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THE RISK OF FOREST FRUITS CONTAMINATION BY HEAVY METALS IN THE HOREHRONIE REGION (SLOVAKIA)

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ABSTRACT

The Horehronie region belongs to the oldest industrial areas in Slovakia. It is contaminated by residual acid tar dumps from times of Petrochema Dubová factory production since 1964. In the presented study, heavy metals (Cu, Cd, Ni, Zn, Pb) were determined in soils and forest fruits (*Vaccinium myrtillus* L., *Vaccinium vitis-idaea* L., *Fragaria vesca* L., *Rosa canina* L., *Rubus idaeus* L.) collected over two years from five locations in the Horehronie using Flame AAS method (Zn, Cu, Ni) and Graphite Furnace AAS method (Cd, Pb). The cadmium content, which was higher than its limit value in all analysed soils, poses the biggest threat. We determined more than 20 times the limit value for cadmium in the sample Veľká Smrekovica (9.17 mg/kg).The contamination factor (C_f) values and the geoaccumulation index (I_{geo}) indicate that this hazardous metal is involved in soil contamination to a large extent. The highest values of the degree of contamination (C_{deg}) and the pollution load index (PLI) were determined in samples from the Ždiarska dolina and the Veľká Smrekovica. The contents of nickel and zinc were higher than the limit values only in the 1st sampling year in the Ždiarska dolina sample (46.30 mg/kg; 138.30 mg/kg). The results showed that soil monitoring and remediation of old environmental burdens are important in the territory of the Horehronie region. The highest permissible amounts for cadmium were exceeded in all types of forest fruits (0.03-0.18 mg/kg FW). The contents of nickel and lead were exceeded only in the sample of rosehips (*Rosa canina*). The contents of zinc and copper were lower than the permissible amounts in all samples. The estimated daily intake (EDI) was lower than the acceptable daily intake of all heavy metals. The bioaccumulation factor (BAF), total hazardous quotient and hazard index of the analysed samples was < 1, which means that the consumption of forest fruits from the Horehronie does not pose a health risk for the consumer.

Keywords: heavy metals, forest fruits, soil contamination, acid tars

INTRODUCTION

Nowadays, the opinion that people should consume more vegetables and fruits is becoming more and more popular. Fruits and vegetables are good sources of vitamins, minerals, fibre and are also beneficial for the proper function of the immune system **(Sharma *et al.*, 2021)**. Bilberries (*Vaccinium myrtillus* L.), wild strawberries (*Fragaria vesca* L.), lingonberries (*Vaccinium vitis-idaea* L.), rosehips (*Rosa canina* L.), and wild raspberries (*Rubus idaeus* L.) are among the most popular types of forest fruits. These species grow almost throughout all of Europe **(Benevenuto *et al.*, 2019; Barkaoui *et al*., 2021; Kowalska, 2021)**. Wild berries are commonly consumed not only in fresh and frozen form but also as dried and canned in yoghurts, drinks, jams or jellies. Forest fruits contain a large number of bioactive compounds, vitamins and minerals that are beneficial for the health of the consumer **(Rajakaruna *et al.*, 2021)**. Some of the known chemoprotective agents present in fruits include vitamins A, C, E, and B9, minerals calcium and selenium. Carotene, lutein, phytosterols such as sitosterol and stigmasterol, triterpene esters, phenolic substances (anthocyanins, flavonols, flavanols, proanthocyanidins, ellagitannins and phenolic acids) are also present **(Nile *et al.*, 2014)**. Forest fruits deserve special attention not only because of their rich and diverse chemical composition but also because of their property of accumulating specific components from soil and air **(Sembratowicz *et al.*, 2008)**. The content of heavy metals in fruit depends on many factors (varietal characteristics, growing season, environmental conditions, place of harvest, etc.) **(Rusinek *et al.*, 2008)**. It is commonly known that plants absorb hazardous elements from contaminated soils or from deposits on plant parts from a polluted environment. High concentrations of heavy metals in the environment raise public concerns about the presence of residual contaminants in the daily diet. Due to potential toxicity and cumulative properties, it is necessary to analyse foods. This will ensure that contaminant concentrations will meet applicable legislative limits **(Sharma *et al.*, 2021)**.
The Horehronie region is one of the most popular tourist destinations in Slovakia due to its favourable geographic position and diverse natural landscape. Horehronie's forests are geographically varied areas. It is necessary to guide and regulate anthropogenic activities that cause environmental pollution for the preservation of valuable mountain areas in the future as well **(Čallag, 2018)**. In the Horehronie, acid tars are the most significant environmental pollutants and causes of contamination since 1964. The acid tars are located in the protection zone of the Low Tatras National Park. They were created as dumps of petroleum waste from the Petrochema Dubová factory, which was exported here for 10 years. The biggest threat is the increasing volume of the water surface of the dumps due to atmospheric precipitation and melting snow from the surrounding mountains. Along the edges of the water surface, we can observe the gradual polymerization of the waste and the formation of solid substances. If these environmental landfills are not dealt with, there is a risk of spillage and penetration of the water phase through the damaged parts of acid tars into the surrounding soil. There are already signs of acid tar spillage and leaks in the dumps' southern and eastern zones. **(Jánová, 2016a)**.
The refining of petroleum products with chemical agents such as sulfuric acid produces by-products – tars. Almost the entire amount of sulfuric acid, hydrocarbons from oil distillates, and newly formed hydrocarbons resulting from oil refining, metal compounds and sulfur compounds permeate the tars. Polycondensation and oxidation processes occur years after being deposited in dumps. These processes change the state and consistency of tars. By increasing the atmospheric temperature during the summer months, the state changes again, making the upper layers of acid tars liquid. The presence of sulfuric acid ranges from 5 to 70%, which causes a strong smell in the surroundings. This type of waste is categorized as dangerous with toxic, teratogenic, carcinogenic and mutagenic effects. Acid tars are also a serious threat to animals and plants **(Janová, 2016b)**.
This study is aimed at determining the danger of soil contamination with heavy metals (Cu, Cd, Ni, Zn, Pb) from the Horehronie region. The degree of soil contamination was determined based on the value of the contamination factor (C_f), degree of contamination (C_{deg}), pollution load index (PLI) and geoaccumulation index (I_{geo}). Another objective of the study was to assess the transfer of heavy metals to forest fruits due to bioaccumulation factor (BAF), estimated daily intake (EDI), total hazard quotient (THQ) and hazard index (HI).

MATERIAL AND METHODS

The Horehronie region is located in the middle of Slovakia. It is bordered in the north by the Low Tatras and in the east by the Slovak Ore Mountains. The western part of the region is bordered by the Kremnické vrchy. Brown forest soils are mainly found in the Horehronie. The eastern part is characterized by rendzina. Podzol is a typical soil type for the Low Tatras. The pseudoglay type of soil is dominant in the middle region. Texture classes of soil are mainly represented by sandy-loamy and loamy soils. **(Čeman, 2010)**.

Sampling and sample preparation

The samples were collected over two years at 5 sampling points (bilberries – Babiná; lingonberries – Zadná hoľa; wild strawberries – Ždiarska dolina; wild raspberries – Veľká Smrekovica; rosehips – Končisté; Figure 1). At each sampling point, 5 average samples of forest fruits (approximately 100 g) and simultaneously 5 average soil samples (approximately 200 g) were collected. Sampling was carried out in accordance with the applicable legislation **(Decree 151/2016)**. The density and distribution of soil sampling from the forest plot must maintain homogeneity. Soil samples were collected to a depth of 25 cm into a pedological probe GeoSampler by Fisher. The top 3 cm is not included in the sample. Organic impurities (leaves, roots) and debris were removed from the soil samples before drying. After drying, the samples were ground (grinding machine VEB Thurm ZG 1) to fine earth (average particle size 0.125 mm), in which the contents of risk metals were determined. The soil samples were stored in polyethylene bags until analysis. Samples of plant material were mechanically cleaned from organic and inorganic impurities immediately after collection. Subsequently, they were washed with distilled water, sliced, and stored in polyethylene bags at a temperature of -18 °C until analysis.

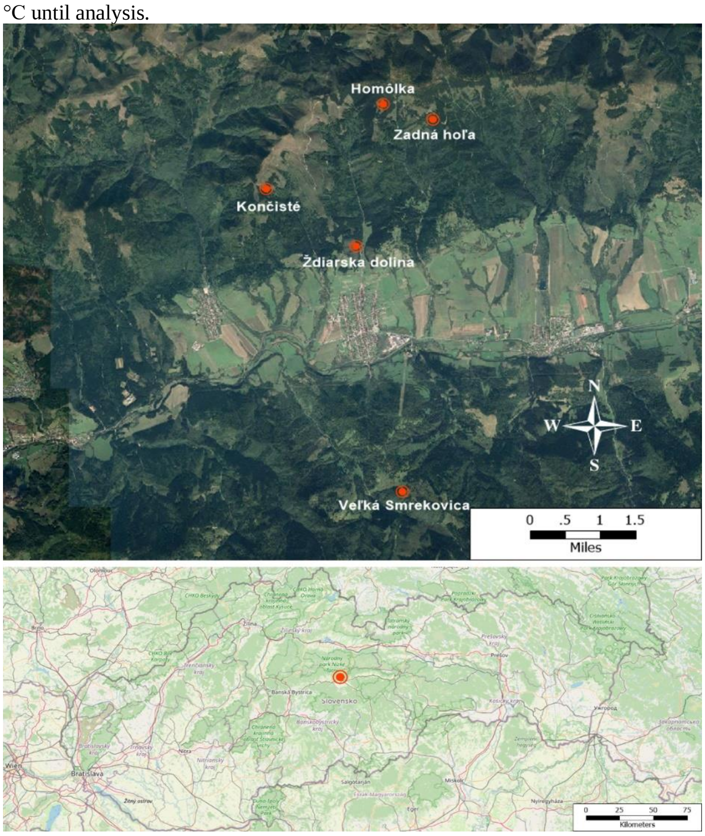

Figure 1 Map of the studied area and sampling sites

Chemical analysis

The contents of heavy metals were determined in soils and fruits samples using Flame AAS method (Zn, Cu, Ni) and Graphite Furnace AAS method (Cd, Pb) (VARIAN AASpectr DUO 240FS/240Z/UltrAA equipped with a D2 lamp background correction system, using an air-acetylene flame, Varian, Ltd., Mulgrave, VIC, AUS). After microwave digestion (70 min, MARS X-Press 5, CEM Corp., Matthews, NC, USA), the total contents of heavy metals, including all metal forms with exception of silicate forms in soil extract by *aqua regia* (1 g fine earth + 10 mL aqua regia, HNO_3, HCl; Merck, Germany) were determined. The fruit samples were mineralised using a closed microwave digestion system (Mars X-Press 5) with conc. HNO_3. Gas flow during analysis: air 13.5 L/min, acetylene 2.0 L/min, Deviation during analysis max 3%. Wavelength (nm) / detection limit (mg/L) / sensitivity (mg/L): 324.8/0.002/0.03 (Cu); 228.8/0.001/0.01 (Cd); 217/0.02/0.1 (Pb); 213.9/0.006/0.008 (Zn); 232/0.008/0.06 (Ni).

Indicators of soil contamination by heavy metals

Contamination factor (C_f) is given by the ratio of the concentration of the given hazardous element in the soil and its background concentration (Eq. 1) **(Islam, Md. S, 2015)**:

$$C_f^i = \frac{C^i}{B^i} \quad (1)$$

C^i: determined concentration of the heavy metals (mg/kg),
B^i: geochemical background concentration of the heavy metals (mg/kg) **(Linkeš *et al.*, 1997)**.

Degree of contamination (C_{deg}) was the sum of contamination factors for all hazardous elements examined (Eq. 2) **(Islam, Md. S, 2015):**

$$C_{deg} = \sum C_f^i \quad (2)$$

Pollution load index (PLI) is defined as the n-th root of the multiplications of the contamination factor (C_f) (Eq. 3) **(Abowaly *et al.*, 2021)**:

$$PLI = \left(C_{f1}^i \times C_{f2}^i \times C_{f3}^i \times \ldots \times C_{fn}^i\right)^{\frac{1}{n}} \quad (3)$$

Geo-accumulation index (I_{geo}) is calculated according to Eq.4. The I_{geo} expresses pollution by comparing the measured levels of trace elements with the background levels originally used for evaluating bottom sediments **(Abowaly *et al.*, 2021)**.

$$I_{geo} = \log_2(C^i 1{,}5xB^i) \quad (4)$$

C^i: concentration of the heavy metals measured in the soil (mg/kg),
B^i: geochemical background concentration of the heavy metals (mg/kg),
1,5: constant (to minimize the effect of potential differences in background values that could be attributed).

Bioaccumulation factor (BAF) expressed transfer of heavy metals from soil to plant (Eq.5) **(Hu, B. *et al.*, 2017):**

$$BAF_i = \frac{C_p^i}{C_s^i} \quad (5)$$

C_p^i: concentration of heavy metals in the plant (mg/kg FW),
C_s^i: concentration of heavy metals in the soil (mg/kg).

Human health risk assessment

Estimated daily intake (EDI) is given by the concentration of the hazardous element in the food, the daily food intake, and the reference body weight (Eq.6) **(Antoine *et al*., 2017)**:

$$EDI = \frac{C.F_{ir}}{BW_a} \; [\mu g/day/kg] \quad (6)$$

C: concentration of heavy metals in the plant (mg/kg FW),
F_{ir}: daily food intake (g/day),
BW_a: reference body weight (70 kg) (Minnesota Department of Health, 2017).
Target hazard quotient (THQ) is defined as the ratio of the daily dose of risk elements to which the consumer may be exposed, and the reference dose of risk elements that can be taken daily for a long period without health risk (Eq.7) **(Antoine *et al*., 2017)**:

$$THQ = \frac{E_{FR}.Ed.F_{ir}.C}{RfD.BW_a.ATn} \; .10^{-3} \quad (7)$$

E_{FR}: exposure frequency to the trace element (365 days),
Ed: exposure duration (70 yrs),
F_{ir}: food ingestion rate (g/day),
C: concentration of heavy metals in the plant (mg/kg FW),
BW_a: reference body weight (70 kg),
RfD: oral reference dose of the trace element (mg),
ATn: averaged exposure time (365 day. 70 yrs),
10^{-3}: unit conversion factor.
The hazard index (HI) is the sum of the individual target hazard quotients of the elements assessed for each food type **(Antoine *et al*., 2017)** (Eq. 8):

$$HI = \sum_{N=1}^{i} THQ_n \quad (8)$$

Statistical analyses

Results were evaluated using descriptive statistical analysis (Microsoft Excel, Redmond, WA, USA) and analysis of variance (ANOVA, multi-range tests, method: 95.0 per cent LSD) using Statgraphics statistical software (Centurion

XVI.I, USA). Spearman's correlation coefficient was used to determine the relationships between the content of hazardous metals in soils and hazardous metals in forest fruits.

RESULTS AND DISCUSSION

Heavy metals content in soil samples

Contaminants found in soils can come from many sources. The bioavailability of heavy metals in the soil is influenced by several factors. These include soil properties – pH value, organic component content, granulation and individual mineral content **(Kicińska & Wikar, 2021)**. The contents of hazardous metals determined in soil samples from individual points of the Horehronie (Ždiarska doilina, Končisté, Babiná, Zadná hoľa, Veľká Smrekovica) are given in Table 1. (The soil extract by *aqua regia*). The sampling points are part of mountain areas, forest ecosystems and adjacent meadows. Limit values aren't established for these kinds of soil systems. The heavy metals' contents have therefore been compared to their limit values - the maximum permissible contents of hazardous substances in agricultural land **(Act No. 220/2004)**. The limit values for copper and lead were not exceeded in a single soil sample. A slight exceedance of the limit value for nickel by 15.8% was determined in the Ždiarska dolina sample. An increased zinc content was also determined in this sample, by 38.3%. The greatest risk is represented by the cadmium content, which was exceeded in all analyzed soil samples. More than 20 times the limit value for cadmium was determined in samples from the Ždiarska dolina and the Končisté. There are statistically significant differences between the contents of hazardous elements in the soils of individual locations.

Table 1 The content of heavy metals in the soil extract of *aqua regia* (mg/kg)

1. sampling year					
location	**Cu**	**Zn**	**Ni**	**Pb**	**Cd**
Babiná	15.80^{ab}	53.80^{ab}	26.90^{bc}	51.90^{ab}	2.15^{ab}
Zadná hoľa	12.60^{ab}	48.00^{ab}	11.30^{a}	27.90^{a}	0.74^{a}
Veľká Smrekovica	7.70^{a}	38.00^{a}	21.80^{abc}	57.80^{b}	9.17^{c}
Ždiarska dolina	38.30^{b}	138.30^{b}	46.30^{c}	52.00^{ab}	3.06^{abc}
Končisté	9.90^{a}	28.70^{a}	19.70^{ab}	54.80^{b}	7.66^{bc}
standard deviation	3.14	7.41	1.25	4.88	0.02
2. sampling year					
location	**Cu**	**Zn**	**Ni**	**Pb**	**Cd**
Babiná	20.00^{ab}	65.00^{ab}	38.40^{b}	38.40^{b}	2.98^{bc}
Zadná hoľa	19.80^{ab}	64.80^{ab}	38.50^{b}	39.00^{b}	2.75^{abc}
Veľká Smrekovica	19.70^{ab}	64.80^{ab}	34.80^{ab}	28.70^{a}	1.53^{a}
Ždiarska dolina	19.00^{ab}	61.10^{ab}	33.20^{a}	33.40^{ab}	2.38^{ab}
Končisté	20.30^{ab}	69.00^{ab}	33.80^{ab}	35.20^{ab}	4.48^{c}
standard deviation	4.56	9.82	1.71	3.52	0.03
Limit value AR*	60	100	40	70	0.04

Notes: LSD test, mean ± standard deviation (n = 5), the coefficients (a, b, c) show a statistically significant difference, $p < 0.05$; *for soil extract of *aqua regia* **(Act No. 220/2004)**

Differences in the content of heavy metals within the comparison of years may be due to the fact that samples of forest fruits cannot be collected from the same place every year. Since within the sampling location, the place where the forest fruit grows is different every year, the soil composition in the vicinity of the sample also changes.

Hahn *et al.* (2019) analysed the degree of soil pollution in the forests of Germany near the smelter chimneys. The authors mention that in localities where mining, ore processing and steel production take place, the permissible levels of certain heavy metals have also been exceeded. Cd contents in the analysed forest soils ranged from 2.5-3.8mg/kg.The content of cadmium as an increased risk for the environment can be affected by climate change and the deposition of acid oxides from the air. This leads to soil acidification and further mobilization of naturally occurring heavy metals in the minerals of the parent rock **(Štrbac *et al.*, 2022).**

Indicators of soil contamination by heavy metals

We evaluated the degree of soil contamination by analysis of the Contamination factor, Degree of contamination, Pollution load index, and Geo-accumulation index (Tab 2).

Table 2 Contamination factor (C_f), degree of contamination (C_{deg}), geo-accumulation index (I_{geo}) and pollution load index (PLI)

1. sampling year						
		Babiná	**Zadná Hoľa**	**Veľká Smrekovica**	**Ždiarska dolina**	**Končisté**
C_f	**Cu**	1.11±0.22	0.89±0.22	0.54±0.22	2.70±0.22	0.70±0.22
	Ni	1.47±0.07	0.62±0.07	1.19±0.07	2.53±0.07	1.08±0.07
	Pb	1.28±0.12	0.69±0.12	1.43±0.12	1.28±0.12	1.35±0.12
	Cd	4.38±0.04	1.51±0.04	18.68±0.05	6.23±0.04	15.60±0.04
	Zn	0.84±0.12	0.75±0.12	0.52±0.12	2.15±0.12	0.45±0.12
I_{geo}	**Cu**	-0.43±0.15	-0.76±0.15	-1.47±0.15	0.85±0.15	-1.11±0.15
	Ni	-0.03±0.05	-1.28±0.05	-0.33±0.05	0.75±0.05	-0.48±0.05
	Pb	-0.23±0.08	-1.12±0.08	-3.39±0.08	-0.23±0.08	-0.15±0.08
	Cd	1.55±0.03	0.01±0.03	3.64±0.03	2.06±0.03	3.38±0.03
	Zn	-0.84±0.08	-1.01±0.08	-1.34±0.08	0.52±0.08	-1.75±0.08
C_{deg}		9.08± 0.13	4.45±0.15	22.43±0.23	14.90±0.43	19.17±0.23
PLI		1.50±0.02	0.84± 0.03	1.59±0.16	2.59±0.11	1.48±0.12
2. sampling year						
		Babiná	**Zadná Hoľa**	**Veľká Smrekovica**	**Ždiarska dolina**	**Končisté**
C_f	**Cu**	1.41±0.32	1.39±0.32	1.39±0.35	1.34±0.25	1.43±0.32
	Ni	2.10±0.09	2.10±0.09	1.90±0.13	1.82±0.06	1.85±0.09
	Pb	0.95±0.09	0.96±0.09	0.71±0.08	0.82±0.08	0.87±0.09
	Cd	6.07±0.06	5.60±0.06	3.12±0.74	4.85±0.04	9.12±0.06
	Zn	1.01±0.15	1.01±0.15	1.01±0.14	0.95±0.16	1.08±0.15
I_{geo}	**Cu**	-0.09±0.21	-0.11±0.21	-0.11±0.23	-0.17±0.16	-0.07±0.21
	Ni	0.49±0.06	0.49±0.06	0.34±0.08	0.28±0.04	0.30±0.06
	Pb	-0.66±0.06	-0.64±0.06	-1.08±0.05	-0.87±0.06	-0.79±0.06
	Cd	2.02±0.04	1.90±0.04	1.06±0.50	1.69±0.03	2.61±0.04
	Zn	-0.57±0.10	-0.57±0.10	-0.57±0.09	-0.66±0.11	-0.48±0.10
C_{deg}		11.54 ±0.50	11.07±0.45	8.12±0.60	9.78±0.18	14.34±0.43
PLI		1.77 ±0.14	1.74 ±0.12	1.43 ±0.08	1.56 ±0.02	1.86±0.13

Notes: mean ± standard deviation (n = 5)

To assess the factor of contamination with hazardous elements, we followed the classification according to **Hakanson, L. (1980)**: $C_f < 1$ – low contamination; $1 \leq C_f < 3$ – moderate contamination; $3 \leq C_f < 6$ – considerable contamination; $C_f \geq 6$ – very high contamination. Background values (Bi) according to **Linkeš *et al.* (1997)** were used to calculate C_f (Zn – 64.2; Cu – 14.2; Ni – 18.3; Pb – 40.53; Cd – 0.49). The average C_f values of hazardous elements of the analysed soil samples were variable. The metals Cu, Ni, Pb and Zn showed low to moderate levels of contamination. However, the Cd values showed high contamination with the highest value of 18.676 in the Veľká Smrekovica sample.
The degree of contamination (C_{deg}) was classified according to **Luo *et al*. (2007)**: $C_{deg} < 5$ – low contamination; $5 \leq C_{deg} < 10$ – moderate contamination; $10 \leq C_{deg} < 20$ – considerable contamination; $C_{deg} \geq 20$ – very high contamination. Based on the C_{deg} assessment in the first sampling year, the soil from the sampling point Veľká Smrekovica with a value of 22.428 can be classified as very high contaminated. In the following year, the highest value (14,344 the Končisté point) was classified as considerable contamination. Such a high degree of contamination is mainly caused by the metals Cd and Zn. If the soils were assessed only on the basis of Cu, Ni and Pb, the indicated sampling points would show only low to medium contamination.
To assess soil contamination based on the geoaccumulation index (I_{geo}), a seven-point scale was used according to **Yakun *et al.* (2016)**: $I_{geo} < 0$ – no contamination; $0 < I_{geo} \leq$ - light contamination; $1 < I_{geo} \leq 2$ - slightly moderate contamination; $2 < I_{geo} \leq 3$ - moderate contamination; $3 < I_{geo} \leq 4$ - slightly heavy contamination; $4 < I_{geo} \leq 5$ - heavy contamination; $I_{geo} > 5$ - extremely heavy contamination. The values of I_{geo} sampling points are shown in Table 2. They are characterized by high variability within sampling points and years. Sampling points are classified as no contamination or light contamination with the metals Cu, Ni, Pb and Zn. Cd values range from 0.007 (the Zadná Hoľa point) to 3.638 (the Veľká Smrekovica point). They cause light to slightly heavy contamination levels.
The amount and type of pollution found in soils are closely related to the type of industry and concentrates (ores) processed in a given plant. Strong Zn pollution ($I_{geo} \geq 3$) and moderate Pb pollution ($I_{geo} > 2$) were classified in soils in the area of southern Poland **(Kicińska** & **Wikar , 2021).**
The assessment of soil contamination based on the pollution load index (PLI) is divided according to **Abowaly *et al.* (2021):** 0 < PLI ≤ 1 – unpolluted; 1 < PLI ≤ 2 - moderately polluted to unpolluted; 2 < PLI ≤ 3 - moderately polluted _ 3 < PLI ≤ 4 - moderately to highly polluted _ 4 < PLI ≤ 5 - highly polluted _ 5 ≤ PLI - very highly polluted. This parameter can be used to determine the level of environmental pollution to undertake monitoring activities to improve soil quality. The pollution load index ranged from 0.843 (the Zadná Hoľa point) to 2.594 (the Ždiarska dolina point). The results showed that all five sampling points according to PLI classification were polluted.

Heavy metal content in forest fruits

Another part of our analysis was the determination of the content of hazardous elements in forest fruits. The results (Table 3) obtained were compared with the maximum levels for fruits **(Decree No. 2/1994, Commission Regulation (EU) 2021/1323)**. The concentration values are given in mg/kg fresh weight (FW).

Table 3 The content of heavy metals in plant materials (mg/kg)

1. sampling year					
species	**Cu**	**Zn**	**Ni**	**Pb**	**Cd**
***Vaccinium myrtillus* L.**	0.80[ab]	1.30[a]	0.12[a]	0.06[ab]	0.05[b]
***Vaccinium vitis-idaea* L.**	0.80[abc]	2.00[ab]	0.19[ab]	0.12[b]	0.03[ab]
***Rubus idaeus* L.**	0.70[a]	4.40[bc]	0.12[a]	0.01[a]	0.01[a]
***Fragaria vesca* L.**	1.00[bc]	2.30[abc]	0.23[ab]	0.04[ab]	0.03[ab]
***Rosa canina* L.**	2.93[c]	4.83[c]	0.74[b]	0.01[a]	0.08[b]
standard deviation	0.06	0.05	0.03	0.01	0.01
2. sampling year					
species	**Cu**	**Zn**	**Ni**	**Pb**	**Cd**
***Vaccinium myrtillus* L.**	0.40[a]	1.20[a]	0.10[a]	0.01[a]	0.01[a]
***Vaccinium vitis-idaea* L.**	0.70[abc]	1.30[ab]	0.10[a]	0.01[ab]	0.01[a]
***Rubus idaeus* L.**	0.50[ab]	2.10[abc]	0.10[a]	0.01[ab]	0.04[ab]
***Fragaria vesca* L.**	1.30[bc]	2.40[b0c]	0.20[ab]	0.01[ab]	0.03[ab]
***Rosa canina* L.**	1.70[c]	3.20[c]	0.61[b]	0.34[b]	0.18[b]
standard deviation	0.04	0.04	0.02	0.01	0.01
limit value*	10	10	0,50	0.10/0.20**	0.03

Notes: LSD test, mean ± standard deviation (n = 5), the coefficients (a, b, c) show a statistically significant difference, p <0.05; *for plant materials according to legislation **(Commission regulation (EU) 2021/1317; Decree no. 2/1994)**; **limit value for cranberries

As with the soil samples, the maximum permissible amounts for cadmium were also exceeded in the forest fruits. The nickel content exceeded the established limits in both years in rosehips (*Rosa canina* L.) samples (0.74 mg/kg; 0.61 mg/kg). The lead content has increased over the years. In the rosehip (*Rosa canina* L.) samples (0.34 mg/kg) it was 2 times higher than what is allowed by the applicable legislation. The contents of copper and zinc were lower than the established limit values for these metals. In the 1st sampling year, the level of pollution in individual forest fruit species was in the order rosehips > wild raspberries > wild strawberries > lingonberries > bilberries. The following year, the order was rosehips > wild strawberries > wild raspberries > lingonberries > bilberries. There are statistically significant differences between the contents of risk elements in forest fruits from individual localities ($p < 0.05$).
Vollmannová *et al.* (2014) compared wild and cultivated blueberries and cranberries in their study. Compared to wild blueberries, lead and cadmium values were 50% lower in wild cranberries, while the resulting metal contents are similar to the contents in our samples. Heavy metals were also determined in samples of raspberries and bilberries from Sarajevo and Montenegro **(Antić-Mladenović *et al.*, 2009; Sapcanin *et al.*, 2021)**. The results of studies showed that the contents of the most toxic risk elements (Zn, Fe, Pb, Cd) were below the detection limit in almost all the examined samples. When determining the heavy metals in fruits on peatlands in the Kirov region (Russia), the copper content was the lowest compared to other metals. Similarly, in our samples, the Cu content in wild raspberries was determined to be the lowest in the first sampling year, despite the high contamination of the soil sample (the Veľká Smrekovica) collected near wild raspberries (**Novosyolova *et al*., 2021).**
The forest berry fruit samples analysed by **Shotyk (2020)** were collected from sites marked by bitumen mining in northern Canada. Nickel is one of the valuable indicators of environmental contamination from surface mining. However, the analysis found that the nickel content from contaminated sites is regulated by plant uptake from the soil. Therefore, the determined nickel content in fruit is not a sufficient indicator of environmental pollution.

The health risk associated with the consumption of forest fruits

Table 4 shows the BAF values of the analysed forest fruits. In the first sampling year, BAF values increased in the order of wild strawberry < bilberry < lingonberry < wild raspberry < rose hip. The following year it was bilberry < lingonberry < wild raspberry < wild strawberry < rosehip. A study by **Vukanović *et al.* (2018)** stated higher BAF values in bilberries from Montenegro. The values stated by **Umlaufová *et al.* (2018)** for wild strawberries are similar to our values.
We evaluated the health risks associated with the consumption of forest fruits based on the calculation of the estimated daily intake (EDI), the target hazard quotient (THQ) and the hazard index (HI).
The estimated daily intake (EDI) of heavy metals was determined based on their average concentration in each type of forest fruit sample and the daily intake in grams for the respective species. Data on the consumption of forest fruits were used from the analysis of forest management (bilbierries – 2.3; lingonberries – 0.52; wild strawberries – 0.009; wild raspberries – 0.9; rosehips – 0.05 kg/person/year) **(Assessment of forest functions, 2008)**. The estimated daily intakes of all hazardous metals (Table 4) were lower than the acceptable daily intake (ADI): Cd – 1, Cu – 500, Pb – 3.5, Zn – 11, Ni – 2.8 body weight/day **(Onwukeme & Mgbemena, 2014)**.
Various contaminants can cause carcinogenic and non-carcinogenic effects in our bodies. The target hazard quotient (THQ) is a parameter considered a factor for assessing the non-carcinogenic effects of risk elements in food. A THQ higher than 1 indicates a high risk of non-carcinogenic diseases. If the THQ is less than 1, it means that the consumption of the food does not have a harmful effect on the health of consumers **(Loghman *et al.*, 2022; Basaran, 2022)**. The determined values of the total hazard quotient for the adult population are given in Figures 2 and 3. The resulting THQ values were significantly < 1. The highest value in the first sampling year was in the sample of bilberries (*Vaccinium myrtillus* L.) for Cd 0.004. When we consume a certain type of food, we are exposed not only to one metal but to a group of hazardous metals. Therefore, the hazard index (HI) (Table 4) is determined by summing the THQ values of each element. In both years, we

determined the highest hazard index in the wild raspberry (*Rubus idaeus* L.) sample (0.116). In both years, the values were significantly lower than 1, which means that the average consumption of forest fruits from the Horehronie region does not pose a health risk to the consumer.

Table 4 Estimated daily intake (EDI, µg/day kg bw), bioaccumulation factor (BAF), hazard index (HI)

		Vaccinium myrtillus L.	*Vaccinium vitis-idaea L.*	*Rubus idaeus L.*	*Fragaria vesca L.*	*Rosa canina L.*
1. sampling year						
EDI	**Cu**	0.07	0.02	0.03	0	0
	Ni	0.01	0	0.00	0	0
	Pb	0.01	0	0	0	0
	Cd	0	0	0	0	0.1
	Zn	0.12	0.04	0.16	0	0.01
BAF	**Cu**	0.05	0.06	0.09	0.03	0.43
	Ni	0.00	0.02	0.01	0.01	0.06
	Pb	0	0	0	0	0
	Cd	0	0	0	0.01	0.02
	Zn	0.03	0.04	0.12	0.02	0.245
HI		0.05	0.01	0.12	0	0
2. sampling year						
		Vaccinium myrtillus L.	*Vaccinium vitis-idaea L.*	*Rubus idaeus L.*	*Fragaria vesca L.*	*Rosa canina L.*
EDI	**Cu**	0.04	0.01	0.02	0	0
	Ni	0.11	0.03	0.16	0	0.01
	Pb	0.01	0	0	0	0
	Cd	0	0	0	0	0
	Zn	0	0	0	0	0
BAF	**Cu**	0.02	0.04	0.03	0.07	0.08
	Ni	0	0	0	0.01	0.02
	Pb	0	0	0	0	0.01
	Cd	0	0	0.03	0.01	0.04
	Zn	0.02	0.02	0.03	0.04	0.05
HI		0.06	0.01	0.02	0	0

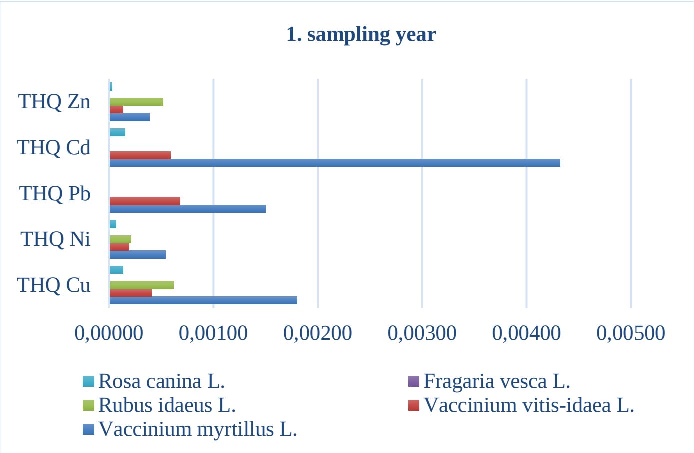

Figure 2 Target hazard quotient (THQ) of heavy metals in forest fruits (1. sampling year)

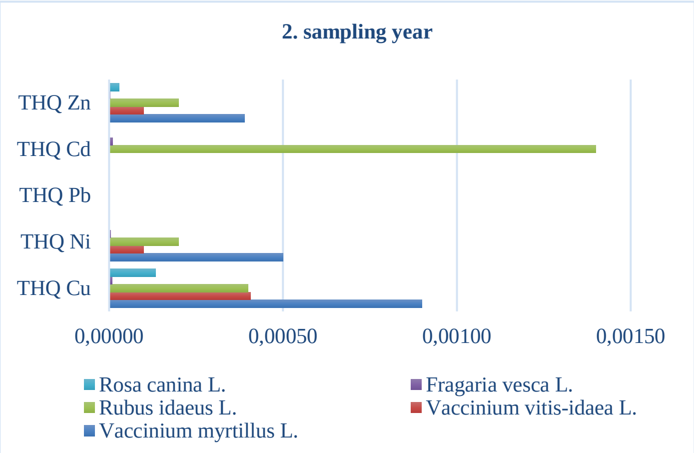

Figure 3 Target hazard quotient (THQ) of heavy metals in forest fruits (2. sampling year)

Spearman's correlation coefficient was used to determine the relationships between the content of hazardous metals in soils and hazardous metals in forest fruits. The content of cadmium, copper and nickel in soils is positively correlated with the content in forest fruits. (Cd – 0.321; Ni – 0.309; Cu – 0.283). The finding of a weak correlation that is statistically significant indicates that a particular exposure has an effect on the resulting values.

CONCLUSION

The biggest source of contamination in the Horehronie region is represented by acid tars of petroleum waste. Heavy metals and other toxic substances accumulate in the soil and transfer to above-ground parts of plants, and forest fruits, which can become risky food for the consumer. The biggest risk in soils and forest fruits is represented by cadmium. Its content exceeded the limit values in all analysed samples. The risk of soil contamination is also confirmed by the values of the indicators of soil pollution. However, the increased level of heavy metals in the soil did not accumulate in forest fruits. The content of hazardous metals (Cd, Ni, Cu) in soils is positively correlated with the content of hazardous metals (Cd, Ni, Cu) in forest fruits. Within the comparison of sampling years, the content of hazardous elements decreases in most forest fruits. Variable contents of contaminants were determined in the soil samples. The mentioned differences may be related to different climatic conditions (amount of precipitation, temperature) in the forest ecosystems during individual sampling years. Our findings indicate that the average consumption of forest fruits from the Horehronie region does not pose a significant health risk to consumers. However, monitoring of forest ecosystems and removal and remediation of old environmental burdens is necessary.

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