



UNIVERSITETI I PRISHTINËS

FAKULTETI I SHKENCAVE MATEMATIKE NATYRORE

Rr. Eqrem Çabej, 10000 Prishtinë, Republika e Kosovës
Tel: +381-38-249-873 • E-mail: fshmn@uni-pr.edu • www.uni-pr.edu

Ref. nr. 4467

Prishtinë, 20/10/2025

Për: Këshillin Mësimor të Departamentit të Biologjisë.

Këshillin e Studimeve të Doktoratës të FShMN-së.

Këshillin e Fakultetit të Shkencave Matematike Natyrore.

Nga: Msc. Alban Hyseni – kandidat në studime të doktoratës në programin Biologji e organizmave dhe ekologji, Departamenti i Biologjisë

Lënda: Kërkesë për formimin e komisionit për vlerësimin e dorëshkrimit të temës së doktoratës

Të nderuar anëtarë të Këshillit,

Në përputhje me Statutin e Universitetit të Prishtinës “Hasan Prishtina” dhe me Rregulloren në fuqi për studimet e doktoratës, ju njoftoj se kam përmbushur të gjitha kushtet e parapara për procedimin e mëtejshëm të dorëshkrimit të temës sime të doktoratës.

Duke u bazuar në këtë, parashtoj kërkesë që të formohet Komisioni për vlerësimin e dorëshkrimit të doktoratës me titull: *“Ndryshimet histopatologjike nga bioakumulimi i metaleve të rënda dhe ndikimet e tjera eventuale antropogjene te bretkosa Pelophylax ridibundus (Pallas, 1771) në Kosovë”*.

Kërkesës i bashkangjes:

1. Kopjen e dorëshkrimit
2. Punimin shkencor nga puna hulumtuese
3. Pëlqimin e mentorit për dorëzimin e dorëshkrimit
4. Dëshmitë e pjesëmarrjes në konferenca
5. Formularin F6.

Prishtinë: 20/10/2025

Kandidati: Alban Hyseni

PARAQITJA E PUNIMIT TË DOKTORATËS ¹	
TË DHËNAT E PËRGJITHSHME	
Doktoranti:	Msc. Alban Hyseni
Adresa:	Rr. Kerem Leton, Mati 1, Prishtinë
Tel./ fax:	+383 44 468 094
E-mail:	alban.hyseni@uni-pr.edu
Emërtimi i studimit:	Biologji e organizmave dhe Ekologji
Udhëheqësi i studimit:	Departamenti i Biologjisë, Fakulteti i Shkencave Matematike Natyrore, Universiteti i Prishtinës "Hasan Prishtina".
TË DHËNAT PËR PUNIMIN E DOKTORATËS	
Titulli në gjuhën shqipe	Ndryshimet histopatologjike nga bioakumulimi i metaleve të rënda dhe ndikimet tjera eventuale antropogjene te bretkosa <i>Pelophylax ridibundus</i> (Pallas, 1771) në Kosovë
Titulli në gjuhën angleze	Histopathological changes from heavy metals bioaccumulation and other possible anthropogeneous impacts in the frog <i>Pelophylax ridibundus</i> (Pallas, 1771) in Kosovo
Fusha e hulumtimit	Biologji e organizmave, Zoologji
DEKLARATA E MENTORIT/BASHKËMENTORIT	

1

UNIVERSITETI I PRISHTINËS "HASAN PRISHTINA"
FAKULTETI I SHKENCAVE MATEMATIKE-NATYRORE
PRISHTINË

shuar me: 20/10/2025			
Nj. org.	Numer	Sasia	Vlera
01	4471	3	

¹ Lutei që ta plotësoni formularin dhe ta dërgoni të nënshkruar me postë elektronike.

Mendim:

Pas përfundimit të fazës hulumtuese në terren dhe në laborator, si dhe pas përpunimit dhe analizës statistikore të rezultateve të arritura, kandidati Msc. Alban Hyseni ka përgatitur dorëshkrimin e temës së doktoratës, i cili përmban 7 kapituj kryesorë (Rezyme, Hyrje, Materiali dhe Metodologjia, Rezultatet, Diskutimi, Përfundimet dhe Literatura) dhe gjithsej 178 faqe, në përputhje të plotë me Rregulloren në fuqi të studimeve të doktoratës në Universitetin e Prishtinës "Hasan Prishtina". Bazuar në punën e kryer hulumtuese, rezultatet e arritura dhe publikimet shkencore të derivuara nga ky studim, vlerësoj se kandidati ka treguar përgatitje të lartë profesionale dhe shkencore. Prandaj, në cilësinë e mentores së temës së doktoratës, konsideroj se Msc. Alban Hyseni i plotëson kushtet për t'iu nënshtruar procesit të vlerësimit të dorëshkrimit të punimit të doktoratës.

Pjesë të hulumtimit të botuara në revista shkencore:

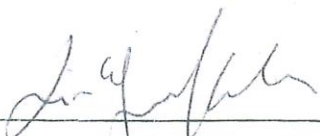
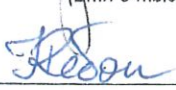
1. Hyseni, A., Bilalli, A., Grapci-Kotori, L., Kastrati, B., Berisha, A., Shala, B., Ristovska, M., Rebok. (2025). *Impact of Industrial Pollution and Genotoxic Effects in Marsh Frog (Pelophylax ridibundus (Pallas, 1771)) Populations in Kosovo*. Journal Polish of Environmental Study, 1–13. DOI: 10.15244/pjoes/207178.

Pjesë të hulumtimit të prezantuara në konferenca shkencore ndërkombëtare:

1. Hyseni, A., Grapci-Kotori, L., Rexhepi, A., Berisha, A., Kastrati, B., Rebok, K., Ristovska, M. (2024). *Micronucleus and nuclear abnormalities in Pelophylax ridibundus (Pallas, 1771); case study of industrially contaminated areas in Kosovo*. 4th European Amphibian Conference, Bordeaux, France.
2. Hyseni, A., Rebok, K., Ristovska, M., Haziri, A., Kastrati, B., Berisha, A., Shala, B., & Grapci-Kotori, L. (2024). *Pollution with heavy metals (Pb, Zn, Ni, Cd) in industrial areas in Kosovo and their impact on the population of the frog Pelophylax ridibundus (Pallas, 1771)*. VI International Agricultural, Biological & Life Science Conference, Edirne, Turkey.

Në përfundim, duke marrë në konsideratë cilësinë shkencore të punës së kryer, rëndësinë e rezultateve të arritura dhe kontributin e dhënë në fushën e biologjisë dhe ekologjisë së organizmave, propozoj formimin e Komisionit për Vlerësimin e Dorëshkrimit të Doktoratës së kandidatit Msc. Alban Hyseni, në përputhje me procedurat dhe dispozitat përkatëse të Universitetit të Prishtinës "Hasan Prishtina".

UNIVERSITETII PRISHTINËS "HASAN PRISHTINA"
F6- Paraqitja e punimit të doktoratës

Vendi, data dhe nënshkrimi	
Në Prishtinë, <u>20 / 10 / 2025</u>	Nënshkrimi <u></u> (Emri e mbiemri i mentorit)
	Nënshkrimi <u></u> (Emri e mbiemri i bashkëmentorit)



UNIVERSITETI I PRISHTINËS

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Ref. nr. 4468

Prishtinë, 20/10/2025

Për: Këshillin Mësimor të Departamentit të Biologjisë.

Këshillin e Studimeve të Doktoratës të FShMN-së.

Këshillin e Fakultetit të Shkencave Matematike Natyrore.

Lënda: Pëlqim për dorëzim të dorëshkrimit për vlerësim të temës së doktoratës të kandidatit Msc. Alban Hyseni me titull: “**Ndryshimet histopatologjike nga bioakumulimi i metaleve të rënda dhe ndikimet e tjera eventuale antropogjene te bretkosa *Pelophylax ridibundus* (Pallas, 1771) në Kosovë**”.

Mendim:

Pas përfundimit të fazës hulumtuese në terren dhe në laborator, si dhe pas përpunimit dhe analizës statistikore të rezultateve, kandidati Msc. Alban Hyseni ka përgatitur dorëshkrimin e temës së doktoratës në përputhje me kërkesat e Rregullores për studimet e doktoratës të Universitetit të Prishtinës “Hasan Prishtina”. Dorëshkrimi përmban gjithsej 7 kapituj (Rezume, Hyrje, Materiali dhe Metodologjia, Rezultatet, Diskutimi, Përfundimet dhe Literatura) dhe 178 faqe.

Në kuadër të këtij hulumtimi, kandidati ka arritur rezultate të rëndësishme shkencore, të cilat janë prezantuar dhe publikuar në revista dhe konferenca ndërkombëtare, siç vijon:

Pjesë të hulumtimit të botuara në formë të punimit shkencor:

1. Hyseni, A., Bilalli, A., Grapci-Kotori, L., Kastrati, B., Berisha, A., Shala, B., Ristovska, M., Rebok. (2025). Impact of Industrial Pollution and Genotoxic Effects in Marsh Frog (*Pelophylax ridibundus* (Pallas, 1771)) Populations in Kosovo. *Journal Polish of Environmental Study (Online First)*,1-13. DOI: [10.15244/pjoes/207178](https://doi.org/10.15244/pjoes/207178)

Pjesë të hulumtimit të botuara në formë të kumtesave ne konferenca shkencore:

1. Hyseni, A., Grapci-Kotori, L., Rexhepi, A., Berisha, A., Kastrati, B., Rebok, K., Ristovska, M. (2024). Micronucleus and nuclear abnormalities in *Pelophylax ridibundus* (Pallas, 1771); case study of industrially contaminated areas in Kosovo. 4th European Amphibian Conference, Bordeaux, France.
2. Hyseni, A., Rebok, K., Ristovska, M., Haziri, A., Kastrati, B., Berisha, A., Shala, B., & Grapci-Kotori, L. (2024). POLLUTION WITH HEAVY METALS (PB, ZN, NI, CD) IN INDUSTRIAL AREAS IN KOSOVO AND THEIR IMPACT ON THE POPULATION OF THE FROG PELOPHYLAX RIDIBUNDUS (PALLAS, 1771). VI. International Agricultural, Biological & Life Science Conference, Edirne, Turkey.

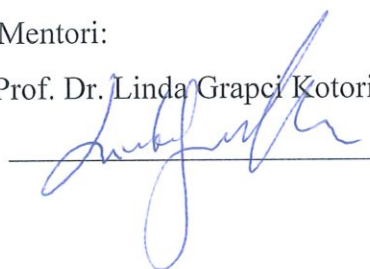
Bazuar në cilësinë e punës kërkimore, rezultatet e arritura dhe publikimet shkencore të realizuara gjatë studimeve, vlerësoj se kandidati Msc. Alban Hyseni i plotëson kushtet për procedimin e mëtejshëm të procesit të mbrojtjes së doktoratës.

Prandaj, propozoj formimin e Komisionit për Vlerësimin e Dorëshkrimit të Doktoratës së kandidatit, në përputhje me procedurat dhe dispozitat përkatëse të Rregullores së Studimeve të Doktoratës.

Prishtinë: 20 / 10 / 2025

Mentori:

Prof. Dr. Linda Grapci Kotori



- 99+
- Mail
- Compose
- Inbox
- Starred
- Snoozed
- Sent
- Drafts
- More

906

Proof of attendance EAC 2024 / External Inbox x



amphiclub2024 <amphiclub2024@sciencesconf.org>
to me

61

[amphiclub2024 : European Amphibian Conference 2024](#)
1-3 Jul 2024 BORDEAUX (France)

Labels

Object : Certificate of attendance to the 4th European Amphibian Conference and validation of continues training hours for ar

To whom it may concern,

As organiser of the 2024 European Amphibian Conference we herein acknowledge the attendance of Alban Hyseni to the 4th
During the course of this 3-day meeting, several platform presentations focused on animal welfare, animal ressources, protocols
presented.

As a guideline, these presentations covered 3,5 hours of the conference.

Yours Sincerely,

The organising committee



Alban Hyseni <alban.hyseni@uni-pr.edu>
to amphiclub2024

Dear

EAC 2024 Organizing Committee,

I hope you are well and work is going well. Please, is it possible for me to receive the certificate of participation in EAC 2024
conference. and to fulfill the condition for defense of the doctoral thesis.

Micronucleus and nuclear abnormalities in *Pelophylax ridibundus* (Pallas, 1771); case study of industrially contaminated areas in Kosovo

Alban Hyseni *¹, Linda Grapci - Kotori¹, Agim Rexhepi², Alban Berisha¹, Blearta Kastrati¹, Katerina Rebok³, Milica Ristovska³

¹ University of Prishtina "Hasan Prishtina", Faculty of Mathematics and Natural Science, Department of Biology – Str. "Mother Teresa" n.n. 10 000 Prishtinë, Kosovo

² University of Prishtina "Hasan Prishtina", Faculty of Agriculture and Veterinary, Department of Veterinary – Boulevard "Bill Clinton" n.n., Prishtinë, Kosovo

³ University "Ss. Cyril and Methodius" University in Skopje – Arkimedova 3, 1000 Skopje Republic of North Macedonia, Macedonia

Industrial pollution caused by the anthropogenic factor constitutes one of the extremely large global problems, directly affecting living beings' populations. Due to their life cycle, limited mobility, skin permeability, life in water and land, amphibians represent the animal group with very high sensitivity and they can be used as bioindicators of environmental pollution. Pollution effects on erythrocyte nuclear morphology in *Pelophylax ridibundus* individuals were investigated. Animals were collected from four polluted industrial sites and one control site in Kosovo: Kishnica, Obiliq, Mitrovica, Drenas (polluted sites) and the Dragash (nonpolluted sites) in spring-summer and autumn-winter season. Blood was taken directly from the heart, by puncture in the ventricular apex; blood smears were prepared according to the standard protocol, stained with May-Grünwald - Giemsa method and observed under a light microscope. The observed changes were: micronuclei, binuclei, vacuolated nuclei and irregularly shaped nuclei. Also, significant changes were found in the frequency of nuclear abnormalities between the research localities, as well as between the research seasons. The present study proves the practical usefulness of the frequency of micronucleation and nuclear abnormalities of *Pelophylax ridibundus* as important evaluative parameters for assessing the environmental health of ecosystems.

Keywords: *Pelophylax ridibundus*, Micronuclei, Nuclear abnormalities, Erythrocytes, Industrial pollution.

*Speaker



V. INTERNATIONAL AGRICULTURAL, BIOLOGICAL
& LIFE SCIENCE CONFERENCE
18-20 September, 2024, Edirne, Turkey



CERTIFICATE OF ATTENDANCE

Alban Hyseni

Organizing Committee of our conference acknowledges

With gratitude participation and contribution VI th International Conference on Agricultural, Biological and Life Science with your "Poster Presentation (Online)" titled "Pollution with heavy metals (Pb, Zn, Ni, Cd) in industrial areas in Kosovo and their impact on the population of Yajlaçın, Pazardag, Palophylax
Chair of Organizing Committee
ridibundus. (Pallas, 1771)"

**POLLUTION WITH HEAVY METALS (PB, ZN, NI, CD) IN INDUSTRIAL AREAS
IN KOSOVO AND THEIR IMPACT ON THE POPULATION OF THE FROG
PELOPHYLAX RIDIBUNDUS (PALLAS, 1771)**

Alban Hyseni^{1,}, Katerina Rebok², Milica Ristovska², Arben Haziri³, Blearta Kastrati⁴,
Alban Berisha⁵, Blendi Shala⁵ & Linda Grapci-Kotori⁶*

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³ Department of Chemistry University of Prishtina

⁴ Department of Biology University "Hasan Prishtina" Kosovo

⁵ Department of Biology University "Hasan Prishtina" Prishtine, Kosovo

⁶ Department of Biology University of Prishtina "Hasan Prishtina", Faculty of Mathematics
and Natural Sciences

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ABSTRACT

Industrial pollution caused by the anthropogenic factor constitutes one of the major global environmental problems. The content of heavy metals Pb, Zn, Ni and Cd in the natural habitats of populations of frogs *Pelophylax ridibundus* was investigated in this study. Water and soil samples were collected from four polluted industrial sites and one control site in Kosovo: Kishnica, Obiliq, Mitrovica, Drenas (polluted sites) and the Dragash-Brezne (control site) in spring- summer and autumn-winter seasons. The investigated areas are characterized by industrial mining activity (Kishnica, Mitrovica), electricity production from coal (Obiliq), and nickel processing (Drenas). Soil samples were dried at 105°C for 48 hours and treated with a mixture of 69% HNO₃ and HClcc in a 1:3 ratio, while the water samples were filtered. Soil samples were dissolved in the microwave digestion at 200°C for 45minutes. Water and soil samples were analyzed using a ISPE.

The results of the study show significancy concentration of heavy metals between the investigated habitats compared to the control site, types of metals, seasonal significance, and difference concentration in water and soil. A greater concentration of metals in the autumn season compared to the spring season has been determined. The values of cadmium in all localities were below the detection limit and did not show seasonal differences, while the concentration values of Pb, Zn, and Ni were higher in the polluted sites compared to the control site. Also, the values show high significance between the water and the substrate, which the concentration of heavy metals is higher in the substrate compared to the water samples.

This study proves the practical usefulness of investigating the types, sources and nature of pollutants in the natyral habitats (water and substrate) of *Pelophylax ridibundus* frogs as important parameters for assessing the investigating the types, sources and nature of environmental health of these ecosystems and the health of frog populations in these areas.

Key words: Key words: Environmental Monitoring, Amphibian Health, Metal Contamination, Seasonal Differences, Industrial Emissions

Original Research

Impact of Industrial Pollution and Genotoxic Effects in Marsh Frog (*Pelophylax ridibundus* (Pallas, 1771)) Populations in Kosovo

Alban Hyseni¹, Astrit Bilalli^{2*}, Linda Grapci-Kotori¹, Blearta Kastrati¹, Alban Berisha¹, Blendi Shala¹, Milica Ristovska³, Katerina Rebok³

¹Department of Biology, Faculty of Mathematics and Natural Science University of Prishtina "Hasan Prishtina", "Mother Teresa" str. n.n. 10 000 Prishtinë, Republic of Kosovo

²Faculty of Agribusiness, University of Peja "Haxhi Zeka", "UÇK" street, 30 000 Pejë, Republic of Kosovo

³Institute of Biology, Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University, Arhimedova 3, 1000 Skopje, Republic of North Macedonia

Received: 7 March 2025

Accepted: 15 June 2025

Abstract

Industrial pollution from anthropogenic activities is a critical global issue, significantly affecting biodiversity. Due to their unique life cycle, limited mobility, permeable skin, and dual habitat in aquatic and terrestrial environments, amphibians exhibit high sensitivity to environmental changes and serve as reliable bioindicators of pollution. This study assesses the impact of industrial pollution on *Pelophylax ridibundus* populations by examining erythrocyte nuclear morphology. Specimens were collected from four polluted industrial sites (Kishnica, Obiliq, Mitrovica, and Drenas) and one control site (Dragash) in Kosovo during the spring and autumn seasons of 2023. Blood samples were obtained via ventricular puncture, and smears were prepared following standard protocols, stained using the May-Grünwald-Giemsa method, and examined under a light microscope. The observed nuclear abnormalities included micronuclei, binuclei, vacuolated nuclei, and irregularly shaped nuclei. Significant variations were identified in the frequency of nuclear abnormalities between the studied locations and across seasons. The findings of this study highlight the practical utility of analyzing micronuclei and nuclear abnormalities in *P. ridibundus* as evaluative parameters for assessing the environmental health of ecosystems exposed to industrial pollution.

Keywords: bioindicators, nuclear abnormalities, environmental monitoring, industrial contamination, amphibian erythrocytes

UNIVERSITETI I PRISHTINËS "HASAN PRISHTINA"
FAKULTETI I SHKENCËVE MATEMATIKE-NATYRORE
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Pranuar me: 20/10/2025			
Nj. org.	Numer	Sasia	Vlera
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*e-mail: astrit.bilalli@unhz.eu

Introduction

Amphibian populations across the globe have experienced significant declines in both numbers and diversity since the 1960s, primarily due to the destruction of their natural habitats [1]. This alarming trend can be largely attributed to the escalating impact of anthropogenic activities on the environment, which has resulted in continuous alterations to the biosphere, an issue recognized as one of the most pressing global challenges of our time [2]. Among the various threats to amphibians, anthropogenic pollution, particularly stemming from agricultural and industrial practices, stands out as a critical concern [3]. Such pollution not only jeopardizes freshwater and marine ecosystems [4] but also poses significant risks to human health [5]. Addressing this multifaceted problem necessitates a comprehensive and integrated approach, employing modern methodologies for biological monitoring to assess and predict changes in natural ecosystems [6].

Environmental pollution in Kosovo presents a major threat to biodiversity, adversely affecting numerous ecosystems and species. From industrial emissions to agricultural runoff, a range of anthropogenic activities contribute to pollution in terrestrial and aquatic environments. The continuing reliance on coal-fired power plants, for instance, results in substantial emissions of particulate matter and heavy metals, adversely affecting both air and water quality. Agricultural practices further exacerbate the situation, as fertilizers and pesticides are commonly overused, leading to nutrient loading and contamination of water bodies. These environmental stressors not only threaten the health of numerous wildlife species, including amphibians, but also have broader implications for human health and ecosystem stability [7-10].

Heavy metals and their by-products represent a category of pollutants with profoundly detrimental effects on living organisms. The issue of heavy metal contamination in wetland ecosystems is a widespread environmental concern of global significance, primarily due to the persistence and bioaccumulation of these pollutants within food chains [11]. Unlike organic contaminants, heavy metals are non-biodegradable, leading to their accumulation in water, soil, sediments, and biota. The accumulation of heavy metals in organisms can result in a range of adverse effects, including cytogenotoxicity, immunotoxicity, hepatotoxicity, nephrotoxicity, and endocrine disruption [12-13].

Amphibians are particularly vulnerable to environmental pollutants compared to other vertebrate groups owing to several biological factors: their eggs lack protective shells, their life cycle is complex, and their skin is permeable [14-15]. Additionally, their limited mobility and intricate life cycles render amphibians among the most sensitive bioindicators of both aquatic and terrestrial pollution, as well as environmental changes [16-17]. Hematological assessments have long

been utilized to investigate the physiology and health of amphibian species, particularly in natural habitats [18]. Non-invasive techniques, such as blood biomarker analysis, have proven effective in revealing the mutagenic effects of pollution. Cytogenetic biomarkers, including nuclear abnormalities in erythrocytes, are essential for evaluating genotoxicity [19-21]. Among these biomarkers, the micronucleus test is favored for its simplicity and reliability in detecting toxicant-induced clastogenic effects [22-24]. This method is capable of assessing the early impacts of prolonged exposure to xenobiotics in both field and laboratory settings [25]. In recent decades, the frequency of micronuclei has been employed to indicate cytogenetic damage across various animal groups, including amphibians [26-28].

Research on the impact of anthropogenic pollution on amphibians in Kosovo is still in its nascent stages, yet it has begun to garner attention due to the alarming decline of these species in the region [29]. One significant study by Kittner et al. [30] highlights the health risks associated with trace metals found in lignite coal, which is prevalent in Kosovo's energy sector. The study underscores that the pollutants released from coal-fired power plants, including heavy metals, contribute to environmental degradation and pose serious health risks to both humans and wildlife, potentially including amphibians [30]. This study's primary objective was to investigate industrial pollution's effects on frog species populations of *Pelophylax ridibundus* in different seasons, sexes, and industrial hotspots within Kosovo. Specifically, this research aims to evaluate the health status of frogs inhabiting these polluted areas and to ascertain whether the pollutants released from industrial activities pose a risk of genotoxic damage in two different seasons (spring and autumn) and both sexes. This study assesses genotoxic effects in frogs from industrial pollution hotspots in Kosovo, focusing on nuclear abnormalities in erythrocytes and heavy metal concentrations in environmental samples. Correlation analyses explore associations between metal exposure and genotoxic markers, providing baseline data for environmental health assessment.

Materials and Methods

Study Area

This study was conducted in four industrial regions and one control site in Kosovo. The industrial sites Kishnica (S-1), Obiliq (S-2), Mitrovica (S-3), and Drenas (S-4) were selected for their significant industrial activities and pollution levels, while Dragash-Brezne (S-5) served as a pristine, uncontaminated control site (Fig. 1).

Industrial regions are directly affected by pollutants from nearby facilities, including heavy metals, ash

by-products from coal combustion, phenols, and other chemicals:

- S-1 (Kishnica) and S-3 (Mitrovica) are impacted by lead (Pb) and zinc (Zn) processing and mining activities.
- S-2 (Obiliq) is affected by lignite extraction and coal combustion.
- S-4 (Drenas) is influenced by the processing of iron (Fe) and nickel (Ni).
- S-5 (Dragash-Brezne) represents a clean, natural ecosystem.

These sites were chosen to represent a gradient of environmental conditions, ranging from heavily industrialized and polluted regions to a pristine natural habitat. This approach enables a comprehensive assessment of the impact of industrial pollutants on local ecosystems.

Water Samples and Analysis

Water samples were collected from the natural habitats of frogs. Five samples were taken from each locality at different points and stored in plastic bottles for laboratory analysis. The samples were filtered, standardized using Zeman's method, and analyzed for metal concentrations using an ICPE Shimadzu.

The analysis was performed with a Plasma Atomic Emission Spectrometer, Shimadzu, ICPE-9820, with ppb detection levels. To assess the health of frog habitats and determine the maximum permissible levels of heavy metals in water and soil, the analysis followed the Kosovo Standard (2009), EU standards, and German standards for water quality.

Soil Samples

Soil samples were taken from frog habitats near industrial sites. Five samples were collected per locality using a hand probe at a 15 cm depth, following Kluge & Wessolek [31] and ISO11466. Samples were sealed, dried at 105°C for 48 hours, and treated with a mixture of 69% HNO₃ and HClcc in a 2:6 ratio. The mixture was processed in a microwave at 200°C for 45 minutes, filtered, standardized to 50 ml with distilled water, and analyzed for metal concentrations using a Plasma Atomic Emission Spectrometer, Shimadzu, ICPE-9820.

Animal Samples

Fifteen marsh frogs for each locality (*Pelophylax ridibundus*; 8 ♂♂ and 7 ♀♀) were collected during May and October 2023, totaling 150 individuals. Frogs were

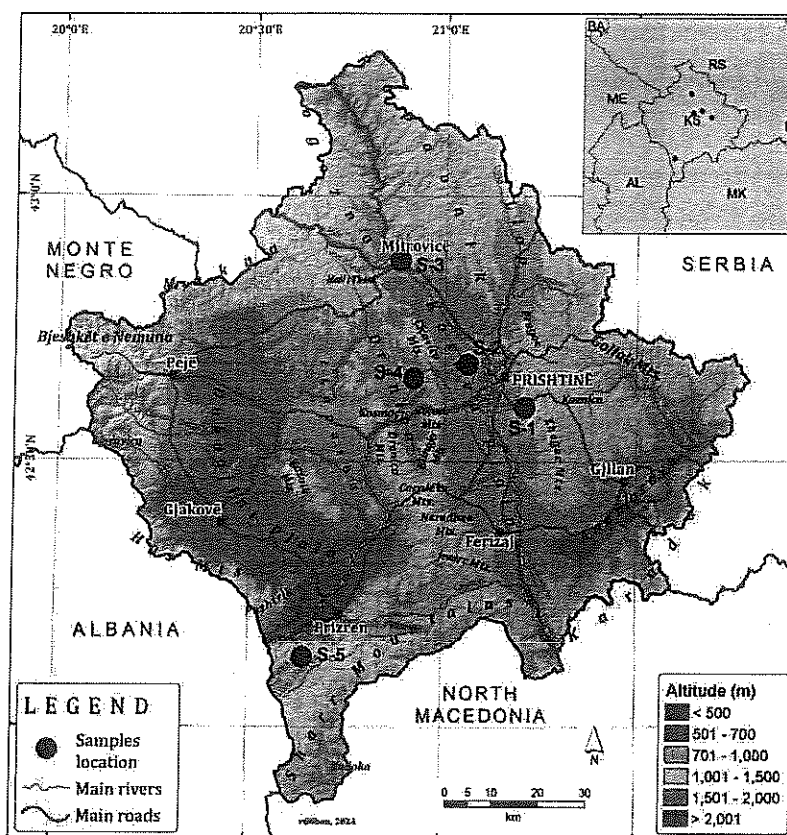


Fig. 1. Map of sampling stations in Kosovo.

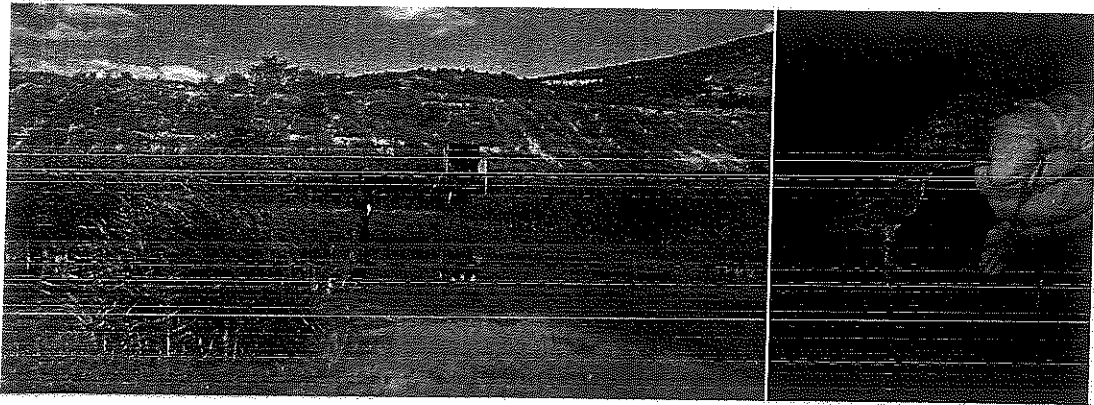


Fig. 2. Fieldwork - frogs captured at the sampling sites.

captured at night with artificial lights, transported in water-filled buckets, and processed in the laboratory. Weight, length, and secondary sexual characteristics were recorded for identification (Fig. 2).

Blood Samples

Hematological analyses were prepared immediately after the frogs were brought to the laboratory. Blood was drawn from the ventricular apex using heparinized syringes, with five smears prepared per frog. The smear preparations were dried at room temperature, fixed, and stained with the May-Grünwald-Giemsa method [32]. The slides were rinsed in distilled water three times for 1 to 3 minutes; Canadian balsam was added to the slides and covered with a cover glass. Approximately 2000-3000 erythrocytes for each preparation were examined for nuclear abnormalities (NAs), including micronuclei (MN), binuclei (BN), vacuolated nuclei (VN), and irregularly shaped nuclei (ISN), following Carrasco et al. [33] and Fenech [34]. Nuclei with small formations of nuclear material separated from the central nucleus were designated as micronuclei (MN); nuclei divided into two parts were designated as binuclei (BN); nuclei with the presence of holes in the nucleus were designated as vacuolated nuclei (VN); and nuclei with larger shapes and a number of anomalies were designated as irregularly shaped nuclei (ISN). Slides were analyzed with a Motic microscope with a 100x lens below immersion. Images were captured with a Motic camera and processed using ImageJ software [35].

Statistical Analyses

Statistical analyses were conducted using Statistica 7 software. ANOVA with Newman-Keuls post-hoc tests evaluated the effects of locality, sex, and season on the parameters investigated. The average frequency of nuclear abnormalities in 2000-3000 labeled erythrocytes, expressed per mile, was calculated for each individual using the following formula:

$$\text{Frequency of NAs}\% = \frac{\text{Number of cells containing NA}}{\text{Total number of labeled cells}} \times 1000$$

Spearman correlation analysis identified relationships between metal concentrations in water, soil, and frog blood parameters. Spearman's rank correlation coefficient (r_s) was calculated using the formula:

$$r_s = 1 - \frac{6 \sum d^2}{n(n^2 - 1)}$$

where: d represents the difference between the ranks of each pair of observations, and n is the number of observations. The significance of the correlation coefficients was determined using a p -value threshold of <0.05 , indicating statistical significance.

Results and Discussion

Water Chemical Analysis

Multiple water samples were read with a Schizmandu-type ICPL with ppb detection levels. The results of the chemical analysis for heavy metal concentrations in water are presented in Table 1. Based on the findings, the water samples from the investigated localities were found to be contaminated with Pb and Zn, while other elements such as Cd, Ni, Co, Mo, Cr, Cu, and As exhibited very low concentrations, all below the detection limit in both the spring and autumn seasons.

- Lead (Pb): The concentration of Pb exceeded the allowed critical limit only in the Kishnica locality during the autumn season. The concentration remained within the permitted limits in other localities in both seasons.
- Zinc (Zn): Zinc concentration exceeded the allowed critical limit only in the Drenas locality during the autumn season. In all other localities, the concentration remained within the permissible limits.

Table 1. Mean metal concentrations¹ of water samples collected from the sampling sites in two different seasons (spring-autumn).

Locality	S-1	S-2	S-3	S-4	S-5
Season	S-A	S-A	S-A	S-A	S-A
Metals					
Pb (mg/L ⁻¹)	0,030 – 0.502	0.104 – 0.036	0.09 – 0.32	0.04 – 0.04	0.003 – 0.043
Zn (mg/L ⁻¹)	0.5 – 7.85	4.38 – 0.12	15.4 – 21.5	106.1 – 225.8	0.2 – 0.11
Ni (mg/L ⁻¹)	nld - nld	nld - nld	nld - nld	nld - nld	nld - nld
Cd (mg/L ⁻¹)	nld - nld	nld - 22.2	nld - nld	nld - 35.5	nld - nld
Co (mg/L ⁻¹)	nld - nld	nld - nld	nld - nld	nld - nld	nld - nld
As (mg/L ⁻¹)	nld - nld	nld - nld	nld - nld	nld - nld	nld - nld
Mo (mg/L ⁻¹)	nld - nld	nld - nld	nld - nld	nld - nld	nld - nld
Cr (mg/L ⁻¹)	nld - nld	nld - nld	nld - nld	nld - nld	nld - nld
Cu (mg/L ⁻¹)	nld - nld	nld - nld	nld - nld	nld - nld	nld - nld

Results are presented as mean values. Localities are indexed with numbers (S-1, S-2, etc.), and seasons are marked as Spring and Autumn. "nld" indicates "not limit detected".

- Cadmium (Cd): In the autumn season, the Cd values in both Obiliq and Drenas were below the allowed critical limit (see Table 1).

Soil Chemical Analysis

The analysis of soil samples revealed contamination with several heavy metals, including Pb, Zn, Ni, Cd, Co, Cr, Cu, and As (Table 2).

- Lead (Pb): The concentration of Pb exceeded the allowed critical limit in the soil samples from Kishnica and Drenas during the autumn season and in Obiliq during the spring season.

- Zinc (Zn): Zinc concentrations were elevated in the soil samples from Kishnica and Obiliq in both seasons but did not exceed the allowed critical limit (Table 2).
- Cadmium (Cd): Cadmium levels were particularly high in the soil samples from Kishnica in the autumn and Drenas in both seasons, surpassing the allowed critical limits.
- Nickel (Ni): The concentration of Ni exceeded the allowed critical limit only in Kishnica during the autumn season and in Obiliq across both seasons.
- Chromium (Cr), Cobalt (Co), and Arsenic (As): The concentrations of these metals were found to be high across all investigated localities. However,

Table 2. Mean metal contents¹ of sediment samples collected from the sampling sites in two different seasons (spring-autumn).

Locality	S-1	S-2	S-3	S-4	S-5
Season	S-A	S-A	S-A	S-A	S-A
Metals					
Pb (mg/kg-1)	35.4 – 113.8	70.3 – 1.68	2.51 – 2.75	37.5 – 65.4	0.13 – 1.34
Zn (mg/kg-1)	146.6 – 232.3	103.9 – 109.0	8.97 – 33.7	65.5 – 73.6	5.5 – 6.8
Ni (mg/kg-1)	15.8 – 72.3	90.3 – 70.1	12.0 – 49.0	13.4 – 16.4	1.76 – 3.6
Cd (mg/kg-1)	0.45 – 5.28	nld – 1.6	nld – 1.49	9.2 – 10.4	nld - nld
Co (mg/kg-1)	793.6 – 1411.6	571.1 – 855.3	946.3 – 569.3	1035.5 – 1812.6	nld – 190.1
As (mg/kg-1)	461.7 – 520.9	3232.3 – 1228.4	973.0 – 1393.3	1900 – 395.5	nld - 224.2
Mo (mg/kg-1)	nld – 316.6	nld - nld	nld - nld	nld - nld	nld - nld
Cr (mg/kg-1)	2558.3 – 4337.4	4910.0 – 4150.0	1306.0 – 1512.7	1803.9 – 6893.6	129.8 – 234.0
Cu (mg/kg-1)	nld – nld	nld – nld	nld - nld	nld - nld	nld - nld

¹Results are presented as mean values. Localities are indexed with numbers (S-1, S-2, etc.), and seasons are marked as Spring and Autumn. "nld" indicates "not limit detected."

only in Kishnica during the autumn season did the concentration of these metals exceed the allowed critical limit for soil samples (Table 2).

Cytohematological Analysis

Various erythrocyte nuclear abnormalities were observed using an optical microscope, including MN, BN, VN, and ISN.

Tables 3, 4, 5, and 6 present the study's results on the frequency of MN and other NAs in frog populations from different localities. The findings reveal significant differences in the investigated parameters between localities and sexes, as well as notable seasonal variations.

Statistical analysis of the univariate test of significance ANOVA/MANOVA showed that the natural factors locality and season alone (or their interactions) have an impact on the frequency of MN, BN, and ISN; while for VN, in addition to the impact of locality and season alone or in interaction, the interaction between season and sex also impacts the frequency of VN.

MN showed the lowest values for this parameter in the control site, in both seasons and for both sexes. In spring, these values are statistically different from those obtained in males from Drenas and females from Drenas and Mitrovica. The statistical difference is stronger and more noticeable in the autumn, though, and practically every site differs from the other for both sexes (Table 3; Fig. 3a).

Regarding BN in spring, only one statistical difference was observed between males from the control site and Mitrovica, while in autumn, the low values for this parameter observed in Drenas and Dragash differ significantly from the other sites in both sexes (Table 4; Fig. 3b).

With the exception of Mitrovica in males and Obiliq in females, the low VN values observed in the control

site during the spring differ significantly from those found in the polluted sites. Furthermore, males from Mitrovica showed significantly different values from those from Obiliq and Kishnica, while females from Drenas differed significantly from all other sites. In autumn, the highest values were recorded in both sexes from Drenas, which are significantly different from all other sites (Table 5; Fig. 3c).

Kishnica had the highest ISN values among males in the spring, which was significantly different from Obiliq's and Dragash's values. In the spring, females from Obiliq showed a significant difference from those from Kishnica, while the lowest values found in Dragash were significantly different from all polluted sites except Obiliq. In the autumn, males from Dragash, Drenas, and Mitrovica, as well as females from Dragash and Drenas, varied greatly from one another and from the other sites (Table 6; Fig. 3d).

In general, all the parameters studied showed seasonal significance. The frequency of MN, BN, and ISN was lower in spring compared to autumn, with the exception of VN, which showed a higher frequency of changes in spring. Significant seasonal differences in the frequency of MN were recorded in the localities of Kishnica, Obiliq, and Drenas for both sexes and female frogs from Mitrovica (Table 3; Fig. 3a), while for BN, only the locality of Kishnica showed significant seasonal differences for both sexes (Table 4; Fig. 3b). Regarding VN, significant seasonal differences were observed for males from Kishnica and Obiliq and females from Mitrovica (Table 5; Fig. 3c). Furthermore, ISN showed significant seasonal differences for both sexes in the localities of Drenas, Mitrovica, and Obiliq (Table 6; Fig. 3d).

The only significant difference between sexes was noticed for the frequency of VN between males and females from Obiliq in spring and Mitrovica in both seasons (Table 5; Fig. 3c).

Table 3. MN¹ (%) from investigated localities. The data are shown for both sexes separately in two sampling seasons: spring and autumn.

Season	MN (%)			
	Spring		Autumn	
Sex	Male	Female	Male	Female
Locality				
Obiliq ^a	2,70 (0,8)*abcde	2,19 (0,6)*abce	9,73 (2,5)*s	7,85 (1,4)*sc
Kishnica ^b	3,72 (1,3)*abcde	3,46 (2,6)*abcde	15,44 (2,1)*b	14,45 (1,7)*b
Mitrovica ^c	3,36 (1,2)*bcde	4,04 (1,5)*abcd	6,41 (1,9) ^c	7,68 (1,0)*sc
Drenas ^d	5,87 (0,9)*abcd	5,46 (1,6)*bcd	9,15 (2,7)*acd	11,23 (2,4)*d
Dragash ^e	0,79 (0,8)*bce	0,44 (0,3)*bc	0,71 (0,4) ^c	0,79 (0,2) ^c

¹Values are expressed as mean (standard deviation). Each locality was marked with a corresponding lowercase letter. Different lowercase letters in the same columns represent differences between sampling sites, i.e., if the letters are present at all sites, then there are no significant differences between them, while when a letter is missing from any of the sites, then that site is significantly different from the others. Stars represent differences between frogs of the same sex between different seasons within the sampling site, according to ANOVA, followed by the Newman-Keuls test.

Table 4. BN¹ (%) from investigated localities. The data are shown for both sexes separately in two sampling seasons: spring and autumn.

Season	BN (%)			
	Spring		Autumn	
	male	female	male	female
Obiliq ^a	3,09 (1,2) ^{abcde}	2,26 (0,5) ^{abcde}	4,45 (2,3) ^{ac}	4,53 (2,0) ^{abc}
Kishnica ^b	1,55 (0,8) ^{*abcde}	1,58 (0,5) ^{*abcde}	7,38 (2,2) ^{*bc}	5,38 (0,9) ^{*abc}
Mitrovica ^c	3,91 (1,6) ^{abcd}	2,93 (0,7) ^{abcde}	6,41 (2,0) ^{abc}	5,58 (4,2) ^{abc}
Drenas ^d	3,36 (0,6) ^{abcde}	3,16 (1,6) ^{abcde}	1,11 (0,6) ^{de}	1,03 (0,4) ^{de}
Dragash ^e	0,45 (0,9) ^{*bde}	0,09 (0,1) ^{abcde}	0,55 (0,3) ^{de}	0,43 (0,2) ^{de}

¹Values are expressed as mean (standard deviation). Each locality was marked with a corresponding lowercase letter. Different lowercase letters in the same columns represent differences between sampling sites, i.e., if the letters are present at all sites, then there are no significant differences between them, while when a letter is missing from any of the sites, then that site is significantly different from the others. Stars represent differences between frogs of the same sex between different seasons within the sampling site, according to ANOVA, followed by the Newman-Keuls test.

Table 5. VN¹ (%) from investigated localities. The data are shown for both sexes separately in two sampling seasons: spring and autumn.

Season	VN (%)			
	Spring		Autumn	
	male	female	male	female
Obiliq ^a	70,11 (45,4) ^{A*abcd}	29,08 (19,4) ^{Babcc}	5,28 (1,5) ^{*abcc}	6,94 (0,8) ^{abco}
Kishnica ^b	68,81 (17,3) ^{*abd}	47,85 (11,9) ^{abc}	24,11 (12,3) ^{*abcde}	28,26 (17,5) ^{abcc}
Mitrovica ^c	32,12 (19,0) ^{Acd}	60,05 (11,8) ^{B*abc}	10,27 (2,0) ^{Aabcc}	11,59 (4,73) ^{B*abcc}
Drenas ^d	57,29 (13,1) ^{abcd}	103,14 (43,5) ^d	53,26 (31,6) ^{bd}	95,49 (8,9) ^d
Dragash ^e	0,72 (0,5) ^{cc}	0,71 (0,3) ^{cc}	6,87 (1,2) ^{abcc}	6,43 (0,8) ^{abcc}

¹Values are expressed as mean (standard deviation). Each locality was marked with a corresponding lowercase letter. Different lowercase letters in the same columns represent differences between sampling sites, i.e., if the letters are present at all sites, then there is no significance between them, while when a letter is missing from any of the sites, then that site is significantly different from the others. Stars represent differences between frogs of the same sex between different seasons within the sampling site, while capital letters represent differences between sexes of a site in the same season, according to ANOVA, followed by the Newman-Keuls test.

The curves in Fig. 3 (a-d) represent the analysis of micronuclei (MN), binucleated cells (BN), vacuolated nuclei (VN), and irregularly shaped nuclei (ISN) frequencies from samples collected at different sites (Obiliq, Kishnica, Mitrovica, Drenas, and Dragash) during spring and autumn, categorized by gender (male and female). Mean values were calculated, and 95% confidence intervals were added as error bars.

Correlation Analysis

Statistical analysis of linear correlations was conducted to examine the relationships between the mean values of the blood parameters and the mean values of metal concentrations in water and metal content in soil. Correlation analysis was performed based on localities and seasons. The data, including

correlation coefficients and significance levels, are presented in Table 7.

Correlation findings:

- MN showed a strong positive correlation with As, Co, Cd, Cr, Pb, and Zn content in sediment.
- BN was positively correlated with Pb and Ni content in sediment.
- ISN was positively correlated with As, Co, Cd, and Pb content in sediment.

These results indicate that higher concentrations of specific heavy metals in sediment are associated with increased frequencies of nuclear abnormalities in the erythrocytes of *Pelophylax ridibundus*.

The analysis of the results obtained from this study indicates the presence of micronuclei (MN) and other nuclear abnormalities (NAs) in erythrocytes of the marsh frog (*Pelophylax ridibundus*) from Kosovo (Fig. 4).

Table 6. ISN¹ (%) from investigated localities. The data are shown for both sexes separately in two sampling seasons: spring and autumn.

Season	Spring		Autumn	
	male	female	male	female
Obiliq ^a	58,10 (14,0)* ^{acde}	52,19 (6,3)* ^{acde}	136,24 (31,0)* ^{ab}	144,09 (8,3)* ^{abc}
Kishnica ^b	123,64 (15,8) ^{bcd}	113,32 (21,9) ^{bcd}	140,91 (26,6) ^{ab}	148,20 (20,6) ^{abc}
Mitrovica ^c	93,77 (26,9)* ^{abcde}	96,77 (8,5)* ^{abcd}	194,51 (42,0)* ^c	194,63 (0,9)* ^{abc}
Drenas ^d	116,76 (14,5)* ^{abcde}	102,23 (53,11)* ^{abcd}	278,01 (30,7)* ^d	290,33 (10,7)* ^d
Dragash ^e	55,98 (74,7)* ^{acde}	33,34 (1,61) ^{ac}	46,84 (3,3)*	47,64 (3,2)*

¹Values are expressed as mean (standard deviation). Each locality was marked with a corresponding lowercase letter. Different lowercase letters in the same columns represent differences between sampling sites, i.e., if the letters are present at all sites, then there is no significance between them, while when a letter is missing from any of the sites, then that site is significantly different from the others. Stars represent differences between frogs of the same sex between different seasons within the sampling site, according to ANOVA, followed by the Newman-Keuls test.

These findings align with the significant heavy metal contamination in the studied areas, particularly from industrial pollution. The chemical analysis of water and soil samples revealed elevated levels of heavy metals such as Pb, Ni, Zn, Cd, Cr, Mn, and Co, sometimes exceeding the critical limits for water quality [36-38].

Notably, elevated concentrations of Pb were found in Kishnica and Drenas during autumn and Obiliq during spring, which is consistent with heavy industrial activities in these regions. Similar seasonal variations in Zn concentrations were observed, with peak levels in Drenas and Kishnica, corroborating findings by Zhelev

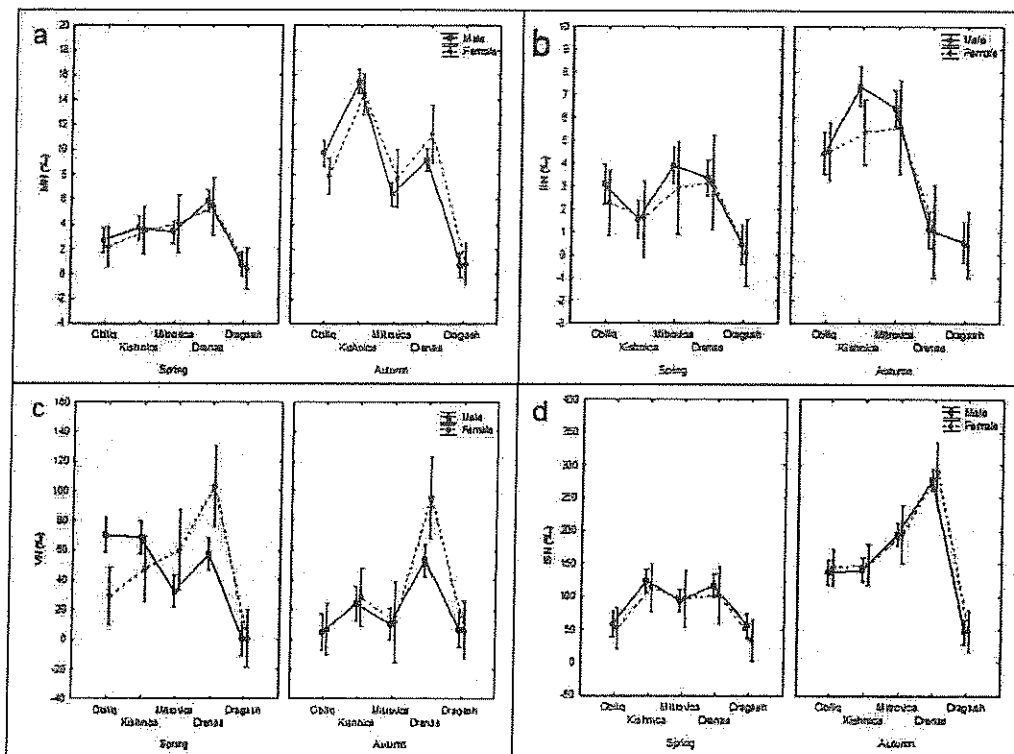


Fig. 3. Comparative analysis of frequencies a) micronuclei – MN; b) binuclei – BN; c) vacuolated nuclei – VN; d) irregularly shaped nuclei – ISN, from polluted and referent sites.

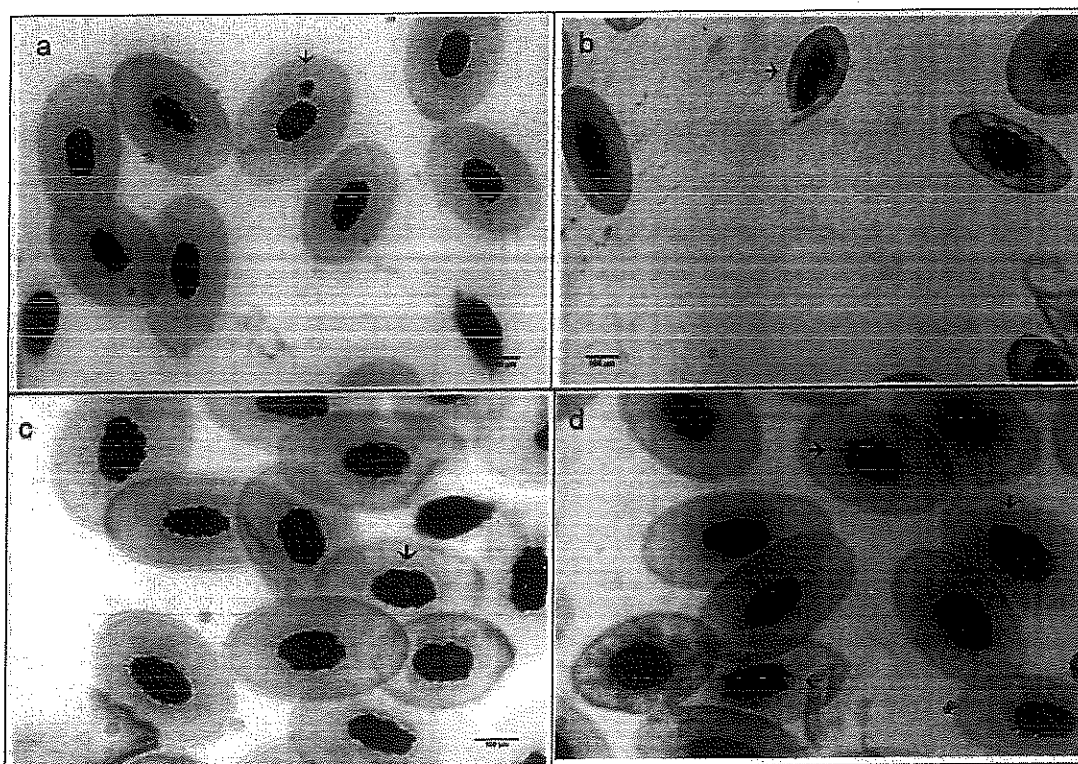


Fig. 4. Light micrograph showing different types of NAs in erythrocytes of marsh frog *Pelophylax ridibundus*: a) micronuclei (MN); b) binuclei (BN); c) vacuolated nuclei (VN); d) irregularly shaped nuclei (ISN).

Table 7. Results from correlation analyses¹ between blood parameters in marsh frog *Pelophylax ridibundus* vs. metal analyses from the water and the sediment.

Variables	Sperman's RhO	p value
Sediment		
MN (‰) vs As (mg/kg ⁻¹)	+ 0.63	<0.05
MN (‰) vs Co (mg/kg ⁻¹)	+ 0.92	<0.001
MN (‰) vs Cd (mg/kg ⁻¹)	+ 0.86	<0.001
MN (‰) vs Cr (mg/kg ⁻¹)	+ 0.67	<0.05
MN (‰) vs Pb (mg/kg ⁻¹)	+ 0.95	<0.001
MN (‰) vs Zn (mg/kg ⁻¹)	+ 0.68	<0.05
BN (‰) vs Pb (mg/kg ⁻¹)	+ 0.63	<0.05
BN (‰) vs Ni (mg/kg ⁻¹)	+ 0.63	<0.05
ISN (‰) vs As (mg/kg ⁻¹)	+ 0.69	<0.05
ISN (‰) vs Co (mg/kg ⁻¹)	+ 0.87	<0.001
ISN (‰) vs Cd (mg/kg ⁻¹)	+ 0.80	<0.01
ISN (‰) vs Pb (mg/kg ⁻¹)	+ 0.79	<0.01

¹Only significant correlations are presented

et al. [39-41] and Dönmez & Şişman et al. [42] regarding heavy metal bioaccumulation and erythrocyte size variations in *Pelophylax ridibundus* inhabiting polluted areas.

Cadmium (Cd) concentrations were notably high in water samples from Obiliq and Drenas during autumn, while soil samples from Kishnica and Drenas also exhibited elevated Cd levels. This aligns with previous research by Cruz-Santiago et al. [43] and Romanova et al. [44], indicating that Cd exposure is a major factor in genotoxic stress in amphibians. Similarly, Ni concentrations, although below detection limits in water, were significantly higher in soil samples, particularly in Kishnica and Obiliq, consistent with findings from previous studies [45-48]. The presence of Cr, Co, and As across all localities further confirms widespread contamination associated with industrial emissions, a phenomenon also reported by Alnoaimi et al. [49] and Patar et al. [50].

A clear seasonal relationship was observed between metal concentrations and nuclear abnormalities in erythrocytes of *Pelophylax ridibundus*. The increase in heavy metal levels during autumn corresponded with significantly higher MN, BN, and ISN values, with Kishnica being the most affected site, followed by Obiliq and Drenas. This pattern is consistent with the findings of Jayawardena et al. [51], who reported that seasonal

variations in industrial pollutants influence genotoxic responses in amphibians. Interestingly, VN values were higher in spring, particularly in males from Kishnica and Obiliq, highlighting potential sex-specific responses to contamination, although no significant overall difference was observed between sexes, as previously noted by Pollo et al. [52] and Zhelev et al. [39-41].

The observed nuclear abnormalities in *Pelophylax ridibundus* agree with previous studies demonstrating the genotoxic effects of industrial pollutants on amphibian populations. Increased frequencies of MN and BN cells have been directly linked to exposure to heavy metals, particularly Cd, as highlighted in research by Özgül et al. [53] and Corredor-Santamaria et al. [54]. Furthermore, Chew et al. [55] emphasized the influence of water quality on amphibian health, reinforcing the necessity for ongoing monitoring of contaminated sites.

It is crucial to consider additional environmental stressors that contribute to nuclear abnormalities, including temperature fluctuations, oxygen availability, and food sources, as discussed by Huang and Peng [56]. Moreover, agricultural pollutants and municipal wastewater discharges likely exacerbate the genotoxic effects observed in this study, as pesticide contamination has also been linked to increased nuclear anomalies [57].

This research highlights that the studied areas are under ongoing pressure from industrial pollution. Persistent contamination could threaten local wildlife without effective mitigation strategies, potentially resulting in species decline or extinction. The study emphasizes the urgent need for environmental policies focused on reducing heavy metal emissions and improving habitat conditions for amphibian populations. Nuclear abnormalities in *Pelophylax ridibundus* from industrially polluted sites in Kosovo highlight the genotoxic impact of heavy metals and the genetic instability they induce. These findings align with previous studies emphasizing the ecological risks of industrial pollution and reinforce the importance of regular biomonitoring to detect early environmental health risks and mitigate potential long-term ecological consequences [58-60].

Conclusion

This study successfully achieved its goal of elucidating the impact of industrial pollution on the frequency of nuclear abnormalities (NAs) in the erythrocytes of *Pelophylax ridibundus*, filling a critical gap in the understanding of amphibian health in polluted aquatic environments in Kosovo. The findings revealed a significant increase in specific NAs, including binucleated and vacuolated and irregularly shaped nuclei, as well as micronuclei, in regions exposed to industrial pollutants compared to the control site. These results underscore the role of *Pelophylax ridibundus* as an effective bioindicator species, highlighting the particular sensitivity of amphibians to chemical pollutants.

Moreover, this study adds to the growing body of literature demonstrating heavy metal's cytotoxic and genotoxic effects on amphibian erythrocytes, reinforcing the significant risks these contaminants pose to amphibian populations. The observed increase in nuclear abnormalities serves as a clear indicator of industrial pollution's detrimental effects on amphibian health, aligning with previous research documenting similar impacts on various aquatic species [61-62].

The implications of this research extend beyond immediate findings, opening avenues for further studies on the expanded use of amphibians in biomonitoring programs. Such initiatives could deepen our understanding of the long-term consequences of industrial contamination on ecosystem health and species conservation. This study emphasizes the necessity for comprehensive monitoring of amphibian populations in polluted environments, as these organisms are not only integral to their ecosystems but also serve as critical indicators of environmental quality. Future research should explore the cumulative effects of multiple pollutants and potential synergistic interactions that may exacerbate genotoxic damage.

Ultimately, this study not only confirms the adverse effects of industrial pollution on *Pelophylax ridibundus* but also underscores the urgent need for continued monitoring and conservation efforts to mitigate the escalating threats posed by environmental contaminants.

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Conflict of Interest

The authors declare no conflict of interest.

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FAKULTETI I SHKENCAVE MATEMATIKE- NATYRORE

DEPARTAMENTI I BIOLOGJISË



Alban A. HYSENI

NDRYSHIMET HISTOPATOLOGJIKE NGA BIOAKUMULIMI I METALEVE
TË RËNDA DHE NDIKIMET E TJERA EVENTUALE ANTROPOGJENE TE
BRETKOSA *PELOPHYLAX RIDIBUNDUS* (Pallas, 1771) NË KOSOVË

PUNIMI I DOKTORATËS

Mentor

Prof. Dr. Linda GRAPCI - KOTORI

Komentor

Prof. Dr. Katerina Rebok

Prishtinë, 2025

UNIVERSITY OF PRISHTINA “HASAN PRISHTINA”
FACULTY OF MATHEMATICAL AND NATURAL SCIENCES
DEPARTMENT OF BIOLOGY



Alban A. HYSENI

**HISTOPATHOLOGICAL CHANGES FROM HEAVY METALS
BIOACCUMULATION AND OTHER POSSIBLE ANTHROPOGENEOUS
IMPACTS IN THE FROGS *PELOPHYLAX RIDIBUNDUS* (Pallas, 1771) IN
KOSOVO**

PhD THESIS

Mentor

Prof. Dr. Linda GRAPCI - KOTORI

Comentor

Prof. Dr. Katerina Rebok

Punimi i Doktoratës me titull “Ndryshimet histopatologjike nga bioakumulimi i metaleve të rënda dhe ndikimet e tjera eventuale antropogjene te bretkosa *Pelophylax ridibundus* (Pallas, 1771) në Kosovë”, është udhëhequr nga Prof. Dr. Linda Grapci Kotori nga Departamenti i Biologjisë, Fakulteti i Shkencave Matematike – Natyrore, Universiteti i Prishtinës “Hasan Prishtina”, Kosovë dhe nga Prof. Dr. Katerina Rebok nga Instituti i Zoologjisë, Fakulteti i Shkencave Matematike – Natyrore, Universiteti i Shkupit “Cyril dhe Methodius”, Republika e Maqedonisë Veriore.

Ky punim është realizuar në kuadër të plan – programit të studimeve të Doktoratës, në drejtimin Biologji e organizmave dhe Ekologji, në Departamentin e Biologjisë, Fakultetin e Shkencave Matematike Natyrore, në Universitetin e Prishtinës “Hasan Prishtina”. Hulumitmi është punuar në Insitutin e Zoologjisë në Fakultetin e Shkencave Matematike Natyrore në Shkup, laboratorin e Zoologjisë në Departamentin e Biologjisë në Prishtinë, në Insitutin e Patologjisë Klinike në Fakultetin e Mjekësisë në Prishtinë dhe në, si dhe në laboratorin e analizave të dheut në Fakultetin e Bujqësisë dhe Veterinës në Prishtinë.

Rezyme

Ky punim i doktoratës është hulumtimi i parë i këtij lloji në Republikën e Kosovës, për amfibet si tregues biologjik të ndotjes së mjedisit. Në këtë punim janë paraqitur të dhëna origjinale dhe të detajuara të ndikimit të ndotjes industriale si rezultat i aktivitetit antropogjen në parametrat e konditiv, histologjik, imunologjik dhe citogjenotoksik te bretkosa *Pelophylax ridibundus* (Pallas, 1771), si dhe në përqëndrimi i metaleve të rënda në ujë dhe përmbajtja e tyre në tokë (dhe), në 5 lokalitete studimore në Republikën e Kosovës. Materiali studimor i mbledhur për këtë punim përfaqëson 4 zona apo hotspote të ndotjes industriale (Kiahnica S – 1, Obiliqi S – 2, Mitrovica S – 3, Drenasi S – 4), dhe 1 zonë të konsideruar zonë të pastërt e cila shërbeu si lokalitet kontroll (Dragashi S – 5).

Mostrat e ujit, mostrat e dheut (nga 5 mostra për secilin lokalitet dhe për secilen sezonë, në total 50 mostra të ujit dhe 50 mostra të dheut), dhe materiali biologjik (nga 15 individ për secilin lokalitet dhe për secilën sezonë, në total 150 individ), është mbledhur në vitin 2023 në muajt Maj dhe Tetor (dy sezona të hulumtimit). Materiali biologjik është përfaqësuar nga individ mashkuj dhe femra për të krahasuar ndikimin e ndotjes në dy gjinit e ndryshme. Lokalitetet e hulumtimit, 4 prej tyre (Kishnica, Obiliqi, Mitrovica dhe Drenasi), përfaqësojnë zona më aktivitet të ndryshëm industrial, dhe 1 zonë kontrolluese (Dragashi), e konsideruar zonë e pastërt nga aktiviteti industrial antropogjen. Janë kryer analiza të ndryshme statistikore për të analizuar ndryshimet në mes të lokaliteteve hulumtuese, sezonave dhe gjinive, si dhe korelacionin e ndryshimeve me metalet e gjetura në habitatet e bretkosave.

Qëllimi i këtij hulumtimi ishte studimi i efekteve negative të ndotjes me metale të rënda dhe aktiviteve të tjera antropogjene në parametrat konditiv, histopatologjik, imunologjik dhe citogjenotoksik te bretkosa *Pelophylax ridibundus* në Kosovë, si dhe përcaktimi i përqëndrimit të metaleve të rënda në ujë dhe përcaktimi i përmbajtjes së metaleve të rënda në mostrat e dheut të mbledhura nga habitatet natyrore të bretkosave. Me qëllim që të kemi rezultatet kredibile shkencore lokalitetet e përzgjedhura për këtë hulumtim përfaqësojnë hotspote të ndotjes nga ndotës të ndryshëm industrial, ndërsa për shkak të veçorive specifike biologjike (mobiliteti i ulët, cikli kompleks jetësor, permeabiliteti i lëkurës etj), si organizëm hulumtues është përzgjedh bretkosa e kënetave *Pelopylax ridibundus*.

Rezultat i këtij studimi është konstatimi i ndotjes më metale të rënda në këto lokalitete dhe konstatimi i efekteve negative të ndotjes së mjedisit në nivel të gjendjes së populacionit, në nivel histopatologjik, imunologjik dhe citogjenotoksik. Po ashtu, rezultatet nga ky studim japin një pasqyrë të qartë të vlefshmërisë së përdorimit të bretkosës së kënetës *Pelophylax ridibundus*, si tregues biologjik të ndotjes së mjedisit. Është regjistruar vlerë e lartë e metaleve të rënda në ujë dhe tokë, me nivel më të lartë të përmbajtjes së metaleve në mostrat e dheut, krahasuar me përqëndrimine metaleve në ujë. Po ashtu, koncentrimi dhe përmbajtja e metaleve në sezonën e vjeshtës ishte me e lartë sesa në sezonën e pranverës për shumicën e metaleve të gjetura në lokalitetet e hulumtuara. Në anën tjetër, frekuenca mikronukleuseve dhe e ndryshimeve të tjera bërthamore në eritrocite, e ndryshimeve histopatologjike dhe imunologjike tregojnë një nivel të lartë ndërmjet lokaliteteve të hulumtimit dhe dallime të rëndësishme në mes të sezonave. Lokalitetet me frekuencën më të lartë të abnormaliteteve bërthamore dhe të cilët treguan dallime të rëndësishme të ndryshimeve histopatologjike ishin Drenasi dhe Kishnica, pasuar nga Obiliqi dhe Mitrovica, ndërsa në lokalitetin e Dragashit frekuenca e ndryshimeve ishte e parëndësishme. Në aspektin sezonal, po ashtu u regjistruan dallime sezonale, me frekuencë më të lartë të mikronukleuseve dhe ndryshimeve të tjera bërthamore, si dhe ndryshimeve histopatologjike dhe imunologjike në sezonin e vjeshtës krahasuar me pranverën. Analiza e korelacionit tregoi lidhshmëri pozitive ndërmjet metaleve të gjetura në lokalitetet e hulumtuara me frekuencën e mikronukleuseve dhe abnormaliteteve bërthamore në eritrocitet e bretkosave.

Rezultatet e këtij studimi paraqesin kontribut të veçantë shkencor në fushën e ekotoksikologjisë mbi përdorimin e ujëtokësorëve si vlerësues të rëndësishëm mjedisor të habitateve natyrore në Kosovë. Andaj, njohja e parametrave hulumtues të këtij grupi shtazor, njohja e ndryshimeve histologjike, ndikimeve sezonale, dhe njohja afinitetit gjinor ndaj ndotjes paraqet një kontribut domethënës për vendin tonë. Këto rezultate nënvizojnë rëndësinë e përdorimit të amfibëve, veçanërisht *Pelophylax ridibundus*, si tregues biologjik të ndotjes mjedisore. Për mJ7 tepër, korelacioni i qëndrueshëm midis ndotjes me metale të rënda dhe dëmtimit gjenetik tek bretkosat, siç tregohet në këtë studim, kontribuon në bazën e rritjes së provave që lidhin ksenobiotikët industrial me efektet gjenotoksike të tyre tek ujëtokësorët.

Fjalët kyçe: Monitoring mjedisor, Bioindikator, Ndotja industriale, Anurat, Organet, Gjaku, Pranverë, Vjeshtë.

Summary

This doctoral dissertation represents the first study of its kind in the Republic of Kosovo, focusing on amphibians as biological indicators of environmental pollution. The paper presents original and detailed data on the impact of industrial pollution, resulting from anthropogenic activity, on the condition, histological, immunological, and cytogenotoxic parameters in the frog *Pelophylax ridibundus* (Pallas, 1771), as well as on the concentration of heavy metals in water and their content in soil (sediment), across five study localities in the Republic of Kosovo.

The study material collected for this research represents four zones or industrial pollution hotspots (Kishnica S – 1, Obiliq S – 2, Mitorvica S – 3, Drenasi S – 4), and one zone considered clean, which served as a control locality (Dragashi S – 5).

Water and soil samples (5 samples per locality and per season, totaling 50 water samples and 50 soil samples), and biological material (15 individuals per locality and per season, totaling 150 individuals), were collected in 2023 during the months of May and October (two research seasons). The biological material included both male and female individuals in order to compare the impact of pollution on both sexes.

The research localities, four of them (Kishnica, Obiliq, Mitrovica, and Drenas) represented areas with different types of industrial activity, while one locality (Dragash) served as a control site, considered free from anthropogenic industrial activity. Various statistical analyses were performed to evaluate differences among study localities, seasons, and sexes, as well as to examine correlations between observed changes and the presence of metals in the frogs' habitats.

The aim of this study was to investigate the negative effects of heavy metal pollution and other anthropogenic activities on the condition, histopathological, immunological, and cytogenotoxic parameters of the frog *Pelophylax ridibundus* (Pallas, 1771) in Kosovo, as well as to determine the concentration of heavy metals in water and their content in soil samples collected from the natural habitats of the frogs.

In order to obtain scientifically credible results, the localities selected for this research represent hotspots of pollution from various industrial sources. Due to its specific biological characteristics such as low mobility, a complex life cycle, and high skin permeability, the marsh frog *Pelophylax ridibundus* (Pallas, 1771) was chosen as the study organism. The correlation analysis showed a

positive relationship between the levels of heavy metals found in the studied localities and the frequency of micronuclei and nuclear abnormalities in the erythrocytes of frogs.

The results of this study represent a significant scientific contribution to the field of ecotoxicology, particularly regarding the use of amphibians as important environmental assessors of natural habitats in Kosovo. Therefore, understanding the research parameters of this animal group, recognizing histological changes, seasonal impacts, and sex-based susceptibility to pollution, provides a meaningful contribution to our country.

These results highlight the importance of using amphibians, especially *Pelophylax ridibundus*, as biological indicators of environmental pollution. Furthermore, the strong correlation between heavy metal pollution and genetic damage in frogs, as demonstrated in this study, contributes to the growing body of evidence linking industrial xenobiotics to their genotoxic effects on amphibians.

Keywords:

Environmental monitoring, Bioindicator, Industrial pollution, Anurans, Organs, Blood, Spring, Autumn