Na)	3,685.53	3,594.6	3,682.06
Phosphorus (P)	130.43	64.75	117
Magnesium (Mg)	18.16	16.53	20.86
Calcium (Ca)	99.36	78.2	135.26
Potassium (K)	191.56	177.2	187.33

* '–' means not detected

No pesticides were were detected in any of the blood samples. The results of the microelement analyses of the blood samples are presented in Table 4. The heavy metals lead, cadmium, chromium, cobalt and nickel were not detected in any blood samples taken from the three sites. Zinc was detected in the blood samples of the animals at all the three sites, although it was not detected in the water samples. Aluminium and manganese were detected in the water samples, but not in any of the blood samples. Barium was found only in the blood samples from Dardanos. According to the microelement analysis, it was determined that the blood samples taken from Gökçeada had the lowest microelement content. Only the copper content was found to be high in the Gökçeada samples. Zinc, magnesium and calcium were found to be high in the blood samples of the animals collected from Dardanos. In the blood samples of animals collected from Bozcaada, the concentrations of iron, sodium, phosphorus and potassium were found to be higher than in the other two regions.

Analysis of micronuclei and other nuclear abnormalities

A total of 8,000 red blood cells were counted in each sample. In addition to micronucleus in erythrocytes, other nuclear abnormalities were also investigated. The typical abnormalities observed in the preparations are shown in Fig. 2. The abnormality percentages of the animals at the three different sites are compared in Table 5. Micronuclei and other nuclear abnormalities, such as blebbed nuclei and notched nuclei, were observed in the samples of each site. The frequency of micronuclei was found to be low at all sites. The frequency of blebbed nucleus was higher than that of the other nuclear abnormalities at all sites. When the abnormality percentages are compared, it is seen that the micronucleus and other nuclear abnormality percentage is the highest in the erythrocytes of Balkan terrapin samples obtained in Dardanos. The second highest percentage of abnormality was seen in the Bozcaada samples. However, the percentage of abnormalities in the Balkan terrapin samples at these two different sites was not significantly different at ≤ 0.05. It was determined that the percentage of micronuclei and other nuclear abnormalities was the lowest in the samples taken in Gökçeada.

DISCUSSION

The life of all aquatic organisms depends on their water habitat. Water quality indices, such as T, pH, EC, DO and heavy metal concentration, etc., are the most basic criteria in terms of revealing the ecological and anthropogenic characteristics of a water body (Zafar et al., 2018). Unlike other

Table 5. Frequencies (%) of micronuclei and other nuclear abnormalities with standard deviations in the red blood cells of Balkan terrapins (*Mauremys rivulata*), collected from the Bozcaada, Gökçeada and Dardanos regions

	Bozcaada	Gökçeada	Dardanos
Micronuclei	0.38 ± 1.00	0.06 ± 0.11	0.93 ± 1.70
Lobed nuclei	0.02 ± 0.02	0 ± 0.00	0.01 ± 0.64
Notched nuclei	1.24 ± 0.37	0.36 ± 0.19	0.83 ± 0.02
Blebbed nuclei	12.38 ± 3.96	6.96 ± 1.16	13.67 ± 3.19
Binucleated cell	0 ± 0.00	0 ± 0.00	0.03 ± 0.07
Kidney-shaped nuclei	0 ± 0.00	0 ± 0.00	0.03 ± 0.07
Total nuclear abnormalities (%)	**14.02 ± 3.62[b]**	**7.38 ± 1.11[a]**	**15.50 ± 3.69[b]**

Mean values marked with an identical superscript are not statistically significantly different at $P \leq 0.05$

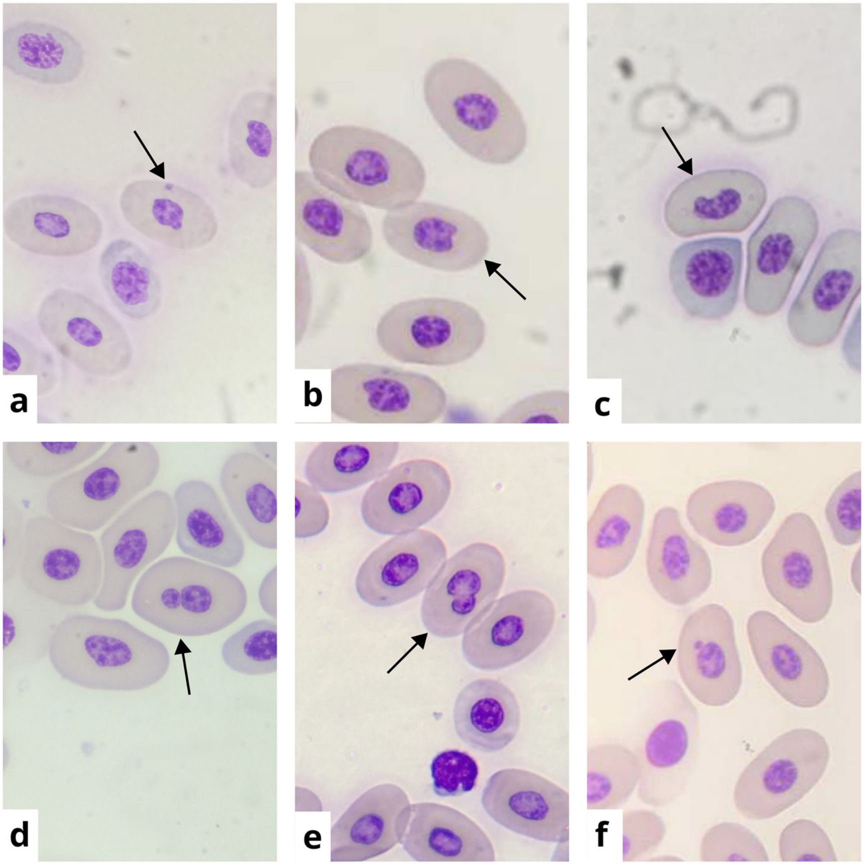

Fig. 2. Peripheral blood smears dyed with Giemsa stain to detect micronuclei and nuclear abnormalities in the erythrocytes of Balkan terrapin (*Mauremys rivulata*) specimens. Micronucleus (a), notched nucleus (b), kidney-shaped nucleus (c), binucleated cell (d), lobed nucleus (e), blebbed nucleus (f)

studies (Hacıoğlu and Dulger, 2009; Gül et al., 2014; Hacıoğlu, 2014; Hacıoğlu Doğru et al., 2019), in this study the physicochemical parameters were found to be within the acceptable limits. In aquatic systems, it is very important to determine the types and density of metals to which humans and animals are exposed, because some metals, such as lead and cadmium, are toxic to living organisms even at quite low concentrations, whereas others (Na, Al, and P) are biologically essential and natural constituents of aquatic ecosystems, and generally become toxic only at very high concentrations (Bahnasawy et al., 2009). In our study, especially high phosphorus and aluminium concentrations are an important evidence of industrial, agricultural and anthropogenic pollution in Bozcaada. Gökçeada appeared to be the cleanest region, as it contained the lowest levels of the investigated metals (except phosphorus). This site probably did not receive many pollutants from agriculture, industry and sewage drains. However, the high phosphorus content may be an indicator of seasonal agricultural drainage into the water system of Gökçeada. The abundance of TC, FC and TE bacteria in freshwater sources is the most important parameter that gives an idea about the pathogens and sanitary risk of these waters for wildlife and human health (Hacıoğlu and Dulger, 2009). We found that especially FC and FS levels were above the limit values in Gökçeada. This is a proof that the Gökçeada water system is under significant pressure in terms of faecal pollution.

The haematological and plasma biochemistry values determined to evaluate the health status of reptiles are affected by many internal and external environmental factors such as season, metabolic activity, reproductive cycle, age, sex, habitat, photoperiod and climate (Hidalgo-Vila et al., 2007; Wack et al., 2012). In this study, the RBC count, Hb concentration and WBC count were determined to be lower in the Dardanos samples. It was found that the RBC count, the WBC count and the MCHC were higher in the samples of animals living in Gökçeada in water of class II–III quality than than at the other sites. In the study conducted by Tosunoğlu et al. (2011), it was determined that Balkan terrapins living in class IV quality (very polluted) water had higher RBC count, WBC count and haemoglobin values. In the same study, MCV and MCH values were found to be higher in class I quality water. Gül et al. (2015) compared the haematological parameters of Balkan terrapin samples at two different sites and found a higher MCH value in samples at the site with high-class water quality and a higher RBC count at the site with the dirty water class; however, the difference was not statistically significant. In the present study, the lowest Hct value was found in Gökçeada among the three sites. It was determined that the blood samples taken from Gökçeada had the lowest microelement contents. It has been reported that the haematocrit value is an index reflecting nutritional and general health status, and a low Hct may be an indicator of anaemia (Peterson, 2002). Hoffman et al. (1981) found that some metals, such as lead, could cause anaemia and a low Hct. In subsequent studies, it was reported that there was no correlation between the metal concentration in tissues and the Hct value in freshwater turtles, and that the metal concentration did not affect the Hct value negatively (Yu et al., 2011). In our study, no negative association was found between microelement concentrations and the haematocrit value. The results of the haematological analyses of Balkan terrapin samples from the three sites are in line with data of the literature reported on the same species (Yılmaz and Tosunoğlu, 2010; Tosunoğlu et al., 2011; Bilgin and Gül, 2019; Çördük et al., 2019).

The results of the analysis of micronuclei and other nuclear abnormalities in the current study suggest that environmental pollutants were capable of attacking erythrocyte DNA, resulting in clastogenic damages. Based on the micronucleus assay, it was determined that micronucleus analysis alone was insufficient, and that micronuclei should be analysed together with other nuclear abnormalities. Similarly, it was reported that micronucleus analysis alone was insufficient to determine the genotoxic effect on *Mauremys rivulata* (Çördük et al., 2019). Guilherme et al. (2008) stated that nuclear abnormality analysis was an alternative to the micronucleus (MN) test because of its high sensitivity in fish with low MN frequency. It was observed that the microelement contents of the water and blood samples were different at the three sites and the lowest microelement content was found in the Gökçeada samples. Heavy metals are significant pollutants for aquatic environments. They have a toxic effect because of bioaccumulation (Gajalakshmi and Ruban, 2014; Wu et al., 2016). Also, the genotoxicity of a water sample may largely be attributed to heavy metals (Ergene et al., 2007; Salles et al., 2016). The MN test results demonstrated the correlation between the accumulation of microelements and the percentage of total nuclear abnormalities in *M. rivulata* erythrocytes. When the results of microelement accumulation and the percentage of micronuclei and other nuclear abnormalities are evaluated together, it can be said that the microelements to which the *M. rivulata* samples were more exposed in their environment, could cause DNA damage.

In conclusion, determination of the effect of different environmental pollutants requires interdisciplinary studies assessing multiple aspects of the ecosystem. This study evaluated the haematological and microbiological properties of *M. rivulata* populations as well as the signs of possible genotoxic effects resulting from their aquatic environment. Monitoring these parameters in terrapins is important for evaluating their health conditions and it can also be used to determine the effect of environmental pollutants.

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