A preliminary study on micronucleus analysis and nuclear anomalies in *Pelophylax ridibundus* (Pallas, 1771) (Amphibia: Anura) specimens collected around Vize (Kırklareli) and Ida Mountains (Çanakkale, Turkey)

Vize ve Kaz Dağı çevresinden toplanan *Pelophylax ridibundus* (Pallas, 1771) (Amphibia: Anura) örneklerinde mikronukluel analizi ve nükleer anomaliler hakkında bir ön çalışma

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Abstract: In this study, a micronucleus analysis was made on the erythrocytes of the *Pelophylax ridibundus* specimens collected around Vize (Kırklareli) and Ida Mountains (Yenice, Çanakkale), nuclear anomalies were detected. For this purpose, 9 (5♂♂, 4♀♀) *P. ridibundus* specimens were collected from Vize in April and 5 (3♂♂, 2♀♀) *P. ridibundus* specimens were collected from Ida Mountains in May 2011. The mean number of micronuclei was calculated as 6±4.17 and frequency as 0.3% in the specimens collected from Vize, while the mean number of micronuclei was calculated as 0.6±0.43 and frequency as 0.03% in the specimens collected from Ida Mountains. As a result of the statistical analyses, it was determined that there was a significant difference in the total number of micronuclei between both localities (p<0.05). Some 4 types of nuclear anomalies, i.e. binucleate, notched, blebbed and small-lobed, were detected in the study. No difference in the total number of nuclear anomalies was detected between the specimens of Vize and Ida Mountains (p>0.31). According to investigation to the results, Vize samples have a higher frequency of micronuclei associated with intensive agricultural activities used genotoxic exposure to pesticides.

Keywords: Pelophylax ridibundus, Micronucleus, Nuclear anomalies, Vize, Ida Mountains

INTRODUCTION

One of the most important causes of reductions in the populations of amphibians distributed around the agricultural areas is pesticide contamination (Sparling et al., 2001). Pesticides are effective on non-target organisms besides target organisms. These chemicals accumulate in different environmental parts (groundwater, surface water and sediment) in time and pose a threat for many species (Finizio et al., 2001).

The micronucleus test (MN) was developed in 1975 by Schmid in order to reveal the effects of genotoxic chemicals on mammalian bone marrow cells. Since then, the MN test has been used to examine the effects of chemicals with a genotoxic effect on different organisms (Orhan et al., 1993; Zhuleva et al., 1996; Villarini et al., 1998, Çavaş and Ergene, 2005; Souza et al., 2006; Saleh and Sarhan, 2007; Çelikler et al., 2008; Bolognesi and Hayashi, 2011). The fact that erythrocytes are nucleated in amphibian specimens increases the usability of the MN test. There are many MN tests and nuclear anomaly studies performed in vitro and in vivo so as to determine genetic damage in amphibians (Zoll-Moreux and Ferrier, 1999; Kryukov, 2000; Saleh and Zeytinoğlu, 2001; Campana et al., 2003; Lajmanovich et al., 2005; Marques et al., 2009; Yin et al., 2009; Ahmad and Saleh, 2010).

Amphibians are the most important natural enemies of agricultural pest insects in aquatic and agricultural ecosystems (Feng et al., 2004). Amphibians are bioindicator living organisms as they are quite sensitive to the changes in environmental conditions, as they spend certain periods of their lives completely in water and as their skin is quite
sensitive (Schuytema and Nebeker, 1999). Pelophylax ridibundus specimens used in our study belong to an amphibian species distributed quite widely in Turkey. Since it is consumed as a food substance, this species has economic significance. *P. ridibundus* specimens are water-dependent throughout their lives. Thus, they are quite sensitive to any chemical change likely to occur in the aquatic ecosystem.

In the study, Vize (Kırklareli) and Ida Mountains (Çanakkale) localities were selected as the study areas. Of the localities concerned, Vize is located in Thrace, where considerable agricultural activities are carried out. Cultivation of sunflowers and cereals are the most intense in this area. Agricultural activities in the region according to the 2006 data year, 12246.5 kg of insecticide, fungicide 64295.75 kg and 238 655 kg of herbicide used (Anonymous, 2008). Moreover, it is quite important that this locality is located within the Meriç Delta, which is reported to have been contaminated as a result of agricultural and industrial activities (Erkmen and Kolankaya, 2005). The Ida Mountains locality, however, was selected as a reference zone that was considered uncontaminated as it was located away from agricultural areas and settlements.

The aim of this study is to determine micronuclei and nuclear anomalies in the *P. ridibundus* specimens collected from Vize, which was considered contaminated in terms of pesticide contamination, and from Ida Mountains, which was considered uncontaminated in terms of pesticide contamination. Our study is important in that it contributes to the development of strategies for the protection of this species which has an essential role in ecological sense and which is economic since it is consumed as a food substance.

**MATERIAL AND METHODS**

In this study, 9 (5♂♂, 4♀♀) *P. ridibundus* specimens from Vize and 5 (3♂♂, 2♀♀) *P. ridibundus* specimens from Ida Mountains (Yenice, Çanakkale) were used as the animal materials. These specimens were anesthetized with ether in order to take blood. Later on, the blood taken from the heart was transferred to heparinized capillary tubes. A drop of the blood samples was put onto the clean slides and smeared as a thin layer. Some 3 preparations were made for each *P. ridibundus* specimen. The smear preparations were dried at room temperature. These preparations were fixed and stained using the May-Grunwald-Giemsa method (Lewis et al., 2006). To determine the frequencies of micronuclei and nuclear anomalies, some 2000 erythrocytes from each preparation were examined under 1000x magnification using a light microscope. Preparations containing significant findings, the camera adapted Olympus BX51 light microscope, photographed using DP2-BSW software. The nuclear anomalies were determined according to Carrasco et al. (1990).

The non-parametric Mann-Whitney U test was used to determine the difference in the numbers of micronuclei and nuclear anomalies between both localities. The significance value was taken as p≤0.05.

**RESULTS AND DISCUSSION**

The mean numbers of micronuclei and nuclear anomalies in the *P. ridibundus* specimens collected from Vize and Ida Mountains are presented in Figures 1 and 2.

The mean numbers and frequency of micronuclei (MN) and nuclear anomalies (NA) were found higher in the *P. ridibundus* specimens collected from Vize than in the specimens collected from Ida Mountains (Table 1).

![Figure 1. The mean numbers of micronuclei and nuclear anomalies in the *P. ridibundus* specimens collected from Vize (N:9)](image1)

![Figure 2. The mean numbers of micronuclei and nuclear anomalies in the *P. ridibundus* specimens collected from Ida Mountains (N:5)](image2)
The micronucleus, notched nucleus, blebbed nucleus, binucleate and lobed nucleus anomalies observed in *P. ridibundus* specimens and normal erythrocyte photographs are presented in Figure 3.

As a result of the Mann-Whitney U test, the difference in the total numbers of micronuclei between the specimens of Vize and Ida Mountains was found substantially significant (*p* ≤ 0.05, *p*=0.00). As a result of the same test, however, the difference in the total numbers of nuclear anomalies between the localities concerned was not found significant (*p*=0.31).

This study revealed the difference between the micronucleus frequencies considered to be based on pesticide contamination in the *P. ridibundus* specimens distributed in Vize and Ida Mountains localities. Findings similar to those in our study were detected in the surveys of the MN test in which the *P. ridibundus* specimens collected from nature were used. According to Ahmad and Saleh (2010), the MN frequency increases in those regions where agricultural pollution is high. Likewise, in his study, Aymak (2010) reports that the MN frequency is higher in the region where the heavy metal pollution is particularly high out of two localities considered uncontaminated and contaminated. Furthermore, he also states in his study that the MN frequency varies by season throughout the year. The collecting of the amphibian specimens in April and May at proximate temperatures in our study prevents a possible seasonal deviation of the MN frequency. Environmental pollutants first show their effects on aquatic organisms. The pollutants concerned can show their effects on many highly organized vertebrate species including human beings by means of the food chain. The genotoxic changes in *P. ridibundus* specimens, which are of economic importance because they are consumed as food and which are exported abroad, should be taken into consideration in this context and investigated in detail by means of other studies. It is thought that similar genotoxic studies will contribute to the development of strategies for the protection of this species. In addition, the making of heavy metal and pesticide analyses in water, sediment and tissue samples in other studies to be performed will contribute to more clearly revealing the genotoxic effects concerned.

### Table 1. The mean numbers and frequency of micronuclei and nuclear anomalies in Vize and Ida Mountains

<table>
<thead>
<tr>
<th>Sampling Site</th>
<th>Number of erythrocytes analysed</th>
<th>Mean±SD (MN)</th>
<th>% Frequency of MN</th>
<th>Mean±SD (NA)</th>
<th>% Frequency of NA</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 2011 Vize</td>
<td>54000</td>
<td>6±4.17</td>
<td>0.3</td>
<td>271.47±108.42</td>
<td>13.57</td>
</tr>
<tr>
<td>May 2011 Ida mountains</td>
<td>30000</td>
<td>0.6±0.43</td>
<td>0.03</td>
<td>283.27±33.64</td>
<td>14.16</td>
</tr>
</tbody>
</table>

### Table 2. The pesticides observed to have been used considerably in Vize (Kırklareli) and their active ingredients

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Active Ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungicide</td>
<td>Prochloraz</td>
</tr>
<tr>
<td>Herbicide</td>
<td>Clodinafop-Propargyl</td>
</tr>
<tr>
<td>Herbicide</td>
<td>Chlorsulfuran</td>
</tr>
</tbody>
</table>

### REFERENCES


Aymak, C. 2010. Determination of heavy metal pollution and its genotoxic effects by using micronucleus test in Rana ridibunda Pallas, 1771 (Ranidae, Amphibia) living in Mersin. Mersin University, Phd Thesis. (in Turkish)


