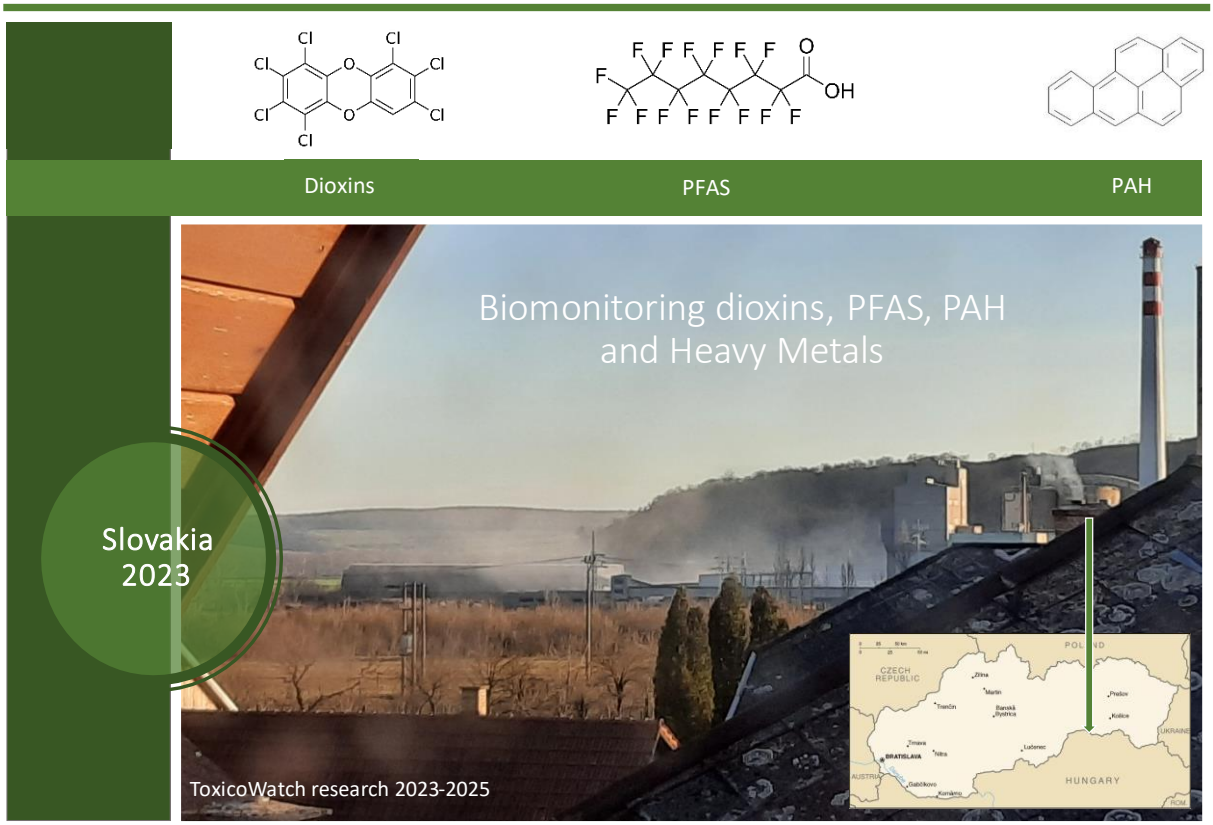
TOXICO WATCH

Biomonitoring research on persistent organic pollutants in the surrounding environment of the Cement plant Turňa nad Bodvou, Slovakia, 2023



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March, 2024

ZERO WASTE EUROPE

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Acknowledgements

Thank you to Zero Waste Europe for enabling this research on persistent organic pollutants (POPs). Special thanks to all the participants in the villages of Dvornìke, Hosťovce, Zádiel, Včeláre and Turňa nad Bodvou for their cooperation and trust in allowing us to analyse their backyard chicken eggs, vegetation, fruit, and roof dust. Your contribution has greatly enhanced our understanding of the environmental health in your communities.

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Table of content

Acronyms

APCD	Air Pollution Control Devices
BAT	Best Available Techniques
BEP	Best Environmental Practice
BEQ	Bioanalytical EQuivalents
BFR	Brominated Flame Retardants
BMI	Body Mass Index
BREF	Best Available Techniques (BAT) Reference Document for Waste Incineration
BBT	Best Available Techniques (BAT)
dl-PCB	Dioxin-Like Polychlorinated Biphenyls
DR CALUX®	Dioxin Responsive Chemical-Activated LUciferase gene eXpression
EFSA	European Food and Safety Authority
GC-MS	Gas Chromatography Mass Spectrometry GC-MS
GenX	Group of fluorochemicals related to of hexafluoropropylene oxide dimer acid (HFPO-DA)
i-PCB	Indicator Polychlorinated Biphenyl
LB	Lower Bound
LOD	Limit of Detection
LOQ	Limit of Quantification
MB	Medium Bound
MWI	Medical Waste Incineration
MSWI	Municipal Solid Waste Incineration
ndl-PCB	Non-Dioxin-Like Polychlorinated Biphenyl (Non-Dioxin-Like PCB)
ng	Nanogram; 10^{-9} gram
OTNOC	Other Than Normal Operating Conditions
PAH	Polycyclic Aromatic Hydrocarbons
PCB	Polychlorinated Biphenyl
PCDD	Polychlorinated Dibenzodioxins
PCDF	Polychlorinated Dibenzofurans
PBDD/F	Polybrominated-dibenzodioxins and furans
pg	Picogram; 10^{-12} gram
POP	Persistent Organic Pollutants
SVHC	Substances of Very High Concern
TCDD	2,3,7,8-tetrachloordibenzo-*p*-dioxine
TDI	Tolerabele Daily Intake = Aanvaardbare Dagelijkse Inname
TEF	Toxic Equivalency Factor
TEQ	Toxic Equivalents
TW	ToxicoWatch
TWI	Tolerable Weekly Intake
UB	Upper Bound (UB)
UPOP	Unintentional POP (Persistent Organic Pollutants)
µg	Microgram 10^{-3} gram
WtE	Waste to Energy (waste incinerator)

Introduction

The civic organisation Zelený živel o.z. representing environmentally conscious residents in Turnianska Kotlina, took the initiative in 2023 to contact Zero Waste Europe and ToxicoWatch (TW) for independent research on the deposition of persistent organic pollutants (POPs) such as dioxins (PCDD/F/dl-PCB), Polycyclic Aromatic Hydrocarbons (PAH) and PFAS, as well as heavy metals in the environment surrounding the cement kiln Cementáreň Turňa nad Bodvou, located in the Košice Region in Slovakia. According to the Turňa nad Bodvou Cement Plant's website[1], the plant is equipped with state-of-the-art BAT/BREV equipment. Waste gases, with a volume flow rate of 165000 m^3/hour are discharged into the air through a fabric filter and subsequently through a chimney with a height of 51.0 meters. The dust separated by the fibre filters is transported as dried clay to the raw material landfill. The conveyor belts used for transporting the clay for crushing within the plant to the sieving station are dust-tight.[2] Cement production ranks among the energy-intensive industries. This plant, supported by EU grants, burns waste materials, ranging from plastic agglomerate, worn-out old used car tyres, and PCB oil-containing waste [3] - as a sustainable alternative to fossil fuels. There are plans to increase waste burning from 65,000 to 115,000 tons annually, a nearly 50% increase. Cement kilns are utilised for the destruction of persistent organic pollutants such as PCBs and PFAS, owing to the higher combustion temperatures they provide.

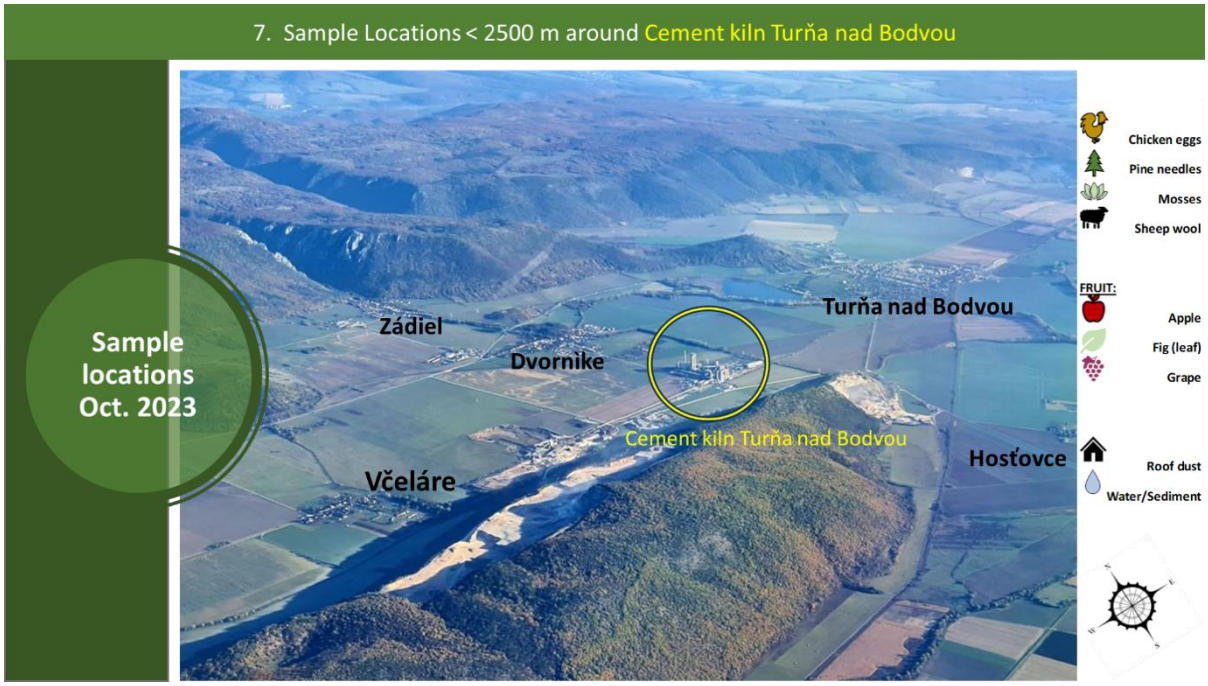
Emissions of pollutants must adhere to the emission limits set by EU Decree No 410/2003 Coll. (Air Act No. 137/2010 Coll. repeals several regulations) as amended for cement rotary kilns and should refer to Industrial Emissions Directive and BREF 2023.[4] It's noteworthy that emissions of dioxins are measured only a few hours per year. The last publication dates to 2018 with limited information on dioxin emissions and lacking detailed TEQ distribution data. No recent data are available on emissions and depositions of other persistent organic pollutants (POPs), like PAH, fluorine compounds (PFAS), and dioxin-like PCBs. This TW research does not (yet) include monitoring of brominated dioxins (PBDD/F) or other halogenated POPs but might be necessary to investigate the emission of these abundant flame retardants.

In this report, TW's research focuses on assessing the environmental impact around the cement kiln Turňa nad Bodvou. We employ biomonitoring techniques using eggs from backyard chickens, as well as analysing fruit and vegetation for dioxins, PFAS, PAH and heavy metals. Alongside cement production, the region of Košice faces additional industrial sources of air pollution. Notably, in Včeláre and Hosťovce quarries, limestone, a basic raw material for cement production, is extracted. Adjacent to the cement kiln are ecologically significant areas, including the Protected Bird Area Slovak Karst (SKCHVÚ 027) and the National Nature Reserve - Zádielska Gorge, which forms part of the Slovak Karst National Park.

[1] https://www.danucem.com/site/2/Turňa -nad-bodvou-cement-plant
[2] Increase in the output of the furnace line VSH, a.s. Turňa nad Bodvou to 3500 tons of clinker per day - OBJECTIVE
[3] *Wastes classified under catalogue numbers 191210, 191211, 19121212, 19121212, 191214 and 160119. In addition, wastes are classified under catalogue numbers 191204 (Plastic agglomerate) and 160103 (Worn tyres).*
[4] *https://eeb.org/wp-content/uploads/2023/04/Upgrading-Europes-air.pdf*

Sampling

This biomonitoring research comprehended the biomarkers: eggs of backyard chickens, eggshells, pine needles (*Picea abies*), mosses (*Bryophyta*), and fruits such as apples, grapes, and figs leaves, as well as the matrices roof dust, sediment, and water. The research area covers the environment of five (5) surrounding villages of the cement kiln within a radius of 2500 meters. The afore-mentioned samples are taken from four (4) locations in Dvornìke, three (3) in Včeláre, three (3) in Hosťovce, two (2) in Zádiel and one (1) in Turňa nad Bodvou.



Eggs

At each egg location, TW collected (2) sets of 6 - 10 fresh eggs, mixed the total contents (egg yolk and white) and stored them in HDPE lab containers in a freezer until analyses in the lab. A questionnaire and a location inspection are conducted at every backyard chicken egg location by the TW team, to identify any potential confounder fact.

Fruit

TW collected samples of 200–300-gram fresh fruit from the fruit trees and shrubs, which were placed in special HDPE-lab bags, and stored in a cool, dry environment.

Vegetation (Mosses /Pine needles)

Vegetation samples, 200–300-grams of fresh pine needles from Pine trees – *Picea abies* and 200–300-gram mosses (*Bryophyta*), were collected from sheds' roofs at the same locations as the egg sampling. Additionally, moss (*Bryophyta*) samples were collected from a rural open field on a hill near Dvornìke. All vegetation samples were stored in HDPE-lab bags, in a cool, dark, and dry environment.

Roof dust

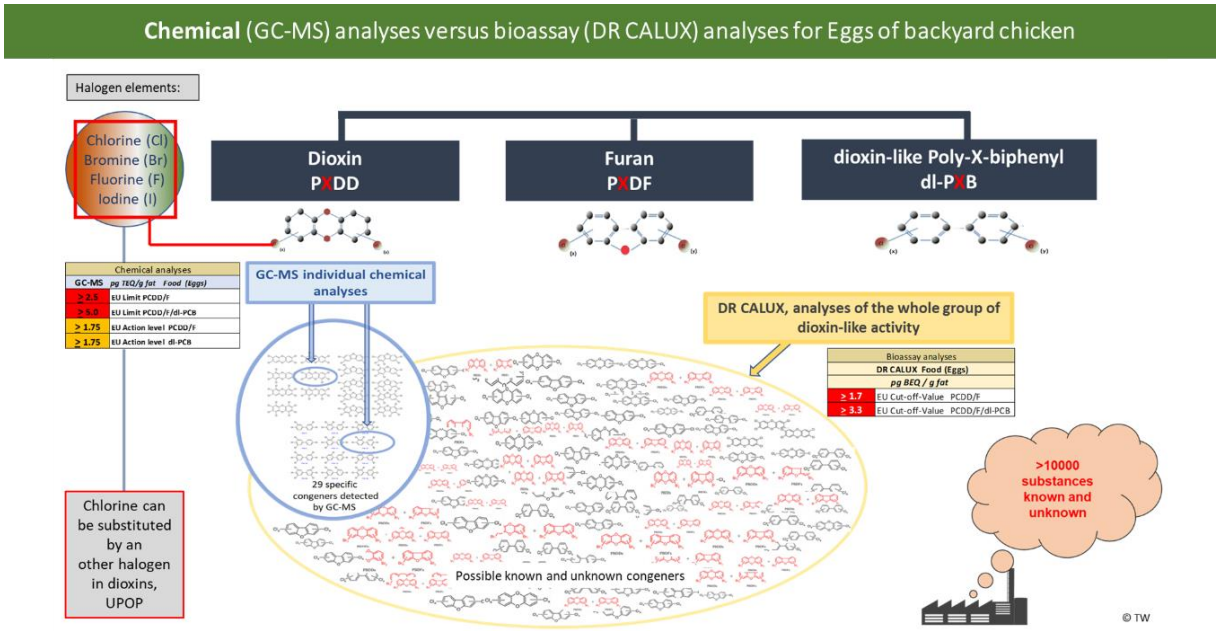
Roof dust samples weighing 50 grams were collected by direct scraping from a roof at location *Dvornìke*. At the location in *Zádiel*, roof dust that had naturally deposited in a metal bowl was sampled.

Water/Sediment

Water and sediment samples, totalling 200 ml water/sediment were collected directly from the downstream floating Bodvou, near the cement kiln using an HDPE lab container and stored cool and in a dark environment.

Analysis methods

The collected samples undergo analysis for persistent organic pollutants (POPs) using both bioassays (CALUX) and chemical analyses. The substances of interest are PCDD/F/dl-PCB (dioxins), Per- and poly-fluoroalkyl Substances (PFAS), Polycyclic Aromatic Hydrocarbons (PAH), and analyses of 6-14 heavy metals: Arsenic, Cadmium, Cobalt, Chromium, Lead, Nickel, Aluminium, Barium, Copper, Manganese, Mercury, Silver, Tin, and Zinc.
In this research, bioassay analysis employs DR CALUX® for dioxins/furans (PCDD/F) and dioxin-like PCBs (dL-PCBs), PAH CALUX for PAH substances, and FITC-T4 for the PFAS. Additionally, DR CALUX®, PFAS CALUX®, FITC-T4 and GC-MS are used for dioxins analysis in eggs, when results from DR CALUX exceed the EU Limits for eggs (1.7 pg BEQ/g fat for PCDD/F and 3.3. pg BEQ/g fat for the sum of dioxins (PCDD/F/dl-PCB)). The analysis is performed by BioDetection Systems in Amsterdam, the Netherlands (NL). BDS is accredited under RvA L401. Chemical analysis for PAH, PFAS and heavy metals are conducted by the accredited laboratory Normec, Groen Agro Control, located in Delft, the Netherlands (NL). PFAS chemical analyses employ LC-MS/MS to detect 24 PFAS, while heavy metals analysis utilises ICP-MS.



Results

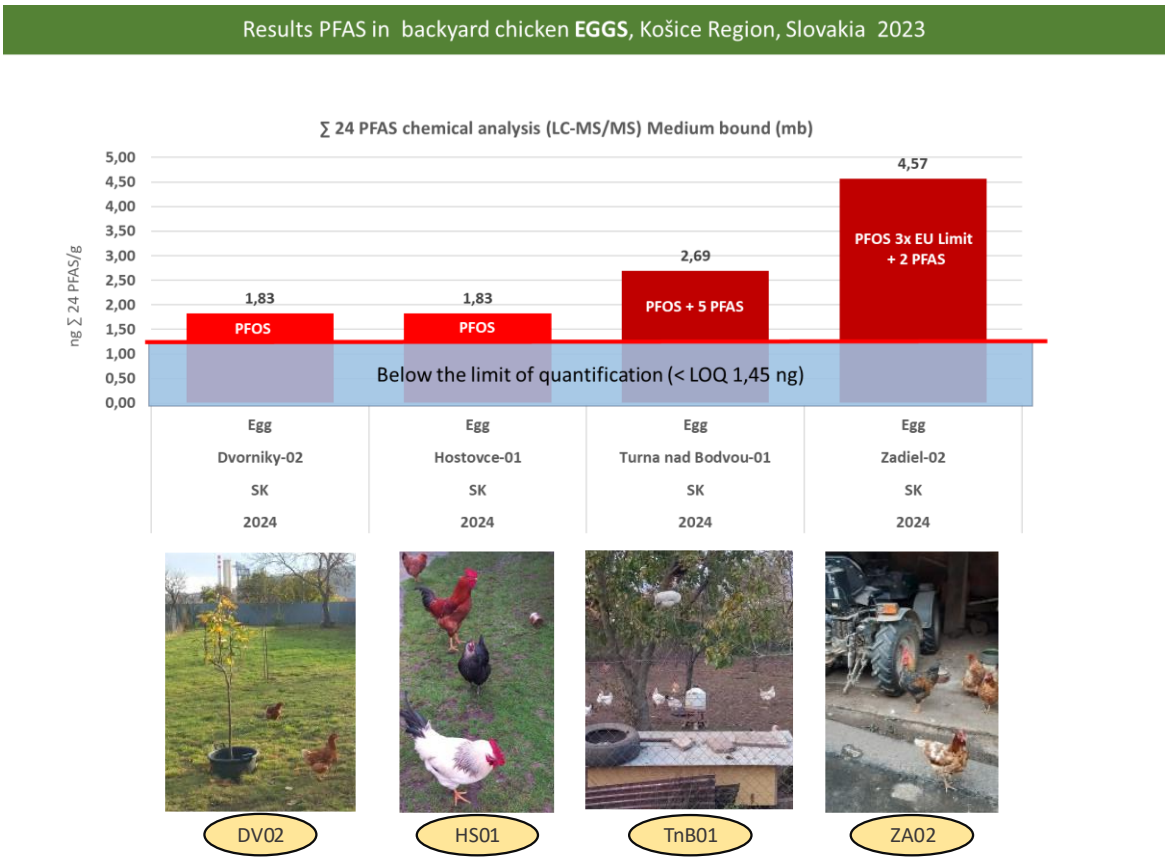
Eggs – Dioxins

In October 2023, TW sampled eggs from backyard chickens in six (6) private locations across five (5) neighbouring villages near the cement kiln. The values with the DR CALUX range from 1.2 – 9.8 pg BEQ/g fat. **Three (3) locations exceeded the EU limit** of 3.3 pg BEQ/g in backyard chicken eggs (DR CALUX), with **4.70 pg** in *Hosťovce*, **4.80 pg** in *Turňa nad Bodvou* and **9,80 pg BEQ/g fat (MB)**[5] in *Zádiel*. The DR CALUX method assesses the toxicity of dioxins, including brominated, fluorinated, and other (mixed) halogenated compounds. Chemical analyses, limited to 29 chlorinated dioxins, found in eggs of *Turňa nad Bodvou* **6.6 pg TEQ/g** and in eggs of location *Zádiel* **8.8 pg TEQ/g**. The levels of dl-PCB are from 0.1 – 6.7 pg TEQ/g. The highest-level dl-PCB is found in *Zádiel*. Chemical analysis confirmed this value with 6.6 pg TEQ/g in *Zádiel* and measured 3.9 pg TEQ/g in *Hosťovce*. Both exceed the EU action limit of 1.7 pg TEQ, where action is needed to determine the source. The congener patterns of dl-PCB closely resemble all these locations.

[5] *The concept which requires using half of the limit of quantification calculating the contribution of each congener*

Eggs – PFAS

Chemical analysis (LC-MS/MS) detected in all the eggs PFAS. The highest concentration of PFAS was also found at location Zádiel-02 with **4.57** µg ∑ 24 PFAS /kg (mb). Notably, the concentration of PFOS, one of the 4 EU-regulated PFAS compounds exceeds the EU limit by 300%: 3.0 µg/kg. In eggs at location Turňa nad Bodvou the PFOS level is 0.75 µg/kg, just below the EU limit. Remarkable is the finding of 6 different PFAS compounds at location Zádiel-02. The PFAS results in eggs are also reported as medium bound (MB).[6]



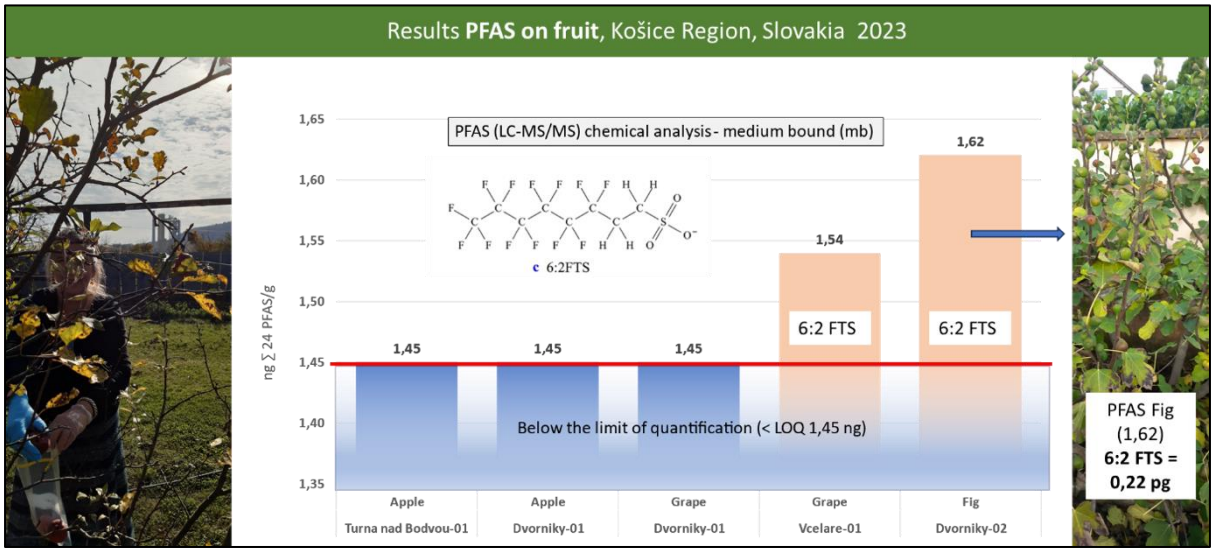
[6] *The concept which requires using half of the limit of quantification calculating the contribution of each congener*

Fruit

Dioxins on fruit in *Turňa nad Bodvou* consist of 0.24 pg TEQ/wet weight (MB) for the sum of dioxins (PCDD/F/dl-PCB) and are just below the EU limit of 0.30 pg TEQ.[7] Other locations measured all below the limit of quantification (<LOQ) for dioxins on fruit.

PFAS was detected in grapes in Včeláre, and fig leaves in *Dvornìke,* with 0.14 and 0.22 ng /gram dw (MB) for 6:2 Fluorotelomer sulfonate (6:2FTS), respectively. In the other locations, no PFAS could be found above the limit of quantification (>LOQ). Although the presence of 6:2 Fluorotelomer sulfonate (6:2FTS) is with great concern, because of the threat of serious health effects, and accumulation potential in people, this PFAS is (still) not included in the EU regulations.

The PAH levels on apples are 2.34 – 19.69 ng Benzo(a)Pyrene equivalent per gram/product with the PAH CALUX. The highest level was found in *Turňa nad Bodvou.* In grapes of *Dvornìke,* and *Včeláre* 19.1 ng and 32.5 ng ∑16 PAH was found with the chemical analysis of GCMS.



Mosses

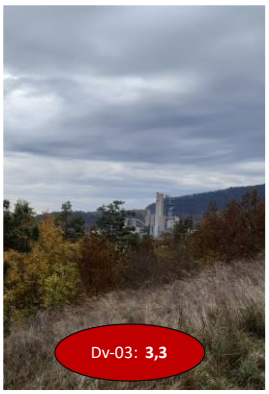
The values of dioxins measured with DR CALUX in mosses at *Dvornìke* are 3.3 pg TCDD eq./g, in mosses at the top of the hill north, and 23.8 pg TCDD eq./g in mosses on a roof 800 meters distance from the plant. Mosses collected from roofs in *Včeláre* dioxins measured 6.4 pg TCDD eq./g, in *Zádiel* 10.8 pg TCDD eq./g, and *Hosťovce* 19.0 pg TCDD eq./g dw (MB). The dioxin in all the moss samples exceeds the limit (for feed) of 0.83 pg TCDD eq./g 88% dry weight (medium bound, MB). High levels of dioxins were detected in all moss samples collected around the cement kiln. The levels of dioxins (PCDD/F/dl-PCBs) in mosses of Slovakia are among the highest observed in international biomonitoring research conducted by TW. Follow-up research in 2024 on moss in this Slovak area will expand to include moss samples from the Slovak Karst National Park region.

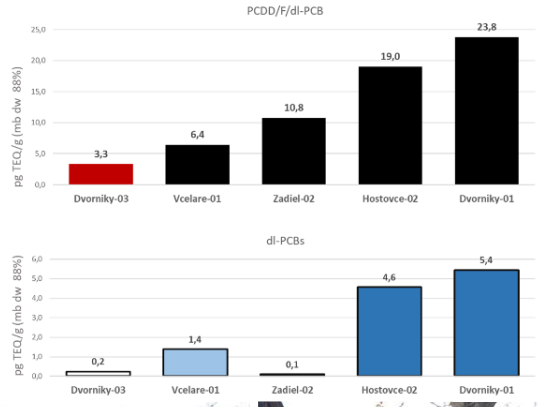
In the mosses of *Hosťovce* and *Dvornìke*, values of 4.6 and 5.4 pg dl-PCB are found. Semi-continuous measurements of the flue gases are needed to determine the amount and patterns of emissions of dl-PCB by the cement kiln. In *Zádiel*, *Dvornìke (Hill North)* and *Včeláre* 0.1, 0.2 and 1.4 pg TCDD eq./g were measured respectively. Notably, mosses exhibited higher levels of dioxins when compared to fruits or pine needles collected from the same locations. This disparity might be attributed to the fact that fruits mature from blossom to ripe fruit within a few months (May-September) and mosses grow continuously throughout the year and can live for many years.

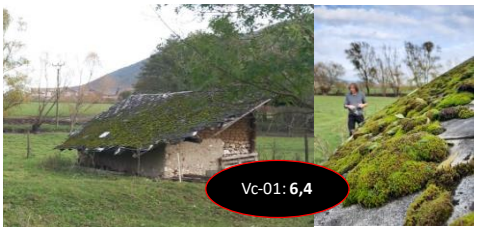
[7] *2013/711/EU*

PAH in mosses varies from 355.4 - 4684.7 ng/g Benzo(a)pyrene equivalent with the PAH CALUX. The chemical analysis tool of the GC-MS on 16 PAH is in the range of 32.5 – 423 ng PAH/g. The lowest level of PAH is found at the top of the hill in *Dvornìke,* and the highest is found in *Hosťovce*. The bioassay PAH CALUX method measures the toxicity of the total PAH instead of 4-16 PAH congeners with chemical analyses (GC-MS).

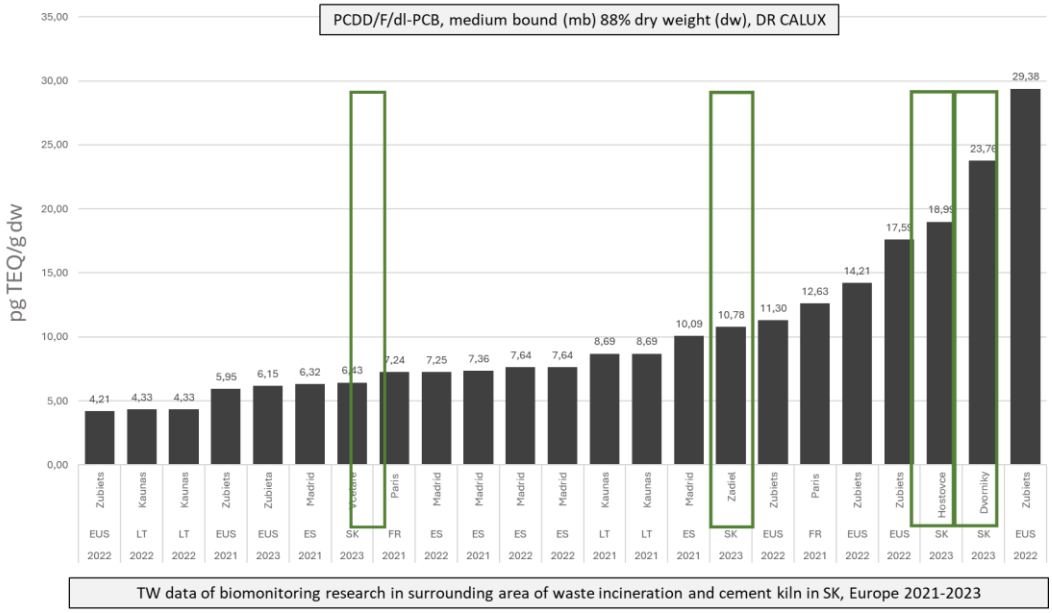
Dioxins in mosses Košice Region, Slovakia 2023





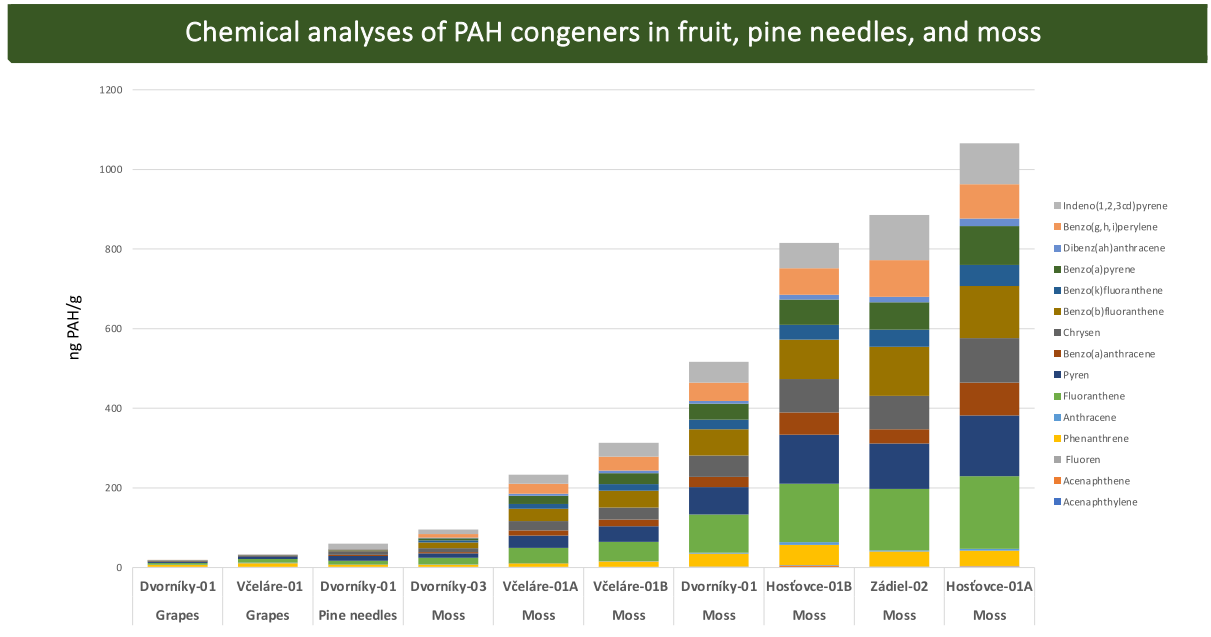


Mosses: Results dioxins, Slovakia 2023 compared with mosses in EU countries



Pine needles

The levels of dioxins in pine needles measured with the bioassay DR CALUX are 0.77 pg TCDD eq./g in *Dvornìke*, 1.52 pg TCDD eq./g in *Zádiel* and 2.85 pg TCDD eq./g in *Hosťovce* and *Včeláre*. PAH levels at these 4 locations in pine needles are 0.08 – 2.16 ng Benzo(a)Pyrene equivalent/g by PAH CALUX. The chemical method of PAH analyses measured a substantially higher level of 60.1 ng ∑ 16 PAH/g in pine needles at a location in *Dvornìke*.



Roof dust

Residents had reported concern about black dust accumulating on their roofs, windows, and windowpanes. In this research, high levels of PAH were found in roof dust sampled directly from a roof at location *Dvornìke*, and from a metal bowl below the roof with naturally deposited dust in *Zádiel*. Dioxin levels in dust are 5.50 TCDD eq./g in *Dvornìke* and 6.30 pg TCDD eq./g in *Zádiel*. The dl-PCBs are 1.20 and 2.20 TCDD eq./g, respectively.

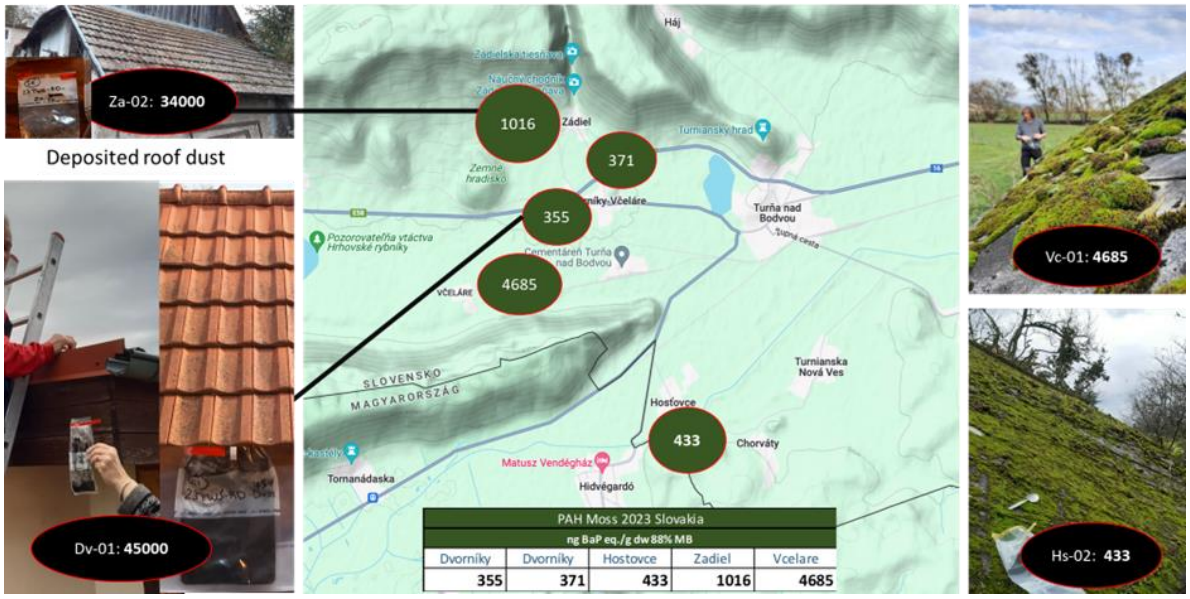
The levels of PAH in *Zádiel* were 34,000 ng and in *Dvornìke* 45,000 ng Benzo(a)Pyrene equivalent per gram. However, on apples and grapes, much lower levels in the range of 0.32 – 2.50 ng Benzo(a)Pyrene equivalent per gram is found in uncleaned fruit samples.

Results (sampling October-November), Košice Region , Slovakia 2023

							Dioxins DR CALUX (mb)			medium bound (mb)
Date	Total	Samples	Location Village	Biomarker	TW-RF-NR	Analyse	PCDD/F	dl-PCB	PCDD/F/dl-PCB	PAH
							DR CALUX	DR CALUX	DR CALUX	PAH CALUX
Sample	loc. / BioMat.				2023	Method	1.7		3.3.	Benzo[a]pyrene equivalent
							pg BEQ (TCDD)/g fat (veg: product)			ng BaP eq./g product
		Roof dust								
30-10-2023	1	Roof dust	Dvornike - Loc. 1		23TWS-RD-Dv01	DR CALUX	5,10	1,20	6,30	
		Roof dust	Dvornike - Loc. 1		23TWS-RD-Dv01	PAH CALUX				45000,00
31-10-2023	2	Roof dust	Zádiel - Loc. 2		23TWS-RD-Za02	DR CALUX	3,30	2,20	5,50	
		Roof dust	Zádiel - Loc. 2		23TWS-RD-Za02	PAH CALUX				34000,00

TW Indicative scale Vegetation / (Feed)			TW Indicative scale Results
DR CALUX (medium bound)			PAH CALUX (mb)
PCDD/F	dl-PCB	(PCDD/F/dl-PCB)	Benzo[a]pyrene equivalent
pg TCDD eq./g dry weight (dw)			ng BaP eq./g product
≥ 2.5	≥ 2.5	≥ 3.32	> 500 ng
≥ 1.0	≥ 1.0	≥ 1.66	> 250 ng
≥ 0.5	≥ 0.5	≥ 0.83	≥ 100 ng
< 0.5	< 0.5	< 0.83	≥ 10 ng
			< 10 ng

Results PAH in roof dust and mosses, Košice Region , Slovakia 2023



PAH Moss 2023 Slovakia

ng BaP eq./g dw 88% MB

Dvorníky	Dvorníky	Hostovce	Zadiel	Vcelare
355	371	433	1016	4685

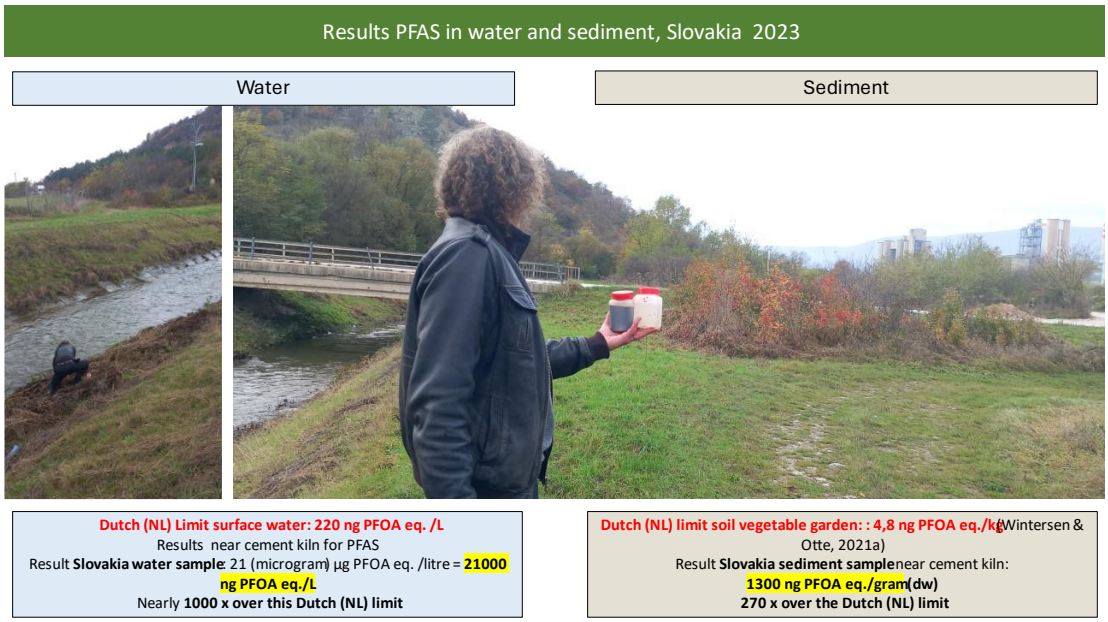
Water / Sediment

A screening test with the FITC-T4 was conducted on water and sediment near the cement kiln. The level of PFAS in water was found to be **21,000 ng PFOA.eq. /l**. This result exceeds the Dutch limit of **0.3 nanograms per litre for PFOA** by **more than a factor of 70,000**.[8] The FITC-T4 is a method that measures the total toxic effect of a mixture of PFAS congeners and is currently used by the Dutch government to screen for PFAS in surface water and inform policy measures for source reduction.

Results (sampling October-November), Košice Region , Slovakia 2023								
Date	Total	Samples	Location Village	Biomarker	TW-RF-NR	Analyse	PFAS: FITC-T4 (mb)	
							Sediment	water
Sample	loc. / BioMat.				2023	Method	ng PFOA eq./ g	µg PFOA eq./ lt
	Water / Sediment							
31-10-2023	1	Water	Hosťovce/border Hungaria/ CK Loc. 1		23TWS-H2O-CK-01	PFAS / FITC-T4		21,00
31-10-2023	2	Sediment	Hosťovce/border Hungaria/ CK Loc. 1		23TWS-SED-CK-01	PFAS / FITC-T4	1,30	

TW Indicative scale	
Bioassay FITC-4 (PFAS)	
Sediment	water
ng PFOA eq./ g	µg PFOA eq./ lt
≥ 0,0768 ng	≥ 1,76 µg
≥ 0,0384 ng	≥ 0,88 µg
≥ 0,0192 ng	≥ 0,44 µg
≥ 0,0096 ng	≥ 0,22 µg
< 0,0048 ng	< 0,22 µg

Sediment sampling downstream showed PFAS levels of **1,300 ng PFOA eq./g (dry weight)** with the method of FITC-T4. The Dutch regulation for soil is set at 0.048 ng PFOA eq./g. The result **greatly surpasses the Dutch regulation for soil**. Further research is necessary on water and sediment samples, as well as upstream samples in the Slovak Karst National Park to find out the extent of the pollution and if it is structural or an accidental disposal. Extended analyses will employ chemical analysis LC-MS/MS and the bioassay ERA-CALUX.



[8] *Smit C.E., Verbruggen E.M.J. (2022). Risicogrenzen voor PFAS in oppervlaktewaterRIVM-briefrapport 2022-0074 C.E. Smit | E.M.J. Verbruggen*

Heavy metals

The results of analyses of heavy metals on mosses (*Bryophyta*) in *Zádiel are* 6293 mg/kg Zinc, 76 mg/kg Lead, 71 mg/kg Nickel, 918 mg/kg Manganese, 22 mg/kg Copper and 2.2 mg/kg Cadmium at location *Zádiel*. More research at reference locations is needed to interpret the results in the context of this region. The heavy metals levels in the mosses are among the highest recorded in biomonitoring research conducted by TW in Europe (2019-2023). In Annexe 7 the results in Slovakia are indicated in boxes for comparative results in Europe. Subsequent samples of mosses will be collected in the nearby Slovak Karst National Park and AGGTELEK National Park, located very close to the cement kiln in Hungary.

In pine needles - *Picea abies* in *Zádiel*, 592 mg/kg of Manganese is found. This result is high, compared to other TW-biomonitoring results in pine needles. Heavy metal analysis of eggshells of backyard chickens found 0.024 mg/kg Lead (Pb), 0.056 mg Nickel (Ni) and no Mercury (Hg) was detected above the limit of detection (< LOD). A relatively high content of Aluminium (Al) of 8.3 mg/kg in eggshells of *Dvornìke* needs to have more attention.

Results Heavy Metals in Pine needles, Mosses and Eggshells

Heavy Metals mg/kg - Medium Bound (mb = LOD/2)

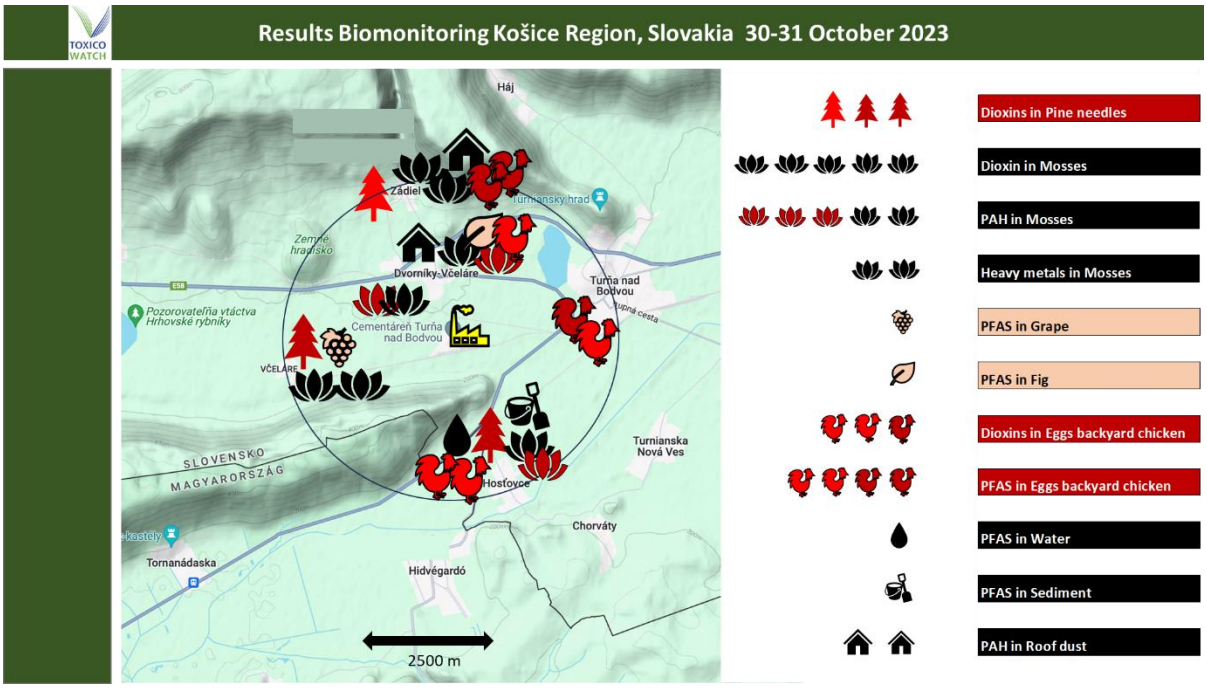
TW-REF-NR	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	As	Al	Ba	Cd	Cr	Co	Cu	Pb	Mn	Hg	Ni	Ag	Sn	Zn
	Arsenic	Aluminium	Barium	Cadmium	Chromium	Cobalt	Copper	Lead	Manganese	Mercury	Nickel	Silver	Tin	Zinc
23TWS-PN-HS02	0,066	99,000	67,000	0,005	0,280	0,061	4,800	0,330	591,000	0,026	0,280	0,005	0,040	41,000
23TWS-PN-VC02	0,083	155,000	61,000	0,011	0,330	0,025	3,100	0,410	13,000	0,028	0,240	0,005	0,053	36,000
23TWS-MOS-HS02	3,900	8789,000	141,000	1,300	23,000	17,000	26,000	47,000		0,086	26,000	0,110	2,200	135,000
23TWS-MOS-ZA01	4,500	14727,000	216,000	2,200	64,000	32,000	22,000	76,000	918,000	0,110	71,000	0,150	3,500	6293,000
23TWS-ES-Dv-02	0,010	8,300		0,005				0,024		0,005	0,056			

Conclusion

The infographic below presents the initial findings from the TW biomonitoring research conducted around the cement kiln Cementáreň Turňa nad Bodvou situated in the Košice Region in Slovakia in 2023. Samples were taken within a radius of 2500 meters around the kiln, in the surrounding of five (5) villages and analysed for persistent organic pollutants (POPs), such as dioxins, PFAS, PAH and heavy metals. Eggs, pine needles, and mosses exhibited high concentrations of dioxins (PCCD/F/dl-PCB), polycyclic aromatic hydrocarbons (PAHs), and per- and poly-fluoroalkyl substances (PFAS). In *Turňa nav Bodvou* six (6) PFAS compounds could be determined in eggs. PFOS level in eggs of location Zádiel- exceeding the EU limit for PFOS by 300%.

Of particular concern are the screening test results in the surface water stream near the cement kiln and sediment for the alarmingly high levels of PFAS. The heavy metal levels in mosses are among the highest recorded in TW-biomonitoring research conducted in Europe (2019-2023). Additionally, elevated levels of PAH were found in dust depositions on the roofs of houses in the villages of *Dvornìke* and *Zádiel*.

Overall, the findings from this initial biomonitoring project raise worrying concerns regarding the presence of dioxins (PCDD/F/dl-PCB), PAH, PFAS and heavy metals in the environment of this region of the Košice. Further research is imperative to comprehend these contaminants' source(s) and deposition patterns.



Annexe

Annexe 1: Analysis methods

The biomarkers underwent analysis for persistent organic pollutants (POPs), like dioxins (PCDD/F/dl-PCB), Per- and polyfluoroalkyl Substances (PFAS), and Polycyclic Aromatic Hydrocarbons (PAH).[9] The analyses were conducted using both bioassays and chemical analyses.

The DR CALUX bioassay® (Dioxin Responsive Chemical Activated Luciferase gene eXpression) was used to quantify dioxins/furans (PCDD/F) and dioxin-like PCBs (DL-PCBs). Results from DR CALUX® are reported in Bioassay Equivalent units, BEQ (pg BEQ/g fat). The term "BEQ" distinguishes results obtained from food samples from those obtained via chemical analysis (Gas Chromatography-Mass Spectrometry GC-MS, pg TEQ/g fat) which are reported in Toxic Equivalence (TEQ) units (pg TEQ/gfat). For non-food biomatrices like mosses or pine needles, results from DR CALUX are expressed in TCDD equivalent per gram of product (TCDD eq./g product) or abbreviated as pg TEQ/g product. The congener of TCDD refers to 2,3,7,8-Tetrachlorodibenzo-p-dioxin, as the most toxic dioxin congener.

Chemical analysis by GCMS is conducted if the BEQ values from DR CALUX exceed the limit of 3.3 pg BEQ/g fat for PCDD/F/dl-PCB or 1.7 pg BEQ/g fat for PCDD/F. This analysis covers 7 dioxins (PCDDs), 10 furans (PCDFs) and 12 dioxin-like polychlorinated biphenyls (DL-PCBs). The maximum limit value for dioxins in eggs is set at 2.5 pg TEQ/g fat for PCDD/F, with the sum of dioxins and dioxin-like PCBs (dl-PCBs) limited to 5 pg TEQ/gram fat).

The action levels for GC-MS analysis of dioxins (PCDD/F) and dioxin-like PCBs (DL-PCBs) in hen eggs, established by 2013/711/EU[10]are set at 1.75 pg TEQ/g fat. See Figure 5. These action levels aid competent authorities and operators in identifying contamination sources and implementing necessary measures for reduction or elimination.

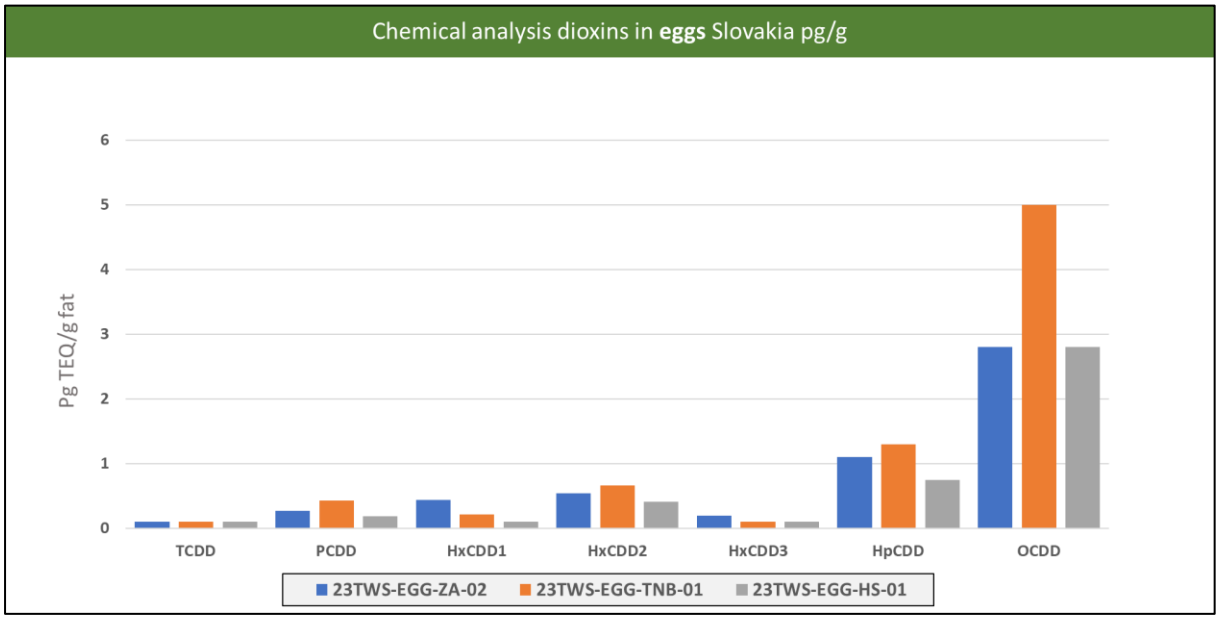
PAH analysis is performed using the PAH CALUX assay, with results expressed in benzo[a]pyrene equivalency (B(a)P). PFAS analyses utilise FITC-T4assay, measuring the binding potency with thyroid hormone thyroxine (T4) and plasma transport protein Transthyretin (TTR) This assay involves fluorescent-labelled thyroxine (FITC-T4), consisting of fluorescein isothiocyanate (FITC) and L-thyroxine (T4), where the measurement is based on fluorescence differences between bound and non-bound FITC-T4 at the TTR-binding site. results from FITC-T4 analysis are reported in µg PFOA equivalent per gram of product (PFPA/g product.

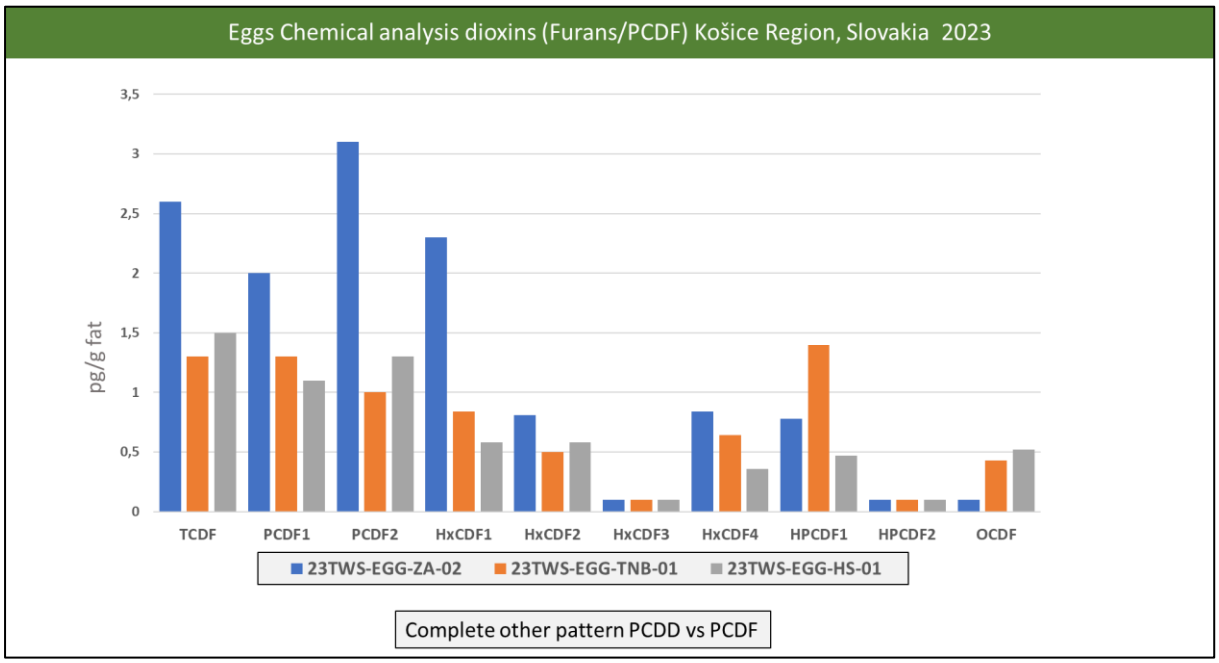
The DR CALUX®, PFAS CALUX®, FITC-T4 and GC-MS analyses on dioxins, are performed by BioDetection Systems, Amsterdam, the Netherlands, accredited under RvA L401.

PFAS chemical analyses were performed on 24 PFAS using LC-LC-MS (A195), PAH with GC-MS/MS and the analyses of heavy metals with ICP-MS (A068+A095) were performed by Normec, Rotterdam NL, the Netherlands.

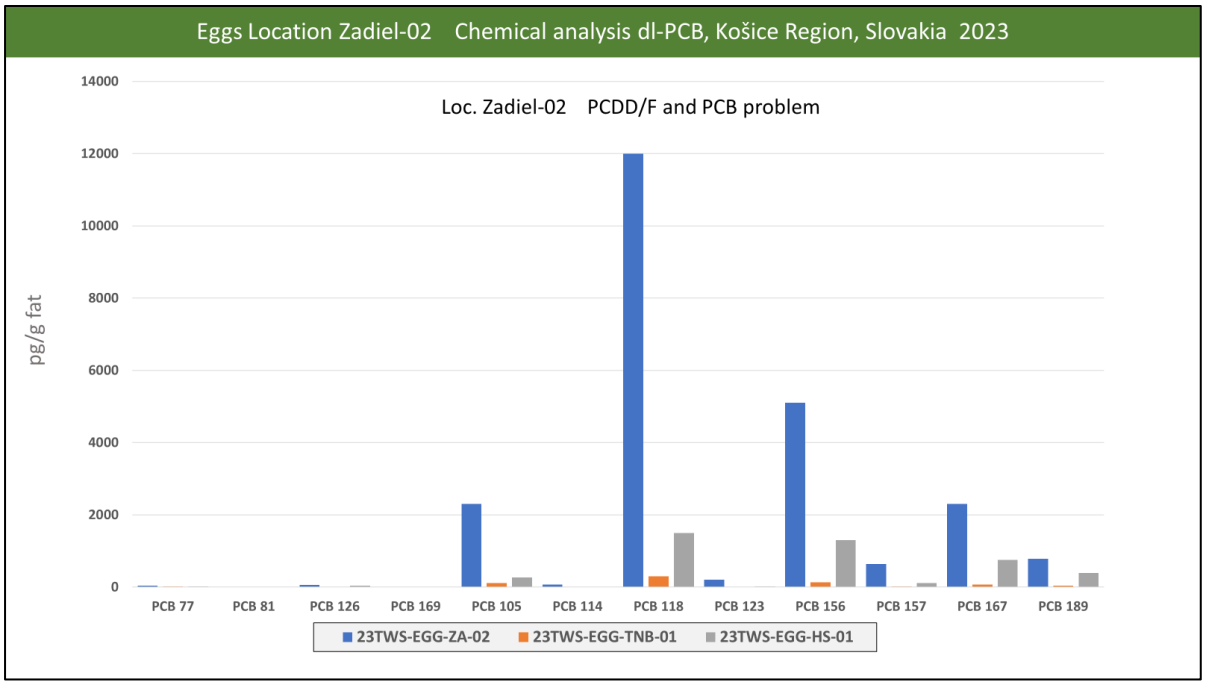
[9] The term POP is used to refer to toxic chemicals that are resistant to degradation processes, travel over long distances, and bioaccumulate in the human body and ecosystems.

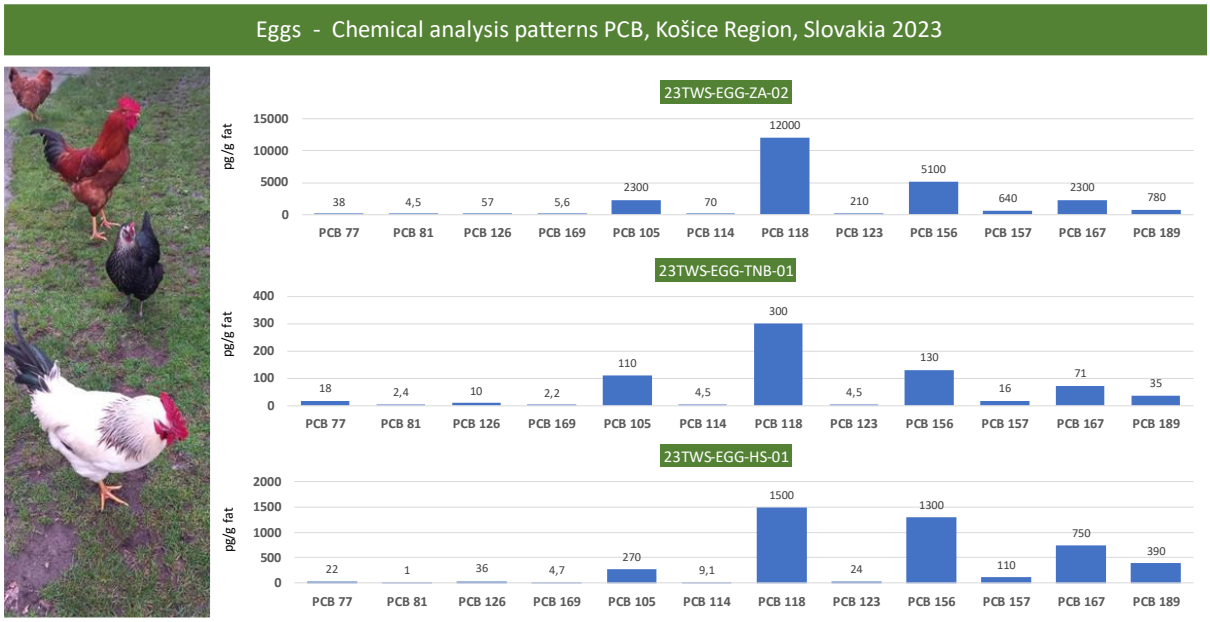
Annexe 2: Results GC-MS analyses on eggs of backyard chicken





Annexe 3: Dl-PCB





Annexe 4: Dioxins and PFAS in eggs

Results Eggs (sampling October-November), Košice Region, Slovakia 2023

Date	Total	Location Village	Biomarker	TW-RF-NR	Analyse	Dioxins DR CALUX (mb) PCDD/F DR CALUX	Dioxins DR CALUX (mb) dl-PCB DR CALUX	Dioxins DR CALUX (mb) PCDD/F/dl-PCB DR CALUX	Dioxins GC-MS (mb) PCDD/F GC-MS-ub	Dioxins GC-MS (mb) dl-PCB GC-MS	Dioxins GC-MS (mb) PCDD/F/dl-PCB GC-MS	PFAS LC-MS/MS ∑ 4 PFAS	PFAS LC-MS/MS ∑ 24 PFAS	Heavy Metals
Sample	loc. / BioMat			2023	Method	1.7		3.3.	2.5		5.0	∑ 4 PFAS	∑ 24 PFAS	14
						medium bound (mb)			1.75	1.75		medium bound (mb)		
Sampling date						pg BEQ (TCDD)/g fat (veg: product)			pg TEQ/g fat (veg: product)			µg / kg - ng/g	µg / kg - ng/g	
30-10-2023	1	Dvornike - Loc. 2		23TWS-Egg-Dv-07	DR CALUX	1,00	0,20	1,20						
		Dvornike - Loc. 2		23TWS-Egg-Dv-07	LC-MS/MS							0,58	1,83	
		Dvornike - Loc. 2 (eggshell)		23TWS-Egg-Dv-07	Heavy Metals									6 HM
30-10-2023	2	Včelâre - Loc. 2		23TWS-Egg-Vc-01	DR CALUX	1,60	0,10	1,70						
30-10-2023	3	Zádiel - Loc. 1		23TWS-Egg-Za-01	DR CALUX	0,65	1,05	1,70						
31-10-2023	4	Zádiel - Loc. 2		23TWS-Egg-Za-02	DR CALUX / GC-MS	3,10	6,70	9,80	2,2	6,6	8,80			
		Zádiel - Loc. 2		23TWS-Egg-Za-02	LC-MS/MS							3,15	4,57	
30-10-2023	5	Turňa nad Bodvou - Loc. 1		23TWS-Egg-TnB-01	DR CALUX / GC-MS	2,70	2,10	4,80	1,3	1,1	2,50			
		Turňa nad Bodvou - Loc. 1		23TWS-Egg-TnB-01	LC-MS/MS							0,98	2,69	
31-10-2023	6	Hosťovce Loc. 1		23TWS-Egg-Hs-01	DR CALUX / GC-MS	2,20	2,50	4,70	1,1	3,9	4,90			
		Hosťovce Loc. 1		23TWS-Egg-Hs-01	LC-MS/MS							0,58	1,83	

						EU regulation (Upperbound - ub)		
						Chemical PFAS (LC-MS/MS)		
TW Indicative scale for Eggs			TW Indicative scale for Eggs			Eggs (1-1-2023)		
DR CALUX			GC-MS			EU Limit	TW Indicative	Heavy Metals
PCDD/F	dl-PCB	(PCDD/F/dl-PCB)	PCDD/F	dl-PCB	(PCDD/F/dl-PCB)	∑ 4 PFAS (EFSA)	∑ 24 PFAS	
pg BEQ / g fat			pg TEQ /g fat			µg / kg - ng/g		
≥ 6.6	≥ 2.5	≥ 10	≥ 7.5		≥ 15.0	≥ 5.1	≥ 5.1	
≥ 3.3	≥ 1.0	≥ 6.6	≥ 5.0		≥ 10.0	≥ 2.4	≥ 2.4	
≥ 1.7	≥ 0.5	≥ 3.3	≥ 2.5	≥ 1.75	≥ 5.0	≥ 1.7	≥ 1.7	
< 1.7	< 0.5	< 3.3	< 2.5	< 1.75	< 5.0	< 1.7	< 1.7	

Annexe 5: Fruit –Dioxins, PAH and PFAS

Sampling October-November, Košice Region , Slovakia 2023

Date	Total	Samples	Location Village	Biomarker	TW-RF-NR	Analyse	Dioxins DR CALUX (mb) PCDD/F DR CALUX	dl-PCB DR CALUX	PCDD/F/dl-PCB DR CALUX	medium bound (mb) PAH PAH CALUX	medium bound (mb) 4 PAH GC-MS/MS	medium bound (mb) 16 PAH GC-MS/MS	medium bound (mb) PFAS LC-MS/MS ∑ 4 PFAS	∑ 24 PFAS
Sample	loc. / BioMat				2023	Method	1.7		3.3.					
							pg BEQ (TCDD)/g fat (veg: product)			Benzo(a)pyrene equivalent ng BaP eq./g product	∑ 4 PAH ng / g	∑ 16 PAH ng / g	medium bound (mb) µg / kg - ng / g	µg / kg - ng/g
	Fruit / Vegetables						FOOD / Fruit/ Apples (Wet Weight / ww)							
30-10-2023	1	Apples (pulp)	Dvornike - Loc. 1		23TWS-APu-Dv01	DR CALUX	0,03	0,03	0,05					
		Apples (pulp)	Dvornike - Loc. 1		23TWS-APu-Dv01	PAH CALUX				0,67				
		Apples (pulp)	Dvornike - Loc. 1		23TWS-APu-Dv01	LC-MS/MS								1,45
		Grape	Dvornike - Loc. 1		23TWS-Grp-Dv01	LC-MS/MS								1,45
		Grape	Dvornike - Loc. 1		23TWS-Grp-Dv01	PAH GC-MS/MS					2,0	19,1		
30-10-2023	2	Apples (pulp)	Dvornike - Loc. 2		23TWS-Apu-Dv02	DR CALUX	0,03	0,03	0,05					
		Apples (pulp)	Dvornike - Loc. 2		23TWS-APu-Dv02	PAH CALUX				0,40				
1-11-2023		Fig	Dvornike - Loc. 2		23TWS_Fig-02-Dv02	LC-MS/MS								1,62
	3	Grape	Včeláre - Loc. 2		23TWS-Grp-Vc01	PAH GC-MS/MS					2,7	32,5		
30-10-2023		Apples (pulp)	Včeláre - Loc. 2		23TWS-APu-Vc03	DR CALUX	0,03	0,03	0,05					
		Apples (pulp)	Včeláre - Loc. 2		23TWS-APu-Vc03	PAH CALUX				0,32				
		Grape	Včeláre - Loc. 2		23TWS-Grp-Vc01	LC-MS/MS								1,54
31-10-2023	4	Apples (pulp)	Turňa nad Bodvou - Loc. 1		23TWS-APu-TnB01	DR CALUX	0,18	0,03	0,21					
		Apples (pulp)	Turňa nad Bodvou - Loc. 1		23TWS-APu-TnB01	PAH CALUX				2,50				
		Apples (pulp)	Turňa nad Bodvou - Loc. 1		23TWS-APu-TnB01	LC-MS/MS								1,45

	TW Indicative scale Vegetation / (Feed) DR CALUX PCDD/F	dl-PCB	(PCDD/F/dl-PCB)	TW Indicative scale PAH CALUX Benzo(a)pyrene equivalent	PAH GC-MS/MS ∑ 4 PAH	TW Ind. Scale PAH GC-MS/MS ∑ 16 PAH	TW Indicative scale PFAS LC-MS/MS ∑ 4 PFAS (EFSA)	∑ 24 PFAS
	pg TCDD eq./g dry weight (dw)			ng BaP eq./g product	ng/g product	ng/g product	µg / kg - ng / g	
	≥ 2.5	≥ 2.5	≥ 3.32	> 500 ng	> 500 ng	> 500 ng	≥ 5,1	≥ 5,1
	≥ 1.0	≥ 1.0	≥ 1.66	> 250 ng	> 250 ng	> 250 ng	≥ 3,4	≥ 3,4
	≥ 0.5	≥ 0.5	≥ 0.83	≥ 100 ng	≥ 100 ng	≥ 100 ng	≥ 1.7	≥ 1.7
	< 0.5	< 0.5	< 0.83	≥ 10 ng	≥ 10 ng	≥ 10 ng	< 1,45	< 1,45
				< 10 ng	< 10 ng	< 10 ng	< 1,45	< 1,45

Annexe 6: Pine needles - Dioxins, PAH, and Heavy Metals

Re+R20+E2:R29+R20+E2:R29+E2:R30+E2:R32+E2:R30+R20+E2:R29														
							Dioxins DR CALUX (mb)			medium bound (mb)	medium bound (mb)			
Date	Total	Samples	Location Village	Biomarker	TW-RF-NR	Analyse	PCDD/F	dl-PCB	PCDD/F/dl-PCB	PAH	4 PAH	16 PAH		
							DR CALUX	DR CALUX	DR CALUX	PAH CALUX	GC-MS/MS	GC-MS/MS	Heavy Metals	
Sample	loc. / BioMat.				2023	Method	1.7		3.3.					
							DR CALUX (dw), 88 %			Benzo(a)pyrene equivalent	∑ 4 PAH	∑ 16 PAH	14	
							pg BEQ (TCDD)/g fat (veg: product)			ng BaP eq./g product	ng / g	ng / g		
		Vegetation Pine needles												
30-10-2023	1	Pine needles - *Picea abies*	Dvornike - Loc. 1		23TWS-PN-Dv01	DR CALUX	0,22	0,56	0,77					
		Pine needles - *Picea abies*	Dvornike - Loc. 1		23TWS-PN-Dv01	PAH CALUX				2,16				
		Pine needles - *Picea abies*	Dvornike - Loc. 1		23TWS-PN-Dv01	PAH GC-MS/MS					14,1	60,1		
30-10-2023	2	Pine needles - *Picea abies*	Včeláre - Loc. 2		23TWS-PN-Vc01	DR CALUX	1,29	1,56	2,85					
		Pine needles - *Picea abies*	Včeláre - Loc. 2		23TWS-PN-Vc01	PAH CALUX				0,79				
		Pine needles - *Picea abies*	Včeláre - Loc. 3 (near CK)		23TWS-PN-Vc02	Heavy Metals							14	
31-10-2023	3	Pine needles - *Picea abies*	Zádiel - Loc. 1		23TWS-PN-Za01	DR CALUX	0,61	0,92	1,52					
		Pine needles - *Picea abies*	Zádiel - Loc. 1		23TWS-PN-Za01	PAH CALUX				0,08				
31-10-2023	4	Pine needles - *Picea abies*	Hosťovce - Loc. 2 (sheep)		23TWS-PN-Hs02	DR CALUX	1,28	1,58	2,86					
		Pine needles - *Picea abies*	Hosťovce - Loc. 2 (sheep)		23TWS-PN-Hs02	PAH CALUX				0,56				
		Pine needles - *Picea abies*	Hosťovce - Loc. 2 (sheep)		23TWS-PN-Hs02	Heavy Metals							14	
							TW Indicative scale Vegetation / (Feed)			TW Indicative scale Results		TW Indicative scale	TW Indicative scale	
							DR CALUX			PAH CALUX	PAH GC-MS/MS	PAH GC-MS/MS	Heavy Metals	
							PCDD/F	dl-PCB	(PCDD/F/dl-PCB)	Benzo(a)pyrene equivalent	∑ 4 PAH	∑ 16 PAH	mos/veg	
							pg TCDD eq./g dry weight (dw)			ng BaP eq./g product	ng/g product	ng/g product		
							≥ 2.5	≥ 2.5	≥ 3.32	> 500 ng	> 500 ng	> 500 ng		
							≥ 1.0	≥ 1.0	≥ 1.66	> 250 ng	> 250 ng	> 250 ng		
							≥ 0.5	≥ 0.5	≥ 0.83	≥ 100 ng	≥ 100 ng	≥ 100 ng		
							< 0.5	< 0.5	< 0.83	≥ 10 ng	≥ 10 ng	≥ 10 ng		
										< 10 ng	< 10 ng	< 10 ng		

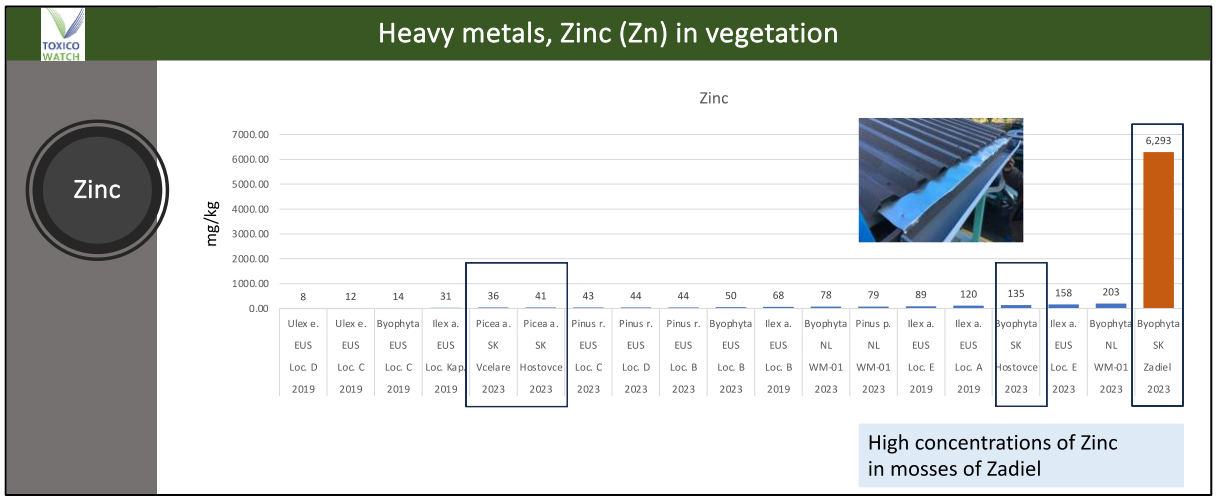
Annexe 7: Results Mosses

The indicative colour bars provided by ToxicoWatch serve as a reference scale. Mosses and pine needles are expressed in 88% dry weight and medium bound (MB).

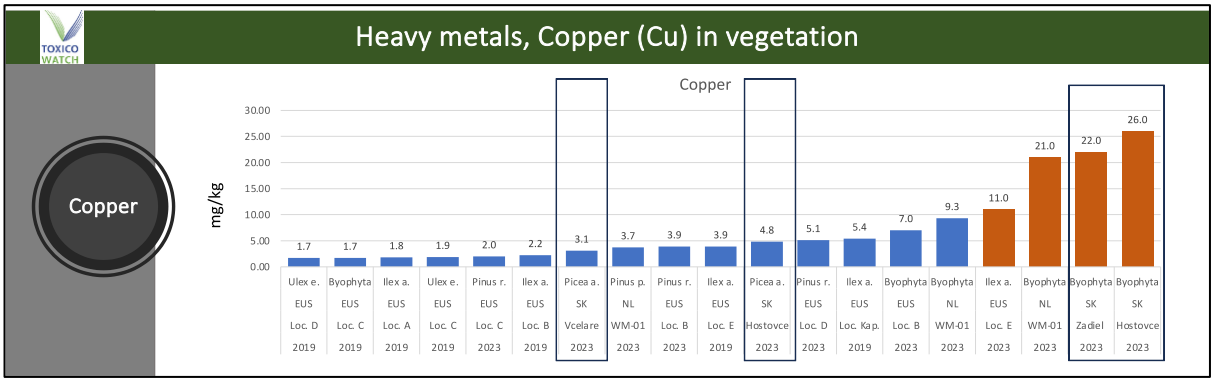
Results (sampling October-November), Košice Region , Slovakia 2023													
							Dioxins DR CALUX (mb)			medium bound (mb)	medium bound (mb)		
Date	Total	Samples	Location Village	Biomarker	TW-RF-NR	Analyse	PCDD/F	dl-PCB	PCDD/F/dl-PCB	PAH	4 PAH	16 PAH	
							DR CALUX	DR CALUX	DR CALUX	PAH CALUX	GC-MS/MS	GC-MS/MS	Heavy Metals
Sample	loc. / BioMat.				2023	Method	1.7		3.3.				14
							pg BEQ (TCDD)/g fat (veg: product)			Benzo(a)pyrene equivalent ng BaP eq./g product	∑ 4 PAH ng / g	∑ 16 PAH ng / g	
		Vegetation / Mosses					FEED / Vegetation /Mosses (Medium bound (mb), 88% Dry Weight/ (dw))						
30-10-2023	1	Mosses Roof	Dvornike - Loc. 1		23TWS-Mos-Dv01	DR CALUX	18,32	5,45	23,76				
		Mosses Roof	Dvornike - Loc. 1		23TWS-Mos-Dv01	PAH CALUX				371,29			
		Mosses Roof	Dvornike - Loc. 1		23TWS-Mos-Dv01	PAH GC-MS/MS					186	516,6	
31-10-2023	2	Mosses ground	Dvornike / Hill		23TWS-Mos-Dv03	DR CALUX	3,08	0,24	3,32				
		Mosses ground	Dvornike / Hill		23TWS-Mos-Dv03	PAH CALUX				355,45			
		Mosses ground	Dvornike / Hill		23TWS-Mos-Dv03	PAH GC-MS/MS					32,6	95,3	
30-10-2023	3	Mosses Roof	Včeláre - Loc. 2		23TWS-Mos-Vc01	DR CALUX	5,05	1,39	6,43				
		Mosses Roof	Včeláre - Loc. 2		23TWS-Mos-Vc01	PAH CALUX				4684,68			
		Mosses Roof	Včeláre - Loc. 2		23TWS-Mos-Vc01	PAH GC-MS/MS					117	313,6	
		Mosses Roof	Včeláre - Loc. 2		23TWS-Mos-Vc01	PAH GC-MS/MS					88,2	232,9	
30-10-2023	4	Mosses Roof	Zádiel - Loc. 1		23TWS-Mos-Za01	Heavy Metals							14
31-10-2023	5	Mosses Roof	Zádiel - Loc. 2		23TWS-Mos-Za02	DR CALUX	10,70	0,09	10,78				
		Mosses Roof	Zádiel - Loc. 2		23TWS-Mos-Za02	PAH CALUX				1016,04			
		Mosses Roof	Zádiel - Loc. 2		23TWS-Mos-Za02	PAH GC-MS/MS					312	885,3	
31-10-2023	6	Mosses Roof	Hosťovce - Loc. 2 (sheep)		23TWS-Mos-Hs02	DR CALUX	14,42	4,57	18,99				
		Mosses Roof	Hosťovce - Loc. 2 (sheep)		23TWS-Mos-Hs02	PAH CALUX				432,69			
		Mosses Roof	Hosťovce - Loc. 2 (sheep)		23TWS-Mos-Hs02	Heavy Metals							14
31-10-2023	7	Mosses Roof	Hosťovce - Loc. 1		23TWS-Mos-Hs01	PAH GC-MS/MS					303	815,3	
		Mosses Roof	Hosťovce - Loc. 1		23TWS-Mos-Hs01	PAH GC-MS/MS					423	1065,9	
							TW Indicative scale Vegetation / (Feed)			TW Indicative scale Results		TW ind. Scale	TW ind. Scale
							DR CALUX			PAH CALUX	PAH GC-MS/MS	PAH GC-MS/MS	Heavy Metals
							PCDD/F	dl-PCB	(PCDD/F/dl-PCB)	Benzo(a)pyrene equivalent	∑ 4 PAH	∑ 16 PAH	
							pg TCDD eq./g dry weight (dw)			ng BaP eq./g product	ng/g product	ng / g	mos/veg
							≥ 2.5	≥ 2.5	≥ 3.32	> 500 ng	> 500 ng	> 500 ng	
							≥ 1.0	≥ 1.0	≥ 1.66	> 250 ng	> 250 ng	> 250 ng	
							≥ 0.5	≥ 0.5	≥ 0.83	≥ 100 ng	≥ 100 ng	≥ 100 ng	
							< 0.5	< 0.5	< 0.83	≥ 10 ng	≥ 10 ng	≥ 10 ng	
										< 10 ng	< 10 ng	< 10 ng	

Annexe 8: Heavy metals

A high amount of Zinc is found in mosses in Zádiel. Maybe this is from zinc-coated (electroplated) gutters or roof-plates. Although Zinc is essential for life, too much Zinc ingestion can result in nausea, vomiting, and diarrhoea.



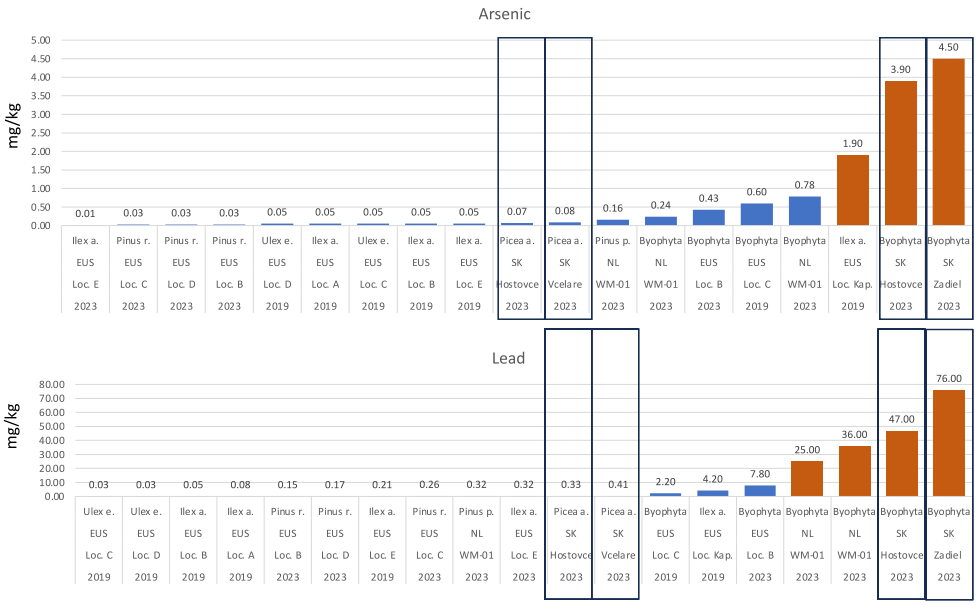
High levels of copper are found in the mosses of Hosťovce and Zádiel, resp. 26 and 22 mg/kg.



Heavy metals, Arsenic (As) and Lead (Pb) in vegetation

Arsenic

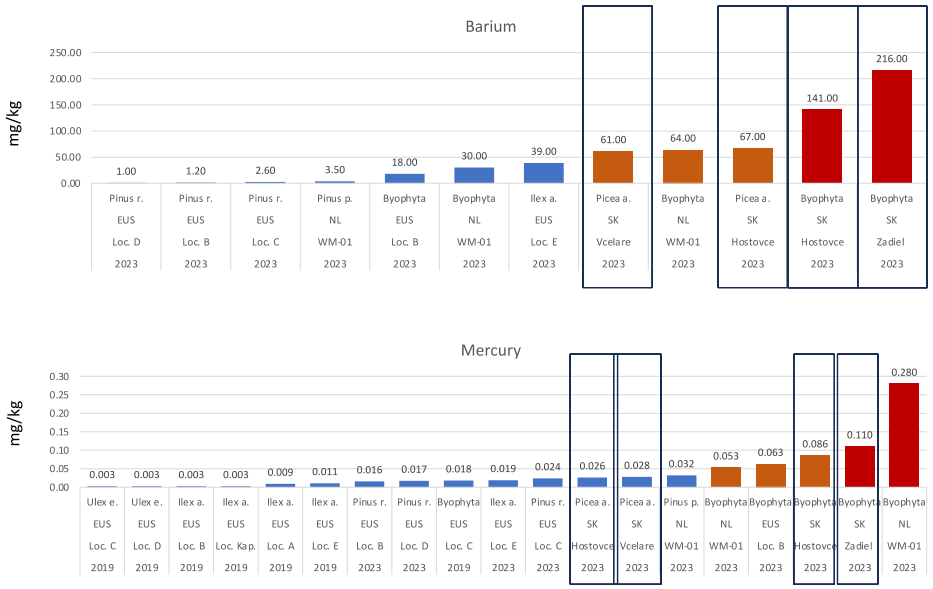
Lead



Heavy metals, Barium (Ba) and Mercury (Hg) in vegetation

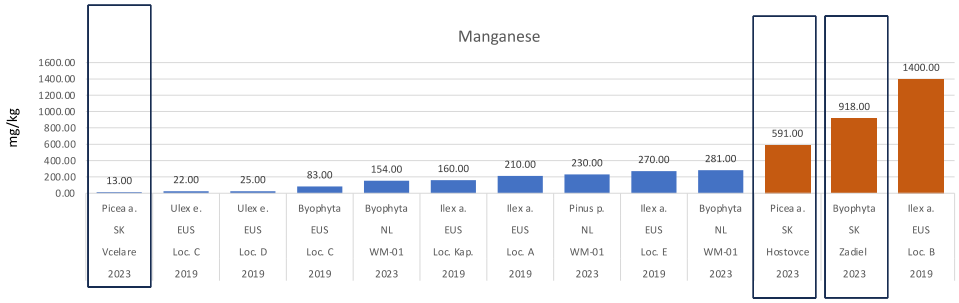
Barium

Mercury

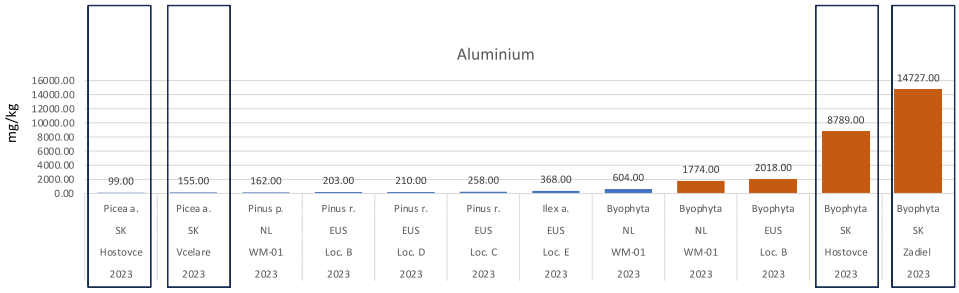


Heavy metals, Manganese (Mn) and Aluminium (Al) in vegetation

Manganese

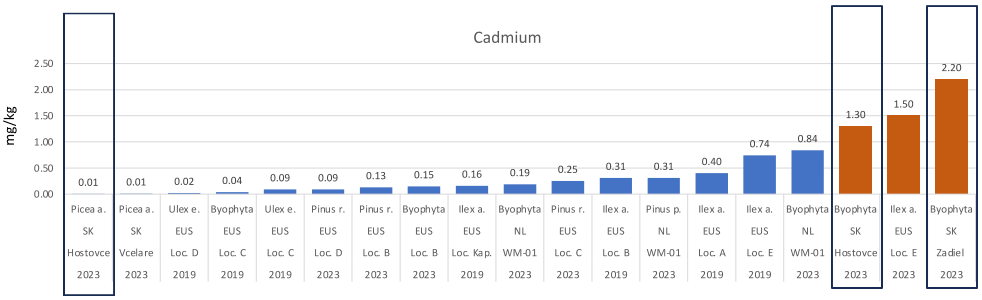


Aluminium

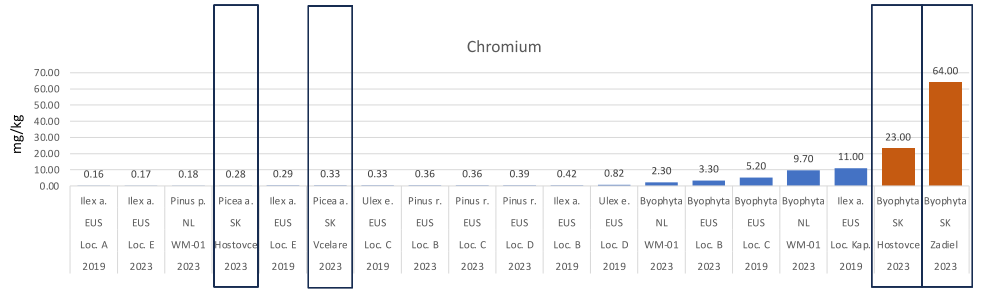


Heavy metals, Cadmium (Cd) and Chromium (Cr) in vegetation

Cadmium

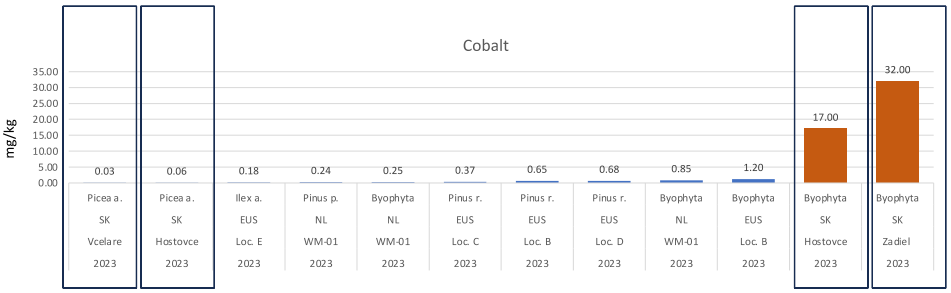


Chromium



Heavy metals, Cobalt (Co) and Nickel (Ni) in vegetation

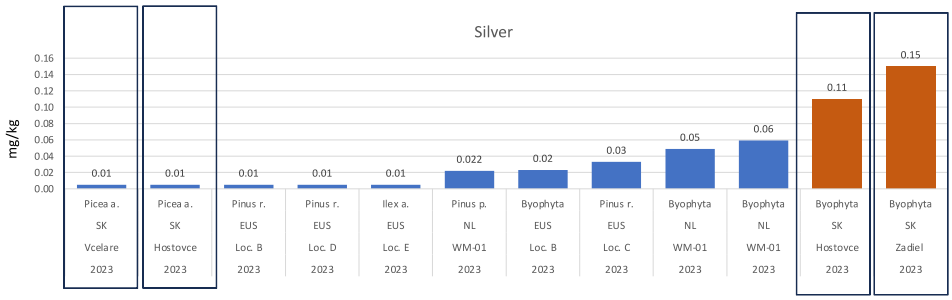
Cobalt



Nickel

Heavy metals, Tin (Sb) and Silver (Ag) in vegetation

Silver



Tin