



Pranuar me: 09.10.2023			
№	Numer	Sasia	Vite
01	5507	2	—

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

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

Prishtinë, dt. 09.10.2023

KË R K E S Ë

Për: Departamentin e Biologjisë
Këshillin e studimeve të doktoratës
Këshillin e Fakultetit të Shkencave Matematike Natyrore

Lënda: Formimi i komisionit për vlerësimin e punimit të doktoratës

Duke u bazuar në Rregullorën Nr. 1/96 për studimet e doktoratës, kërkoj nga organet e lartëcekura të FSHMN-së që të formojnë komisionin për vlerësimin e dorëshkrimit të punimit të doktoratës me titull: **"BIOAKUMULIMI DHE BIOMAGNIFIKIMI I Pb, Zn, Ni NGA TOKA NË ZINXHIRIN TROFIK HITHI-KËRMILL SI DHE EFEKTI I TYRE NË DISA PARAMETRA TË STRESIT OKSIDATIV TE KËRMILLI *Helix pomatia* NË TRI LOKALITETE TË KOSOVËS"**

Kërkesës ja bashkangjes:

Punimin shkencor

Dëshmitë e pjesmarrjes në konferenca shkencore

Kopjen e dorëshkrimit

Pëlqimin nga mentori

F6

Kandidati:

Msc. Mentor Bici

Pranuar me: 09.10.2023			
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Lënda: Pëlqim nga Mentori për dorëzimin e dorëshkrimit të punimit të doktoratës me titull:
"BIOAKUMULIMI DHE BIOMAGNIFIKIMI I Pb, Zn, Ni NGA TOKA NË ZINXHIRIN
TROFIK HITHI-KËRMILL SI DHE EFEKTI I TYRE NË DISA PARAMETRA TË STRESIT
OKSIDATIV TE KËRMILLI *Helix pomatia* NË TRI LOKALITETE TË KOSOVËS" të
kandidatit **Msc. Mentor Bici**.

Mendim:

Kandidati **Msc. Mentor Bici** ka ofruar të dhëna të reja mbi ndikimin e ndotjes industriale në tri lokalitetet e Kosovës (Obiliq, Drenas dhe Mitrovicë) me metale të rënda Pb, Zn dhe Ni duke pasqyruar nivelet e koncentimeve të tyre në tokë të këtyre zonave, si dhe bioakumulimin dhe biomagnifikimin e tyre përgjatë zinxhirit ushqimor hithi-kërmill dhe ndikimit të tyre në disa parametra të stresit oksidativ në hepatopankreas të kërmillit *Helix pomatia.L.* Në këtë punë hulumtuese kandidati ka mbledhur 1200 mostra të dheut, 120 mostra të hithit *Urtica dioica* dhe 120 mostra të kërmillit *Helix pomatia* në tri lokalitetet e cekura më lartë si dhe 30 mostra të dheut, hithit dhe kërmillit në lokalitetin kontrollë Brezne-Opojë. Analizat statistikore treguan vlera të larta të koncentimeve të metaleve të rënda Pb, Zn dhe Ni në tokë në zonat e hulumtuara dhe gjithashtu u argumentua bioakumulimi dhe biomagnifikimi nga bima e hithit e cila ka treguar një potencial për fitoremedim-pastrim të tokave nga metalet e rënda. Po ashtu u konstatua ndikimi i tyre në rritjen e koncentimeve të parametrave të stresit oksidativ duke shkaktuar karbonilim të

proteinave, rritje të peroksidimit të lipideve si malondialdehidit dhe rritjen e koncentrimëve të proteinave totale. Gjatë kësaj periudhe të hulumtimit nga një pjesë e rezultateve të doktoratës kandidati Msc. Mentor Bici ka publikuar një punim shkencor në revistën e cila është e indeksuar në SCOPUS, e poashtu ka marr pjesë në tri konferenca shkencore me karakter ndërkombëtar.

Punimi shkencor:

Bici, M., Mazreku, I., Hyseni, B., Halili, J., Krasniqi, Y., Bislimi, K. (2023). Effect of Lead, Nickel, and Zinc Pollution in Some Parameters of Oxidative Stress in Hepatopancreas of Snail *Helix pomatia* L. in Power plant of Obiliq. *Journal of Ecological Engineering*, 24(9), 385-395. <https://doi.org/10.12911/22998993/169471>.

Konferencat shkencore:

Mentor Bici¹, Jeton Halili², Kemajl Bislimi^{3*} “The potential of the nettle plant *Urtica dioica* L. in phytoremediation of soils polluted by heavy metals”. International Conference on New Achievements in Science, Technology and Arts” –ICNA-STA 4-5 May 2023, Prishtina, Kosovo.

Mentor BICI¹, Hazbije SAHITI², Muhamet ZOGAJ³, Ilir MAZREKU⁴, Jeton HALILI⁵, Kemajl BISLIMI^{6*} “TRANSFER OF LEAD (Pb) FROM SOIL IN FOOD CHAIN NETTLE - SNAIL FROM HEAVY METAL POLLUTION OF MINING ACTIVITY IN DRENAS”. International Scientific Conference BRIDGE2022 09.12.2022

Mentor Bici¹, Kemajl Bislimi^{2*} “POLLUTION FROM SOME HEAVY METALS CAUSED BY MINING ACTIVITY AND THEIR EFFECT IN OXIDATIVE STRESS OF HEPATOPANCREAS OF SNAIL *HELIX POMATIA* L.”- 8th International Congress on Applied Biological Sciences 14-16 September 2023 University of Prishtina, Kosova.

Andaj, nga ajo që u tha më lartë, kandidati **Msc. Mentor Bici** ka përmbushur të gjitha kushtet për dorëzimin e temës së doktoratës.

Prof. Dr. Kemajl Bislimi:



UNIVERSITETI I PRISHTINËS "HASAN PRISHTINA"

F6- Paraqitja e punimit të doktoratës

PARAQITJA E PUNIMIT TË DOKTORATËS ¹	
TË DHËNAT E PËRGJITHSHME	
Doktoranti:	Msc. Mentor Bici
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Emërtimi i studimit:	Biologji e organizmave me ekologji
Udhëheqësi i studimit:	FSHMN- UNIVERSITETI I PRISHTINËS
TË DHËNAT PËR PUNIMIN E DOKTORATËS	
Titulli në gjuhën shqipe	BIOAKUMULIMI DHE BIOMAGNIFIKIMI I (Pb, Zn, Ni) NGA TOKA NË ZINGJIRIN TROFIK HITHI-KËRMILL SI DHE EFEKTI I TYRE NË DISA PARAMETRA TË STRESIT OKSIDATIV TE KËRMILLI HELIX POMATIA NË TRI LOKALITETE TË KOSOVËS
Titulli në gjuhën angleze	BIOACCUMULATION AND BIOMAGNIFICATION OF (Pb, Zn, Ni) FROM SOIL TO THE TROPHIC CHAIN NETTLE-SNAIL AS WELL AS THEIR EFFECT ON SOME PARAMETERS OF OXIDATIVE STRESS OF SNAIL HELIX POMATIA IN THREE LOCALITIES OF KOSOVO
Fusha e hulumtimit	EKOTOKSIKOLOGJI
DEKLARATA E MENTORIT/BASHKËMENTORIT	

1

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

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

Kandidati **Msc. Mentor Bici** ka ofruar të dhëna të reja mbi ndikimin e ndotjes industriale në tri lokalitetet e Kosovës (Obiliq, Drenas dhe Mitrovicë) me metale të rënda Pb, Zn dhe Ni duke pasqyruar nivelet e koncentimeve të tyre në tokë të këtyre zonave, si dhe bioakumulimin dhe biomagnifikimin e tyre përgjatë zinxhirit ushqimor hithi-kërmill dhe ndikimit të tyre në disa parametra të stresit oksidativ në hepatopankreas të kërmillit *Helix pomatia.L.* Në këtë punë hulumtuese kandidati ka mbledhur 1200 mostra të dheut, 120 mostra të hithit *Urtica dioica* dhe 120 mostra të kërmillit *Helix pomatia* në tri lokalitetet e cekura më lartë si dhe 30 mostra të dheut, hithit dhe kërmillit në lokalitetin kontrollë Brezne-Opojë. Analizat statistikore treguan vlera të larta të koncentimeve të metaleve të rënda Pb, Zn dhe Ni në tokë në zonat e hulumtuara dhe gjithashtu u argumentua bioakumulimi dhe biomagnifikimi nga bima e hithit e cila ka treguar një potencial për fitoremedim-pastrim të tokave nga metalet e rënda. Po ashtu u konstatua ndikimi i tyre në rritjen e koncentimeve të parametrave të stresit oksidativ duke shkaktuar karbonilim të proteinave, rritje të peroksidimit të lipideve si malondialdehidit dhe rritjen e koncentimeve të proteinave totale. Gjatë kësaj periudhe të hulumtimit nga një pjese e rezultateve të doktoratës kandidati Msc. Mentor Bici ka publikuar një punim shkencor në revistën e cila është e indeksuar në SCOPUS, e poashtu ka marr pjesë në tri konferenca shkencore me karakter ndërkombëtar.

Punimi shkencor:

2

Bici, M., Mazreku, I., Hyseni, B., Halili, J., Krasniqi, Y., Bislimi, K. (2023). Effect of Lead, Nickel, and Zinc Pollution in Some Parameters of Oxidative Stress in Hepatopancreas of Snail *Helix pomatia L.* in Power plant of Obiliq. *Journal of Ecological Engineering*, 24(9), 385-395. <https://doi.org/10.12911/22998993/169471>.

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Vendi, data dhe nënshkrimi

Në Prishtinë,

09 10 2023

Nënshkrimi



(Emri e mbiemri i mentorit)

Nënshkrimi

(Emri e mbiemri i bashkëmentorit)

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Msc. MENTOR BICI

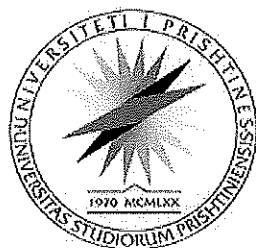
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PARAMETRA TË STRESIT OKSIDATIV TE KËRMILLI HELIX POMATIA NË
TRI LOKALITETE TË KOSOVËS**

PUNIMI I DOKTORATËS

Mentori:
Prof. Dr. Kemajl Bislimi

Prishtinë, 2023

UNIVERSITY OF PRISHTINA
FACULTY OF MATHEMATICAL – NATURAL SCIENCES



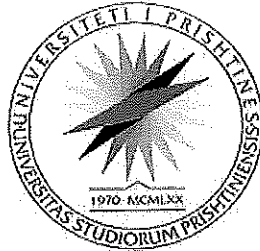
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**BIOACCUMULATION AND BIOMAGNIFICATION OF Pb, Zn, Ni FROM SOIL
TO THE TROPHIC CHAIN NETTLE-SNAIL AS WELL AS THEIR EFFECT ON
SOME PARAMETERS OF OXIDATIVE STRESS OF SNAIL HELIX POMATIA
IN THREE LOCALITIES OF KOSOVO**

DOCTORAL THESIS

Prishtina, 2023

UNIVERSITETI I PRISHTINËS
FAKULTETI I SHKENCAVE MATEMATIKE-NATYRORE



Msc. MENTOR BICI

**BIOAKUMULIMI DHE BIOMAGNIFIKIMI I Pb, Zn, Ni NGA TOKA NË
ZINXHIRIN TROFIK HITHI-KËRMILL SI DHE EFEKTI I TYRE NË DISA
PARAMETRA TË STRESIT OKSIDATIV TE KËRMILLI HELIX POMATIA NË
TRI LOKALITETE TË KOSOVËS**

PUNIMI I DOKTORATËS

Mentori:
Prof.Dr. Kemajl Bislimi

Prishtinë, 2023

REZYMEJA

Metalet e rënda janë të njohur si ndotës potencial të mjedisit, për shkak të toksicitetit dhe qëndrueshmërisë së tyre në ekosistem.

Hulumtimet kanë treguar se burimet dhe origjina e këtyre metaleve të rënda në mjedis është nga shpërlarjet e shkëmbinjëve nga kushtet klimatike, shpërthimeve vullkanike, ngrohjes globale dhe aktivitetve antropogjene përfshirë këtu industrializmin, fabrikat e përpunimit të xeheve, plastikës dhe aktivitetet bujqësore.

Minierat dhe përpunimi industrial për nxjerrjen e mineraleve nga burimet e tyre dhe aplikimet e tyre të mëvonshme për zhvillim industrial, bujqësor dhe ekonomik gradualisht ka çuar në një rritje të përqendrimit të këtyre elementëve në mjedis dhe prishjen e cikleve të tyre biogjeokimike.

Ndotja e ujërave dhe ekosistemeve tokësore me metale të rënda toksike janë një problem mjedisor aktual dhe me shqetësim për shëndetin publik.

Rëndësi të madhe ka njohja e aftësisë bioakumuluese të organizmave të ndryshëm siq janë bimët, shtazët dhe njeriu, si dhe translokimi i tyre përgjatë zingjirit ushqimor.

Edhe vendi ynë Kosova nuk mund të mos preket nga ndotja industriale me metale të rënda si pasojë e lirimt të tyre nga aktivitetet minierare si ajo e Kishnicës, Zveqanit, Ferronikelit në Drenas si dhe Termocentralet Kosova A dhe B, të cilat cilësohen si hot-spote mjedisore apo pika të nxehta të nxehta të ndotjes si dhe aktivitetet tjera antropogjene.

Andaj, përmes këtij punimi i cili bazohet në mbledhjen e mostrave 1200 për dheun, 120 mostra të hithit dhe 120 mostra të kërmillit në tri lokalitete të Kosoves- Obiliq, (TC Kosova A), Drenas (Ferronikeli) dhe Mitrovicë (Fabrika e akumulatorëve) dhe 30 mostra të dheut, hithit (*Urtica dioica*) dhe 30 mostra të kërmillit (*Helix pomatia*), është bërë matja e përqendrimit të disa metaleve të rënda (Pb, Zn, Ni) në tokë si dhe bioakumulimin dhe translokimin e tyre nga dheu tek bima e hithit (*Urtica dioica*) dhe shtaza – kërmilli *Helix pomatia* dhe ndikimin në aspektin toksik të tyre në disa paramtera biokimikë të stresit oksidativ si: MDA, karbonilimi i proteinave dhe proteinave totale.

Fjalët kyqe: bioakumulim, biomagnifikim, stres oksidativ, hepatopankreas, kërmill, hithi.

RESUME

Heavy metals are known as potential environmental pollutants, due to their toxicity and persistence in the ecosystem.

Research has shown that the sources and origin of these heavy metals in the environment is from rock washes from climatic conditions, volcanic eruptions, global warming and anthropogenic activities including industrialization, ore processing factories, plastics and agricultural activities. Mining and industrial processing for the extraction of minerals from their sources and their subsequent applications for industrial, agricultural and economic development has gradually led to an increase in the concentration of these elements in the environment and the disruption of their biogeochemical cycles.

Pollution of water and terrestrial ecosystems with toxic heavy metals is a current environmental problem of public health concern.

It is of great importance to know the bioaccumulative capacity of different organisms such as plants, animals and humans, as well as their translocation along the food chain.

Even our country Kosovo cannot be affected by industrial pollution with heavy metals as a result of their release from mining activities such as that of Kishnica, Zveqan, Ferronikel in Drenas as well as Kosova A and B thermal power plants, which are considered hot-spots environmental or pollution hot spots as well as other anthropogenic activities.

Therefore, through this work which is based on the collection of 1200 soil samples, 120 heather samples and 120 snail samples in three localities of Kosovo - Obiliq, (TC Kosova A), Drenas (Ferronikel) and Mitrovica (Battery Factory) and 30 soil samples, stinging nettle (*Urtica dioica*) and 30 snail (*Helix pomatia*) samples, the concentration of some heavy metals (Pb, Zn, Ni) in the soil was measured, as well as their bioaccumulation and translocation from the soil to the nettle plant (*Urtica dioica*) and the animal - the snail *Helix pomatia* and the impact on their toxic aspect on some biochemical precursors of oxidative stress such as: MDA, carbonylation of proteins and total proteins.

Key words: bioaccumulation, biomagnification, oxidative stress, hepatopancreas, snail, nettle.



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Ibrahim M.A. Al-Salman

J. Ecol. Eng. 2023; 24(10):1-12

DOI: <https://doi.org/10.12911/22998993/169459>

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Effect of Lead, Nickel, and Zinc Pollution in Some Parameters of Oxidative Stress in Hepatopancreas of Snail *Helix pomatia* L. in Power plant of Obiliq

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ABSTRACT

Large quantities of heavy metals come from anthropogenic sources, including coal combustion, furthermore, these heavy metals can cause oxidative stress in terrestrial animals, such as snails, which acquire these metals through food from storage plants in industrial and coal-burning-polluted areas. In this research, we measured the impact and distribution of power plant TC Kosovo as activity on the concentrations of heavy metals (Pb, Zn, Ni) in the locality of Obiliq, as well as their effect on oxidative stress parameters such as carbonyl proteins, malondialdehyde (MDA), and total proteins in the hepatopancreas of the snail *Helix pomatia* L. We collected 120 soil samples and 12 snails from concentric circles around the pollution point at distances of 1 km, 2 km, and 5 km. Relevant methods were then applied, and the samples were measured using a spectrophotometer and flame absorber Analyticyena. Results shown that the heavy metals bioaccumulated from snail shells (*Helix pomatia* L.), and concentrations of these metals influenced an increase in the levels of oxidative stress parameters, including protein carbonylation, MDA, and total proteins in the hepatopancreas.

Keywords: heavy metals, pollution, oxidative stress, hepatopancreas, snail.

INTRODUCTION

Kosovo heavily relies on coal-fired power plants (97%) for its electricity generation (BB 2014). The two main lignite-fired units, Kosovo A and Kosovo B, have been operational since 1983 and are located in Kastriot (Obiliq), merely 13 kilometers away from Kosovo’s capital, Pristina (Fig. 1). These power plants are known to emit sulfur dioxide and particulate matter, making them a significant source of pollution in Europe (IQAir, 2019).

Heavy metals have garnered considerable attention as environmental contaminants. Current research focuses on ecotoxicological challenges

associated with this phenomenon, which is influenced by human activities such as mining, traffic, intensive agriculture, and others that release particles into the soil or air, especially during dry and windy weather (Olayinka et al., 2011), coal burning in power plants, oil burning, nuclear power plants (Bradl, 2002).

The concentration of heavy metals in soil is determined primarily by the chemical properties of the soil and the distance from the contamination source. The extent of heavy metal accumulation and absorption by plants depends on the type and concentration of heavy metals, as well as the plant and animal species involved in the food chain (Jolly et al., 2013).

The quality of the environment, particularly soil and water, is intricately linked to the quality of agricultural products, which in turn impacts human health (Sun et al., 2019). Heavy metal soil contamination is a significant environmental issue that poses health risks (Shakeri et al., 2009). Potentially contaminated soils can be found in various locations, including old landfills (especially those that accepted industrial waste), industrial areas where chemicals may have been dumped, or downwind areas from industrial sites (Aubum, 2000).

Snail meat has long been regarded as a high-quality food source due to its high protein content and relatively low lipid content. Snails play a crucial role in the food chain, serving as a link through which heavy metals can be translocated from one organism to another (Mooney et al., 2002).

Traditionally, researchers have utilized snails to study the accumulation of pollutants. By assessing the internal concentrations of contaminants, such as metals, following a predetermined exposure period, researchers can determine the snails' capacity for accumulation, the bioavailability of contaminants, and the intensity of their transfer from the environment to food and/or soil (Gimbert et al., 2006). The foot and viscera are two important components to consider in snails. The viscera, which include the kidney, hepatopancreas and heart. Pollutant concentrations in the hepatopancreas and kidney exhibit dose-dependent increases and are correlated with the bioavailability of pollutants to the organism and environmental concentrations (Cœurduassier et al., 2002). These organisms are often chosen as sentinels due to their limited

toxic response and their ability to reflect tissue levels. Studying the impact of metals and other pollutants on organism physiology contributes to the development of toxicological studies that can be employed as tools for environmental assessment (Baroudi et al., 2020).

The propensity of animal organs, such as those in snails (*Helix pomatia*), to accumulate heavy metals can vary. Organs with high metabolic activity, such as the hepatopancreas and digestive system, are particularly susceptible (Dallinger, 1993; Menta and Parisi, 2001).

According to Mohammadein et al. (2013), the ecotoxicological approach described in this study holds relevance for understanding the ecological impact of various pollutants on ecosystems and human health. The obtained results from assessing the bioaccumulation and histological responses of the common snail can provide valuable insights for monitoring soil pollution caused by heavy metals. Elevated levels of Pb in plants can disrupt normal metabolic pathways, interfere with specific cellular enzymes, and hinder photosynthesis. Generally, high concentrations of heavy metals can induce oxidative stress, DNA damage, and disruptions to metabolic processes (Olowoyo et al., 2015).

MATERIALS AND METHODS

Soil samples and snails of the species *Helix pomatia* L were used as materials to investigate the impact of heavy metal pollution in soil and

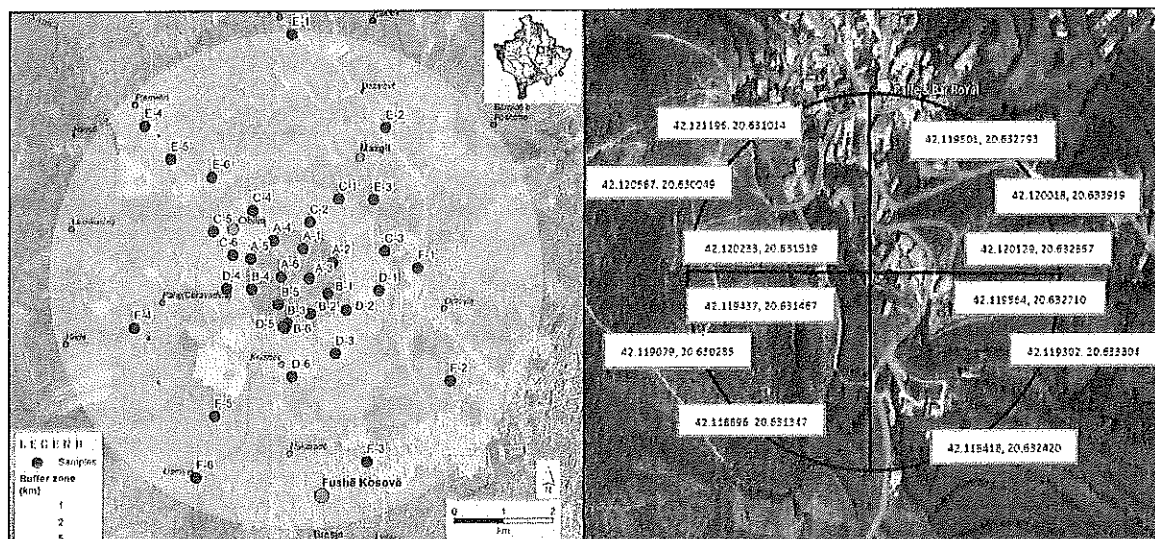


Figure 1. Schematic representation of the sample collection at the polluted locality TC Kosova A Obiliq and unpolluted site Brezne-Opoja

its effect on oxidative stress in the hepatopancreas in the municipality of Obiliq. A total of 120 soil samples and 36 snails were collected based on concentric circles with radii of 1 km, 2 km, and 5 km from the contamination point at Power Plant TC Kosovo A. These concentric circles were further divided into four geographical areas: northwest, northeast, southeast, and southwest. Additionally, 30 soil samples and 10 snails were collected from the unpolluted locality of Brezneo-Poja. The obtained results were analyzed using statistical software such as Minitab, and statistical methods including Tuckey Kramer and ANOVA were employed for data analysis.

Soil samples were collected using a hand probe at a depth of 15 cm in the pristine areas surrounding the Kosova A power plant. The sampling was conducted at distances of 1 km, 2 km, and 5 km during the summer-autumn period of 2020. The purposive sampling method, as described in BBodSchV (1999), Theocharopoulos et al. (2001) was employed for sample collection. The coordinates for sample showed in Figure 1. These sampling points were distributed across four geographical areas. Using this method, 10 soil drillings were performed at three selected points within each geographical area of the radius circle. The collected soil from the drillings was then mixed according to DIN ISO11466 (1995) standards. The soil samples were transported to the laboratory, ground in a soil mill, and placed in glass cups. They were dried in a thermostat at 105°C for 48 hours to remove moisture. After drying, 0.3 g of each soil sample was weighed and treated with concentrated 69% HNO₃ and HCl (Merck Millipore) reagents in a 2:6 ratio in Teflon columns. The samples were then digested in the analyticyena TOP wave microwave at 200°C for 45 minutes. The contents were filtered and transferred to normal 50 ml glassware using distilled H₂O. For the analysis of metals such as Pb, Zn, and Ni, Merck Millipore ICP multi-element standard solution 11355 was used, and the samples were read using two types of flame absorbers: Thermo and Contra AAA.

Additionally, snail samples were also collected at the same place and time, focusing on older individuals. The shells of the snails were separated from their bodies and washed with distilled H₂O. The shells were then dried in a thermostat at 105°C for 24–48 hours and ground using a Philips kitchen mixer. A 0.5 g sample of the ground shell was treated with Merck Millipore reagents, which

included 69% ultra-pure nitric acid (HNO₃) in a 1:3 ratio with Lachner H₂O₂ 30%. The samples were digested in a microwave at 200°C for 45 minutes. After digestion, the contents were filtered in normal 50 ml glassware. The samples were normalized with distilled H₂O, and the metals Pb, Zn, and Ni were read using flame absorber types Analyticyena Contra AAA.

The bioaccumulation coefficient (BCF) was calculated using the standard formula:

$$BCF = C_{plant\ parts} / C_{soil}$$

where $C_{plant\ parts}$ – represents the metal concentration in the plant or animal tissue, measured in mg/kg dry weight, C_{soil} – represents the metal concentration in the soil, measured in mg/kg dry weight.

The hepatopancreatic samples for oxidative stress parameters in the vineyard snail *Helix pomatia* were treated using standard methods protocol. A total of 36 live snail samples from the locality were selected. The shells of these snails were removed and used for the measurement of heavy metals, along with soil and heath samples. The hepatopancreas was then extirpated from these individuals. The hepatopancreas samples were weighed and homogenized in phosphate buffer at a ratio of 9:1, which means adding 9 ml of phosphate buffer per 100 g of living tissue. The homogenized samples were placed in 1.5 ml Eppendorf tubes and stored in a refrigerator at -80°C.

The samples were centrifuged at 3600 revolutions for 15 minutes, and from the supernatant, 1.5 ml of the contents were pipetted and placed in normal test tubes. Reagents were added to the test tubes for further analysis.

To measure lipid peroxidation, specifically malondialdehyde (MDA) in the hepatopancreas, the TBARS (thiobarbituric acid reactive substances) method was applied. The reagents, including 0.75% thiobarbituric acid (TBA), 30% trichloroacetic acid (TCA), and 5M HCl, were prepared. These reagents were added to the test tubes containing the sample, following the sequence: TBA (1.5 ml) + TCA (1 ml) + HCl (0.2 µl) + sample (250 µl). The contents were placed in a water bath and kept at 95°C for 45 minutes. After this time, the contents turned purple. A portion (1 ml) of the contents was pipetted and placed in the cuvette of the spectrophotometer, and the absorbances at 360 nm and 450 nm were read. The obtained values were recorded in the experiment's database.

To measure the carbonylation of proteins in the hepatopancreas, the 2,4-dinitrophenylhydrazine (DNPH) method was applied. Reagents were prepared by dissolving 17.20 g of 2,4-dinitrophenylhydrazine in 41 ml of 2.5M HCl. In the test tubes, 1 ml of the sample was pipetted, and 4 ml of 10 mmol DNPH was added at room temperature for 1 hour. This allowed the acid to react with the contents and bind the carbonyl groups. After 1 hour, 5 ml of 20% TCA were added to the samples, which were then centrifuged and vortexed. A mixture of 6 ml of ethanol and ethyl acetate in a 1:1 ratio was added, followed by centrifugation at 6000 revolutions for 5 minutes. The supernatant was carefully discarded, and the bottom was saved. The samples were vortexed every 15 minutes until the supernatant was dissolved. The supernatant was washed three times with 6 ml of ethanoethyl acetate, vortexed, and centrifuged. The bottom was then dissolved in 1 ml of 6M guanidine hydrochloride in a water bath at 37°C for 10 minutes. The absorbances were read, and a blank acid test was performed at 360 nm using a spectrophotometer. The obtained values were recorded in the experiment's database.

For the measurement of total proteins in the hepatopancreas of the snail, the Lovri's method was applied. The first step in this method was the preparation of BSA (bovine serum albumin) standard at a concentration of 1 mg/ml. Standard BSA is prepared by adding the following solutions:

- 2% Na₂CO₃ in 0.1 N NaOH is pipetted into a 50 ml eluizer plate,
- 1% NaK Tartrate in H₂O 0.5 ml,
- 0.5% CuSO₄·5H₂O in H₂O 0.5 ml,
- Reagent I: 48 ml of A, 1 ml of B, 1 ml of C,
- Reagent II: 50 µl Folin-Phenol [2 N].

The total protein measurement procedure is done in such a way that BSA is placed in 0, 5, 10, 20 µL concentrations in the 5 wells of the 96 well plate (ELISA), while distilled water is placed in one well as a blind test. In the other wells, the samples are placed and then 50 µL of 2N phenol foil is added to all of them and then they are read in two types of absorbances, 360 nm and 450 nm. The obtained values are recorded on the special paper of the apparatus.

RESULTS

The concentration of heavy metals Pb, Ni and Zn in soil and shell samples are shown in Table 1.

According to these results, it can be observed that the concentrations of the three metals in the analyzed soil samples increase as the distance from the point of contamination increases. Additionally, these metal concentrations show significant differences when compared to the control samples. Specifically, the concentration of metal Ni in the shell samples was found to be low in our cases after converting from mg/kg to µg/kg.

Referring to Table 1, the findings indicate that the concentration of Pb is below the standard limit. However, the level of Ni in the soil exceeds the standard values by five times. As for Zn, it is within the limit according to the UK standard. However, it exceeds the standard values in Germany, reaching 290 mg/kg.

These results highlight the presence of heavy metal pollution in the soil, particularly with elevated levels of Ni and Zn, which surpass the permissible limits set by certain standards. The data suggests a potential environmental concern and the need for further investigation or remediation measures to address the heavy metal contamination in the study area.

Based on Table 2, it is evident that the concentrations of metals in the control samples from the locality show significant differences ($p < 0.001$) compared to the contaminated site in Obiliq. This indicates that the levels of heavy metals vary significantly between the two locations in all types of analyzed samples. Furthermore, the elevated concentrations of these heavy metals have influenced the parameters of oxidative stress in the hepatopancreas of the snails. Specifically, high values of protein carbonylation, malondialdehyde (MDA), and total proteins were recorded. These results are presented in Table 3 and Figure 3, providing a visual representation of the impact of heavy metal contamination on the oxidative stress parameters.

The high values of protein carbonylation and MDA indicate oxidative damage to proteins and lipids, respectively, in the hepatopancreas of the snails. The increase in total proteins suggests a potential response of the snails to counteract the oxidative stress caused by heavy metal exposure.

These findings highlight the detrimental effects of heavy metal pollution on the oxidative stress parameters in the snail hepatopancreas and emphasize the need for further investigation and potential mitigation strategies to address the ecological consequences of heavy metal contamination in the study area.

Table 1. Average concentration of metals in soil and shell of samples analyzed in polluted locality Obiliq and unpolluted locality Brezne-Opoja

Soil/Shell samples 1 km				
Concentration of Metal	Soil	RSD%	Shell	RSD%
Pb (mg/kg)	32.6	0.4	11.6	1.4
Ni (mg/kg)	186.7	5.1	2.8 (µg/kg)	2.4
Zn (mg/kg)	161.5	3.3	44.5	5.6
Soil/Shell samples 2 km				
Concentration of Metal	Soil	RSD%	Shell	RSD%
Pb (mg/kg)	77.3	1.3	11.1	1.38
Ni (mg/kg)	214.9	0.4	1.58 (µg/kg)	2.5
Zn (mg/kg)	230.8	13.2	40.7	4.3
Soil/Shell samples 5 km				
Concentration of Metal	Soil	RSD%	Shell	RSD%
Pb (mg/kg)	90.4	1.4	10.3	1.29
Ni (mg/kg)	252.2	0.5	2.3 (µg/kg)	2.1
Zn (mg/kg)	290.9	9.6	40.9	6.34
Significant of 1:2:5 km	P<0.05		P<0.05	
Soil/Shell samples control site				
Sample/Metal	Soil	RSD%	Shell	RSD%
Pb (mg/kg)	9.23	0.9	0.09	0.02
Ni (µg/kg)	25.1	1.3	0.011	0.05
Zn (mg/kg)	46.2	0.62	0.025	0.09
Significant with pollution site	p<0.001		p<0.001	

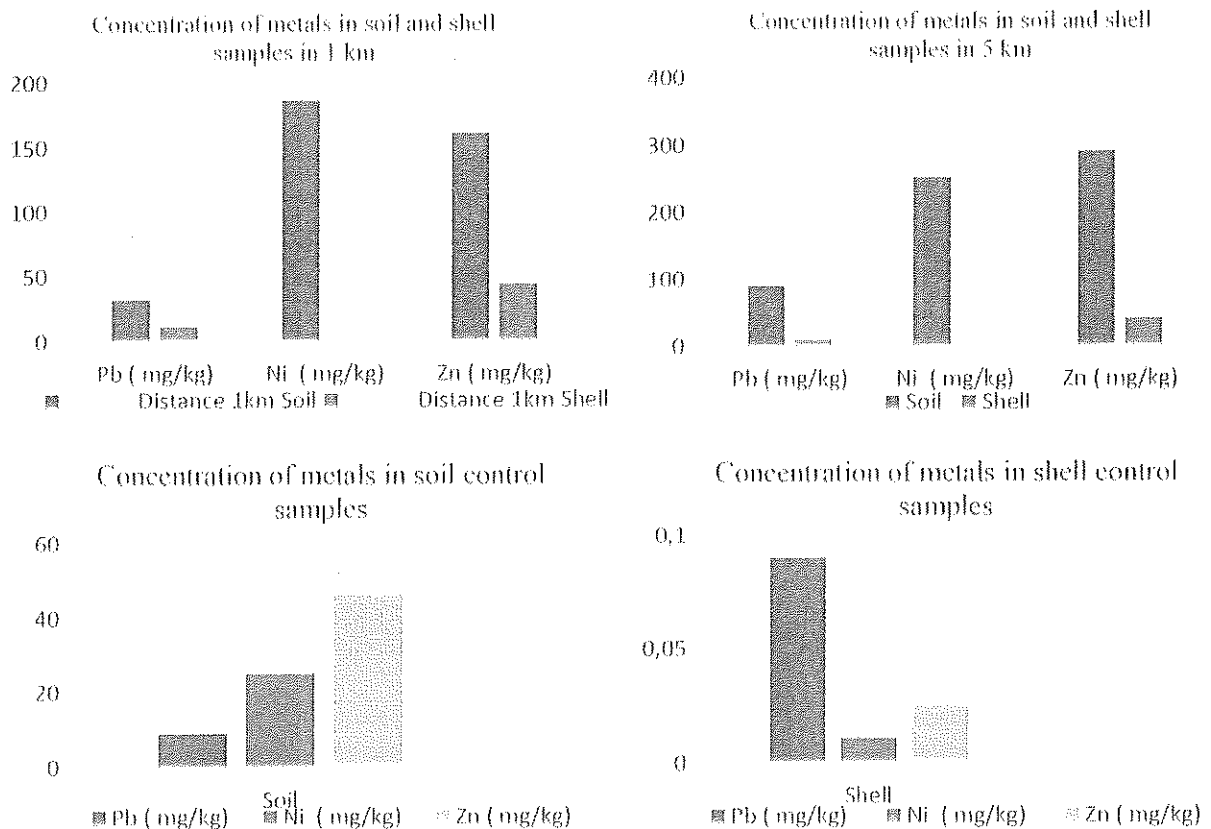


Figure 2. Concentration of metals Pb, Ni, Zn in soil and shell samples of distance 1, 2, 5 km in polluted and unpolluted area

Table 2. Average concentration of carbonyl proteins in polluted site Obiliq

Hepatopancreas samples in three distances 1, 2, 5 km and control samples				
Samples	Coordinates	Weight (g)	Abs	Conc. (µmol/L)
A1	North sample	0.389	1.231	55.3
B1	South sample	0.512	0.972	43.5
C1	North sample	0.461	1.443	64.9
D1	South sample	0.313	1.469	66.1
E1	North sample	0.528	0.157	79.2
F1	South sample	0.372	1.889	85.2
A4	North sample	0.432	1.883	84.9
B4	South sample	0.523	1.18	53.1
C4	North sample	0.298	0.528	23.3
D4	South sample	0.451	0.477	21.1
E4	North sample	0.426	0.311	13.4
F4	South sample	0.365	0.312	13.5
			Average	50.29
			SD	27.1
Unpolluted site				
1	North sample	0.236	0.086	3.23
2	North sample	0.324	0.017	0.9
3	South sample	0.421	0.028	0.59
4	South sample	0.382	0.104	0.41
			Average	1.54
			SD	1.27

Table 3. Average MDA in polluted site Obiliq and unpolluted site Opoja

Hepatopancreas samples in three distances 1, 2, 5 km of MDA				
Samples	Coordinates	Weight (g)	Abs	Conc. (µmol/L)
A1	North sample	0.389	0.115	73.7
B1	South sample	0.512	0.3	19.2
C1	North sample	0.461	0.215	13.7
D1	South sample	0.313	0.255	16.3
E1	North sample	0.528	0.196	12.5
F1	South sample	0.372	0.208	13.3
A4	North sample	0.432	0.261	16.7
B4	South sample	0.523	0.4	25.6
C4	North sample	0.298	0.155	99.1
D4	South sample	0.451	0.275	17.6
E4	North sample	0.426	0.281	18.1
F4	South sample	0.365	0.298	19.1
			Average	28.74
			STD	27.6
Unpolluted site Opoja				
1		0.236	0.065	7.1
2		0.324	0.053	4.2
3		0.421	0.038	1.9
4		0.382	0.103	6.5
5		0.354	0.092	2.5
			Average	4.44
			SD	2.07

Based on Figure 3, it is evident that the highest concentration of protein carbonylation is observed in the southern part of the country, whereas the northern part exhibits comparatively lower levels. This trend in protein carbonylation values aligns with the concentrations of metals, which are also notably higher in the southern part of the polluted area. The correlation between protein carbonylation and metal concentrations suggests a potential link between heavy metal pollution and oxidative stress in the snail hepatopancreas.

These graphical representations provide visual evidence supporting the notion that the southern region, characterized by higher metal concentrations, experiences increased oxidative stress in the snail population, as indicated by elevated levels of protein carbonylation. This correlation reinforces the understanding that heavy metal pollution can contribute to oxidative damage and stress in organisms, potentially leading to adverse ecological consequences. Further analysis and research are necessary to

fully understand the underlying mechanisms and the specific impacts of heavy metal pollution on the oxidative stress parameters in different regions. These findings emphasize the importance of addressing and managing heavy metal pollution to safeguard the environmental health and biodiversity of the affected areas.

The Figure 3 demonstrates a highly significant difference ($p < 0.001$) between the polluted and control areas in terms of heavy metal concentrations. Furthermore, the elevated metal concentrations have significantly impacted lipid peroxidation and total protein levels in the snail hepatopancreas. These results are also provided in Table 4 and Figure 4. From the Figure 3 we see that we have a high concentration of 28.74 $\mu\text{mol/L}$ of the MDA parameter compared to the control locality of 4.44 $\mu\text{mol/L}$. From the Figure 4 we see that we have a high concentration of 19.85 $\mu\text{g}/100 \mu\text{L}$ of the total protein parameter compared to the control locality 9.77 $\mu\text{g}/100 \mu\text{L}$.

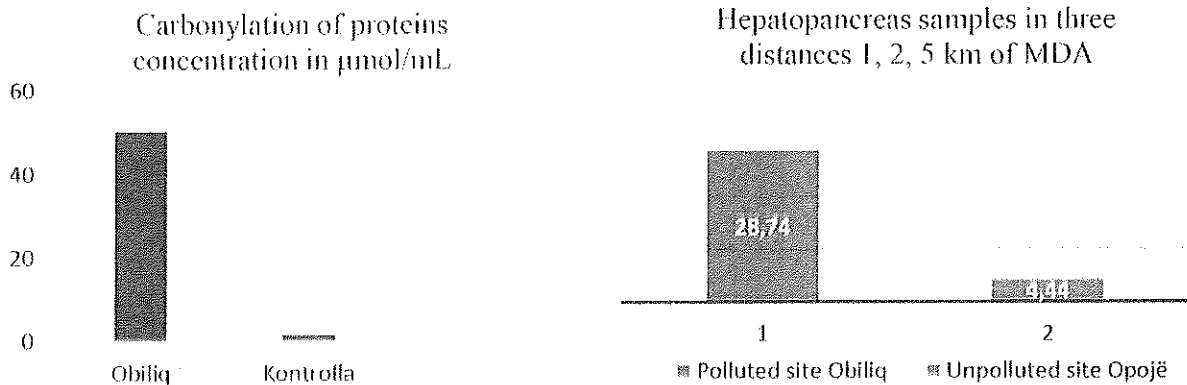


Figure 3. Concentration of protein carbonylation and MDA in polluted area Obiliq and unpolluted area Brezne-Opoja

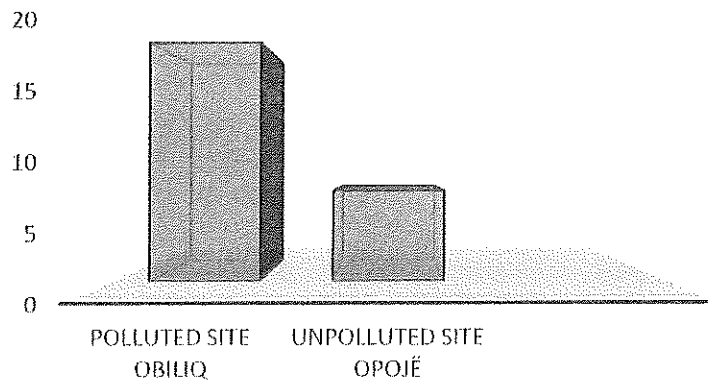


Figure 4. Concentration of total protein in polluted area Obiliq and unpolluted area Brezne-Opoja

Table 4. Concentration of total protein $\mu\text{g}/100\mu\text{L}$ in polluted site Obiliq and unpolluted site Opoja

Total proteins in hepatopancreas at polluted site Obiliq					
Sample	Weight (g)	Total ($\mu\text{g}/100\mu\text{L}$)	Absorbance	450 nm	630 nm
A1O	0.389	14.38753	0.323	0.043067	0.030762
A4O	0.512	13.98664	0.314	0.041867	0.029905
B1O	0.461	11.44766	0.257	0.034267	0.024476
B4O	0.313	17.59465	0.395	0.052667	0.037619
C1O	0.528	21.91537	0.492	0.0656	0.046857
C4O	0.372	22.49443	0.505	0.067333	0.048095
D1O	0.432	10.06682	0.226	0.030133	0.021524
D4O	0.523	10.46771	0.235	0.031333	0.022381
E1O	0.298	15.10022	0.339	0.0452	0.032286
E4O	0.451	40.04454	0.899	0.119867	0.085619
F1O	0.426	39.28731	0.882	0.1176	0.084
F4O	0.365	21.42539	0.481	0.064133	0.04581
Average		19.85			
STD		9.77			

Control samples Brezne Opoja				
Sample	Total	Abs	450 nm	630 nm
1	5.43	0.122	0.0163	0.0116
2	4.54	0.102	0.0136	0.0097
3	11.67	0.262	0.0349	0.025
4	8.69	0.195	0.026	0.0186
5	7.31	0.164	0.0219	0.0156
Average	7.53			
STD	2.52			
O:B	Obiliq: Brezne			
				Significance $p < 0.05$

DISCUSION

Our results indicate elevated concentrations of Pb, Zn, and Ni at a distance of 5 km from the pollution source in the Obiliq locality, particularly in the southwest region of the country. These findings align with previous research, which reported significantly higher lead and zinc concentrations near the Kosovo A power plant compared to European standards (Velju et al., 2008). Studies by Bajraktari et al. (2020) have also shown that pollution from coal fly ash in the vicinity of the Thermal Power Plants (Kosovo A and B) leads to metal stratification at different distances and significant bioaccumulation. Additionally, it has been observed that snail tissue tends to have higher metal content during autumn and winter seasons compared to summer (Radwan et al., 2010).

The Roman snail, *Helix pomatia*, serves as an important bioindicator of long-term metal accumulation in contaminated environments due to its ability to concentrate heavy metals in its body. The snail's hepatopancreas, in particular, is sensitive to heavy metals and often accumulates

elevated levels of Pb, Ni, Zn, Cu, and Cd, which are significantly higher than those found in lower soil trophic levels. Consequently, the snail hepatopancreas can be utilized for monitoring the bioavailability of heavy metals in soil Nica D. et al., 2012. Histopathological changes observed in target animals, such as vacuolated hepatocytes, dilated central veins, compressed blood sinusoids, and congestion in fish liver, can be attributed to the direct harmful effects of heavy metals on edible organs (Elwasify et al., 2021).

The impact of ultrafine particles of TiO_2 and other heavy metals on various toxicological parameters of *Helix aspersa* has been demonstrated. These particles affect the antioxidant enzyme system (CAT, GST, glutathione) and lead to elevated levels of protein carbonylation and lipid peroxidation in the kidney and digestive gland. The activation of antioxidant metabolism in *Helix aspersa* is evident through the examination of various toxicity biomarkers (Khene et al., 2017). Roman snails, such as *Helix pomatia*, have the ability to accumulate relatively high concentrations of heavy metals, particularly in the hepatopancreas,

while surviving in such environments. Therefore, they can serve as suitable animal models for monitoring heavy metal-contaminated environments (Çarkaj et al., 2022).

While research on this topic is limited, the results of our study align with other authors' findings regarding the implication of analyzed metals in oxidative stress. Chronic exposure of snails to a mixture of Pb, Zn, Ni, and heavy metal dust has been found to cause changes in enzymatic activities and the development of oxidative stress, as evidenced by increased catalase activity and lipid peroxidation (Atailia et al., 2016). High activity of antioxidant enzymes (GPX, catalase, glutathione-S-transferase) along with elevated levels of protein carbonylation and MDA have been recorded in the hepatopancreas of *Helix pomatia* snails (Guessasma et al., 2020; Freire et al., 2011). Furthermore, heavy metals can inhibit antioxidant enzymes and disrupt membrane structure and function through binding to ligands, including protein cysteinyl and histidyl groups (Yeboah et al., 2021). Metal ions enriched in the snail hepatopancreas can induce oxidative stress by activating xanthine oxidase and heme oxidases or by inhibiting the respiratory chain, electron transport chain, and enzymatic reactions. These effects can be detrimental and even fatal for organisms (Jena et al., 2009).

Research by (Xie et al., 2016), has shown variations in the modes of action of different metal ions in lipid peroxidation. Excessive production of reactive oxygen species (ROS) under heavy metal stress can lead to attack on the double bonds of unsaturated fatty acids in membrane phospholipids, resulting in lipid peroxidation and increased MDA content, as observed with Pb and Cd.

Antioxidant enzymes can be inactivated by heavy metals through the formation of covalent bonds, primarily between the metals and sulfhydryl groups of proteins (Ercal et al., 2001). Copper-induced increase in glutathione-S-transferase (GST) activity may be attributed to an increased utilization of glutathione (GSH) in conjugation processes involved in the metabolism of lipid hydroperoxides and carbonyl compounds resulting from the peroxidation of cellular membranes caused by copper (Canesi et al., 1999). Proteins play a crucial role in cellular structure and energy provision, particularly under stress conditions (Radwan et al., 2008). Exposure to chemical substances such as heavy metals and pesticides can lead to increased energy consumption and

organelle disintegration in snails, thereby promoting protein production (El Gohary et al., 2021).

Understanding these mechanisms is crucial, as indicated by research from Liu et al., (2022), which emphasizes the varying contributions of Cd and Pb in their interactions depending on the dosage and duration of exposure in the hepatopancreas of *M. nipponense*. Regardless of concentration, both Cd and Pb equally contribute to their interaction effects. Hence, when establishing water quality criteria and implementing ecological restoration measures in aquatic environments, the interaction effects of combined pollutants and their dynamic changes over time and concentration should be comprehensively considered to enhance the scientific basis of water management and environmental protection.

CONCLUSIONS

Based on our findings, it can be concluded that the activity of energy production through coal burning in the Kosova A thermal power plant located in the Municipality of Obiliq results in the release of heavy metal pollutants at concentrations exceeding the limits set by EU standards. The Roman snail (*Helix pomatia*) bioaccumulates these metals from the contaminated soil and plants it consumes, making it an effective bioindicator of environmental pollution. The accumulation of these metals in the snail's shell leads to oxidative stress in the hepatocytes of the snail's hepatopancreas, which poses a potential health concern for the public. This is particularly alarming considering that snails are consumed as a food source. Therefore, the pollution caused by the elevated concentrations of heavy metals in the shell and tissues of *Helix pomatia* not only highlights the environmental impact of the thermal power plant but also raises concerns regarding public health risks associated with the consumption of contaminated snails.

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The potential of the nettle plant *Urtica dioica* L. in phytoremediation of soils polluted by heavy metals

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Abstract

In this research project, we measured the potential of plant (*Urtica dioica*, L) in phytoremediation of soil polluted by heavy metals (Pb, Zn, Ni) from activity of mining Trepça Complex in Mitrovica.

Climatic factors such as winds, rains, and temperatures are believed to be major contributors to the spread over time and space of heavy metals in the environment. Large quantities of these metals come from natural and anthropogenic sources including mining activity, agriculture, pesticide use, industrialization. These inorganic pollutants are deposited in the soil, water, and atmosphere in various forms of complexes and are thus transmitted from plants, animals to humans.

Soil samples, nettle plant (*Urtica dioica*, L), were collected from the selected pollution source of mine Trepça complex at distances of 1km, 2km, and 5km in the radius circles divided into four geographical areas. Also the control samples are collected in unpolluted site Opeja- Dragash municipality. The samples were digested in microwave at 200°C for 45 min and have been read in two types of absorbers Thermo and Contra AAA.

Higher concentrations of Pb, Zn, and Ni were recorded in the southern parts of the country compared to that control with significant differences ($p < 0.01$). Bioaccumulation and biomagnification levels of these heavy metals have also been recorded in the roots, stalks, and leaves of the stinging nettle plant (*Urtica dioica*, L).

The results show that the stinging nettle plant has translocated larger amounts of these heavy metals especially Pb along with the vegetative organs wherefrom these they are carried in the snail shell, which is fed on the stinging nettle plant.

Also, results shown that the nettle plant *Urtica dioica* can be used in phytoremediation process of soil pollution from heavy metals.

Keywords: soil, bioaccumulation, mine complex, metals, stinging nettle.

TRANSFER OF LEAD (PB) FROM SOIL IN FOOD CHAIN NETTLE-SNAIL FROM HEAVY METAL POLLUTION OF MINNING ACTIVITY IN DRENAS"

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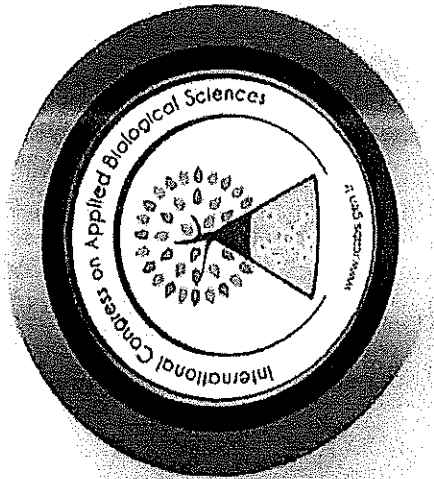
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In this research project we measured the impact of heavy metal pollution from mining activity of "Ferronikel" in Municipality of Drenas Republic of Kosovo.

Climatic factors: winds, rains are believed to be major contributors to the spread over time and space of heavy metals in the lands around the mine. Samples of soil, nettle *Urtica dioica* and shell of snails *Helix pomatia*, are collected around the mining area distances with radius circle 1km, 2km and 5km. The concentration of Pb metal was measured by means of the Contra AAA flame atomic absorber.

Our results shown that the stinging nettle plant, has a high affinity in the bioaccumulation and translocation of heavy metal of Lead (Pb) in vegetative organs roots, stalks and leaves. This metal also have affinity of translocation in the shell of sanils wich feed with leaf of nettle. High concentrations of lead have been recorded even at high distances from the "Ferronickel" mine. Translocation of Pb along the nettle-snail food chain exceeds the value <1 in all three distances. So that, translocation from food chains affect the health of the human which they live in these environments.

Keywords: Bioaccumulation, Translocation, Heavy Metal, Pollution, Environment Health



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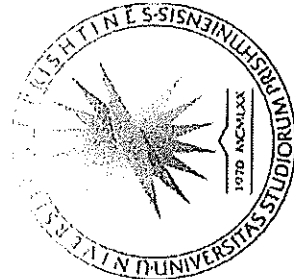
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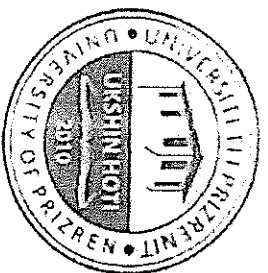
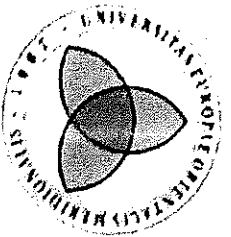
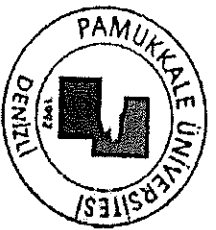
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