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FERRONICKEL MINING POLLUTION: HEAVY METAL ACCUMULATION, OXIDATIVE STRESS, AND BIOINDICATOR POTENTIAL OF *HELIX POMATIA* (L.) SNAILS IN DRENAS, KOSOVO

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Key words: heavy metals, pollution, oxidative stress, hepatopancreas, snail.

Abstract

Heavy metal contamination from mining activities poses significant environmental and public health risks. This study investigates the impact of the Ferronikel mine in Drenas, Kosovo, on soil heavy metal concentrations (Pb, Zn, Ni) and their subsequent bioaccumulation in *Helix pomatia* (L.) snails. 120 soil and 120 snail samples were collected at 1 km, 2 km, and 5 km radial distances from the mine, and heavy metal concentrations were measured using atomic absorption spectrometry. Oxidative stress biomarkers (protein carbonylation, malondialdehyde, and total protein) were analyzed in snail hepatopancreas tissue. Results revealed elevated heavy metal concentrations in soil and snail shells near the mine, exceeding permissible limits for Ni. Oxidative stress parameters were significantly increased in snails from contaminated sites, suggesting a direct link between heavy metal exposure and physiological damage. These findings highlight the potential of *H. pomatia* (L.) as a bioindicator for heavy metal pollution and emphasize the need for stringent environmental monitoring and mitigation strategies in mining areas to safeguard public health.

The results of this research show that there is a high concentration of these metals in the soil in polluted areas. Therefore, they bioaccumulate in the snail shell and cause oxidative stress in the hepatocytes of the tissue snail *H. pomatia* (L).

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Introduction

Heavy metals are gaining a lot of attention as environmental contaminants due to their capacity to infiltrate the food chain through polluted soil, their bioaccumulation in plants and animals, and their transfer to humans, which is currently being researched in ecotoxicological issues. Human activities such as mining, traffic, intensive agriculture, and others can cause air pollution by releasing particles in the soil or dust. This is especially true when the weather is dry and windy (OLAINKA et al. 2011), additionally, it also affects the plant and animal species that make up the food cycle of the ecosystem (JOLLY et al. 2013).

Their environmental quality is inextricably linked to the quality of agricultural goods, which in turn has an impact on people's health. As a result, soil and water-related environmental challenges are crucial for environmental contamination from heavy metals (SUN et al. 2019). The most important environmental issue is heavy metal soil contamination, which greatly impacts people's health including neurological, cardiovascular, and nephrological (SHAKERI et al. 2009).

Industrial sources of heavy metal include operations that process metal in refineries, burning coal in power plants, burn oil, have nuclear power plants, use high-tension lines, process plastics, textiles, and microelectronics, preserve wood, and process paper (BRADL 2002).

Urban and agricultural soils may become contaminated with heavy metals as a result of mining, manufacturing, and the usage of synthetic items (such as pesticides, paints, batteries, industrial waste, and the application of residential or industrial sewage to the ground).

Bioaccumulation is the buildup of absorbed chemicals in an organism over time, whereas the biomagnification is the increase in the concentration of these chemicals in each organism up the food chain (CARSON 2023).

Old landfills (especially those that accepted industrial wastes), old orchards that used insecticides with arsenic as an active ingredient, fields that had previous applications of wastewater or municipal sludge, areas in or around mining waste piles and tailings, industrial areas where chemicals may have been dumped on the ground, or areas downwind from industrial sites are examples of places where potentially contaminated soils may be found (AUBUM 2000).

Snail meat has a long history of being valued as a premium meal. Snail meat has a high protein level and a comparatively low lipid content used for food by humans and carnivores and this is a link in the food chain through which heavy metals are translocated from one link to another (MOONEY et al. 2002).

Researchers have traditionally used snails to analyze the buildup of pollutants. The assessment of internal concentrations of heavy metal contamination following a predetermined exposure period of heavy metal determines the bioaccumulation of contaminants, such as metals, and permits the possibility of determining the snails' capacity for accumulation, their bioavailability, and the intensity of the transfer of contaminants from the environment, food, and/or soil (Gimbert et al. 2006).

In terms of snail anatomy the foot and the viscera are two important components to take into account. The kidney, hepatopancreas, heart, and a portion of the genital system that extends into the foot are among the viscera, which are the organs of the snail shell. Essentially, the foot is made up of the nervous system and the first section of the digestive tract. Pollutant concentrations in the hepatopancreas and kidney increase proportionally with exposure, reflecting both the pollutants' bioavailability to the organism and their levels in the environment (Coeurdasier et al. 2002). Snail organisms are also chosen as sentinels due to their limited toxic response or reduced ability to control their tissue levels. Studying the impact of metals and other pollutants on organism physiology contributes to the development of many toxic studies which can be used as an environmental evaluation tool (Broudi et al. 2020). Within bioindicator species, like the *H. pomatia* (L.) snail, the accumulation of heavy metals can differ across organs, with those exhibiting high metabolic rates, such as the hepatopancreas and digestive tract, being particularly prone to elevated concentrations (Dallinger 1993, Menta and Parisi 2001).

Excessive (Pb) in plants affects normal metabolic pathways by interfering with particular cellular enzymes and may also prevent plants from photosynthesizing. In general, high quantities of heavy metals can cause oxidative stress, damage to DNA, and disruptions to the metabolic processes (Joshua et al. 2015). Generally, based on the research Mohammadein et al. (2013). The ecotoxicological approach described in the current study may have relevance to the ecological impact of several pollutants on the ecosystem and human health. Results obtained from bioaccumulation and histological responses of this common snail can give a useful indication in monitoring soil pollution by heavy metals.

The territory of the municipality of Drenas lies in the central part of Kosovo, in the valley of Drenica, 32 km from Pristina. Its territory lies between the Plain of Kosovo and that of Dukagjin and is a connecting bridge between these two regions. The municipality of Drenas has an area of 275.63 km2 with an altitude of: 575 m, the lowest point, and 1072 m, the highest point. The slope of the terrain is 10–35%. The latitude where the municipality lies is 42°32", longitude latitude is 20°64". The territory of

Drenas municipality is not uniform; it is generally composed of flat, hilly and mountainous areas. The concentrations of heavy defects according to a project financed by UNDP for the monitoring of PM10 and the determination of heavy defects in the area of Drenas, exceedances of the allowed values were recorded only for the metal Nickel (Ni). Within this project, the concentration of heavy metals in PM10 has been evaluated, for the metals Arsenic (As), Cadmium (Cd), Chromium (Chr), Copper (Cu), Mercury (Hg), Nickel (Ni), Lead (Pb), Zinc (Zn) and Iron (Fe).

According to the results from the analysis of the filters, exceedances of the allowed values (20 $\mu g/m^3$) were recorded only for the metal Nickel (Ni), in 12 cases, mainly during October, November, and December 2015 in all four sampling locations (Çikatovo and old, Çikatovo e Re, Gllobar and Lagja e Feronikeli) (UNDP/IHMK. 2015).

Materials and Methods

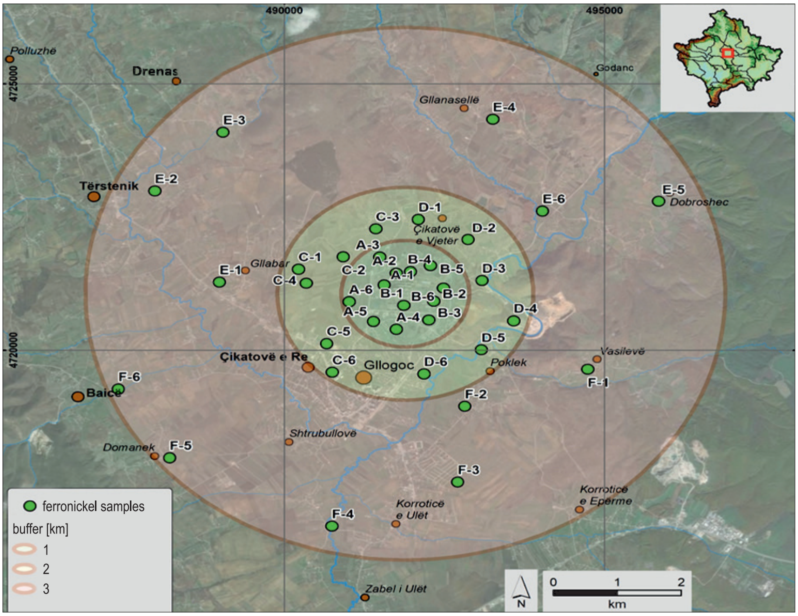
Applied methods

Soil samples and snail *H. pomatia* (L.) were used as material for researching the impact of heavy metal pollution in soil and their effect on oxidative stress in hepatopancreas at the locality of the Municipality of Drenas in Kosovo.

Samples of soil (120), and 120 snails were collected according to radius circles 1 km, 2 km, and 5 km from the point of contamination "Ferronikel" area. Concentric circles were divided into 4 geographical areas northwest, northeast, southeast, and southwest. Samples were collected in natural soils at a depth of 0–15 cm, and an average sample was prepared from 10 separate samples. Snails are also collected in natural habitats around the Ferronikeli mine. In addition, 30 soil samples and 10 snails were collected in the unpolluted locality Brezne – Opoja (served as a control group). Samples were collected in the period summer–autumn 2023. Statistical analysis of the data was conducted using Minitab software, employing Tukey–Kramer post-hoc tests and ANOVA to assess statistical significance. Also, the schematic figures of points with coordinates are made from the ARCmap software program.

Soil samples were collected within a 1 km radius circle centered at coordinates N 42.6821537°, E 21.0884580° and S 42.673995°, E 21.094527°. Additional samples were collected at coordinates N 42.6910814°, E 21.0972603° and N 42.6590480°, E 21.0857567° within a 2 km radius. For the 5 km radius, samples were collected at N 42.720892°, E 21.085643°, divided across four geographical areas.

a



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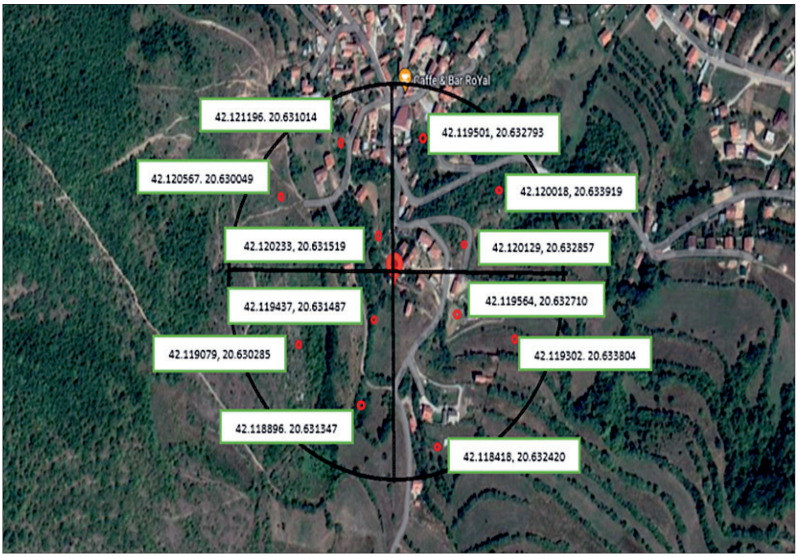

Fig. 1. Schematic representation of the sample collection points at the polluted locality Drenas a) and unpolluted site Brezne (Opoja region) b) in Kosovo

Explanations: Figure 1*a*, points: A1–A3 – Ibër Lepenc Street, northeast; A4–A6 – Mehmet Grainca Street, northwest; B1–B3 – Abedin Nika Street southeast; B4–B6 – Mehmet Grainca Street, southwest in 1 km; C1–C3 – Rrustem Grainca Street, northeast; C4–C6 – Halil Bajraktari street, northwest; D1–D3 – Ferronikel neighborhood; southeast; D4–D6 – Bajram Bajraktari Street, southwest in 2 km; E1–E3 – Istref Hoti Street northeast; E4–E6 Iber Lepenc Street; northwest; F1–F3 – Poklek; Vasileve; Korroticë e epërme; F4–F6- Korroticë e ulët, Domanek, Baincë, southwest in 5 km

Source: own study, in these two areas based on the ARCmap software program and Google Maps

This method involves from three selected points in a geographical area of radius circles are made by 10 drilling of the soil which is then mixed (*Soil quality*… DIN ISO11466. 1995). These soil samples were brought to the laboratory, ground in a soil mill and placed in glass cups and dried in a thermostat at 105°C for 48 h in order to remove moisture. They were then weighed 0.3 g after dehydration and treated with 69% nitric acid (HNO_3) and hydrochloric acid (HCl). (Merck Millipore) reagents concentrated in a 2:6 ratio in teflon columns and digested in the analyticyena TOP wave microwave at 200 C for 45 min. The contents were filtered and placed in normal 50 ml glasses in distilled H_2O. Merck Millipore ICP multi-element standard solution 111355 for metals: Lead (Pb), Zinc (Zn), and Nickel (Ni) are applied for analysis in two flame types of absorbers Thermo and Contra AAA.

In addition, snail samples were collected at the same time and place, according to the selection where previous individuals were used. The shell samples were separated from the body of snail and were washed in distilled H_2O, dried in a thermostat at 105°C for 24–48 h, then ground with a Philips kitchen mixer and 0.5 g of the sample was treated with Merck Millipore reagents; nitric acid 69% ultra-pure (HNO_3) in report 1:3 Lachner hydrogen peroxide (H_2O_2) 30% and digested in microwave at 200°C for 45 min. The contents were filtered and placed in normal glasses of 50 mL, normalized with distilled H_2O, and the metals Pb, Zn, and Ni were read in flame absorber type Analyticjena Contra AAA.

The bioaccumulation coefficient is calculated using the standard formula:

$$\mathrm{BCF} = \frac{\mathrm{C}_{pp}}{\mathrm{C}_{s}}$$

where:
C_{pp} – metal concentration in plant or animal tissue [mg/kg dry weight]
Cs – concentration in soil [mg/kg in dry weight].

Applied methods for oxidative stress

Hepatopancreatic samples for oxidative stress parameters in the vineyard snail *H. pomatia* (L.) were treated according to standard methods protocol. Thus, 36 samples of live snails per km of the locality, their shells were removed and used for the measurement of heavy metals together with soil and heath (plant) samples, then the hepatopancreas were collected from these individuals, which was then weighed and homogenized in phosphate buffer at a ratio of 9:1 mL that is (9 mL of phosphate buffer per 100 g of living tissue) and were placed in 1.5 mL eppendorf tubes and stored in a refrigerator at -80°C. The samples were centrifuged at 3600 revolutions per minute (RPM) for 15 minutes and from the supernatant 1.5 mL of the contents were pipetted and placed in normal test tubes to which the aforementioned reagents were added.

For the measurement of lipid peroxidation, namely malondialdehyde (MDA) in the hepatopancreas, the TBARS method was applied, according to which 0.75% thiobarbituric acid (TBA), then 30% trichloracetic acid (TCA) and 5M HCl are first prepared. The contents are added to the test tubes as follows: (TBA 1.5 mL + TCA 1 mL + HCl 0.2 µL + sample 250 µL). The contents are placed in a water bath and kept at 95°C for 45 min. After this time the contents turn purple. 1 mL of the contents are pipetted and placed in the cuvette of the spectrophotometer and the absorbances at 360 and 450 nm are read. The obtained values of the samples are recorded in the database of the experiment (Sytar et al. 2021).

To measure the carbonylation of proteins in the hepatopancreas, the 2.4 dinitrophenylhydrazine method was applied, where according to this method, the reagents are prepared: 17.20 g of 2.4 dinitrophenylhydrazine is dissolved in 41 mL of 2.5M HCl. Then 1 mL of sample is pipetted into the test tube and 4 mL of 10 mmol dinitrophenylhydrazine (DNPH) is added to it at room temperature for 1 hour so that the acid reacts with the contents and binds the carbonyl groups. After 1 hour, 5 mL of TCA 20% are added to the samples, they are centrifuged and vortexed, 6 mL of ethanol and ethyl acetate are added in a 1:1 mL ratio, they are centrifuged at 6000 RPM for 5 min. The supernatant is carefully discarded and the bottom is retained. The samples are vortexed every 15 min until the supernatant is dissolved. The supernatant is washed three times, vortexed and centrifuged in 6 mL ethanolethyl acetate. Then the bottom is dissolved in 1 mL of 6M guanidine hydrochloride in a water bath at 37°C for 10 min. The absorbances are then read and a blank acid test is performed at 360 nm in a spectrophotometer. The obtained values are recorded in the database of the experiment (Stefek 1993).

While for the measurement of total proteins in the hepatopancreas of the snail, Lovri's method was applied. According to this method, the preparation of BSA standard 1mg/mL is done first.

Standard BSA is prepared by adding the following solutions:

a) 2% Na_2CO_3 in 0.1 N NaOH is pipetted into a 50 mL eluizer plate;
b) 1% NaK tartrate in H_2O 0.5 mL;
c) 0.5% $CuSO_4$. 5 H_2O in H_2O 0.5 mL;
d) reagent I: 48 mL of A, 1 mL of B, 1 mL of C;
e) 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 ELISA plate, 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 (LOWRY 1990).

Results

The results of this research were calculated with the programs Minitab, Tuckey Kramer- ANOVA.

Average data of heavy metals Pb [mg/kg], Ni [μg/kg] and Zn [mg/kg], reported as dry weight values, in all sample types: soil and snail shells from Drenas and Opoja are reported in (Table 1).

Table 1

Summary table of average concentration of metals in soil and shell of samples analyzed in polluted locality Drenas

Metal/Soil	Concentration 1 km *A*	Concentration 2 km *B*	Concentration 5 km *C*	Significance
Pb	126.45 ±1.5 n.s	105.89 ±1.3*	89.46 ±0.9*	*A* : *B* n.s *B* : *C* < 0.05 *A* : *C* < 0.05
Ni	1053 ±3.2***	217.9 ±2.1*	522 ±0.42**	*A* : *B* < 0.001 *B* : *C* < 0.05 *A* : *C* < 0.01
Zn	188.3 ±1.8 n.s	132.3 ±1.6 n.s	145.8 ±1.7 n.s	*A* : *B* n.s *B* : *C* n.s *A* : *C* n.s
Metal/Shell	concentration 1 km *A*	concentration 2 km *B*	concentration 5 km *C*	significance
Pb	9.87 ±1.6**	31.16 ±2.2 n.s	50.75 ±2.5 n.s	*A* : *B* < 0.01 *B* : *C* n.s *A* : *C* < 0.01
Ni	0.42 ±0.9 n.s	0.17 ±0.2 n.s	0.29 ±0.8 n.s	*A* : *B* n.s *B* : *C* n.s *A* : *C* n.s
Zn	42.83 ±2.1 n.s	47.22 ±1.4 n.s	48.1 ±3.2 n.s	*A* : *B* n.s *B* : *C* n.s *A* : *C* n.s

From Table 1 we can see that for all three metals, when the concentration at 1 km was compared with 5 km, a significant $p < 0.05$ was recorded. While comparing 1 km with 2 km, then 2 km with 5 km, no significance was recorded.

Table 2

Summary table of metals in uncontaminated site Opoja

Soil/Shell samples control site				
Sample/metal	soil	SD	shell	SD
Pb [mg/kg]	9.23	0.9	0.09	0.02
Ni [mg/kg]	25.1	1.3	0.011 [μg/kg]	0.05
Zn [mg/kg]	46.2	0.62	0.025	0.09
Significant with pollution site	Pb, Ni, Zn 1–2–5 km; control $p < 0.001$		Pb, Ni, Zn 1–2–5 km; control $p < 0.001$	

The results from Table 2 show that in the analyzed soil samples, the concentrations of the three metals Pb, Zn, and Ni increase as they move away from the point of contamination. Also, the concentration of these metals has significant differences with the control samples. In our cases, we calculated the concentration of metal Ni by converting from mg/kg to μg/kg which recorded low values in shell samples.

According to Table 3 we can see that the value of Pb is below the standard limit, while the level of Ni exceeds 5 times the standard values in the soil. While Zn according to Table 3 standard is in the limit.

Table 3

Standard values according to European Directive 86/278/EEC

Metals	Soil [mg/kg of dry soil]		
	A	*B*	*C*
Arsenic (As)	30	55	80
Barium (Ba)	200	625	2000
Cadmium (Cd)	3	12	25
Chromium (Cr)	300	600	800
Cobalt (Co)	20	240	300
Copper (Cu)	200	300	500
Nickel (Ni)	300	600	800
Lead (Pb)	200	300	600
Mercury (Hg)	1.5	5	10
Molybdenum (Mo)	10	40	200
Tin (Sn)	20	50	300
Zinc (Zn)	300	500	1000
Selenium (Se)	2	100	200

From Table 3 of the locality control we see that the concentrations of metals have significant differences ($p < 0.001$) in all types of analyzed samples with contaminated site Drenas in our cases.

These heavy metals concentrations have also influenced the parameters of oxidative stress in the snail hepatopancreas, in which high values of protein carbonylation, malondialdehyde and total proteins were recorded, which are presented in Table 5 and Table 6 and graphical form.

Table 4

Table of average concentration of carbonyl proteins in polluted site Drenas

Hepatopancreas samples in three distances 1, 2, 5 km				
Samples	coordinates	weight [mg]	abs	conc. [µmol/L]
A1	North sample	0.397	0.170	70.4
B1	South sample	0.531	0.342	14.8
C1	North sample	0.621	1.400	62.9
D1	South sample	0.474	1.623	73.2
E1	North sample	0.527	0.675	30.3
F1	South sample	0.426	1.056	47.3
A4	North sample	0.472	1.592	716
B4	South sample	0.437	0.434	19.2
C4	North sample	0.533	0.345	15.4
D4	South sample	0.491	0.383	16.7
E4	North sample	0.413	0.318	13.8
F4	South sample	0.468	0.342	14.9
Average				91.24
STD				19.10

Table 5

Table of average concentration of carbonyl proteins in unpolluted site Opoja

Unpolluted site			
Sample	weight [mg]	abs	conc. [mol/L]
1	0.236	0.086	3.23
2	0.324	0.017	0.9
3	0.421	0.028	0.59
4	0.382	0.104	0.41
5	0.354	0.072	2.58
Average			1.54
SD			1.27

These results are presented graphically and we see that the highest concentration of protein carbonylation is higher in the southern part of the country than the northern part, these values correlate with the concentrations of metals which are also high in the southern part of the polluted area.

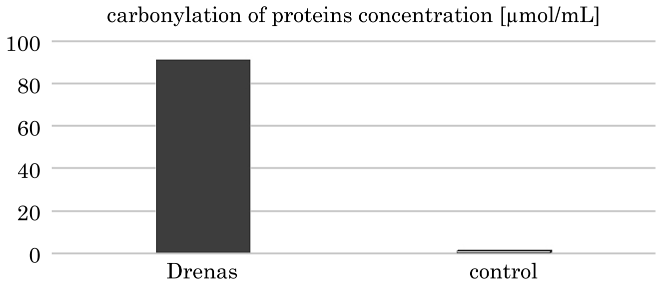

Fig. 2. Concentration of protein carbonylation in polluted area Drenas and unpolluted area control – Opoja

From Figure 1. we see that we have a highly significant difference $p < 0.001$ between the polluted and control area.

The concentration of these heavy metals has also influenced the high values of lipid peroxidation (MDA) and total proteins in snail hepatopancreas, these results are presented in Table 6 and Table 8.

Table 6

Summary table of average MDA in polluted site Drenas

Hepatopancreas samples in three distances 1, 2, 5 km of MDA				
No. of samples	coordinates	weight [mg]	abs	conc. [μmol/L]
A1	North sample	0.397	0.044	128
B1	South sample	0.531	0.119	176
C1	North sample	0.621	0.082	152
D1	South sample	0.474	0.073	46
E1	North sample	0.527	0.047	103
F1	South sample	0.426	0.104	166
A4	North sample	0.472	0.186	107
B4	South sample	0.437	0.239	153
C4	North sample	0.533	0.066	42
D4	South sample	0.491	0.206	132
E4	North sample	0.413	0.097	169
F4	South sample	0.468	0.230	147
Average				126.75
STD				44.9

Table 7

Summary table of average concentration of MDA in unpolluted site Opoja

Unpolluted site			
Sample	weight [mg]	abs	conc. [µmol/L]
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

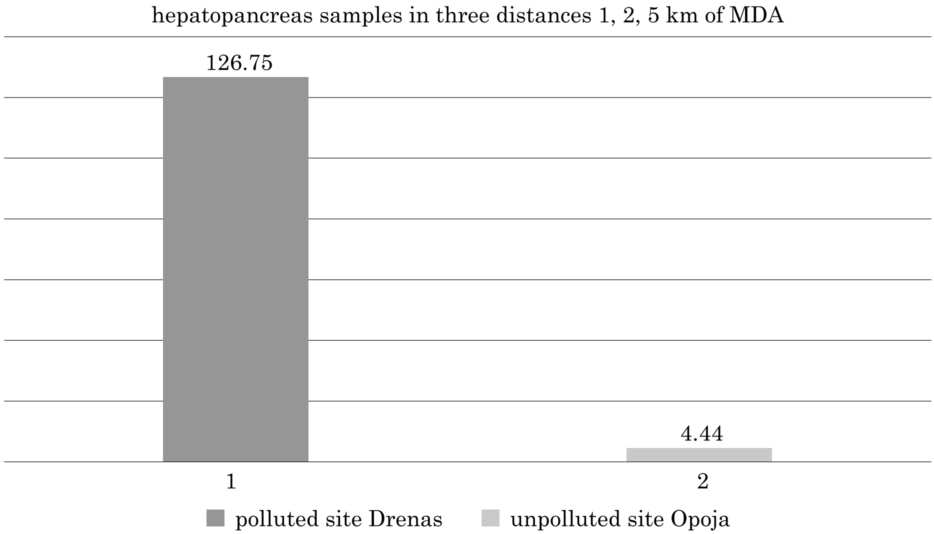

Fig. 3. Concentration of MDA µmol/L in polluted area Drenas and unpolluted area Opoja

Table 8

Summary concentration of total proteins in polluted site Drenas

Total proteins in hepatopancreas at polluted site Drenas					
Sample	weight [mg]	Total [ug/100 uL]	absorbance	450 nm	630 nm
1	2	3	4	5	6
A1O	0.397	23.25167	0.522	0.0696	0.0497
A4O	0.531	19.73274	0.443	0.0591	0.0422
B1O	0.621	18.44098	0.414	0.0552	0.0394
B4O	0.474	16.6147	0.373	0.0497	0.0355

cont. table 8

1	2	3	4	5	6
C1O	0.527	9.131403	0.205	0.0273	0.0195
C4O	0.426	12.82851	0.288	0.0384	0.0274
D1O	0.472	16.39198	0.368	0.0491	0.0350
D4O	0.437	20.62361	0.463	0.0617	0.0441
E1O	0.533	11.80401	0.265	0.0353	0.0252
E4O	0.491	22.49443	0.505	0.0673	0.0481
F1O	0.413	12.78396	0.287	0.0383	0.0273
F4O	0.468	29.66592	0.666	0.0888	0.0634
Mesatarja	–	17.81	–	–	–
STD	–	5.54	–	–	–

Table 9

Summary concentration of total proteins in unpolluted site Opoja

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	significance		
STD	2.52			
D : B	Drenas: Brezne		$p < 0.05$	

Discussion

Our results show high concentrations of metals lead (Pb), zinc (Zn), nickel (Ni) at a distance of 5 km from the concentric circle in Drenas locality from the center of pollution “Ferronikel” mine. Also our research shows the influence of climatic and seasonal factors on the distribution of metals in the soil, since the measurements were made during the early summer-late autumn season. Measurements show high concentrations moving away from the source of pollution as well as in the southwest exposure of the country. These measurements correspond with those of other authors, according to VELIU et al. (2008), states that the air quality research in the vicinity of TC Kosova A has shown that the annual measure of lead (Pb) and zinc (Zn) concentrations compared to European standards are significantly higher.

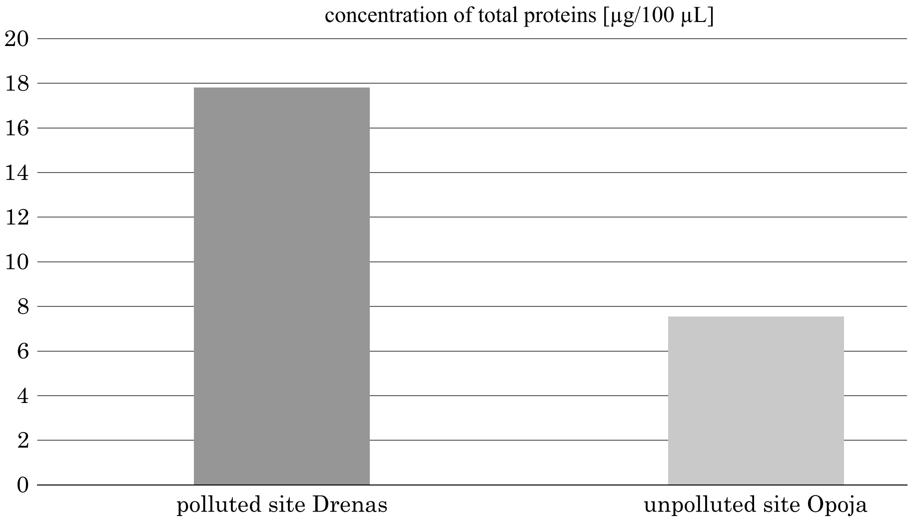

Fig. 4. Concentration of total protein in polluted and unpolluted site

Also, research by BAJRAKTARI et al. (2020) shows that pollution through coal fly ash in the industrial area of Thermal Power Plants (Kosova A and B) causes stratification of metals at different distances and their significant bioaccumulation from soil to snails. So this research also stated for pollution same as we did.

Study from RADWAN et al. (2010) shown that the maximum metal content in snail tissue is reached in autumn and winter and the lowest levels in summer.

The Roman snail (*H. pomatia*) is an important bioindicator of the accumulation of long-term exposure to metals in contaminated environments because of its remarkable capacity to concentrate heavy metal HMs in its body. The snail's hepatopancreas was discovered to be particularly sensitive to HM and frequently accumulated quantities of lead (Pb), nickel (Ni), zinc (Zn), copper (Cu), and cadmium (Cd) that were many times higher in the soil-snail chain link than at lower soil trophic levels. This organ can therefore be utilized to monitor the bioavailability of heavy metal HM in soil (NICA et al. 2012).

Research on this problem is few however, regarding the implication of these analyzed metals in oxidative stress, the results of our research are similar to those of other authors where according to ATAILIA et al. (2016) chronic exposure (5 mg/kg) of snails to the combination of Pb, Zn, Ni heavy metal dust cause changes in enzymatic activities and are also responsible for the development of oxidative stress, which is manifested by increased catalase activity and lipid peroxidation (MDA).

High activity of antioxidant enzymes (glutathione peroxidase (GPX), catalase, glutathione-S-transferase, and high levels of protein carbonylation and lipid peroxidation (MDA) have been recorded in the hepatopancreas of the snail *H. pomatia* (GUESSASMA et al. 2020).

Research by XIE et al. (2016) showed that there were differences in the modes of action of different metal ions in Lipid Peroxidation LPO. The reason for this phenomenon was that Reactive Oxygen Species ROS generated by heavy metal stress could not be removed over time, and the double bonds of unsaturated fatty acids in membrane phospholipids were attacked by excess ROS. Consequently, this resulted in lipid peroxidation, and the MDA content increased accordingly due to the heavy metals Pb and Cd.

Due to the creation of covalent bonds, which exist primarily between heavy metals and sulhydryl groups of the proteins, antioxidant enzymes can be inactivated as a result of direct binding of heavy metals to the active sites of the enzymes (ERCAL et al. 2001). Based on a research CANESI et al. (1999), revealed that the copper-induced increase in Glutathione S-transferases GST activity may be a result of an increased use of GSH in conjugation processes involved in the metabolism of lipid hydroperoxides and carbonyl compounds created by the peroxidation of cellular membranes caused by the copper.

Additionally, proteins play a key role in the structure of the cell. Also, they provide energy while under a lot of stress (RADWAN et al. 2008). In handled snails, the toxic effects of chemical substances such as heavy metals and pesticides may result via increased energy consumption and/or organelle disintegration, which may promote protein production (EL GOHARY et al. 2021).

The importance of knowing these mechanisms is demonstrated by research LIU et al. (2022) which states that the contribution of Cd and Pb in their interactions varies with the dose and duration of exposure in the hepatopancreas of Macrobrachium nipponense. Cadmium (Cd) and lead (Pb) contribute equally to their interaction effects, regardless of concentration. According to recent study demonstrated that zinc oxide nanoparticles (ZnO NPs) have toxic effects on *Helix aspersa*, posing great challenges to the environment. Therefore, the misuse of nanomaterials may have relevant and negative effects on the environment and human health. Industrial applications of ZnO NPs need to be monitored and regulated. Helix aspersa is an excellent bioindicator of nanoecotoxic efficacy (ABDEL-AZEEM et al. 2021).

According to the health risk assessment, infants and children under the age of ten have significant non-carcinogenic hazards associated with lead consumption. There is an urgent need for appropriate control and protection techniques to stop pregnant women, kids, and newborns from being exposed to lead (PERERA et al. 2021). The issue of metals in the environment poses a significant threat to both human and ecological health, and their removal from the environment is a worldwide topic that requires attention (KUMAR et al. 2022).

Conclusions

Based on our results, we can conclude that the activity of energy production from the drilling of soil from activity of minning "Ferronikel" in the Municipality of Drenas causes pollution with heavy metals specially for Ni with high concentrations above the rate allowed according to EU standards.

H. pomatia snails bioaccumulate these metals from the soil and plants in shell that grow in these areas, therefore they can serve as efficient bioindicators in environmental pollution.

These metals accumulated in the shell cause oxidative stress in the hepatocytes of the hepatopancreas in snail and may represent a problem in the public health of people since it is used for food and for trophic level cycle.

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