



# BIOACCUMULATION AND BIOMAGNIFICATION FROM SOIL IN BIOTA NETTLE-SNAIL (*URTICA DIOICA* L. AND *HELIX POMATIA* L.) OF HEAVY METALS (Pb, Zn, Ni) POLLUTION OF MINING ACTIVITY IN MITROVICA.

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## Abstract.

In this research project, we measured the impact and distribution of the activity of minning Trepça Complex in Mitrovica on the concentrations of heavy metals (Pb, Zn, Ni) in soil, plant (*Urtica dioica*, L) and shell of snail (*Helix pomatia*).

Large quantities of these metals come from natural and anthropogenic sources including mining activity, agriculture, pesticide use, industrialization, and inadequate disposal of mineral waste and artificial fertilizers. 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.), and snail (*Helix pomatia*, 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 Opoja-Dragash municipality. The samples were digested in microwave at 200°C for 45 min and have been read in flame absorber Analyticjena 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.) as well as in snail shells (*Helix pomatia*, 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 whereas snail *Helix pomatia* can be used like bioindicator of heavy metal pollution and these heavy metals can contribute to anatomical and morphological changes of these types of organisms or even their extinction.

**Key Words:** soil, bioaccumulation, mine complex, metals, stinging nettle, snail.

## 1. INTRODUCTION

The enrichment and pollution of heavy metals in cultivated lands are of serious importance to many sectors of society and scholars from numerous disciplines as the global community focuses more on heavy metal pollution of soil (Li *et al.*, 2018). Additionally, because the heavy metal is difficult to decompose but simple to accumulate in the soil-plant system, it not only harms the growth and development of plants and animals but also poses a threat to human survival and health (Xu *et al.*, 2017).

Heavy metals are gaining a lot of attention as environmental contaminants due to their capacity to infiltrate

the food chain through polluted soil, and their bioaccumulation in plants and animals, as well as their transfer to humans, which is currently being researched in ecotoxicological difficulties. 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 (Olayinka *et al.*, 2011).

The main factors determining the concentration of heavy metals in the soil are the chemical properties of the soil and the distance from the source of contamination. The amount of heavy metals accumulated and absorbed

by plants is dependent on the kind and concentration of heavy metals, as well as the plant and animal species that make up the food chain (Jolly *et.al.*, 2013).

Mineral resource development has resulted in immense material wealth, but it has also resulted in several ecological and environmental problems, including soil, groundwater, and air pollution (Zhang *et.al.*, 2021). As the basis of agriculture, soil and water play an irreplaceable role, heavy metal contamination has become a severe environmental problem in mining areas. 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 (Sun *et.al.*, 2019). Heavy metals are one of the most damaging groups discharged into the soil due to their capacity to remain. Heavy metals can naturally accumulated by organisms that live and feed on the soil, its components and products, such as land snails and plants including *Urtica dioica L.* and they pass those metals on to other organisms in the food chain (Baroudi, 2020).

Metal exposure through drinking water or absorption through the skin and food with polluted plants is linked to the gastrointestinal tract as a result of industrial mining activities near contaminated soils (WHO, 2007).

In today's climate, heavy metal toxicity and persistence are important environmental problems. Due to both natural and anthropogenic activity, including illegal mining and extensive mineral prospecting in mining zones, heavy metals accumulate in our environment. As a result, a lot of rubbish is produced, which causes the discharge of dangerous elements into the environment, especially heavy metals (AbduYusuf *et.al.*, 2013).

Furthermore, the geochemical properties of both the ore and the bedrock and base rocks handled during mineralogical processes determine the degree and extent of heavy metal contamination. (Iavazzo *et.al.*, 2012).

Additionally, research showed that Pb and Zn had the highest enrichment, with most values exceeding the maximum allowable limits established by various countries, and they are responsible for nearly all of the total potential ecological harm in the analyzed locations (98.64%) (Sharhabil *et.al.*, 2021).

The environmental influence on the food chain, on the other hand, has received very little attention. The most prevalent plants, leaves, and herbs in the researched area include common nettles, which are widely utilized as a herb and food additive in the local population's diet and are ranked among the most popular foods worldwide due to high vitamin and mineral content (Manara *et.al.*, 2012). Administering a complicated investigation involving heavy metal pollution analyses in soil water, air, plant and animal life in agricultural production areas and virgin soils it's very important would reveal their dynamic evolution over time, resulting in more accurate results in the future (Zejnnullahu *et.al.*, 2017).

Heavy metals, particularly Pb, Zn, and Ni, bioaccumulated in the shell and tissues of snails, and this bioaccumulation is more common in industrial areas and along urban highways. These are sites that could be poisoned as a result of human activities (Salih *et.al.*, 2021).

The issue of soil pollution has gotten progressively worse in recent years. The issue of soil heavy metal pollution and the risks it poses to human health has gained attention both domestically and internationally (Zhang *et.al.*, 2018).

Our daily lives now contain more pollution, which has detrimental effects on our health. All types of pollution should be avoided, but heavy metals cause the most devastating, irreversible effects. Because the heavy metals cannot be broken down, they enter the food chain directly and then, through the process of bioaccumulation, reach higher-level consumers. Due to bio-magnification, which causes major illnesses and a high mortality rate in both humans and animals, there is a higher metal concentration than is safe (Ali *et.al.*, 2019). So due to several factors, including industrialization and urbanization, the contamination of soil and water with heavy metals like Cd, Pb, Ni, Cu, and Fe is growing every day. Lead (Pb), Cadmium (Cd), and Nickel (Ni) are a few examples of potential heavy metals that can collect in various areas of a plant and living things are not essential elements, but usually accumulate in vegetative parts and biomagnificate from root to stalk and leaf with >1 factor translocation value (Bislimi *et.al.*, 2021).

## 2. MATERIALS AND METHODS

Soil samples, nettle *U. dioica* and snail *H. pomatia* were collected to assess heavy metals (Pb, Zn and Ni) around the mining complex "Trepça" in Mitrovica-(Kosovo) and in the locality Brezne-Opoja, at a mountainous plain in the south of Kosovo (1380 m a.s.l.), considered unpolluted, as control site (Fig. 1). The material was collected during the summer-autumn period of 2022. The area was split into 4 geographical areas: northwest, northeast, southeast, southwest, with imaginary circles in 1 km, 2 km, 5 km; 12 stations were selected in total, three in each area (Fig. 2above). 10 material samples were collected in each sampling site, of soil, nettle and snails, in total 120 samples. Additionally 10 soil samples, nettles and snails were collected separately (30 samples in total) in the control site of Brezne-Opoja (Fig. 2below).

### 2.1. Applied methods

Soil samples were collected by hand probe at a depth of 15cm in the virgin lands (BBodSchV, 1999; Theocharopoulos *et al.*, 2001; ICP Forest, 2006). According to this method from three selected points in a geographical area of radius circles are made by 10 drilling of the soil which is then mixed (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 48h in order to remove moisture. They were then weighed 0.3g and treated with 69% HNO<sub>3</sub> and HCl (Merck Millipore) reagents concentrated in a 2: 6 ratio in teflon columns and digested in the analyticjena TOP wave microwave at 200°C for 45 min. The contents were filtered

and placed in normal 50 ml glasses in distilled H<sub>2</sub>O. Merck Millipore ICP multi-element standard solution 111355 for metals Pb, Zn, Ni are applied for read in flame absorber Analyticjena Contra AAA.

The results were elaborated with the Minitab® 19 - statistical software; Tukey-Kramer Test; ANOVA software for Excel.

*Table 1. Tabular presentation of sampling coordinates in polluted area Mitrovica.*

Sample 1km	Coordinates Longitude/latitude	Sample 2km	Coordinates Longitude/latitude	Sample 5km	Coordinates Longitude/latitude
A-1	42.888164, 20.876495	C-1	42.893895,20.872635	E-1	42.914962, 20.847280
A-2	42.885627, 20.876433	C-2	42.895612, 20.880263	E-2	42.884845, 20.849683
A-3	42.884754, 20.872694	C-3	42.889717, 20.862067	E-3	42.901131, 20.875776
A-4	42.879640, 20.875086	C-4	42.876235, 20.870940	E-4	42.865755, 20.842945
A-5	42.877887, 20.879609	C-5	42.871629, 20.878372	E-5	42.862546, 20.853245
A-6	42.876169, 20.879767	C-6	42.872765, 20.870154	E-6	42.860030, 20.871870
B-1	42.889274, 20.884839	D-1	42.891130, 20.889069	F-1	42.899272, 20.895742
B-2	42.885823, 20.884823	D-2	42.888093, 20.898035	F-2	42.892261, 20.900291
B-3	42.883980, 20.886070	D-3	42.872763, 20.888766	F-3	42.907941, 20.899325
B-4	42.878127, 20.883554	D-4	42.873746, 20.894270	F-4	42.871483, 20.917629
B-5	42.880025, 20.888173	D-5	42.876718, 20.899870	F-5	42.864013, 20.905076
B-6	42.877518, 20.885971	D-6	42.878023, 20.898153	F-6	42.868542, 20.889433

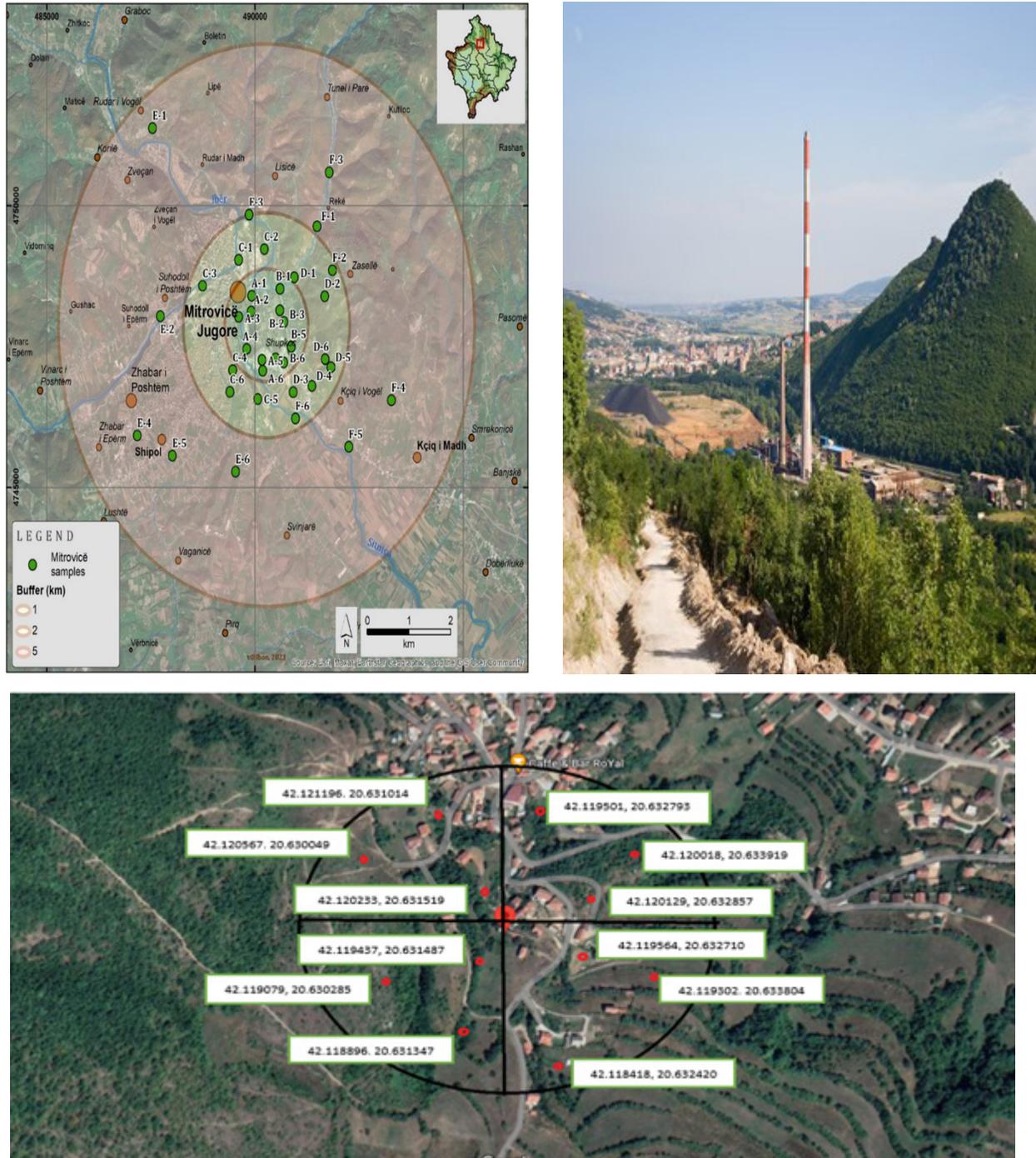


Figure 1. Schematic representation of the sample collection at the polluted locality Mitrovica and unpolluted locality Brezne-Opoja.

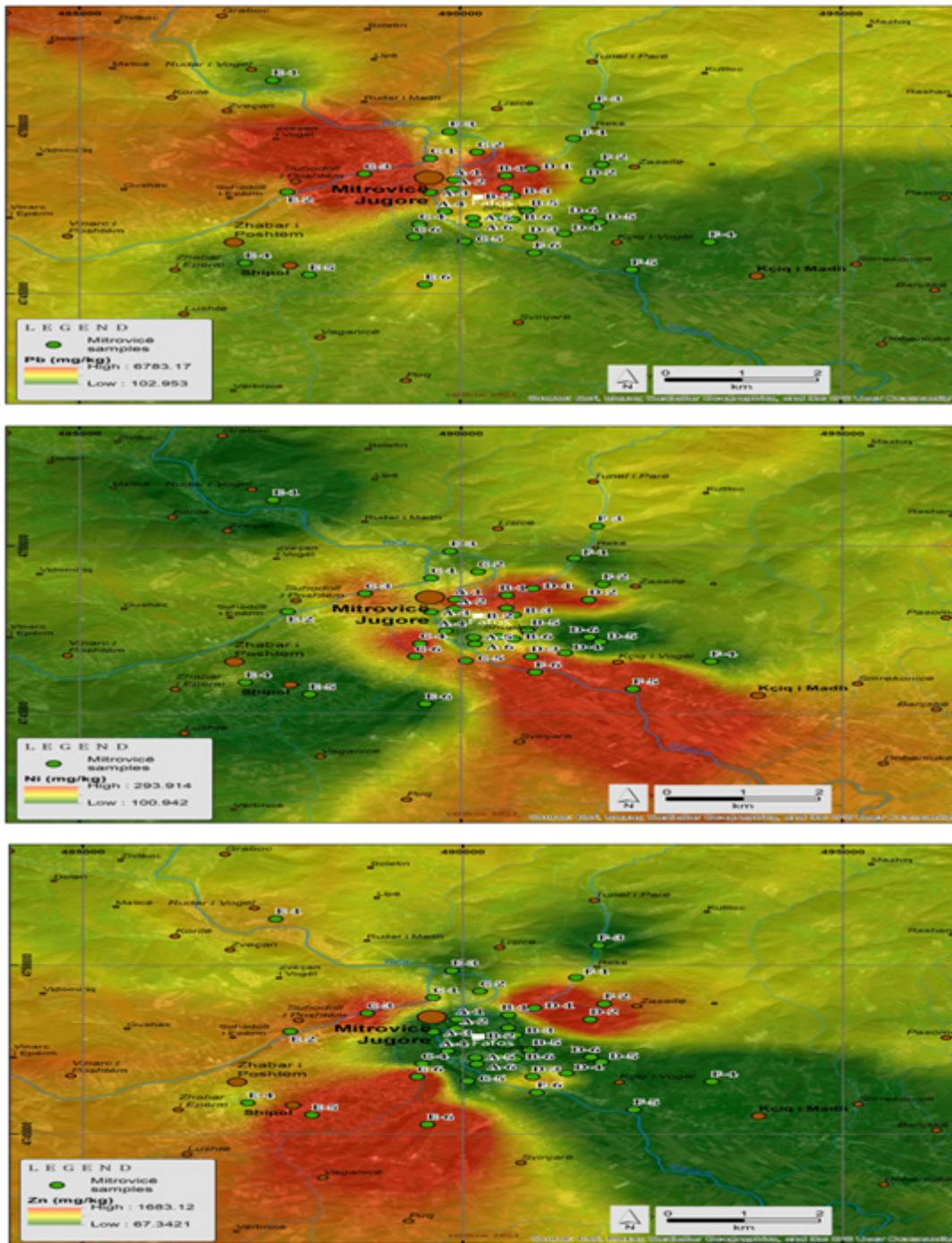


Figure 2. Map representation of the concentration distribution of three heavy metals Pb Zn Ni in polluted area Mitrovica.

From figure 2 we see that the concentration of the analyzed metals in the soil is more pronounced in the south-western part, which has also affected the plant and animal organism samples analyzed, affecting the accumulation of these metals.

Samples of nettle and snail 4-6 years old material (Cowie *et.al.*, 2009) was collected in the same habitats. The nettle vegetative organs (rhizomes, stalk, leaves) and the snail shells were separated and washed in distilled

H<sub>2</sub>O, dried in 105°C for 24-48 h, ground with a Philips kitchen mixer. 0.5 g of the sample was treated with nitric acid 69% ultra pure (HNO<sub>3</sub>) and Lachner hydrogen peroxide H<sub>2</sub>O<sub>2</sub> 30% in report 1:3 and digested in microwave at 200°C for 45 min. The content was filtered and placed in 50 ml normal glasses, normalized with distilled H<sub>2</sub>O. The metals Pb, Zn, Ni were analyzed in flame absorber (Analyticaena Contra AAA).

The bioaccumulation coefficient is calculated using the standard formula:

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

C<sub>plant parts</sub>= metal concentration in plant or animal tissue, mg/kg dry weight

C<sub>soil</sub>= concentration in soil mg/kg in dry weight.

The translocation factor TF is calculated with formula  $TF = C_{plant\ shoot} / C_{plant\ root}$ , whereas the enrichment factor (EF) is calculated with formula  $EF = C_{plant} / C_{soil}$  (Galal & Shehata, 2015).

### 3. RESULTS

Average data of heavy metals (Pb, mg/kg, Ni, µg/kg, Zn, mg/kg), reported as dry weight values, in all sample types: soil, nettle parts (rhizomes, stems and leaves), overall nettle plant, and snail shells from Mitrovica and Opoja are reported in table 2, High values of nickel were recorded in soil samples and vegetative organs; therefore we reported it in mg/kg for nettle and shell samples in all cases. Concentration, bioaccumulation factor (BCF), transfer factor (TF) and enrichment factor (EF) of heavy metals from soil to rhizomes, from rhizomes to stems and leaves, and from leaves to shells in Mitrovica (1 km, 2 km and 5 km) are reported in table 2,3,4,5.

Table 2. Summary table of average concentration of metals in all types of samples analyzed in polluted locality Mitrovica and control site Opoja

Mitrovica 1km										
Nettle/Snail										
Sample/ Metal	Soil	RSD%	Root	RSD%	Stalk	RSD%	Leaf	RSD%	Shell	RSD%
Pb	2203.4	2.1	131.79	3.6	17.03	1.7	32.29	1.6	7.09	1.3
Ni	166.9	1.3	18.48	1.2	17.38	1.9	16.64	1.2	0.036	0.3
Zn	72.68	0.9	187.33	4.5	163.96	5.2	159.12	2.3	31.77	1.6
Mitrovica 2km										
Nettle/Snail										
Sample/ Metal	Soil	RSD%	Root	RSD%	Stalk	RSD%	Leaf	RSD%	Shell	RSD%
Pb	1338.6	1.7	24.54	2.3	3.79	2.8	3.25	1.3	57.25	2.5
Ni	184.5	2.2	13.4	1.1	18.15	2.3	14.08	1.1	0.021	0.2
Zn	885.3	1.9	74.33	3.5	76.98	4.1	62.46	2.4	31.71	1.9
Mitrovica 5km										
Nettle/Snail										
Sample/ Metal	Soil	RSD%	Root	RSD%	Stalk	RSD%	Leaf	RSD%	Shell	RSD%
Pb	471.3	1.4	16.39	1.4	4.67	3.6	6.06	1.5	50.18	2.3
Ni	166.6	1.1	0.24	0.74	0.089	0.4	0.176	0.5	0.011	0.1
Zn	538.3	1.5	25.17	1.6	26.96	2.6	19.67	1.4	40.38	2.1
Significant of 1:2:5 km	P<0.05									

Summary table of samples of control locality- Opoja										
Sample/Metal	Soil	RSD%	Root	RSD%	Stalk	RSD%	Leave	RSD%	Shell	RSD%
Pb	9.23	0.9	0.16	0.12	0.078	0.035	0.012	0.09	0.09	0.02
Ni	25.1	1.3	0.021	0.019	0.034	0.013	0.054	0.026	0.011	0.05
Zn	46.2	0.62	0.042	0.021	0.026	0.018	0.045	0.017	0.025	0.09
Significant with pollution site	p<0.001		p<0.001		p<0.001		p<0.001		p<0.001	

The results from table. (1) show that in the analyzed soil samples, the concentrations of the three metals Pb, Zn, Ni increase as they move away from the point of contamination. Also the concentration of these metals has significant differences with the control samples.

According to table.(2) we see that the value of Pb is very high of the standard limit according of DE standard and AI of Agency for the protection of nature, while the level of Ni exceeds 5 times the standard values in the soil according of DE standard. Also Zn according to the DE standard and AI exceeds of the limit values at 290 (mg/kg). The samples as follows (in mg/kg) Pb>2203.4, Ni >166.9, Zn >72.68 in 1km, while for 2km Pb>1338.6, Zn>885.3, Ni>184.5, while in 5 km Pb>471.3, Zn>538.3, Ni>166.6.

The concentration of Pb along this nettle-snail food chain, specifically in the shell, is explained by the fact that these areas along the Industrial Complex of the Trepça mine are heavily polluted with lead. From our results, it can be seen that the concentration of Pb in the soil samples along three km exceeds many times the allowed values for soil. Respectively Pb= 2203.4 mg/kg in 1 km, Ni =166.6 mg/kg in 2km, Zn =885.3 mg/kg of 2km in soil samples. Whereas, in the shell samples we can see that the high concentration of Pb is registered in 2 km when Pb= 57.25, whereas the low concentration Ni=0.036, 0.021, 0.011 mg/kg in three distances.

Whereas the concentration of Zn in three distances is Zn 31.77, 31.71, 40.38 mg/kg.

In this case the factors such as spread, winds and antropogenic activities, transport of soil from minning, have an impact in distribution of these metals. Relatively high content of Pb absorbed preferentially by the nettle is serious for the animal health that graze it or the man health itself; young shoots are traditionally harvested and used as food, to make tasty ‘burek’, ‘lakror’ or ‘pispilit’; its leaves are also commonly traded as medicinal plants. The same considerations can be given also for the snail; its preferential bioaccumulation of Ni and Zn can be harmful for other biota in its upper food chain (birds and mammals), and man itself; it is commonly harvested and used as tasty food, and also traded to pharmaceutical or cosmetic industry.

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 Mitrovica in our cases.

When the translocation coefficient was calculated, we saw that in some cases, for example, in the parts of the nettle plant, the root, stalk, leaf and shell, Pb and Zn in some cases reach the coefficient <1 which means that it is biomagnified along the food chain. These results are the same as those of other authors (Nica *et al.*, 2012); (Salih *et al.*, 2021), while in some other cases it is at the limit of the coefficient 1. While for Ni very low translocation values were recorded in our cases.

Table 3. Bioaccumulation and concentration of heavy metals from soil to root, root to stalk, stalk to leaf of *Urtica dioica* L. and leaf to shell of *Helix pomatia* in the contaminated site (Mitrovica) 1km

Metal	Sample	Concentration ppm	BCF	EF	TF
<b>Pb</b>	Soil	2203.4		26.84	
	Root	131.79	0.060	8.79	
	Stalk	17.03	0.0077	405.47	0.13
	Leaf	32.29	0.015	504.53	0.25
	Shell	7.09	0.0032	590.83	0.23
<b>Zn</b>	Soil	72.68		1.57	
	Root	187.33	2.57	3229.82	
	Stalk	163.96	2.25	3903.81	0.73
	Leaf	159.12	2.19	2040	0.85
	Shell	31.77	0.44	96.27	0.20
<b>Ni</b>	Soil	166.9		1.16	
	Root	18.48	0.11	1680	
	Stalk	17.38	0.10	1931.11	0.94
	Leaf	16.64	0.099	1188.57	0.90
	Shell	0.036	0.00022	2.76	0.0021

Table 4. Bioaccumulation and concentration of heavy metals from soil to root, root to stalk, stalk to leaf of *Urtica dioica* L. and leaf to shell of *Helix pomatia* in the contaminated site (Mitrovica) 2km.

Metal	Sample	Concentration ppm	BCF	EF	TF
<b>Pb</b>	Soil	1338.6		25.49	
	Root	24.54	0.018	1636	
	Stalk	3.79	0.0028	90.24	0.15
	Leaf	3.25	0.0024	50.78	0.13
	Shell	57.25	0.043	4770.83	17.61
<b>Zn</b>	Soil	885.3		20.54	
	Root	74.33	0.084	1281.55	
	Stalk	76.98	0.087	1790.23	1.03
	Leaf	62.46	0.071	800.77	0.84
	Shell	31.71	0.036	406.54	0.51
<b>Ni</b>	Soil	184.5		1.13	
	Root	13.4	0.073	1218.18	
	Stalk	18.15	0.098	1210	1.35
	Leaf	14.08	0.076	220	1.05
	Shell	0.021	0.00011	1.62	0.0014

Table 5. Bioaccumulation and concentration of heavy metals from soil to root, root to stalk, stalk to leaf of *Urtica dioica* L. and leaf to shell of *Helix pomatia* in the contaminated site (Mitrovica) 5km.

Metal	Sample	Concentration ppm	BCF	EF	TF
<b>Pb</b>	Soil	471.3		8.73	
	Root	16.39	0.035	1092.66	
	Stalk	4.67	0.0099	111.19	0.28
	Leaf	6.06	0.013	94.69	0.37
	Shell	50.18	0.11	4181.66	8.28
<b>Zn</b>	Soil	538.3		18.56	
	Root	25.17	0.053	433.97	
	Stalk	26.96	0.057	626.98	1.07
	Leaf	19.67	0.042	252.18	0.78
	Shell	40.38	0.086	122.36	2.05
<b>Ni</b>	Soil	166.6		1.18	
	Root	0.24	0.00053	21.82	
	Stalk	0.089	0.00019	9.9	0.37
	Leaf	0.176	0.00037	12.57	0.73
	Shell	0.011	0.000023	0.85	0.045

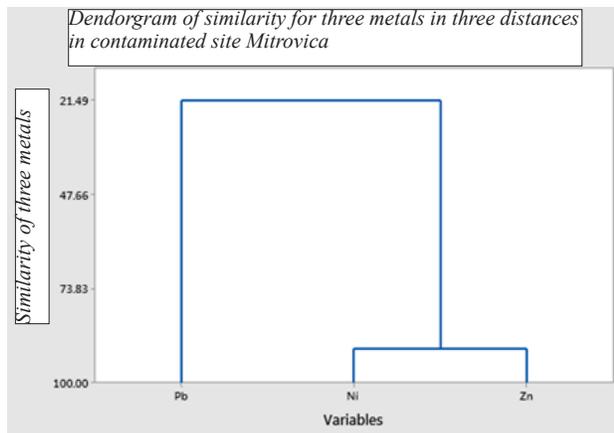


Figure 3. Dendrogram of cluster value and similarity for three metals in three distances in contaminated site Mitrovica.

From figure (3) we see that the dendrogram graph shown the cluster value of these heavy metals in the soil of pollution area.

#### 4. DISCUSSION

The current study showed that the ecological and environmental problems are often caused from the mining industry, including soil, groundwater, and air pollution (Zhang *et al.* 2019). Soil and water play an irreplaceable role for the agriculture; heavy metal contamination has become a serious environmental issue in mining regions, related with the quality of agricultural goods, which in turn has an impact on human health. Therefore, soil and water-related environmental challenges are crucial role in agriculture (Linhua and Songbao 2019). HMs may have long-term effects on human health by the consumption of apple fruit and other plants with a real possibility that these elements (Cd, Pb, Ni, and Cr) could enter the food chain (Imeri *et al.* 2019). Pb and Zn often exceed the maximum permissible limits set by various countries, and they are accountable for virtually all of the potential ecological impact in the studied sites (98.64%) (Yahya *et al.* 2021). Pb, Cd and Ni are a few examples of not essential elements but usually accumulate in vegetative parts and biomagnify from root to stems and leaves with >1 factor translocation value (Bislimi *et al.* 2021). Additionally, their potential bioavailability in products near contaminated soils is demonstrated extremely high (Zogaj *et al.* 2014). Also, emphasize that the nettle plant (*U. dioica*) bioaccumulate and translocate the heavy metals and can serve as a base plant in the phytoremediation and soil amendment from heavy metal pollution process exclusively for Pb as confirmed also by Bislimi *et al.* (2021). Çarkaj *et al.* (2022) also emphasizes that the garden snail can accumulate relatively high concentrations of heavy metals and survive, making it good model for the biomonitoring of heavy metals in the environment.

Heavy metals, particularly Pb, Zn, and Ni, bioaccumulate in the shells and tissues of snails, and this bioaccumu-

lation is more common in industrial areas and along urban highways (Salih *et al.*, 2021). Our findings for dispersion of these pollutants are same with the findings of (Genchi *et al.*, 2020) which concluded that lead is a transition element that is widely dispersed in the environment, including the air, water, and soil. It could come from both anthropogenic activity and natural sources. Also the findings of (Gardiner *et al.*, 1995) concluded that the concentration of these metals in the air, soil, or water, the plant species, bioavailability, cation exchange capacity, pH, vegetation season, climatic condition, age and nutrition from animals like snails, mammals and humans all affect the concentration of heavy metals in the plant components.

To fully understand the human risk associated with heavy metal pollution in general, it is essential to expand the current research to other plant species (cereals, fruits, and other vegetables), locations, and routes of metal exposure; for example, consumption of animal foods (meat, milk, and eggs), drinking water, or contact with air (Filimon *et al.* 2021).

#### 5. CONCLUSIONS

From our results of this research we can conclude that, soil pollution with heavy metals comes as a result of their release from the chimneys of the mining “Trepca Industrial Complex”, traffic and ash dump.

We also conclude that climatic factors such as wind, wind rosettes and fly ash in a southerly direction contribute to the spread of these metals.

From the values obtained from this research, a trend of the bioaccumulation and translocation ability of the nettle plant is observed in our cases.

We can also emphasize that the nettle plant *Urtica dioica* can serve as a base plant in the phytoremediation and soil amendment from heavy metal pollution process exclusively for Pb.

*Helix pomatia* snails can be used as bio indicators for measuring pollution with heavy metals and their impact on the transfer processes of these metals in other food chains.

Since these two types of organisms are used for human consumption, there is a strong possibility that this sensitive link in the trophic chain will be contaminated and have major consequences for environment and public health.

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