© Environmental Toxicology and Ecology
2025, Vol. 5 (1)
ISSN: 2757-9719

Research Article

Investigation of Genotoxic Effect Potential of Water Pollution in Büyük Akgöl on *Scardinius erythrophthalmus* and *Perca fluviatilis*

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Article Info

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Article history

Received: 11/04/2025

Accepted: February 30/04/2025

Available online:02/05/2025

Keywords:

Genotoxicity, *Scardinius erythrophthalmus*, *Perca fluviatilis*, water pollution, heavy metals

Anahtar Kelimeler:

Genotoksisite, Scardinius erythrophthalmus, Perca fluviatilis, su kirliliği, ağır metaller

How to Cite: G. Meredova, P. Göç Rasgele, Ş.G. Kırankaya " Investigation of Genotoxic Effect Potential of Water Pollution in Büyük Akgöl on *Scardinius erythrophthalmus* and *Perca fluviatilis*", *Environmental Toxicology and Ecology, c. 5, sayı. 1, ss. 32-41, 2025.*

DOI: 10.59838/etoxec.1674401

ABSTRACT

This study aims to evaluate heavy metal accumulation and related genotoxic effects on two species, the omnivorous *Scardinius erythrophthalmus* and the carnivorous *Perca fluviatilis*, living in Büyük Akgöl within the borders of Sakarya province. In this context, the genotoxic effect potential was evaluated using micronucleus (MN) and erythrocyte nuclear abnormalities methods in peripheral blood samples taken from fish. In addition, the levels of heavy metals such as Al, Fe, Zn, Cu, Mn, Pb, Cd, As, Ni, Co, Cr and Hg in muscle tissues were analyzed. According to the obtained data, significant genotoxic responses reflecting the effects of environmental pollutants were observed in both species. While the MN rate increased up to 16% in *S. erythrophthalmus* individuals, this rate remained at 1.80% in *P. fluviatilis*. In addition, nuclear abnormality frequencies also differed among species. Heavy metal analyses revealed that heavy metals including Zn, Pb, Fe, Cu, Al and Cr accumulated at relatively high levels in the muscle tissue in both species. These results suggest that pollution in Büyük Akgöl induces biological effects at genetic level and that heavy metal contamination may constitute a potential threat to fish health. In this context, it is recommended that use of agricultural chemical in agricultural areas surrounding Büyük Akgöl be limited and that domestic and industrial wastes be effectively regulated. Long-term, seasonal biomonitoring studies should be conducted on species at different trophic levels, such as *P. fluviatilis* and *S. erythrophthalmus*, in order to monitor genotoxic effects caused by heavy metals. In addition, considering that metals accumulated in fish can be transferred along the food chain from plankton to predatory fish, ecosystem-based protection strategies should be developed.

Büyük Akgöl'deki Su Kirliliğinin *Scardinius erythrophthalmus* ve *Perca fluviatilis* Üzerindeki Genotoksik Etki Potansiyelinin Araştırılması

ÖZET

Bu çalışma, Sakarya ili sınırlarında yer alan Büyük Akgöl'de yaşayan omnivor tür olan *Scardinius erythrophthalmus* ve karnivor tür olan *Perca fluviatilis* üzerinde ağır metal birikimi ve buna bağlı genotoksik etkileri değerlendirmeyi amaçlamaktadır. Bu kapsamda, balıklardan alınan periferik kan örneklerinde mikronukleus (MN) ve eritrosit çekirdek anomalileri yöntemleri kullanılarak genotoksik etki potansiyeli değerlendirilmiştir. Ayrıca, kas dokularında Al, Fe, Zn, Cu, Mn, Pb, Cd, As, Ni, Co, Cr ve Hg gibi ağır metallerin düzeyleri analiz edilmiştir. Elde edilen verilere göre, her iki türde de çevresel kirleticilerin etkilerini yansıtan anlamlı genotoksik yanıtlar gözlemlenmiştir. *S. erythrophthalmus* bireylerinde MN oranı %16'ya kadar çıkarken, *P. fluviatilis*'de bu oran %1,80 düzeyinde kalmıştır. Ayrıca çekirdek anomali frekansları da türler arasında farklılık göstermiştir. Ağır metal analizleri, Zn, Pb, Fe, Cu, Al ve Cr gibi ağır metallerin her iki türün kas dokusunda nispeten yüksek seviyelerde biriktiğini ortaya koymuştur. Bu sonuçlar, Büyük Akgöl'deki kirliliğin genetik düzeyde biyolojik etkilere neden olduğunu ve ağır metal kirliliğinin balık sağlığı açısından potansiyel bir tehdit oluşturabileceğini göstermektedir. Bu bağlamda, Büyük Akgöl çevresindeki tarım alanlarında kullanılan tarımsal kimyasalların kullanımının sınırlandırılması ve evsel ile endüstriyel atıkların etkin şekilde denetlenmesi önerilmektedir. Ağır metallerin neden olduğu genotoksik etkileri izleyebilmek amacıyla *P. fluviatilis* ve *S. erythrophthalmus* gibi farklı trofik seviyelerdeki türler üzerinde uzun vadeli, mevsimsel biyolojik izleme çalışmaları yapılmalıdır. Ayrıca, balıklarda biriken metallerin planktondan yırtıcı balıklara kadar olan besin zinciri boyunca taşınabildiği göz önünde bulundurularak, ekosistem temelli koruma stratejileri geliştirilmelidir.

1. INTRODUCTION

Ecotoxicology is a scientific discipline that analyzes the sources of environmental pollutants, the toxic effects of these pollutants on ecosystem components, and the ecological consequences of these effects at a multi-level. This field is not limited to examining biological responses at the species level, but also aims to evaluate the disruptions caused by these effects at the ecosystem level and to develop strategies for protecting ecosystem integrity [1]. Currently, chemical pollutants originated from expanding industrial, agricultural and urban activities pose serious threats to ecosystem health. In this context, ecotoxicology emerges as a multidisciplinary field that investigates the effects of toxic substances on individual organisms, population dynamics, natural communities, and entire ecosystems. Aquatic ecosystems, in particular, are extremely sensitive to environmental pollutants due to their high biodiversity and the fact that they form the basis of many food chains [2].

Among aquatic organisms, fish are particularly susceptible to the adverse effects of toxic pollutants, making them important indicators of environmental contamination [3]. The selection of model organisms is of great importance in biomonitoring studies. In particular, it is recommended to select fish species that occupy different ecological niches, belong to distinct taxonomic groups, and exhibit broad geographical distributions. Among these species, the omnivorous rudd (*Scardinius erythrophthalmus* L., 1758), which has an important place in the fish communities of many water resources in Eastern and Central Europe and is also found in Türkiye, and the carnivorous perch (*Perca fluviatilis*) stand out [3-4]. The ability of these fish species to adapt to different feeding habits, rapid adaptation to sudden changes in the environment [5], high resistance to diseases and rapid reproduction make them valuable organisms for toxicological research [6].

Perca fluviatilis belongs to the Percidae family and is an ecologically important predator, both commercially and in terms of fisheries [7]. Several studies have investigated the potential genotoxic effects of water pollution on *Perca fluviatilis* populations inhabiting rivers in various countries. In studies using micronucleus assay, nuclear damage levels were investigated in *P. fluviatilis* fish caught from the Odra River in the Czech Republic [8], rivers in the Rivne Region in Ukraine [9], the Desenka River [10], and the Nemunas River in Lithuania [11] and the sensitivity level of this species to environmental stress factors was reported. In addition, heavy metal accumulation levels were investigated by ICP-OES analysis of muscle tissue in samples collected from the Međuvršje Reservoir located in the West Morava River Basin in western Serbia [12].

Scardinius erythrophthalmus is one of the widespread species belonging to the Leuciscidae family [7]. As an omnivorous pelagic species, the rudd predominantly occupies macrophyte-rich zones in aquatic ecosystems, and its foraging behavior has been reported in some studies to exert adverse effects on macrophyte biomass [13]. These characteristics render it a suitable model organism for comprehensive ecotoxicological assessments of aquatic ecosystems, particularly in urban water bodies The tolerance of this species to variations in oxygen, salinity, and gas conditions, along with its ability to adapt to atypical dietary sources, highlights its physiological adaptability [14]. Nuclear disorder levels were investigated in *S. erythrophthalmus* fish caught from the Desenka River in Ukraine [10]. In addition, several studies have focused on the effects of toxicant exposureon morphological, physiological and biochemical parameters in this species [3], on its histological and biochemical responses to environmental pollutants [15-17], and on the impacts of anthropogenic pollution on enzyme activity [14].

In ecotoxicological studies, micronucleus test [18] and erythrocyte nuclear abnormality test are among the most widely employed assays for the assessment of genotoxic effects. These tests are also biomarker tools used to determine whether xenobiotic agents cause DNA damage by causing changes in chromosomes or disruptions in the mitotic process [19]. By reflecting the genetic-level effects of environmental pollutants, these genotoxicity assays offer valuable insights for ecosystem health monitoring and are widely employed in both in vivo and in vitro settings across a range of taxa, particularly aquatic organisms [20].

The objective of this study was to assess the genotoxic effects of environmental pollutants in Lake Büyük Akgöl through the application of micronucleus and erythrocyte nuclear abnormality assays in the erythrocytes of *Scardinius erythrophthalmus* and *Perca fluviatilis*. Furthermore, heavy metal concentrations in the muscle tissues of these model species were quantified.

2. MATERIALS AND METHODS

2.1. Study Area and Fish Sampling

Büyük Akgöl is a shallow lake located in the Ferizli district of Sakarya province, in the north-western part of Türkiye's Marmara Region, at coordinates 41°01′ K and 30°33′ E. The lake covers an area of 3.6 km² with an average depth of 1.5 meters and has a drainage basin of 47 km². Field studies were carried out in November 2023. Fish samples were caught by local professional fishermen using gillnets with different mesh sizes. In order to perform micronucleus assay and heavy metal analyses, five fish samples were taken from each fish species.

2.2. Micronucleus Assay

The collected fish were anaesthetized using clove oil. Subsequently, peripheral blood was carefully drawn from the caudal venous sinus using heparinized syringes. The blood from each fish was spread across three separate slides and left to air dry for 24 hours. After this drying period, the slides were fixed with pure ethanol and stained with 5% Giemsa for 10 minutes. The samples were then observed under a light microscope, and the number of micronuclei was counted and recorded in 1,000 erythrocytes per slide. A total of 3,000 erythrocytes were randomly selected for analysis from each sample. For the MN assay micronuclei were identified according to the criteria established by Kirsch-Volders et al. [21].

2.3. Erythrocyte Nuclear Abnormality Assay

Blood samples were taken from preparations prepared for the MN assay. The other nuclear morphological alterations observed in erythrocytes were thoroughly examined using an Olympus® CX21 light microscope. Abnormalities in both cellular and nuclear structures were counted and visually documented. To evaluate nuclear deformations caused by environmental pollutants, the erythrocytes were categorized into specific morphological classes. These included segmented nuclei, kidney-shaped, lobed, notched nuclei, binucleated cells, and karyolysis. The classification criteria were based on the studies conducted by Canedo et al. [22] and Braham et al. [23].

2.4.Heavy Metals Analysis of Fish Tissue

After fish were anesthetized using clove oil, muscle tissue samples were taken using a plastic knife. Tissue samples were stored at -20ºC until analyzed and heavy metal analyses were carried out at DUBIT (Scientific and Technological Research and Application Center)

For heavy metal analysis, 0.45–0.60 g of muscle tissue was weighed and placed into microwave digestion vessels. Initially, 1 mL of H_2O_2 was added to Teflon containers and allowed to react for 1 minute. Subsequently, 6 mL of HNO_3 was added, and the vessels were sealed. The samples were then subjected to digestion for 20 minutes using a Milestones Microwave Digestion System. Following digestion, the samples were allowed to cool within the device for 10–15 minutes. The digested fish tissue samples were filtered through 45 µm pore-size filters into 50 mL falcon tubes and diluted to a final volume of 13 mL with deionized water. The concentrations of heavy metals in the tissue samples were then measured in triplicate using a ThermoScientific X Series ICP-MS instrument. Concentrations of heavy metals such as aluminum (Al), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), lead (Pb), cadmium (Cd), arsenic (As), nickel (Ni), cobalt (Co), chromium (Cr), mercury (Hg) were determined in muscle tissues.

2.5.Statistical Analysis

The frequency of micronucleus and other nuclear abnormalities in erythrocytes of *Scardinius erythrophthalmus* and *Perca fluviatilis* were analyzed with the Kruskall-Wallis test, one of the non-parametric tests, using SPSS 20.0 software.

3. RESULTS

This study was conducted to evaluate the presence of possible genotoxic effects on the aquatic ecosystem in Büyük Akgöl located in the lower part of Sakarya River Basin. Micronucleus (MN) test and erythrocyte nuclear abnormalities analyses were applied to determine the genotoxic effects of environmental pollution around the lake on fish. Data obtained from two different fish species, *Scardinius erythrophthalmus* and *Perca fluviatilis*, were used in the study. MN frequencies were determined as a result of microscopic examinations and the findings are presented in Figure 1.

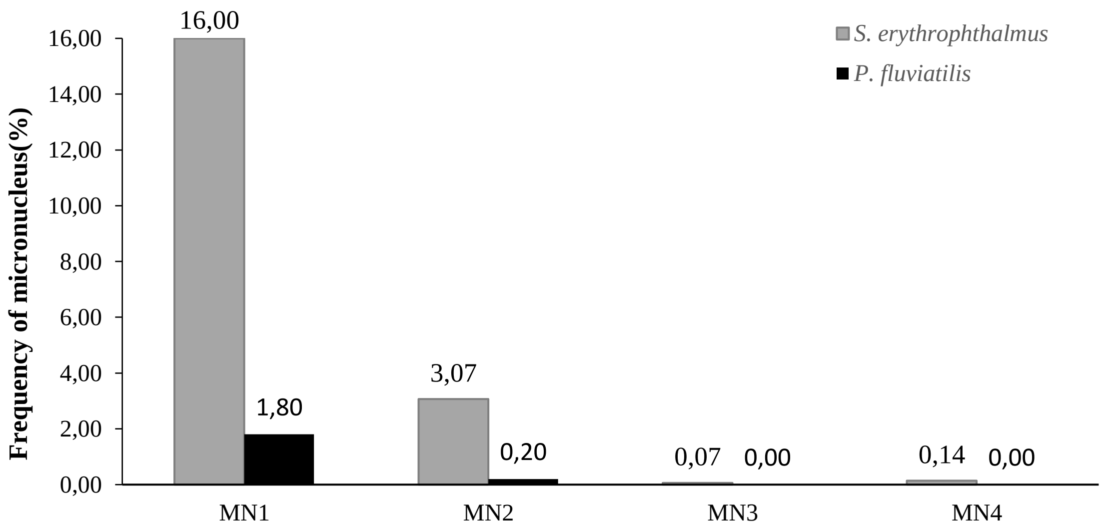

Figure 1. Frequency of different types of micronucleus abnormalities in *Scardinius erythrophthalmus* and *Perca fluviatilis*

According to the data obtained from the micronucleus test showed significant differences between the two fish species. While the frequency of one micronucleus cell (MN1) was detected at 16.00% in *S. erythrophthalmus* erythrocytes, the same form was observed at only 1.80% in *P. fluviatilis* erythrocytes. Similarly, the frequency of two micronucleus cells (MN2) was found at 3.07% in rudd, and this rate remained at 0.20% in perch erythrocytes. The frequencies of three and four micronucleus cells (MN3 and MN4) were detected only at 0.07% and 0.14% in *S. erythrophthalmus* individuals, respectively, while these abnormalities were not observed in *P. fluviatilis* individuals. These data suggested that *S. erythrophthalmus* species carried nuclear abnormalities at higher frequencies compared to *P. fluviatilis* in all micronucleus frequencies.

The data obtained from the erythrocyte nuclear abnormality test were analyzed with statistical methods and the data related to the results are presented in Figure 2. The highest rate in *P. fluviatilis* erythrocyte cells was observed as "kidney shaped" nucleus form with 1.60%; this form was not detected at all in *S. erythrophthalmus* erythrocyte cells. "Lobed nuclei" abnormality was detected in 0.13% in *S. erythrophthalmus* and 0.40% in *P. fluviatilis*. "Notched nuclei" type was only seen in 0.27% in *S. erythrophthalmus* and was not found in perch individuals. Similarly, "Segmented nuclei" form was detected in 0.20% in rudd and was not observed in *P. fluviatilis*. "Binucleated" cells were detected only in *S. erythrophthalmus* individuals at a rate of 0.13%.

As a result, while nuclear abnormalities such as "notched", "segmented" and "binucleated" were observed in *S. erythrophthalmus* individuals, these forms were not detected in *P. fluviatilis*; on the other hand, a high rate of "kidney shaped" form was observed, which is specific to *P. fluviatilis*.

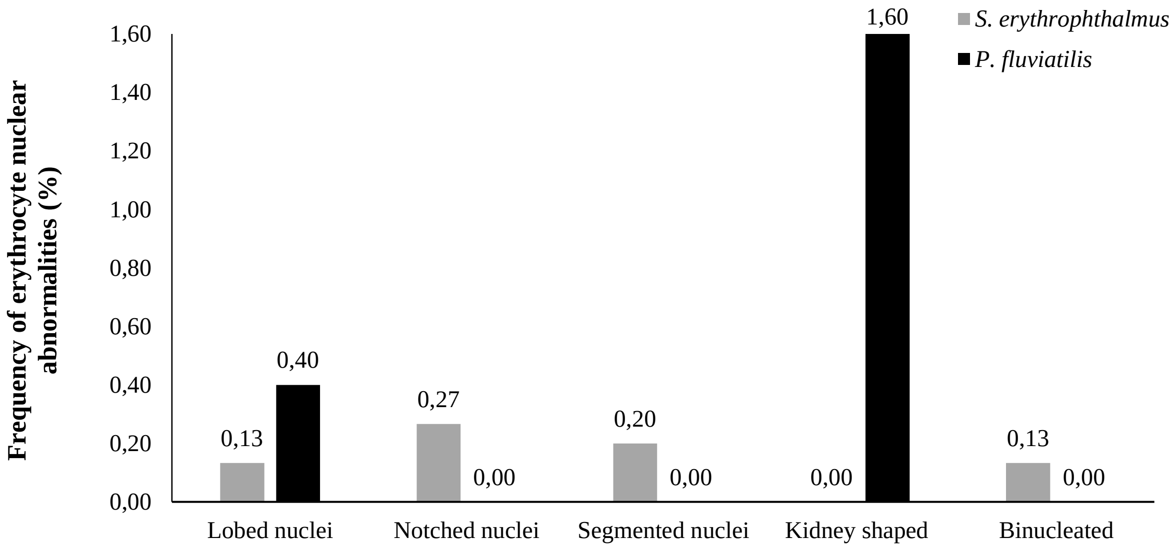

Figure 2. Frequency of erythrocytic nuclear abnormalities observed in *Scardinius erythrophthalmus* and *Perca fluviatilis*

The results of heavy metal analysis of muscle tissue are presented in Figure 3. As seen in the graph, the accumulation levels of some metals differ in both species. The highest concentration in *S. erythrophthalmus* muscle tissue was determined for zinc (Zn) with 6.03 mg/kg. This was followed by aluminum (Al) 3.47 mg/kg and iron (Fe) 2.39 mg/kg, respectively. On the other hand, zinc (Zn) showed the highest value in *P. fluviatilis* muscle tissue with 8.134 mg/kg, followed by copper (Cu) 5.002 mg/kg and iron (Fe) 2.334 mg/kg. These findings reveal that the heavy metals detected in muscle tissue accumulate at different rates according to species and that especially zinc, copper, lead, aluminum and iron elements were at remarkable levels.

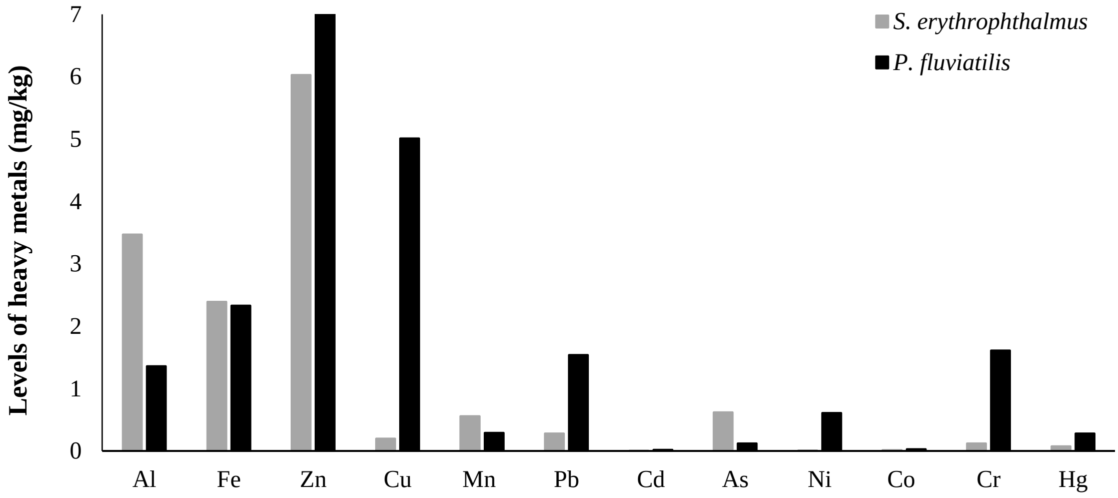

Figure 3. Concentrations (mg/kg) of heavy metals detected in the muscle tissues of *S. erythrophthalmus* and *P. fluviatilis*

4. DISCUSSION

In this study, the frequencies of nuclear abnormalities observed in erythrocytes of *S. erythrophthalmus* and *P. fluviatilis* species emerged as a significant biological indicator in assessing the potential genotoxic effects of environmental factors. The detection of nuclear abnormalities at varying frequencies in both species reveals species-specific differences in sensitivity to pollutants present in the aquatic environment. The combined use of nuclear abnormality analysis with micronucleus testing allows for a more comprehensive determination of the damage caused by environmental stress factors at the genetic level.

According to the micronucleus and erythrocyte nuclear abnormality analyses performed on peripheral blood cells taken from fish, it was revealed that the genotoxic effect levels between *S. erythrophthalmus* and *P. fluviatilis* species living in Büyük Akgöl differed significantly. As a result of the evaluation of micronucleus (MN) and nuclear abnormalities, it was understood that *S. erythrophthalmus* individuals were exposed to a higher level of genotoxic effect compared to *P. fluviatilis* individuals. The results of the present study were compared with other studies investigating the potential genotoxic effects of water pollution on *S. erythrophthalmus* and *P. fluviatilis* in rivers located in different countries.

Řehulka & Bradík, [8] conducted a study to evaluate the mutagenic effects of chemical substances on *P. fluviatilis* in three water reservoirs located in the Odra River basin in the Czech Republic and revealed that micronucleus and erythrocyte nuclear abnormalities showed differences in genotoxicity levels among species. Particularly, *P. fluviatilis* has a high MN frequency, which is consistent with the "kidney shaped" nuclear anomaly and general nuclear abnormality frequencies observed in *P. fluviatilis* individuals in our study, supporting that *P. fluviatilis* may be a potential biomarker against environmental stress factors. Biedunkova et al., [10] reported that nuclear anomalies observed in fish in the Desenka River were hidden below the spontaneous mutagenesis limit in all species. This situation indicates that genotoxic stress in the region is at a low level. Comparison rates between species show that *S. erythrophthalmus* has a low average nuclear abnormality (0.92±0.11‰), while *P. fluviatilis* species have a slightly higher average (1.69±0.29‰). Biedunkova et al., [10] suggest that this difference may be due to *S. erythrophthalmus* being more resistant to activity stress behaviors or lower exposure, while *P. fluviatilis* may be slightly more sensitive to discharge. In our study, it is seen that *P. fluviatilis* was more resistant to activity stress, while *S. erythrophthalmus* was more sensitive. Differences in diet, habitat use and ecological niche can be played consistently in this diversity [10]. Klimenko and Biedunkova [9] stated that *P. fluviatilis* living in small rivers showed higher nuclear damage and morphological deterioration compared to those living in medium-sized rivers. Bagdonas et al. [11] reported that *P.*

fluviatilis showed high sensitivity to micronucleus test. This situation is consistent with the detection of relatively high rates of specific abnormalities such as "kidney shaped" nucleus in *P. fluviatilis* individuals despite the low rates of general nuclear abnormalities in our study. These findings support that *P. fluviatilis* can be considered as a sensitive bioindicator species in biological monitoring of environmental genotoxic agents.

There are studies on heavy metal analysis in *S. erythrophthalmus* and *P. fluviatilis* muscle tissue. Tokatlı and Ustaoğlu [24] reported that estimated daily intake (EDI) values for Li, B, Cr, Co, Se, Tl, and Pb in edible muscles of *S. erythrophthalmus* and *P. fluviatilis* from the delta of Maritsa River (Türkiye) generally exceeded the permitted daily dose limit. Tokatlı et al. [25] stated that the levels of cadmium, lead, nickel, and chromium detected in muscle tissues of all fish, including *S. erythrophthalmus* and *P. fluviatilis* in Gala Lake and Meriç River (Türkiye), were well above human consumption limits. Çetin et al. [26] reported that heavy metals, especially Zn, Pb, and Cd, accumulated at high levels in *P. fluviatilis* in Altınyazı Reservoir; Pb reached 6.01 mg/kg and Cd reached 0.25 mg/kg. These findings indicate the presence of serious heavy metal pollution in the lake. Similarly, in this study conducted in Büyük Akgöl, it was observed that metals Zn was found at high levels in the analyses performed on *P. fluviatilis* and *S. erythrophthalmus* species, and toxic metals such as Cu (5.002 mg/kg), Fe (2.334 mg/kg) were prominent especially in *P. fluviatilis*. From this perspective, there are similarities in terms of different heavy metal accumulations in the studies. The accumulation of heavy metals in muscle tissue may increase depending on factors such as the feeding habits of the fish, the pollution level of the environment in which it lives and the chemical properties of the metal [27, 28]. In addition, the high accumulation of Zn and Al in *S. erythrophthalmus* reveals that heavy metal retention varies between species. In this study, micronucleus and erythrocyte nucleus abnormalities were determined in *S. erythrophthalmus* and *P. fluviatilis* erythrocytes. It is thought that these abnormalities may be due to the higher environmental pressure caused by local pollution sources (agricultural activities, pesticides, domestic waste, heavy metal accumulation) in Büyük Akgöl. In fact, in the study conducted by Durmaz [29] in Büyük Akgöl, water analyses conducted between 2016 and 2017 revealed that heavy metals such as arsenic, mercury, selenium and cadmium in the lake exceeded the regulation limits. The fact that the water is at the IV and V class quality level in most stations indicates that the lake ecosystem is exposed to serious pollutants. The pollution in question is based on sources such as intensive agricultural activities, arsenic-containing pesticide use, domestic waste discharges and atmospheric transport. In addition, uncontrolled fishing and direct discharge of lake water into the Sakarya River are also evaluated among other factors that increase the existing pollution load. Similarly, in the study of Aras and Arslan [30], the water quality of Büyük Akgöl was classified as "inappropriate" according to the water quality index (WQI). In the same study, it was reported that heavy metals such as Pb, Hg, Cd, Se and Al, as well as turbidity values, were above both WHO and national limits. Agricultural inputs, industrial wastes, pesticide use and pollutants carried by drainage waters come to the fore as pollution sources.

5. CONCLUSION

In this study, two different fish species (*Scardinius erythrophthalmus* and *Perca fluviatilis*) obtained from the Büyük Akgöl ecosystem were examined and heavy metal accumulation and nuclear abnormalities were evaluated. The findings obtained show that various heavy metals may accumulate in the muscle tissu in both species and this leads to genotoxic effects. In *P. fluviatilis* individuals, especially Zn, Cu, Fe and Cr accumulation was prominent; in *S. erythrophthalmus* individuals, Zn, Al and Fe accumulation was prominent. In micronucleus analyses, while the total micronucleus rate reached 19.07% in *S. erythrophthalmus* individuals, this rate was determined as 1.80% in *P. fluviatilis*. It is thought that these abnormalities may have occurred as a result of exposure to environmental pollutants such as heavy metals, pesticides and domestic waste rather than spontaneous mutations in the Büyük Akgöl environment.

Acknowledgments

Authors thanks to thank Havva ÖZER and Fatma DEMİR for their contributions to laboratory and field studies.

Funding

This study is supported by Duzce University Project Number: DÜBAP- 2023.05.01.1409.

The Declaration of Conflict of Interest/ Common Interest

No conflict of interest or common interest has been declared by the authors.

Authors' Contribution

The first author contributed 45%, the second author 35%, the third author 20%.

The Declaration of Ethics Committee Approval

Since the fish used in this study were supplied by fishermen, they did not require ethics committee approval or any special permit.

The Declaration of Research and Publication Ethics

The authors of the paper declare that they comply with the scientific, ethical and quotation rules of ETOXEC in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Environmental Toxicology and Ecology and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Environmental Toxicology and Ecology.

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