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Haematological and Genotoxicological Effects of Water Quality on *Pelophylax bedriagae* (Camerano, 1882) (Anura: Ranidae) and *Mauremys rivulata* (Valenciennes, 1833) (Testudines: Geoemydidae) in Çanakkale, Türkiye

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Abstract: Haematological and genotoxicological parameters of the Levant green frog *Pelophylax bedriagae* (Camerano, 1882) and the Western Caspian turtle *Mauremys rivulata* (Valenciennes, 1833) were studied. These species inhabited two sites with differing water quality in the Sarıçay Stream passing through the city centre of Çanakkale, Türkiye. We examined whether the two species were affected by the water quality classes identified based on physical and chemical properties of the water. Statistically significant differences were found between the two sites in terms of the frequencies of micronucleus, lobbed, notched, kidney-shaped and blebbed nuclei, as well as total nuclear abnormality in *Pelophylax bedriagae*. Moreover, a higher rate of nuclear abnormalities was observed at the more polluted site, classified as water quality class IV. Statistically significant differences were observed in the haematocrit value, percentages of lymphocytes, eosinophils, heterophils and basophils, erythrocyte width and frequencies of lobbed and kidney–shaped nuclei as well as the heterophil/lymphocyte ratio, often used as a stress indicator, in *Mauremys rivulata*. In this species, it was determined that parameters other than lymphocyte and eosinophil percentages were higher in specimens from the more polluted site.

Key words: *Pelophylax bedriagae*, *Mauremys rivulata*, MICRONUCLEUS TEST, Blood Parameters, Çanakkale.

Introduction

Overpopulation, urbanisation, industrialisation, water pollution due to chemical wastes, pesticides use and other environmental pollutants are the main reasons of the formation of environmental problems (Doğan 2002, Uysal & Tosunoğlu 2012, Çördük et al. 2018, Gül et al. 2019). In addition, the discharge of domestic and industrial wastes into streams without treatment increases the extent of pollution and has become a great threat to aquatic life (Egemen 2000, Selvi 2006).

The presence of many pollutants affecting the quality of Çanakkale's water resources (Akbulut et al. 2006). The causes of water pollution in the province are waste generated by the city and the wastewater discharged by industrial enterprises located in the coastal regions (Ilgar 2000, Akbulut et al. 2006). Agricultural activities surrounding the Atikhisar Dam, the largest source of drinking water

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in Çanakkale and Sarıçay Stream were observed to result in the entry of pesticides used in these areas into the river (Kaya 2007). In addition, it was stated that the concentrations of certain heavy metals detected in the coastal streams within the province exceeded the recommended threshold values (Ilgar 2000). The impacts of heavy metal concentrations in Sarıçay Stream on certain macroinvertebrate and frog species have been assessed, but their effects on other aquatic species remain unknown (Selvi 2006, Kaya 2007, Boran & Gül 2021).

Anthropogenic pollutants have a direct impact on the hematology of living organisms, resulting in alterations in the form and function of both erythrocytes and leukocytes (Beynon et al. 1992, Browne 2004). Previous studies reported that heavy metals exhibited toxic effects, including immune system impairment and haematological disorders, such as erythrocyte destruction, leukocytosis and deviations in haematocrit, haemoglobin, MCV and MCH values in aquatic animals (Javed & Usmani 2012, Zhelev et al. 2017, Wijesinghe 2018). Thus, it was reported that blood profiles were good indicators for determining the health status of species (Ivanc et al. 2005). Haematological parameters play a crucial role in assessing environmental risks and health effects associated with potentially toxic chemicals and serve as important indicators for implementing precautionary measures and acting as early warning signals in polluted areas (Salinas et al. 2015, Pollo et al. 2016, Zhelev et al. 2017).

Physical, chemical and biological environmental pollutants found in the ecosystems of living organisms can damage their genetic materials (Çördük et al. 2018). In recent years, the Micronucleus Test, which is one of the in vitro genotoxicity tests and is used to assess the impact of environmental pollutants on various species, has been increasingly employed to detect nuclear abnormalities such as micronucleus, kidney-shaped nucleus, lobbed nucleus, notched nucleus, and blebbed nucleus (Ergene et al. 2007, Guilherme et al. 2008, Napierska 2009, Strunjak–Perovic et al. 2010, Dar et al. 2016, Çördük et al. 2018, Özgül et al. 2020).

This study focused on the effects of water quality on *P. bedriagae* and *M. rivulata* in the Sarıçay Stream, an area known for its exposure to environmental pollutants. Haematological and genotoxicological analyses were conducted to determine the impacts. This study is the first to reveal the effects of pollutants in this stream on some aquatic herptile species, specifically in terms of genotoxicology and hematology parameters.

Materials and Methods

P. bedriagae (Levant Green Frog) and M. rivulata (Western Caspian Turtle) specimens used in this study were collected from the two sites of Sarıçay Stream located in Çanakkale province (Fig.1C). Previous studies found that the Atikhisar Dam was classified as Class I, indicating high water quality, while the Sarıçay Stream fell within the Class II–III category, representing moderate water quality. Sarıçay, a stream passes through the city center, is polluted due to industrial and domestic waste. It was reported that the pollution in the stream increased towards the city center with previous studies (Ilgar 2000, Akbulut 2006, Kaya 2007). For this reason, the part of Sarıçay close to the city centre was selected as Site I (40° 8’21.35” N, 26°28’54.29” D, Fig. 1A) and the Atikhisar Dam was selected as Site II (40° 7’58.37” K, 26°30’35.08” D, Fig. 1B).

Totally, 41 adult specimens were captured from the two sites: 10 *P. bedriagae* and 11 *M. rivulata* from Site I, whereas 10 *P. bedriagae* and 10 *M. rivulata* from Site II. The field studies were carried out between May and August 2022. Frog and terrapin specimens were collected with the help of scoops and tunnel–nets. Necessary permissions were obtained from the Ethics Committee of Animal Experiments of Çanakkale Onsekiz Mart University (Decision no. 2021/06–13). After haematological analyses were performed, the animal specimens were returned to their biotopes.

The pH, dissolved oxygen and temperature of the water samples taken monthly in the study sites were measured using an ecological kit (Hach–Lange, HQ40D, USA). They were used to determine the water quality classes. In Türkiye, the inland waters are divided into four groups in terms of water quality according to the Water Pollution Control Regulation (Anonymous 2004, Table 1). In addition, certain heavy metal concentrations of water samples were analysed in Çanakkale Onsekiz Mart University Science and Technology Application and Research Centre.

A total of 2 mL of blood was taken from the individuals taken to laboratory for haematological analyses with the help of a 5 mL syringe with a diameter of 21-gauge needle on the day of capturing (Ballard & Cheek 2003, Thrall et al. 2004). The blood samples required for the analyses were taken from the middle abdominal veins of the frogs and from the dorsal caudal veins of the terrapins (Wright & Whitaker 2001, Ballard & Cheek 2003, Thrall et al. 2004).

Within the scope of haematological analyses, red blood cell (RBC) and white blood cell (WBC)

Fig. 1. Study sites. A. Site I, near the city centre. B. Site II, near the Atikhisar Dam. C. Location of the two sites (https://maps.google.com).

count, haemoglobin (Hb) and haematocrit (HCT) values, erythrocyte indices (mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)), leukocyte formula and erythrometric parameters were determined (Atatür et al. 1999, Gül et al. 2015). For genotoxicological analyses, the frequencies of micronucleus, kidney–shaped nucleus, lobbed nucleus, notched nucleus and blebbed nucleus as well as binucleate cells in the blood were detected using the Micronucleus Test (Gül et al. 2019). Additionally, concentrations of certain heavy metals in the blood were measured to assess their presence and potential genotoxic effects.

The standard values of the obtained data were analysed using Microsoft Excel, IBM SPSS Statistics 26 and R Project for Statistical Computing programs. In statistical analyses, Shapiro–Wilk's Test for Normality and the Levene's Test for Equality of Variances were used. The Mann–Whitney U non–parametric test was used to determine whether there was a difference in the haematological parameters between the sites. The Spearman correlation test was used to determine whether there was a correlation between water parameters and some haematological parameters. In all cases, $P \leq 0.05$ was considered statistically significant. Since there was no significant difference between genders in individuals of *P. bedriagae* and *M. rivulata*, female and male specimens were evaluated together.

Table 1. Water quality classes based on physicochemical parameters according to the Water Pollution Control Regulation (Anonymous 2004).

Parameters	Water Quality Classes			
	I	II	III	IV
pH	6.5–8.5	6.5–8.5	6.0–9.0	<6.0–9.0<
Dissolved Oxygen (mg/L)	8	6	3	<3
Temperature (ºC)	25	25	30	<30<

Results

Physicochemical Parameters of Water Samples

In Türkiye, the inland waters are divided into four groups in terms of water quality according to the Water Pollution Control Regulation (Table 1): high–quality waters (Class I), moderate–quality waters (Class II), polluted waters (Class III) and highly polluted waters (Class IV). Taking into account the pH, dissolved oxygen and temperature values measured in the study areas, it was determined that Site I was in Class IV (Fig.1A), while Site II was in Class I-II (Fig.1B). As a result of the water quality analyses performed on the samples taken from both sites in each month (May-August), some physicochemical parameters and heavy metal concentrations were determined. Mean values for four months of detected physicochemical parameters and heavy metal concentrations were given in Table 2. According to the results obtained from the water analyses, it was found that Ba, Cd, Fe, Mn, and Zn metals were in higher concentrations at Site I and that Co and Ni metals were higher at Site II.

Haematological parameters

The hematological parameters, percentages of leukocytes and erythrometric parameters of *P. bedriagae* at the two sites are given in Table 3 in detail. There were no statistically significant differences ($P \geq 0.05$).

In *M. rivulata,* there were statistically significant differences between the two sites for haematocrit, percentages of lymphocyte, eosinophile and heterophile, H/L ratio and erythrocyte width (Table 4). Results of Mann–Whitney U Test were as follows: haematocrit (U: 12,000, W: 78,000, Z:–3,039, P: 0,002), percentage of lymphocyte (U: 27,000, W: 82,000, Z: –1,977, P: 0,048), percentage of eosinophile (U: 16,000, W: 71,000, Z: –2,749, p: 0,006), percentage of heterophile (U: 8,000, W: 74,000, Z: –3,324, P: 0,001), percentage of basophile (U: 23,000, W: 89,000, Z: –2,270, P: 0,023), H/L ratio (U: 6,000, W: 72,000, Z: –3,452, P: 0,001), erythrocyte width (U: 27,000, W: 82,000, Z: –1,972, P: 0,049) (Table 4). No statistically significant difference was found for the other haematological parameters ($P \geq 0.05$).

Genotoxicological parameters

According to the results from the examination of the blood smears of *P. bedriagae*, it was observed that there were statistically significant differences between the two sites for frequencies of micronucleus, lobbed nucleus, notched nucleus, blebbed nucleus and total nuclear abnormalities (Table 5). Results of Mann–Whitney U Test: frequencies of micronucleus (U: 21,000, W: 76,000, Z: –2,423, P: 0,015) kidney–shaped nucleus (U: 17,000, W: 72,000, Z: –2,543, P: 0,011), lobbed nucleus (U: 15,000, W: 70,000, Z: –2,864, P: 0,004), notched nucleus (U: 4,500, W: 59,500, Z: –3,442, P: 0,001), blebbed nucleus (U: 4,500, W: 59,500, Z: –3,442, P: 0,001) and total nuclear abnormalities (U: 10,000, W: 65,000, Z: –3,026, P: 0.002). However, there was no statistically significant differences for binucleate frequency between two sites ($P \geq 0.05$).

Considering the two sites, in *M. rivulata*, there were statistically significant differences for frequencies of lobbed nucleus and kidney–shaped nucleus (Table 5). Results of Mann–Whitney U Test: frequency of lobbed nucleus (U: 26,000, W: 92,000, Z: –2,456, p: 0,014) and frequency of kidney–shaped nucleus (U: 16,000, W: 82,000, Z: –2,851, p: 0,004). There was no significant difference between the two sites in terms of the other nuclear abnormalities and total nuclear abnormality frequency ($P \geq 0.05$) (Fig. 2).

Heavy metal concentrations in blood samples

According to heavy metal analyses on the *P. bedriagae* specimens, it was found that Co, Pb, Fe, and Cr metals were in higher concentrations at Site I, which was more polluted, and Hg, Cu, Zn and Ni metals were higher at Site II. In the *M. rivulata* specimens, Pb, Fe and Cr metals were in higher concentrations at Site I, which was more polluted and Co, Cu and Zn metals were higher at Site II. As a result it was found

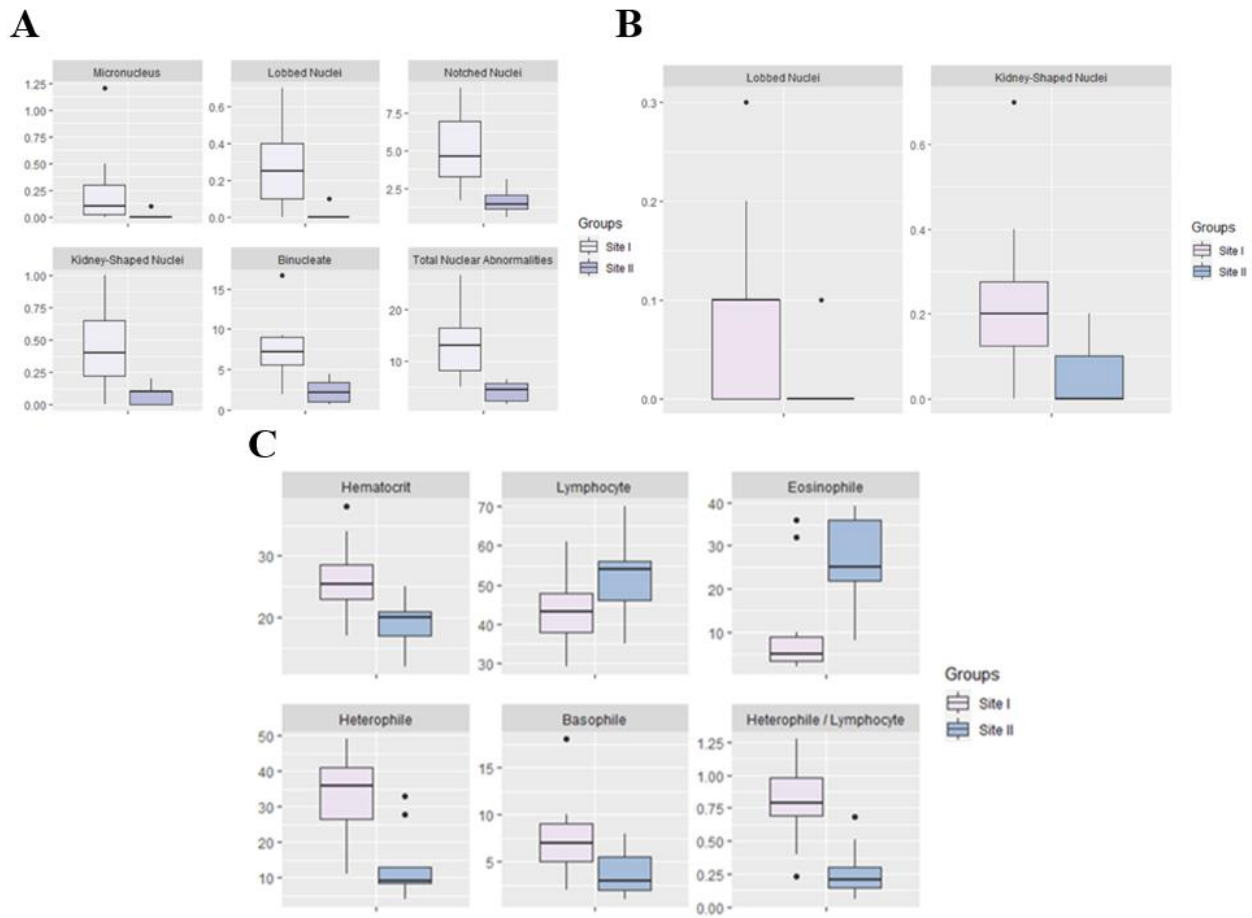

Fig. 2. Haematological and genotoxicological parameters with statistically significant differences in *Pelophylax bedriagae* (A) and *Mauremys rivulata* (B–C) as box–plots.

Table 2. Mean values of some physicochemical parameters (Mean ± SE) and heavy metal concentrations of the water samples taken from both sites.

Parameters	**Site I (Class IV)**	**Site II (Class I–II)**
pH	5.76±0.37	6.87±0.33
Dissolved Oxygen (mg/L)	2.64±1.53	13.79±2.22
Temperature (ºC)	18.10±2.45	25.47±2.83
Barium (ug Ba/L)	68.21 ug/L	40.07 ug/L
Cadmium (ug Cd/L)	0.87 ug/L	0.71 ug/L
Cobalt (ug Co/L)	0.98 ug/L	1.12 ug/L
Iron (ug Fe/L)	67.58 ug/L	10.63 ug/L
Manganese (ug Mn/L)	204.30 ug/L	1.46 ug/L
Nickel (ug Ni/L)	6.39 ug/L	8.83 ug/L
Zinc (ug Zn/L)	4.24 ug/L	2.95 ug/L

that the., the concentration of all heavy metals (except for Pb) was higher in specimens of *P. bedriagae* than in specimens of *M. rivulata* (Table 6).

The Spearman correlation test was employed to investigate the potential relationship between the pH and dissolved oxygen levels of the habitats for both species and various haematological parameters, including erythrocyte size, nucleocytoplasmic ratio, hematocrit, total nuclear abnormalities and H/L or N/L values (Fig. 3).

There was a strong negative correlation between the total nuclear abnormalities and the pH

Table 3. Descriptive statistics of haematological parameters of *P. bedriagae* at both sites.

Haematological parameteres	Site I (Class IV) N:10			Site II (Class I–II) N:10		
	Min–Max	Mean	SE	Min–Max	Mean	SE
Haemoglobin (g/dl)	4.00–12.60	7.13	0.76	4.80–9.00	6.88	0.41
Haematocrit (%)	11.00–53.00	30.70	3.62	19.00–43.00	27.70	1.99
RBC (1mm^3)	270.000–530.000	408.000	28394.05	290.000–640.000	485.000	34968.24
WBC	4.000–8.300	6.520	438.63	4.600–7.700	5.930	306.61
MCV (fl)	407.41–1656.25	772.25	111.76	429.69–862.07	590.12	47.18
MCH (pg)	12.12–39.38	18.07	2.47	10.91–17.93	14.48	0.75
MCHC (%)	16.19–50.91	25.43	3.40	20.80–29.09	25.08	0.91
Lymphocyte (%)	38–71	53.20	3.69	45–76	59.70	3.19
Monocyte (%)	3–16	9.90	1.27	4–20	10.50	1.58
Eosinophile (%)	1–25	8.50	2.56	2–28	11.20	2.71
Neutrophile (%)	1–39	16.20	4.09	2–30	10.50	2.46
Basophile (%)	3–24	12.20	2.20	1–12	7.10	1.01
N/L (%)	0.01–0.81	0.35	0.09	0.03–0.66	0.19	0.05
Erythrocyte Length (µm)	18.50–31.00	23.66	0.11	19.00–29.00	23.20	0.09
Erythrocyte Width (µm)	5.00–16.10	15.04	0.37	11.00–21.00	14.68	0.10
Nucleus Length (µm)	7.00–14.00	9.81	0.06	7.00–12.00	9.59	0.04
Nucleus Width (µm)	4.00–8.00	5.91	0.04	4.00–8.00	5.66	0.04
Erythrocyte Size (µm^2)	90.27–373.93	282.44	9.05	183.69–436.85	269.25	2.67
Nucleus Size (µm^2)	25.12–81.64	46.04	0.54	23.55–72.22	43.00	0.46
EL/EW (µm)	0.18–4.60	1.63	0.01	1.20–2.25	1.59	0.00
NL/NW (µm)	1.18–2.54	1.67	0.01	1.28–2.50	1.71	0.01
NR (µm)	0.01–0.38	0.16	0.00	0.09–0.22	0.16	0.00

Abbreviations: RBC: Red Blood Cell Count, WBC: White Blood Cell Count, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration, N/L: Neutrophile/Lymphocyte, EL/EW: Erythrocyte Length/Erythrocyte Width, NL/NW: Nucleus Length/Nucleus Width, NR: Nucleocytoplasmic Ratio, Min–Max: Minimum–Maximum, SE: Standard Error).

Table 4. Descriptive statistics of haematological parameters of *M. rivulata* at both sites (* Parameters that have significant differences between two sites).

Haematological parameters	Site I (Class IV) N:10			Site II (Class I–II) N:11		
	Min–Max	Mean	SE	Min–Max	Mean	SE
Haemoglobin (g/dl)	3.50–8.00	6.06	0.43	3.00–7.40	5.47	0.43
Haematocrit (%)*	17.00–38.00	26.50	1.91	12.00–25.00	19.13	1.04
RBC (1mm^3)	320.000–700.000	516.000	41424.63	220.000–610.000	439090.91	34124.22
WBC	3.300–6.700	5.680	305.06	2.500–8.100	5372.73	501.65
MCV (fl)	348.48–843.75	538.69	51.34	250.00–732.14	466.30	46.10
MCH (pg)	83.33–202.94	124.36	13.68	62.29–236.36	134.51	16.19
MCHC (%)	12.06–40.58	23.73	2.25	19.00–41.11	28.57	1.82
Lymphocyte (%)*	29–61	43.90	2.90	35–70	52.64	3.10
Monocyte (%)	1–8	4.30	0.74	0–9	4.36	0.76
Eosinophile (%)*	2–36	10.50	3.99	8–39	26.36	3.20
Heterophile (%)*	11–49	33.70	3.82	4–33	12.82	2.76
Basophile (%)*	2–18	7.60	1.40	1–8	3.82	0.84
H/L (%)*	0.22–1.27	0.80	0.10	0.05–0.68	0.25	0.05
Erythrocyte Length (µm)	16.0–23.0	19.14	0.05	16.0–23.0	19.386	0.058
Erythrocyte Width (µm)*	9.5–14.0	11.45	0.04	9.5–15.0	11.820	0.042
Nucleus Length (µm)	5.5–9.0	6.90	0.02	5.0–10.0	6.919	0.031
Nucleus Width (µm)	4.0–6.5	5.12	0.02	4.0–7.0	5.197	0.028
Erythrocyte Size (µm^2)	125.60–241.78	172.36	0.92	131.880–239.032	180.035	0.916
Nucleus Size (µm^2)	18.84–42.39	27.86	0.22	15.700–43.960	28.358	0.235
EL/EW (µm)	1.34–2.21	1.67	0.00	1.266–2.157	1.648	0.007
NL/NW (µm)	1.07–1.75	1.35	0.00	1.000–2.000	1.342	0.007
NR (µm)	0.109–0.233	0.161	0.00	0.087–0.240	0.157	0.001

Abbreviations: RBC: Red Blood Cell Count, WBC: White Blood Cell Count, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration, H/L: Heterophil/Lymphocyte,EL/EW: Erythrocyte Length/Erythrocyte Width, NL/NW: Nucleus Length/Nucleus Width, NR: Nucleocytoplasmic Ratio, Min–Max: Minimum–Maximum, SE: Standard Error).

Table 5. Descriptive statistics of micronucleus test findings of *P. bedriagae* and *M. rivulata* (Mean±SD). *Parameters that have significant differences between two sites in *P. bedriagae*. **In both species.

Nuclear Abnormalities	*Pelophylax bedriagae*		*Mauremys rivulata*	
	Site I (Class IV) N:10	**Site II (Class I–II) N:10**	**Site I (Class IV) N:10**	**Site II (Class I–II) N:11**
Micronucleus (%)*	0.26±0.36	0.02±0.04	0.02±0.04	0.04±0.06
Lobbed Nucleus (%)**	0.26±0.23	0.02±0.04	0.09±0.09	0.00±0.03
Notched Nucleus (%)*	5.06±2.37	1.66±0.78	3.04±1.37	3.16±1.48
Kidney–Shaped Nucleus (%)**	0.43±0.33	0.08±0.07	0.24±0.19	0.05±0.06
Blebbed Nucleus (%)*	7.47±4.08	2.32±1.38	2.38±1.83	1.65±1.07
Binucleate (%)	0.02±0.04	0.00±0.00	0.0±0.031	0.00±0.00
Total Nuclear Abnormalities (%)*	**13.03±6.63**	**4.10±1.96**	**5.78±3.06**	**4.92±2.05**

Table 6. Mean heavy metal concentrations in the blood of samples taken from both sites. Undetectable concentrations are marked by dash.

Parameters	*Pelophylax bedriagae*		*Mauremys rivulata*	
	Site I (Class IV)	**Site II (Class I–II)**	**Site I (Class IV)**	**Site II (Class I–II)**
Mercury (mg/kg)	0.01	0.02	0.01	0.01
Cobalt (mg/kg)	0.58	–	0.35	0.44
Copper (mg/kg)	8.52	10.32	6.41	6.66
Manganese (mg/kg)	–	–	–	–
Zinc (mg/kg)	8.54	11.01	0.95	1.39
Lead (mg/kg)	0.21	–	0.88	0.69
Nickel (mg/kg)	–	0.19	–	–
Iron (mg/kg)	5.21	0.37	0.66	0.24
Chromium (mg/kg)	0.47	0.01	0.03	–

(r=–0.694, p=0.001) and dissolved oxygen levels (r=–0.694, p=0.001) in *P. bedriagae*. In addition, it was found that there was a moderate positive relationship between the total nuclear abnormalities and the N/L ratio (r=0.445, p=0.049). In *M. rivulata*, there were strong negative correlations between the pH levels, the haematocrit value (r=–0.680, p=0.001) and the H/L ratio (r=–0.772, p=0.001). A strong negative correlation was determined between the dissolved oxygen levels, the haematocrit value (r=–0.680, p=0.001) and the H/L ratio (r=–0.772, p=0.001). Moreover, there was a moderate positive correlation between the haematocrit and the H/L ratio (r=0.552, p =0.015).

Discussion

Areas near to the city center in Sarıçay Stream were generally more polluted physically, chemically and biologically, while the pollution levels gradually decreased from the city centre to the Atikhisar Dam (Ilgar 2000, Odabaşı 2005, Akbulut et al. 2006, Kaya 2007). In the field studies, solid wastes and presence of sewage disposal into the stream were observed at Site I. Therefore, it was estimated that the dissolved oxygen level may have decreased due to this pollution. Considering heavy metal concentrations, it was found that levels of Ba, Cd, Fe and Zn were higher at Site I and that Co and Ni levels were higher at Site II. Cobalt and nickel can naturally occur in high concentrations in certain surface waters. Dissolution of volcanic rocks and silty sedimentary rocks in streambeds can contribute to their higher concentration (Barałkiewicz & Siepak1999). Therefore, this data provides an explanation for higher concentrations in Site II.

Previous studies showed that the number of erythrocytes increased in polluted areas (Zhelev et al. 2013, Tosunoğlu et al. 2015); however, there was no statistically significant difference found be-

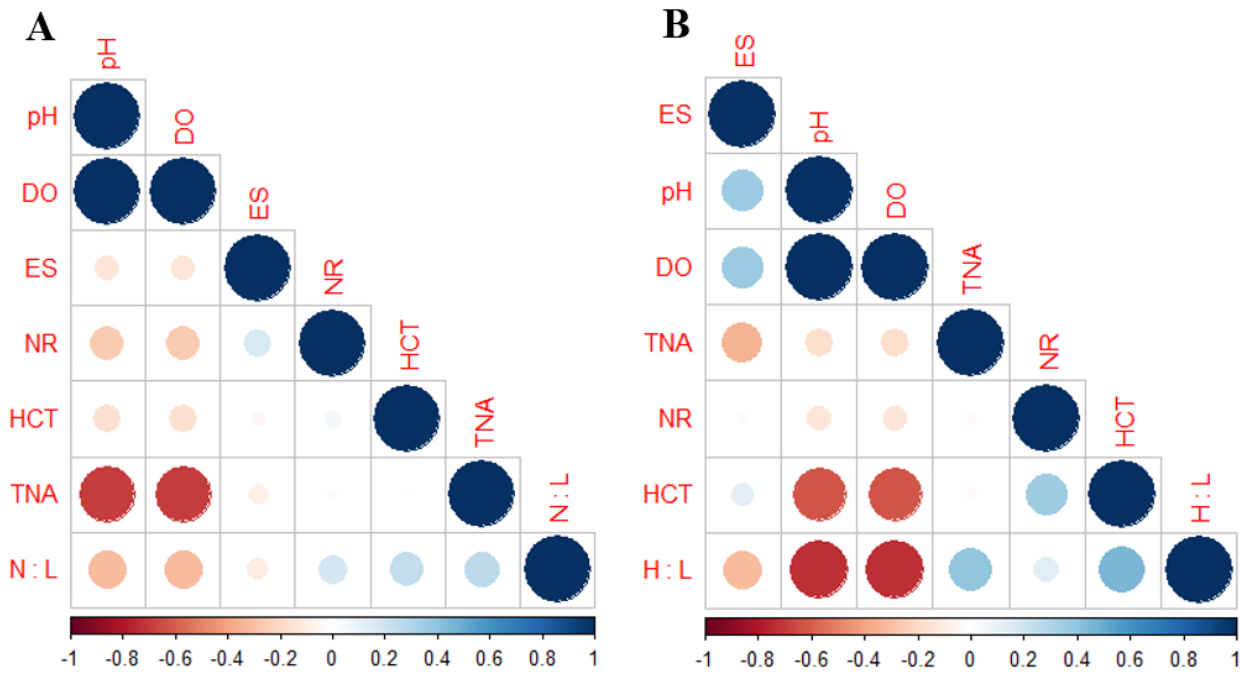

Fig. 3. A correlogram showing the relationships between some haematological parameters and physicochemical water parameters in *P. bedriagae* (A) and *M. rivulata* (B). Dark blue dots indicate strong positive correlations (r=1.0), while dark red dots indicate strong negative correlations (r=–1.0). *Abbreviations:* DO: Dissolved Oxygen, ES: Erythrocyte Size, NR: Nucleocytoplasmic Ratio, HCT: Haematocrit, TNA: Total Nuclear Abnormalities, N:L: Neutrophils/Lymphocytes, H:L: Heterophils/Lymphocytes.

tween the two sites in the present study. Fierascu et al. (2018) reported that erythrocytopenia may be observed in individuals exposed to pesticides. It is believed that the reason for these changes in haematological parameters observed in polluted areas is indicative of anaemia and disorders in the immune system due to the presence of xenobiotics in ecosystem and hypoxia, which can disrupt oxygen transport to tissues (Zhelev et al. 2018). It was reported that the percentage of monocytes increased in polluted areas (Zhelev et al. 2013, Peskova et al. 2019), while basophils decreased (Zhelev et al. 2013), however, in the present study, there was no statistically significant difference between the two sites.

Hematological parameters obtained from *M. rivulata* were in accordance with the findings reported for polluted areas (Gül et al. 2015). It was reported that increase in haematological parameters in polluted areas may be evidence of acceleration of metabolism to provide an active toxin output (Zhelev et al. 2017). Leukocyte profiles are useful for evaluation of stress since they are related to stress hormone levels. Thus, the ratio of heterophils or neutrophils to lymphocytes (H/L or N/L) is often used to assess stress in vertebrates as haematological and a larger H/L or N/L ratio indicates an increase in stress levels (Davis et al. 2008). In the present study, it was found that this ratio was higher at Site I which is more polluted. In addition, in the present study the percentages of heterophils and basophils in the individuals located in the polluted area were higher, while the percentages of lymphocytes and eosinophils in the individuals located in the unpolluted area were higher. It was suggested that taken from the natural environment often suffer from parasites and, thus, may have a higher eosinophil count (Zhang et al. 2011). Lymphocytosis can occur due to wound healing, inflammation, parasitic infections (Mead & Borysenko 1984) and viral diseases (Campbell 2004, Zhang et al. 2011). Increase in the number of heterophils may be due to increased metabolic activity, competition, feeding and mating behaviour that cause tissue damage and inflammation (Terry 2006, Chi et al. 2013). Although it is known that basophils in turtle species have an immune capacity, similar to basophils or mast cells in mammals, their numbers in the peripheral blood of many species vary (Zhang et al. 2011).

When the previous studies about erythrometric parameters of *P. bedriagae* were examined, the data obtained from the present study are consistent with the literature predominantly (Zhelev et al. 2017, 2021, Şahin 2019). Previous studies determined that the nucleocytoplasmic ratio was higher in unpolluted

areas than polluted areas (Şahin 2019). However, in the present study, no difference was found between the two sites in terms of the nucleocytoplasmic ratio. It was reported that the nucleocytoplasmic ratio increased depending on the season, especially in spring, compared to other months (Zhelev et al. 2016).

There are no detailed studies regarding morphometric changes of blood cells of *M. rivulata* due to environmental parameters. In the transport of oxygen and carbon dioxide, which is one of the most important functions of erythrocytes, the ratio of surface area to size is a determining factor in terms of the passage of these gases to tissues. It was proven by studies that small–sized erythrocytes offer a higher gas exchange compared to large–sized ones (Parto et al. 2013). The data from the previous studies provide an explanation for the observation that terrapins living in environments with low dissolved oxygen levels have smaller erythrocytes.

It was stated that pollution leads to more DNA damage in nucleus (Aymak 2010, Çördük et al. 2018, Şişman et al. 2021) and the results of the present study also support this data. Based on this information, it is possible to say that Site I was more polluted and that *P. bedriagae* was affected by this pollution. The findings of the current study indicate that the frequencies of micronucleus in *M. rivulata* were higher compared to previous studies (Metin et al. 2008, Gül et al. 2019). The number of micronucleus is a marker of genetic damage in adult animals and an increase in their number indicates impaired cell health. However, Zuniga–González et al. (2000) suggested in their studies that the presence of micronucleus may increase in young individuals of some species due to immature reticuloendothelial system.

In previous studies regarding the concentration of heavy metals in the blood of frog species, it was observed that the Fe concentration was higher in *Pelophylax ridibundus* and that the Cu concentration was higher in *Pelophylax lessonae*. Moreover, in terms of the biological accumulation coefficient, it was found that higher accumulation in the blood of *P. ridibundus* was in Cu, Fe and Zn metals (Romanova et al. 2021). Çördük et al. (2022) found that among three sites compared in their study, in *M. rivulata* specimens taken from the area where microelements in water were in higher concentration, only Fe metal was higher in the blood compared to the other two sites, while Cu and Zn metals were higher at the other two sites. As a result of our findings, the concentration of heavy metals in the P. bedriagae individuals was higher than the M. rivulata individuals in all heavy metals except Pb.

In terms of the correlation between the physicochemical properties of water (pH and dissolved oxygen) and haematological parameters, the results may support the finding that DNA damage is more common in polluted areas with low pH and dissolved oxygen levels. In contrast, in a study conducted by Zhelev et al. (2022), it was observed that the polluted area that has more genotoxic damage had a more suitable pH level for the normal development of amphibians than reference site. According to our findings, it can be stated that haematocrit values and the H/L ratio tend to increase as pH and dissolved oxygen levels decrease. Hypoxia, which is characterised by a decrease in dissolved oxygen levels in aquatic ecosystems, can arise because of the introduction of biological and chemical wastes caused by human activities (Keleştemur 2012). However, in recent studies, it was reported that hypoxia was accompanied by low pH values. For this reason, many organisms exposed to hypoxic stress also need to cope with low pH values, known as acidification (Gobler & Bauman 2016, Tomasetti & Gobler 2020).

Conclusions

With this study, the effects of different water parameters and heavy metal accumulations on *Pelophylax bedriagae* and *Mauremys rivulata* were revealed. By comparing total nuclear abnormalities and heavy metal accumulations in the blood between the species, it can be inferred that *P. bedriagae* is more significantly impacted by water pollution. This suggests that *P. bedriagae* could potentially serve as a bioindicator species for monitoring environmental pollutants.

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